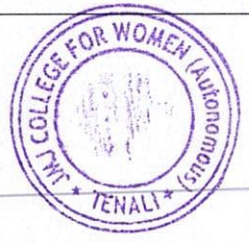
3.4.3

Full Length Research Papers Published in the Journals Notified on UGC Care List

2020 - 21

2020-2021						
30.	Geometri Feature extraction for detacting carcinoma in three dimensional MR images through mechine learning algorithms	Sr.F.Amul Mary	Computer Science	Advances in Computational and Bio Engineering Learning and Analytics in Intelligent Systems	Aug.2020	DOI:10.1007/978-3-030-46943-6_45
31.	Pivotal Role of Supramolecular interactions towards the stebility of Na -1, 2-bis (tetrazol – 5yL) ethane coordination polymer	A.Revathi	Computer Science	Journal of Molecular Structure	Oct.2020	1226 (2021) 129376
32.	Antioxidant antimicrobial DNA binding and cleavage studies of novel Co (II), Ni (II), and cu(II) complexes of N, O donar Schiff basis: Synthasis and spectral characterization	Dr.V.Sumalatha	Chemistry	Journal of Molecular Structure	Nov.2020	**ISSN:** 129606
33.	Sustainable Eco-Friendly Polymer: Applications and Future Scopes	Suresh Undavally	Botany	ITJ of Multidisciplinary Educational Research (Peer Reviewed andReferred Journal)	Nov.2020	ISSN 2277 – 7881
34.	Digital Marketing - An Intermediary to Commerce and Digital Technologies	Dr.G.Sudhakaraiah& Dr. M.Sambasivudu	Commerce	Psychology and Education Journal	Nov.2020	ISSN: 00333077



35.	Review on Indian English Language and Literature	Dr.N.Vimala Devi,	English	Journal of Information and Computational Science	Nov. 2020	ISSN 1548-7741
36.	Biodegradable Super Absorbent Nano Polymer properties and its Applications	Suresh Vundavalli	Botany	International Journal of Agriculture and Environmental Science	Dec.2020	ISSN 2394-2569
37.	Karshakuni Sowseelyamu	Dr.B.Mary Kumari	Telugu	IOSR Journal of Humanities and Social Science	Jan.2021	ISSN 2279-0837
38.	Development and Validation of Stability indicating rp – hpie method for quantitative estimation of levofloxacin injection 5 mg /ml dosage form	Dr. K.Geetha Bhavani	Chemistry	Currect Trends in Biotechnology and Pharmacy	Jan.2021	ISSN: 0973-8916 (P)
39.	Fungal Alkaline Proteases: Applications and Properties	Dr.Ch.Sarojini	Zoology	International Educational and Research Journal IERJ	Jan.2021	E-ISSN: 2454-9916
40.	A case study on infection of trematodes pathogenicity in wild channastriata Collected from various fresh water ponds in	Mrs. M. Aruna	Zoology	International Journal of Fisheries and Aquatic Studies	Feb.2021	ISSN: 2347 -5129 (o)

	Guntur Dt. Rural, semi rural and urban areas					
41.	Max 30100 / 30102 Sensor Implementation to Viral Infection Detection based on Sp 0.2 and Heartbeat Patte	Mrs. A.Asha Priya Darsini	Computer Science	Annals of RSCB	Feb.2022	ISSN: 1583-6258
42.	Water Pollution: Psycgological effect on Human Health & Live Reporting Using IoT Technology	Mrs.CM Anitha	Physics	Turkish Journal of Computer and Mathematics Education	Feb.2021	ISSN: 2847-2852
43.	Bio accumulation of lead and Cadmium and its impact on fresh water cat fish, heteropneustesfossilis	Dr.P.Bujjamma	Zoology	International journal of Creative Research Thought	Feb.2021	ISSN 29-05-2024
44.	Percentage Concentration of Nucleotides in Genome Data of SARS-CORONA Viruses	Sr. Amul Mary	Computer Sciences	International Journal of Advanced Research in Engineering and Technology	Feb.2021	ISSN 0976-6480 Print 0976-6499 Online
45.	Advanced ICT Tools for Implementation of the Complex Calculative Study	Mrs. N.Devi Priya	Computer Science	IT in Industry	Mar.2021	ISSN: 2204 -0595 (P)
46.	Using of Technology to Improve Communication and Soft Skills of ESL learners	Dr.Sr.Shiny K.P	English	Research Journal of English	Mar.2021	ISSN 2456-2696
47.	Solving Knapsack Problem By using Super-Increasing Sequence Method	K.Subbanna	Mathematics	Buttetin Monumental	Mar.2021	0007-473X

48.	Ground Water Quality Analysis of Srikakulam District Andhra Pradesh, India for Domestication and Agricultural Practice	Suresh Vundavalli	Botany	Turkish Online Journal of Qualitative Inquiry(TOJQI)	Mar.2021	ISSN: 767-781
49.	Exploration of Alienated Self in Thrity Umrigar's If Today Be Sweet	S. Mary Sophia Rani	English	International Journal of Advances in Engineering and Management (IJAEM)	May.2021	2395-5252
50.	Evaluation of anti-inflammatory activities of isorhamnetin 3-O-α-L-(6''-E-p-coumaroyl)-rhamnoside isolated from indigofera tinctoria	M.Sathiyaseelan	Chemistry	Research J. Pharm. and Tech	Jan.2020	0974-3618 (p); 0974-360X (e)
51.	Investigation on Co(II), Ni(II), Cu(II) and Zn(II) complexes derived from Quadridentate salen-type schiffbase:Structure , characterization , DNA interactions, anti oxidant , proficiency and biological evaluation	Ms. V. Sumalatha	Chemistry	Chemical Data Collection	May.2020	https://doi.org/10.1016/j.cdc.2020.100434

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Geometric Feature Extraction for Detecting Carcinoma in Three Dimensional MR Images Through Machine Learning Algorithms

F. Amul Mary and S. Jyothi

Abstract Quantitative analysis of Anatomical structures in CT scan and MRI scan images using Artificial Intelligence (AI) techniques requires extraction of physical features, be it geometric or parametric, pertaining to Volumes and shapes of some parts of the human body. Anatomical features of a human body could be visualized as spatial distributions of basic geometric features like points, straight lines, curves, surfaces, textures and skeleta. To detect superficial and volumetric features of three dimensional images are always been a difficult task. There is certain amount of progress made in this area and found in the literature. The above said problem is addressed in this paper and some of the Machine Learning Algorithms are proposed to detect edges and skeletal of three dimensional images. These features lead to detection of tumours, either benign or malignant [1, 2].

Keywords Machine learning algorithm · 3D image processing · Feature extraction · 2.5D processing · 3D processing

1 Introduction

Processing three dimensional images has always been a subject of interest owing to various requirements in medical imaging, subsurface seismic imaging, solid modeling, and Computer Aided Design. Spectral domain techniques and spatial domain techniques are the two major approaches adopted in solving problems related to 3-D image processing, pattern recognition and analysis and 3-D printing, to name a few. Recently there has been an urge to make use of Artificial Intelligence and Machine Learning in processing and classification of medical images. More precisely, 3-D medical images like MR and CT scan images are being analysed for locating tumors

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in various parts of the human body. Moreover, it has become a dire necessity to judge whether a tumor is benign or malignant. By the time it is concluded that the tumor is malignant, the patient goes to the fourth stage of cancer and it turns out to be a terminal disease for the patient. With advancement in basic sciences and technology, it should be possible to predict well in advance the possibility of a tumor turning out to be malignant. In such cases, Artificial Intelligence and Machine Learning techniques have been found to be more reliable, provided data available is genuine.

In this context, it is imperative to precondition image data so that such predictive techniques work with a high level of credibility and robustness. All these amount to saying that the inputs to be given to the AI (Artificial Intelligence) engine are all genuine. An AI engine admits two types of inputs (i) correlated data and (ii) raw and uncorrelated data. Textual data need not be correlated, whereas spatial information data like multidimensional images are highly correlated. It should be noted that an image could also be represented as knowledge, which could be uncorrelated like a csv (comma separated values) file.

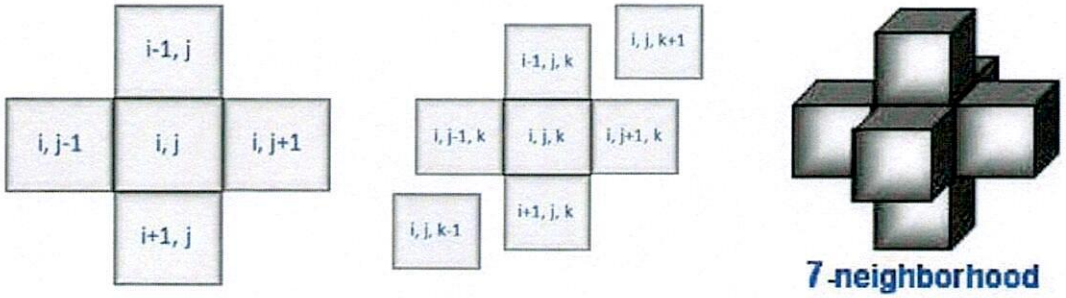
This paper focuses on developing techniques for detecting geometric features like edges and skeletal forms in medical images, especially in MR images. Quantificational measures of these geometric features are used for prediction purposes. Section 2 describes 2-D and 3-D algorithms for detecting edges and skeletal forms. Section 3 demonstrates the application of the algorithms on a real time MR image data called "Cetautomatix". Section 4 provides statistical parametric values of the original MR image and other processed versions.

2 Algorithms for Extracting 2-D and 3-D Edges and Skeleta

As outlined earlier, any AI based machine learning algorithm meant for data analysis or classification would invariably use features of the data under investigation. Textual data, be it a raw data or a formatted data, is classified based on certain parametric features like distance metric, eigen values, principal components and statistical details. Spatial data like images which are totally neighbourhood correlated are classified based on geometric features and their quantificational measures. Alternatively, image data can also be viewed as uncorrelated textual data known as knowledge, which could be used by classifiers.

This paper focuses on extraction of geometric features from 2-D and 3-D image data. Edges and skeletal forms are two fundamental geometric features and their measures are used by various classifiers.

2-D edge detection involves identification of edge pixels having maximum gradients [3, 4, 5]. The given 2-D digital image is segmented by threshold based quantization scheme. Then the boundaries of quantized regions are extracted [6, 7]. Similarly, 3-D edge detection involves identification of edge voxels having maximum gradients. The 3-D digital image is segmented by a threshold based quantization scheme.



2-D five neighbourhood | 3-D seven neighbourhood | 3-D 7-neighbourhood voxels

Fig. 1 2-D and 3-D scanning windows

At that moment boundaries of the regions are extracted. On the other hand, skeletonization is a boundary removal process, which is the complementary operation of edge detection.

The 2-D and 3-D scanning windows used for the purpose of edge detection and skeletonizationare shown in Fig. 1.

2-D edge detection algorithm

Gray images are the ones whose RED, GREEN and BLUE components of a pixel are of the same value and this algorithm addresses processing of 2-D gray images [8, 9, 10, 11].

Algorithm

At every scanning position, the 3 × 3 sub image spanned by the five neighbourhood scanning window is checked. Maximum and minimum values among the five pixels are found. The gray distance D is calculated by subtracting the minimum value from the maximum value. D is compared with a user defined threshold T, which decides the segmentation of the image. A machine learning algorithm could evaluate T which is image dependent. If D is less than or equal to T then the central pixel value is assigned the value '0'. If D is greater than T then the scanning window is moved to the next pixel. By doing this the boundary of the sub image covered by the 2-D window is detected. This procedure is repeated for every pixel in the image. The overall effect is edge detection of the image.

The pseudocode is given below

Input: 2-D image, user defined threshold is T.
Output: Edge detected version of 2-D image.
Steps:

Step 1: Read 2-D data and place pixel values in 1-D array called *input_array.*

Step 2: Copy *input_array* to *output array.*

Step 3: Repeat sliding the 5-neighbourhood window over the image (*input_array*)

{

Step 3(a): find the Maximum Gray value *GMax;*

Step 3(b): find the Minimum Gray value *GMin;*

Step 3(c): find the difference D = *GMax – GMin;*

Step 3(d): if (D <= threshold) then assign zero value to the central pixel in

output_array else slide the 5-neighbourhood

} until the scanning window spans the whole image.

Step 4: Pass the *output array* to display.

2-D Skeletonization Algorithm

Edge detection is boundary detection, where as skeletonization is boundary removal operation [12, 13].

Algorithm

At every scanning position, the 3 × 3 sub image spanned by the five neighbourhood scanning window is checked. Maximum and minimum values among the five pixels are found. The gray distance D is calculated by subtracting the minimum value from the maximum value. D is compared with a user defined threshold T, which decides the segmentation of the image. A machine learning algorithm could evaluate T which is image dependent. If D is less than or equal to T then all the five boundary pixel values are assigned the value '0'. If D is greater than T then the scanning window is moved to the next pixel. By doing this the boundary of the sub image covered by the 2-D window is deleted. This procedure is repeated for every pixel in the image. The overall effect is one boundary removal of the image. Repeated application of this procedure causes removal of all boundaries that yield 2-D skeletal form.

The pseudocode is given below

Input: 2-D image, user defined threshold is T.
Output: Skeletal form of 2-D image

Steps:

Step 1: Read the 2-D data and place pixel values in 1-D array called *input_array*

Step 2: copy *input_array* to *output_array*

Step 3: Repeat sliding the 5-neighbourhood window over the image (*input_array*)

{

Step 3(a): find the Maximum Gray value GMax;

Step 3(b): find the Minimum Gray value GMin;

Step 3(c): find the difference D = GMax-GMin;

Step 3(d): if(D <= threshold) then assign zero value to all five boundary pixels in *output_array* else slide the 5-neighbourhood

} until the scanning window spans the whole image

Step 4: subtract *output_array* from *input_array*

Step 4(a): if results of subtraction is not '0'
then copy *output_array* and repeat step1 to step 4
else *output_array* is treated as output image.

Step 5: Pass the *output_array* to display

3-D edge detection algorithm

MR images are gray images and hence RED, GREEN and BLUE components of a voxel are of the same value and this algorithm addresses processing of 3-D gray images [14, 15, 16, 17, 18].

Algorithm

At every scanning position, the 3 × 3 × 3 sub image spanned by the seven neighbourhood scanning window is checked. Maximum and minimum values among the seven voxels are found. The gray distance D is calculated by subtracting the minimum value from the maximum value. D is compared with a user defined threshold T, which decides the segmentation of the 3-D image. A machine learning algorithm could evaluate T which is image dependent. If D is less than or equal to T then all the seven voxel values are assigned the value '0'. If D is greater than T then the scanning window is moved to the next voxel. By doing this the boundary of the sub image covered by the 3-D window is detected. This procedure is repeated for every voxel in the image. The overall effect is edge detection in the 3-D image.

The pseudocode is given below

Input: 3 - D image, user defined threshold is T
Output: Edge detected version of 3-D image

Steps:

Step1: Read the 3-D data and place the voxel values in 1-D array called *input_array*

Step 2: Copy the *input_array* to *output_array*

Step 3: Repeat sliding the 7-neighbourhood window over the image (*input_array*)

{

Step 3(a): Find the Maximum Gray value GMax;

Step 3(b): Find the Minimum Gray value GMin;

Step 3(c): Find the difference D = GMax – GMin;

Step 3(d): if(D <= threshold) then assign zero value to all seven cells in *output_array;* else slide the 7-neighbourhood

} until the 3-D window spans the entire 3-D image

Step 4: Pass the *output_array* to *Volume Renderer ( )* method.

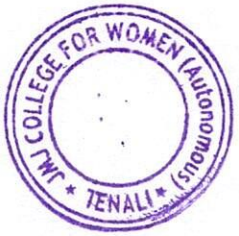
3-D skeletonization algorithm

3-D Edge detection is boundary detection, where as 3-D skeletonization is boundary removal operation.

Algorithm

At every scanning position, the 3 × 3 × 3 sub image spanned by the seven neighbourhood scanning window is checked. Maximum and minimum values among the seven voxels are found. The gray distance D is calculated by subtracting the minimum value from the maximum value. D is compared with a user defined threshold T, which decides the segmentation of the image. A machine learning algorithm could evaluate T which is image dependent. If D is less than or equal to T then all six boundary voxel values are assigned the value '0' but the central voxel value is retained. If D is greater than T then the scanning window is moved to the next voxel. By doing this the boundary of the sub image covered by the 3-D window is deleted and central voxel retained. This procedure is repeated for every voxel in the image. The overall effect is one boundary removal of the image. Repeated application of this procedure causes removal of all boundaries that yield ultimate 3-D skeletal form.

The pseudocode is given below



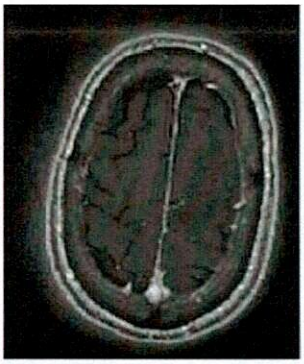
Input: 3-D image, user defined threshold is T
Output: Skeletal form of 3-D image.
Steps:

Step 1: Read the 3-D image and place voxel values in 1-D array called *input_array;*
Step 2: Copy *input_array* to *output_array;*
Step 3: Repeat sliding the 7-neighborhood window over the image(*input_array)* {
Step 3(a): find the Maximum Gray value *GMax*;
Step 3(b): find the Minimum Gray value *GMin*;
Step 3(c): find the difference D = *GMax -GMin*;
Step 3(d): if(D <= threshold)then assign zero value to all six boundary voxels in *output_array* and retain central voxel value;
else slide the 7-neighborhood
} until the scanning window spans the whole image
Step 4: Subtract *output_array* from *input_array*
Step 4(a): if result of subtraction is not '0',
then copy *output_array* to *input_array* and repeat **Step 1** to **Step 4**
else *output_array* is treated as output image
Step 5: Pass the *output_array* to Display

For example, let us consider the 3-D edge detection algorithm and apply it on a sample MR brain image shown in Fig. 2. The processed image clearly shows the brain tumor at the centre of the brain.

In practice, 2-D algorithms are applied on a 3-D image slice by slice and the processed slices assembled to present it as a 3-D image. The purpose of this paper is to demonstrate the usefulness of applying 3-D algorithms on a 3-D image in one

MR image: Brainix
Source: osirix.com
Width: 256
Height: 256
Depth: 256
Image type: Gray image
MR System: 2 Tesla GE MRI
Patient: Unknown
Preprocessing: Sectioned
Diagnosis: Brain tumor
Nature: Unknown
Result: Possibly brain tumor


MR image of a human brain


Tumor detected at the centre

Fig. 2 Tumor detected using 3-D edge detection algorithm

go and present it a solid model. A real time MR image called "cetautomatix" is used for verifying the algorithms given in Sect. 2 of this paper.
MR data details: MR Image
Name: Cetautomatix;
Source: osirix.com
Width: 256
Height: 256
Depth: 114,

3 Feature Extraction of 3-D images Using 2-D and 3D Algorithms

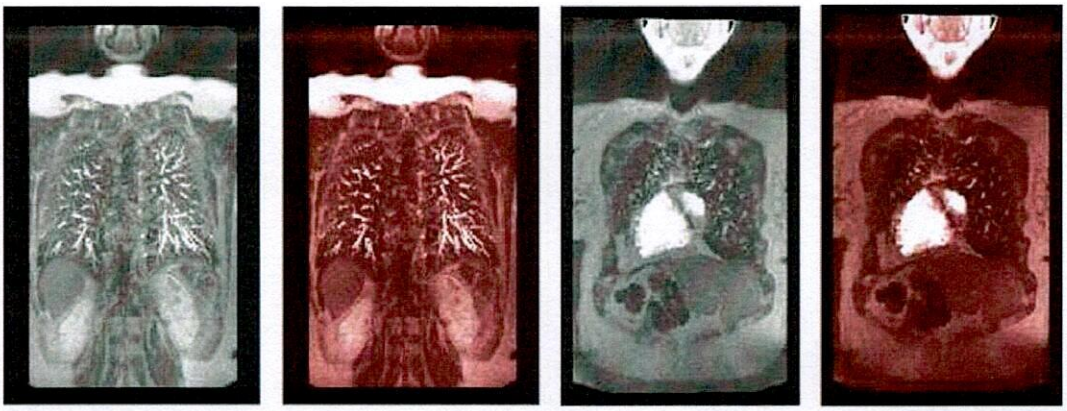
2.5-D Processing of cetautomatix image
Figure 3 shows the original MR image of cetautomatix and its different views both in gray and color.

2-D edge detection algorithm is applied on this image and the results shown in Fig. 4

2-D skeletonization algorithm is applied on this image and the results are shown in Fig. 5.

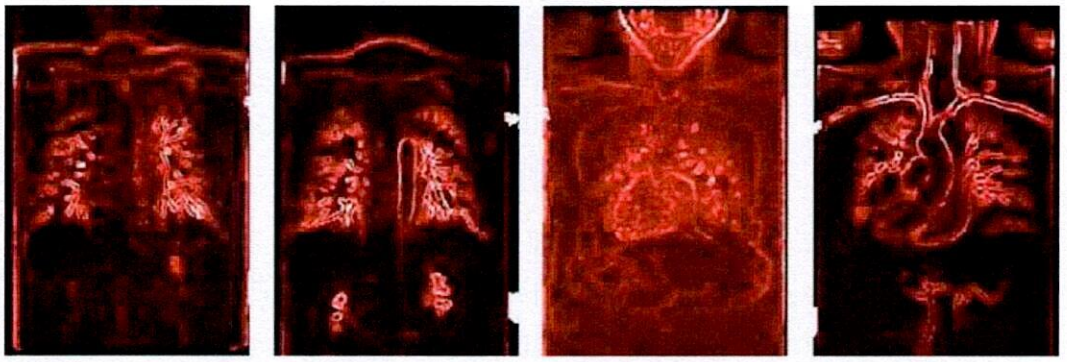
3-D Processing of cetautomatix image
3-D edge detection algorithm is applied on the image shown in Fig. 3 and the results shown in Fig. 6.

3-D skeletonization algorithm is applied on the image shown in Fig. 3 and the results shown in Fig. 7.



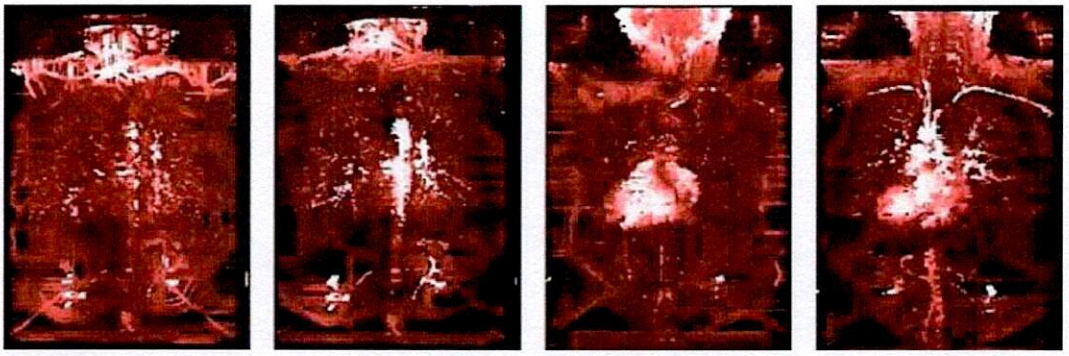
Gray Image (Front View) | Color Image (Front View) | Gray Image (Rear View) | Color Image (Rear View)

Fig. 3 MR image cetautomatix and different views



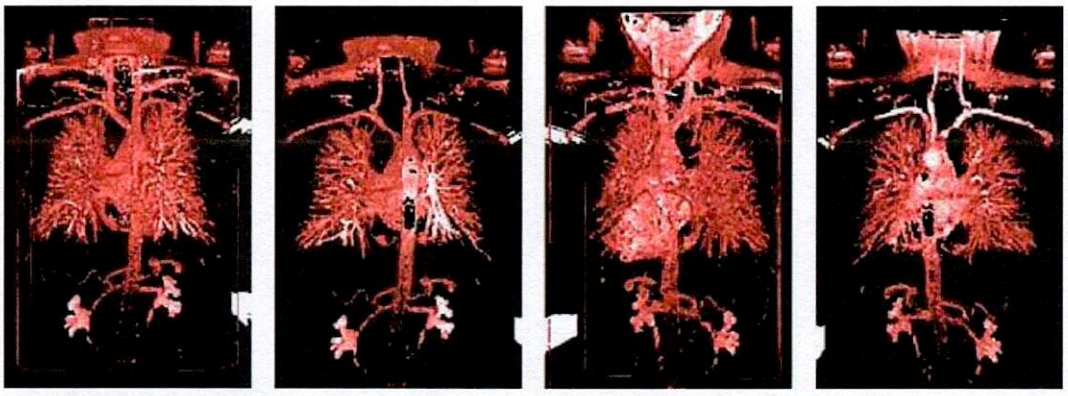
2.5D contour Front view (T=40) | 2.5D contour Front view (40-100) | 2.5D contour Rear view | 2.5D contour Rear view (40-100)

Fig. 4 2.5D edge detected cetautomatix image



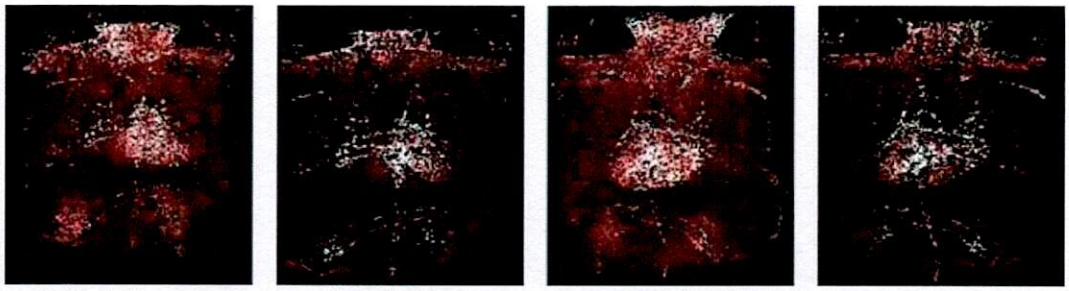
2.5D skeleton Front view (T=40) | 2.5D skeleton Front view (40-100) | 2.5D skeleton Rear view | 2.5D skeleton Rear view (40-100)

Fig. 5 2.5D skeletonized cetautomatix image



3D contour Front view (T=40) | 3D contour Front view (40-100) | 3D contour Rear view | 3D contour Rear view (40-100)

Fig. 6 3-D edge detected cetautomatix image



3D skeleton Front view (T=40) | 3D skeleton Front view (40-100) | 3D skeleton Rear view | 3D skeleton Rear view (40-100)

Fig. 7 3-D skeletonized cetautomatix image

4 Comparative Analysis of 2.5-D and 3-D Feature Extraction Algorithms

Parameters	Cetautomatix MR image	2.5-D edge detected image	3-D edge detected image	2.5-D skeletonized image	3-D skeletonized image
Pixels count	22509	27429	26937	28980	28500
Pixels without black	22503	27426	26905	26560	24581
Red Min	0	0	0	0	0
Red Max	255	255	255	255	255
Red Mean	103.519	67.3649	102.1479	65.709	42.0808
Red standard deviation	68.870	48.0589	60.254	79.216	47.0709
Red median	104	54	98	16	30
Red total count	22509	27429	26937	28980	28500
Green Min	0	0	0	0	0
Green Max	255	255	255	255	253
Green mean	103.519	13.6949	38.257	30.5839	18.4607
Green standard deviation	68.870	34.687	56.325	48.0644	33.2558
Green median	104	2	19	2	5
Green total count	22509	27429	26937	28980	28500
Blue Min	0	0	0	0	0

(continued)

(continued)

Parameters	Cetautomatix MR image	2.5-D edge detected image	3-D edge detected image	2.5-D skeletonized image	3-D skeletonized image
Blue Max	255	255	255	255	253
Blue mean	103.519	14.680	38.844	31.0038	19.02649
Blue standard deviation	68.870	34.8206	56.101	48.1467	33.61179
Blue median	104	3	19	3	5
Blue total count	22509	27429	26937	28980	28500
Saturation Min	0	0	0	0	0
Saturation Max	0	1	1	1	1
Saturation mean	0	0.861	0.687	0.6382	0.52028
Saturation standard deviation	0	0.175	0.222	0.35136	0.352738
Saturation median	0	0.925	0.690	0.600	0.52549
Luminance Min	0	0	0	0	0
Luminance Max	1	1	1	1	0.992
Luminance Mean	0.405	0.157	0.273	0.187	0.11726
Luminance standard deviation	0.270	0.1548	0.220	0.2426	0.15348
Luminance median	0.407	0.1098	0.223	0.03137	0.0666
Y Min	0	0	0	0	0
Y Max	1	1	1	1	0.992
Y Mean	0.405	0.115	0.223	0.15957	0.09877
Y standard deviation	0.270	0.14559	0.2189	0.21918	0.1433
Y median	0.407	0.0666	0.1647	0.0196	0.047
Cb Min	−0.001	−0.1078	−0.092	−0.088	−0.0568
Cb Max	−0.0019	0.0098	0.0058	0.0137	0.00588
Cb mean	−0.0019	−0.03557	−0.043	−0.02439	−0.01649

(continued)

(continued)

Parameters	Cetautomatix MR image	2.5-D edge detected image	3-D edge detected image	2.5-D skeletonized image	3-D skeletonized image
Cb standard deviation	1.159	0.01728	0.0196	0.0277	0.0134
Cb median	−0.0019	−0.0333	−0.049	−0.0098	−0.0137
Cr Min	−0.0019	−0.0176	−0.0215	−0.02549	−0.0137
Cr Max	−0.0019	0.3078	0.2568	0.249	0.1941
Cr mean	−0.0019	0.10319	0.12329	0.06709	0.04435
Cr standard deviation	1.159E−10	0.0534	0.0589	0.08189	0.042669
Cr median	−0.00196	0.09607	0.143	0.02156	0.041176
Red Min WB	1	0	0	0	0
Red Max WB	255	255	255	255	255
Red mean WB	103.547	67.372	102.269	71.6967	48.7898
Red standard deviation WB	68.858	48.05637	60.1868	80.1108	47.34539
Red median WB	104	54	98	27	39
Red Total count WB	22503	27426	26905	26560	24581
Green Min WB	1	0	0	0	0
Green Max WB	255	255	255	255	253
Green mean WB	103.547	13.696	38.3028	33.3706	21.4039
Green standard deviation WB	68.858	34.688	56.343	49.27158	34.918
Green median WB	104	2	19	4	7
Green total count WB	22503	27426	26905	26560	24581
Blue Min WB	1	0	0	0	0
Blue Max WB	255	255	255	255	253

(continued)

(continued)

Parameters	Cetautomatix MR image	2.5-D edge detected image	3-D edge detected image	2.5-D skeletonized image	3-D skeletonized image
Blue mean WB	103.547	14.6819	38.890	33.828	22.0599
Blue standard deviation WB	68.8587	34.822	56.118	49.333	35.2555
Blue median WB	104	3	20	5	8
Blue ttal count WB	22503	27426	26905	26560	24581
Saturation Min WB	0	0	0	0	0
Saturation Max WB	0	1	1	1	1
Saturation mean WB	0	0.861	0.688	0.696	0.603
Saturation standard deviation WB	0	0.1749	0.221	0.3069	0.3069
Saturation median WB	0	0.92549	0.694	0.69019	0.6000
Luminance Min WB	0.0039	0	0	0	0
Luminance Max WB	1	1	1	1	0.992
Luminance mean WB	0.406	0.1574	0.273	0.2044	0.1359
Luminance standard deviation WB	0.270	0.15488	0.220	0.2464	0.15738
Luminance median WB	0.4078	0.1098	0.2235	0.0588	0.09019
Y Min WB	0.0039	0	0	0	0
Y Max WB	1	1	1	1	0.992
Y Mean WB	0.406	0.1151	0.223	0.1741	0.1145
Y Standard deviation WB	0.270	0.1455	0.2189	0.22335	0.14839

(continued)

(continued)

Parameters	Cetautomatix MR image	2.5-D edge detected image	3-D edge detected image	2.5-D skeletonized image	3-D skeletonized image
Y median WB	0.4078	0.0666	0.1647	0.0431	0.0627
Cb Min WB	−0.0019	−0.1078	−0.092	−0.088	−0.0568
Cb Max WB	−0.0019	0.0098	0.00588	0.0137	0.00588
Cb mean WB	−0.0019	−0.03558	−0.043189	−0.0264	−0.0188
Cb standard deviation WB	1.159E−10	0.01728	0.0195	0.0281	0.0130
Cb median WB	−0.0019	−0.0333	−0.049	−0.0098	−0.0176
Cr Min WB	−0.0019	−0.0176	−0.02156	−0.02549	−0.0137
Cr Max WB	−0.0019	0.3078	0.25686	0.249019	0.1941
Cr Mean WB	−0.0019	0.1032	0.1234	0.073388	0.0517
Cr standard deviation WB	1.15959E−10	0.05343	0.05879	0.0827	0.0414
Cr median WB	−0.0019	0.09607	0.143	0.0294	0.05294

5 Conclusion

This paper has introduced 2-D and 3-D algorithms for detecting edges and skeletal form in 3-D images. A sample MR image of cetautomatix is used for testing the algorithms. Comparative analysis is made in terms of statistical parameters of various images.

References

1. Z. Yu-qian, G. Wei-hua, C. Zhen-cheng, T. Jing-tian, L. Ling-yun, Medical images edge detection based on mathematical morphology, in 27th *Annual International Conference Engineering in Medicine and Biology Society*, pp. 6492– 6495, 01–04 September 2005
2. R.D. da Silva, R. Minghim, H. Pedrini, 3D edge detection based on Boolean functions and local operators. Int. J. Image Graph **15**(1), 155003-1-155003-21 (2015)
3. R. Deriche, Using Canny's criteria to derive a recursively implemented optimal edge detector. Int. J. Comput. Vis. **1**, 167–187 (1987)

4. Y.J. Zhang, Quantitative study of 3D gradient operators. Image Vis. Comput. **11**(10), 611–622 (1993)
5. O.A. Zuniga, R.M. Haralick, Integrated directional derivative gradient operator. IEEE Trans. Syst. Man Cybern. **17**(3), 508–517 (1987)
6. P. Bhattacharya, D. Wild, A new edge detector for gray volumetric data. Comput. Biol. Med. **26**(4), 315–328 (1996)
7. Marek Brejl, Milan Sonka, Directional 3D edge detection in anisotropic data: detector design and performance assessment. Comput. Vis. Image Understand. **77**(9), 84–110 (2000)
8. A.B. Watson, A.J. Ahumada, A hexagonal orthogonal-oriented pyramid as a model of image representation in visual cortex. IEEE Trans. Biomed. Eng. **36**(1), 97–106 (1989)
9. H.K. Liu, Two-and three-dimensional boundary detection. Comput. Graph. Image Process. **6**, 123–134 (1977)
10. S.W. Zucker, R.A. Hummel, A three-dimensional edge operator. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. PAMI-3, no. 3, pp. 324–331, May 1981
11. M.T. Hueckel, An operator which locates edges in digitized pictures. J. ACM **18**, 113–125 (1971)
12. L.M. Luo, C. Hamitouche, J.L. Dillenseger, J.L. Coatrieux, A moment-based three-dimensional edge operator. IEEE Trans. Biomed. Eng. **40**(7), 693–703 (1993)
13. C. Bδhnisch, P. Stelldinger, UllrichKφthe, Fast and accurate 3D edge detection for surface re construction. *LNCS 5748*, pp. 111–120, Springer, 2009
14. M. Popescu, R.T. Tanasie, Graph-based volumetric data segmentation on a hexagonal-prismatic lattice, in *Proceedings of the Federated Conference on Computer Science and Information Systems*, pp. 745–749 (2012)
15. D.G. Morgenthaler, A. Rosenfeld, Multidimensional edge detection by hyper surface fitting. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. PAMI-3, no. 4, pp. 482–486, July 1981
16. O. Monga, R. Deriche, A new three dimensional boundary detection, in *Proceedings of International Conference on Pattern Recognition,* Paris (1986)
17. O. Monga, R. Deriche, J.-M. Rocchisani, 3D edge detection using recursive filtering: application to scanner images. *CVGIP: Image Understanding*, vol. 53, no. 1, pp. 76–87 (1991)
18. G.R. Chandra, T. Sultana, G. Sathya, E.G. Rajan, 3D edge detection of medical images using mathematical morphological and cellular logic array processing techniques, in *IEEE International Conference on Systems, Man, and Cybernetics (SMC)*, Seoul, pp. 2156–2160 (2012)

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Pivotal role of supramolecular interactions towards the stability of Na-1,2-bis(tetrazol-5-yl) ethene coordination polymer

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ABSTRACT

The article presents the design and synthesis of 2D Na-1,2-bis(tetrazol-5-yl) ethene (H_2bte) coordination polymer and highlights the influence of supramolecular interactions in the self-assembly process towards the formation of thermodynamic product. By grinding the linker H_2bte and NaOH followed by crystallization in H_2O/MeOH solvent at room temperature gives homogeneous compound $[Na_2(bte)(H_2O)_7]_n$ in quantitative yields. The compound is characterized by IR spectroscopy, PXRD, thermogravimetric, calorimetric analyses and unambiguously characterized by single crystal X-ray diffraction. The compound contains 3-connected $Na_4(H_2O)_{14}$ tetramer units as building blocks which are connected by trans bte^{2-} linkers in planar, dipodal fashion to form 2D sheets. Robust hydrogen bonding interactions exists between the adjacent 2D sheets through terminal and bridged water molecules present in the tetramer to form dense 3D supramolecular framework. Hirshfeld surface analyses have been studied to gain deep insights into the strength and contributions of hydrogen bonding interactions exhibited by the molecular structure for the formation of stable phase.

1. Introduction

Self-assembly of coordination polymers (CPs)/Metal organic frameworks (MOFs) are subjected to intense research area over the past two decades [1–4]. Exercise on fine control over the design and synthesis of these extended architectures is crucial part towards the reproducibility of physical properties in terms of crystallinity, particle size, morphology, and surface porous properties [5,6]. Earlier knowledge on the underlying mechanism of self-assembly process facilitate to achieve the anticipated product phase by preventing the metastable kinetic product [7,8]. The synthetic chemistry of these materials has been developing rapidly to obtain materials with unique properties and applications [9–11]. The quest for construction of coordination polymers with desired assemblies is subjected to judicious selection of synthetic strategies [12,13] as well as the subtle parameters like geometric restrictions imposed by the linker and metal, template molecules and supramolecular interactions [14–16].

The most efficient parameter in nucleation of the coordination networks to form kinetic and thermodynamic products are supramolecular interactions displayed by molecular structure, through coordinated and lattice solvent molecules [17]. Among all the non-covalent interactions, hydrogen bonding has a prominent role in the assembly of coordination networks [18]. Two distinct roles played by the hydrogen bonding interactions are, either restricting the dimensionality of the network by blocking the coordinating groups [19] or enhancing the stability of the network through directional interactions [20–23]. Both roles are operated simultaneously or cooperatively to enhance the stability of the molecular structure [24]. Das et al. reported the polyoxometalate based ion-pair compound, wherein, the supramolecular interactions displayed by the octamolybdate anion restricts the formation of Cu-1,4-bis[2-(2-*pyridyl*)*imidazol*-1-*ylmethyl*]benzene (1,4-bpimb) network and stabilizes in discrete metallo-macrocycle by

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mediating the conformation of ligand to unfavorable *cis* conformation [25]. Generally, the lower dimensional coordination polymers are stabilized by non-covalent interactions such as hydrogen bonding and $\pi-\pi$ stacking etc. [26]. Ghosh et al. reported host guest supramolecular chemistry in which 2D supramolecular sheets involved in $\pi\cdots\pi$ and C–H···N hydrogen bonding interactions with the guest 4,4′-azobis(pyridine) molecules thereby achieves the additional stability [27]. Coordination polymers with metal centers having greater number of solvent water molecules in their coordination sphere often displays supramolecular isomerism with both kinetic and thermodynamic products [28]. Thermodynamic products are stabilized by various intermolecular interactions to achieve the local minima before the global minima is reached on the reaction coordinate [29].

In an attempt to prepare the Na-bte coordination polymers, in our previous article, we isolate the meta stable kinetic products during crystallization of final thermodynamically stable products [30]. The factors like crowdedness in metal coordination sphere, ligand conformations and supramolecular interactions drive the formation of final products. Isolation of meta stable phases indicates their stability and also generates the question on purity of final compounds. In this context we continue our studies through different synthetic procedures to study the self-assembly of Na-bte coordination polymers. Only Na and Zn coordination polymers based on H_2bte linker are reported in the literature prompts us to explore the linker [31,32]. The synthetic procedure was modified by adopting the mechanical grinding process to obtain the compound $[Na_2(bte)(H_2O)_7]_n$ as homogeneous product. The product formed does not change its composition in reaction conditions even after few days reveals the thermodynamic stability of the compound. The compound was structurally characterized by single crystal X-ray diffraction and non-covalent interactions exhibited by molecular structure are studied by Hirshfeld surface analyses. Thermal stability and bulk purity of the compound were also additionally characterized.

2. Experimental section

2.1. Materials and methods

All chemicals are commercially available reagents of analytical grade and were used without further purification. The ligand H_2**bte** was synthesized according to the procedure reported by Sharpless et al. as shown in Section 1 of ESI [33].

2.2. Synthesis of $[Na_2(bte)(H_2O)_7]_n$

H_2bte (8.2 mg, 0.05 mmol) and NaOH (1 mg, 0.025 mmol) was grinded in a mortar to obtain fine powder which was then dissolved in water (1.5 mL) and MeOH (300 μL to enhance solubility) in a glass vial. The solution was swirled to complete dissolution and the vial was sealed and kept in a heating bath adjusted to 85 °C for 3 days. The solution was filtered and crystallized at room temperature to obtain colorless block crystals in 2 days. The crystals were collected by filtration, washed with cold water and dried in air (89% based on sodium) Anal. Calc. for $C_4H_{16}N_8Na_2O_7$ (**1**): C, 14.37; H, 4.82; N, 33.52. Found: C, 14.10; H, 4.23; N, 33.19 IR (KBr, cm^{-1}): 3333, 2388, 1618, 1484, 1410, 1330, 1210, 1163, 1089, 1031, 950, 818, 731.

2.3. Physical measurements

Differential scanning calorimetry (DSC) was carried out on a Mettler Toledo DSC3+ instrument and thermogravimetric analysis (TGA) was carried out on a TA Q500 instrument, using dry nitrogen atmosphere, at a rate of 20 mL/min. About 0.5–1 mg samples were sealed in aluminum pans for DSC measurements with heating rate of 10 °C/min, between 25 °C and 450 °C. Infrared spectra of solid samples were obtained on Bruker Tensor 27 system spectrophotometer in ATR mode. X-ray powder diffraction (PXRD) measurements were performed on a Rigaku SmartLab diffractometer at 60 kV, 30 mA and CuK_α radiation (λ = 1.5406 Å), with a scan speed of 1°/min and a step size of 0.02°

Table 1
Crystallographic data and refinement parameters of Compound $[Na_2(bte)(H_2O)_7]_n$.

CCDC Number	2,019,007
Empirical formula	$C_4H_{16}N_8Na_2O_7$
Formula weight	334.20
Temperature/K	110.15
Crystal system	Triclinic
Space group	P-1
a/Å	7.0792(2)
b/Å	9.3198(2)
c/Å	11.6282(3)
α/°	108.2250(10)
β/°	96.9450(10)
γ/°	100.3380(10)
Volume/Å^3	703.89(3)
Z	2
ρ_{calc}g/cm^3	1.5767
μ/mm^{-1}	0.191
F(000)	348.3
Crystal size/mm^3	0.25 × 0.22 × 0.15
Radiation	Mo K_α (λ = 0.71073)
2Θ data collection/°	3.76 to 57.16
Index ranges	$-9 \le h \le 8$, $-12 \le k \le 12$, $-15 \le l \le 15$
Reflections collected	12,109
Independent reflections	3555 [R_{int} = 0.0267, R_{sigma} = 0.0256]
Data/restraints/parameters	3555/0/232
Goodness-of-fit on F^2	1.124
Final R indexes [$I>=2\sigma$ (I)]	R_1 = 0.0286, wR_2 = 0.0735
Final R indexes [all data]	R_1 = 0.0324, wR_2 = 0.0825
Largest diff. peak/hole / e Å^{-3}	0.29/−0.32

Table 2
Geometrical parameters of the O–H···O and O–H···N hydrogen bonds (Å, °) involved in 3D supramolecular framework of the title compound. *D* = donor; *A* = acceptor.

S. No	D-H···A	d(D...A) (Å)	<(DHA) (°)
1	O1– H1a··· N4	2.8885(13)	176.1(15)
2	O1– H1b··· O2	2.8515(12)	167.1(17)
3	O2– H2a··· O5	2.7806(12)	151.9(15)
4	O3– H3b··· N5	2.8973(13)	172.2(16)
5	O5– H5b··· N1	2.8986(13)	162.7(15)
6	O6– H6a··· O3	2.8577(13)	171.0(16)
7	O7– H7b··· N2	2.7736(13)	176.8(16)

2.4. Single crystal X-ray diffraction measurement

Single crystal x-ray diffraction measurements performed on Bruker-Apex Duo diffractometer with IμS micro-focus using MoK_α radiation. Measurements were carried out at 110 (2) K on crystals coated with a thin layer of amorphous oil. The structures were solved by direct methods and refined by full-matrix least-squares, using standard crystallographic software: SHELXT-2014, SHELXL-2014 [34,35]. The TOPOS software package was used to analyze the topological features of the available framework solids [36]. The crystal data of the compound is displayed in Table 1 and hydrogen bonding parameters in Table 2. Thermal ellipsoidal plot diagram of the compound was shown in figure S1 in supporting information. Complete list of bond lengths and bond angles are shown in section-6 of supporting information

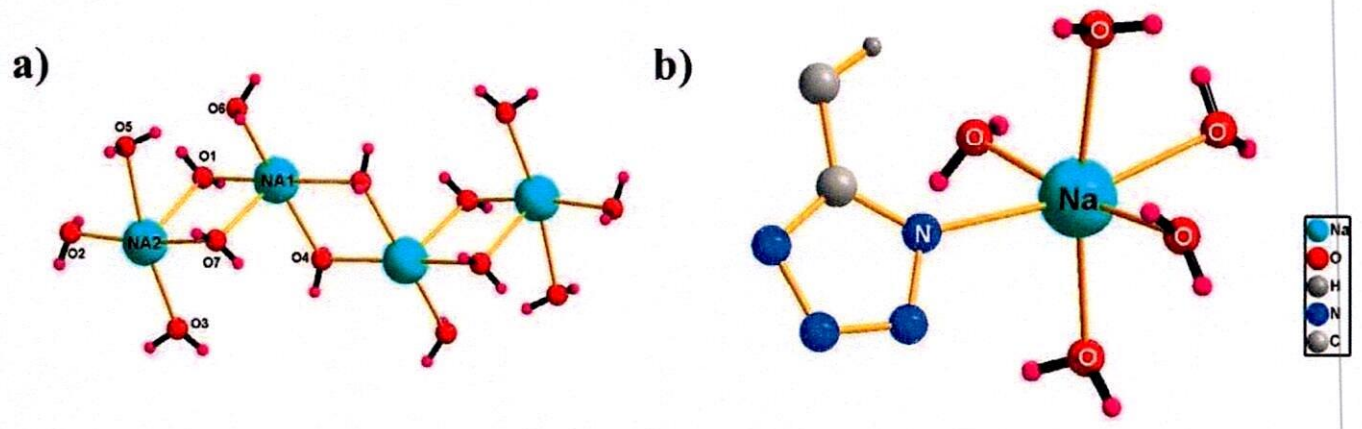

Fig. 1. a) Representation of Na tetramer observed in the compound (excluding the bte^{2-} linkers) b) The coordination environment around the Na(I) metal center.

3. Results and discussion

By grinding stoichiometric ratio of H_2bte, NaOH and dissolving in mixed solvent system $MeOH/H_2O$ followed by heating the reaction mixture for 3 days at 85 °C gives the title compound $[Na_2(\mathbf{bte})(H_2O)_7]_n$ in quantitative yields. The compound was crystallized as colorless block shaped crystals in triclinic space group P-1. This phase was characterized by 2D polymer with discrete $Na_4(H_2O)_{14}$ tetramers as building blocks.

Two crystallographically independent Na atoms constitute the tetramer in which two symmetrically equivalent Na atoms (Na2) resides as terminal atoms and other two atoms (Na1) in bridging positions of tetramer as shown in the Fig. 1a. The terminal Na2 atoms reside in octahedral $NaNO_5$ coordination sphere composed from one N atom from the bte^{2-} molecule along with three terminal and two bridging aqua ligands. On the other hand, the bridging Na1 atoms also stays in $NaNO_5$ octahedral coordination sphere in which the N atom from linker bte^{2-}, four bridging and one terminal aqua ligand comprises the coordination sphere (Fig. 1b) Analyses of coordination geometries around Na center through SHAPE 2.1 program reveals that both the Na centres are present in slightly distorted octahedral environment (O_h symmetry)(detailed geometric analyses described in section S7 ESI) [37]. The Na-O bond distances ranging from 2.508(1) Å to 2.348(2) Å are in good accordance with the Na-H_2O bond lengths found in the literature [38]. Overall, six aqua ligands bridge the four Na atoms in a zig-zag manner to form a tetramer. These tetramers linked to each other by fully deprotonated bte^{2-} in a dipodal planar fashion, in trans form (with torsion angle of 180 ° (C1–C4–C4–C1) between two tetrazole rings).

Both the tetrazole rings of the bte^{2-} linker coordinates to the Na atoms in μ_1-coordination mode through nitrogen atoms (Na(1)–*N*(6): 2.495(1) Å, Na(2)–N3: 2.477(1) Å) exhibiting dipodal coordination connectivity. The bte^{2-} linkers coordinated to terminal Na atoms i.e. Na2 centers connects the tetramers along crystallographic *b*-axis to form 1D chains. These chains are in turn connected to each other along *c*-axis through another set of bte^{2-} linkers which are coordinated to bridged Na atoms to form a 2D sheets along the *bc* plane as shown in Fig. 2a. The dimensions of the void spaces created in the planar sheets are 18.164×9.195 Å and the Na-bound both bridging and terminal water molecules are protruded into these void spaces. The topology of the framework was obtained by reducing the Na tetramer to 3-connected node resulting in a 3-c, uninodal network with point symbol (6^3) (Fig. 2b).

The key structural factors in the formation of title compound as stable form is due to coordination restrictions imposed by the metal as well as the hydrogen bonding interactions. The sodium coordination sphere contains only one bte^{2-} linker and five aqua ligands, ($NaNO_5$) which reduces maximum strain in the sodium coordination environment. The other factor which directs the formation of stable product is robust hydrogen bonding between the 2D sheets of the compound. In the formation of tetramer fourteen water molecules are involved in which six molecules are bridging and eight molecules are terminally attached. Three types of strong O–H···O interactions exhibited by both terminal and bridging water molecules, connect the adjacent 2D sheets (*bc* plane) along crystallographic *a* and *c* axis to form 3D supramolecular framework.

The terminal water oxygen atoms (O1) attached to the Na2 atoms connects the tetramers of adjacent sheets through $O-H\cdots O$ (O1– H1B···O2, $d_{O\cdots O}$ = 2.851(12) Å, hydrogen bonding to form $R^2_2(8)$ ring along c-axis. The connectivity is further strengthened along the same axis by oxygen atoms of the terminal water molecules connected to Na2 atom through O2-H2a···O5, $d_{O\cdots O}$ = 2.780 (12) Å interactions as shown in Fig. 3a. The oxygen atom O6 of the terminal water molecule connected to the bridging Na1 involves in hydrogen bonding interaction with O3 (O6– H6A···O3, $d_{O\cdots O}$ = 2.857(13) Å) connected to the terminal Na2 atom of tetramer present in adjacent sheet along the *a* axis resulting in the ladder like connectivity (Fig. 3b). Overall, each tetramer connects to four different tetramers from adjacent sheets through twelve $O-H\cdots O$ interactions to form a 2D supramolecular sheet along crystallographic *ac* plane as shown in the Fig. 4a. These 2D supramolecular sheets are connected through bte^{2-} linkers to form a 3D robust supramolecular framework, and its topological representation was shown in Fig. 4b. All the eight terminal water molecules connected to both Na atoms involves in hydrogen bonding with the adjacent sheets.

The 3D supramolecular framework formed due to $O-H\cdots O$ interactions are further reinforced by O–H···N interactions involving both terminal as well as bridging water molecules. Several O–H···N interactions with $d_{O\cdots N}$ distances ranging from 2.77 to 2.95 Å exists between the adjacent 2D sheets as shown in the figure S3, ESI. Along with these interactions the compound is additionally stabilized by $\pi\cdots\pi$ interactions between the tetrazole rings with intermolecular c.g···c.g distance of 3.727 Å (figure S4, ESI).

The Na(I) coordination polymers based on bte^{2-} are reported very few in the literature which are listed in Table 3. As seen in the table, entry 1 and 5 reaction procedures produce only thermodynamic products. On the other hand, the other reaction conditions give both kinetic and thermodynamic products. The presence of five water molecules along with bte^{2-} in the Na coordination sphere in the thermodynamic products merely represents like most stable $Na(H_2O)_6$ entity. The coordination sphere in these compounds have only one exchangeable organic linker with wa-

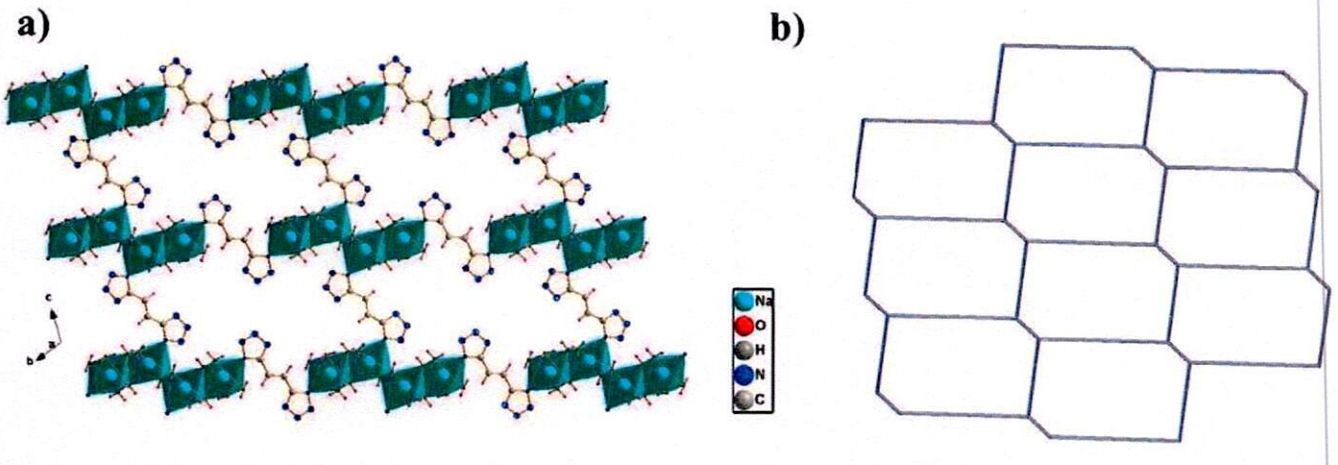

Fig. 2. a) 2D coordination polymer formed due the connectivity of Na tetramers and bte^{2-} linkers and b) Topological representation of (6,3) connected network of the compound.

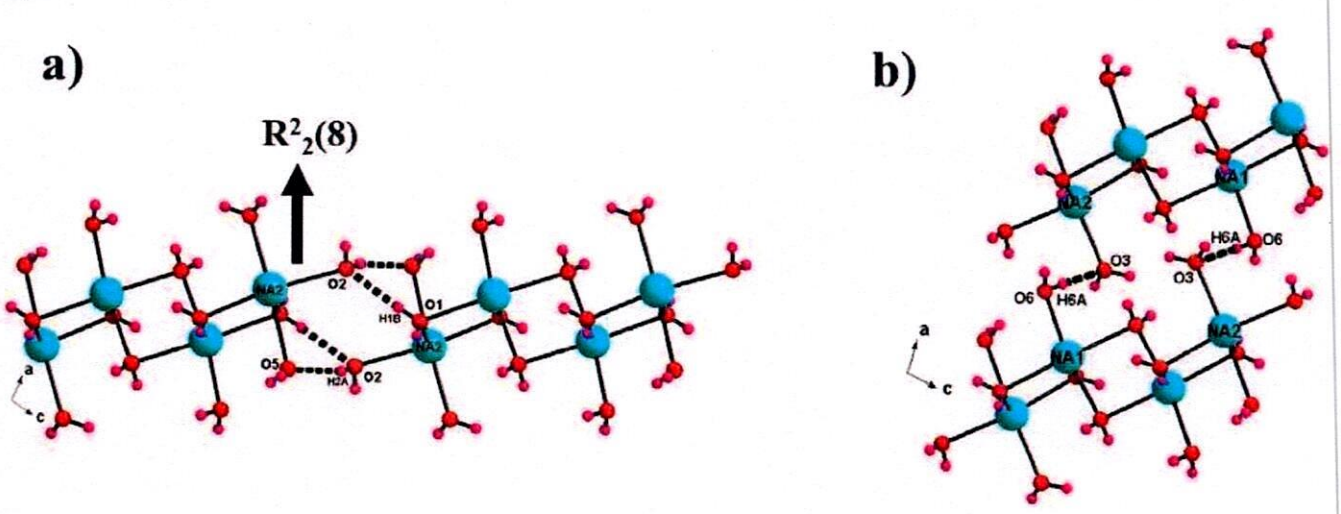

Fig. 3. Three types of O–H···O interactions observed between the water molecules coordinated to the tetramers and their connectivity towards (a) crystallographic *c*-axis and (b) *a*-axis.

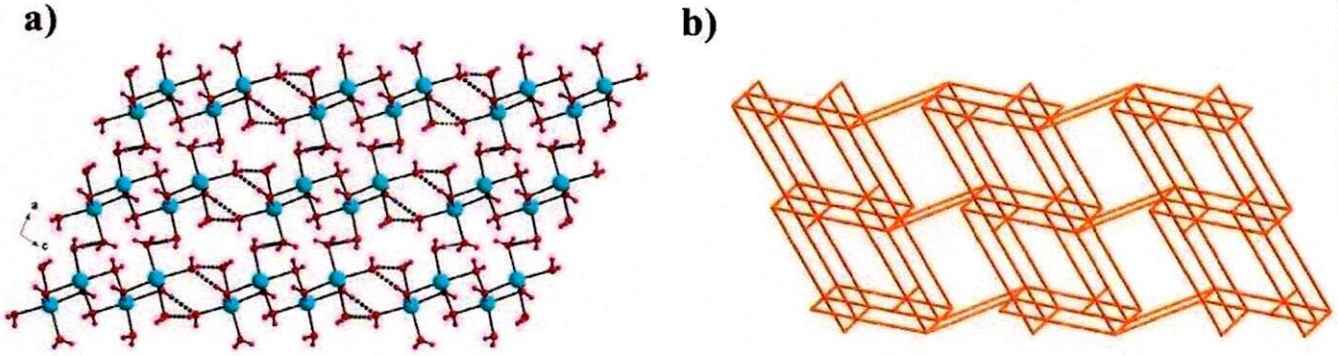

Fig. 4. (a) 2D- supramolecular Na-(H_2O) sheet formed due to $O - H\cdots O$ interactions along the ac plane (b) topological representation of 3D supramolecular framework formed due to the $O - H\cdots O$ interactions.

Table 3
Table displaying the different products formed through different synthetic procedures in literature based on Na-bte polymers.

S. No	Reaction Conditions	Products		Ref.
		Kinetic	Thermodynamic	
1	1,2-dicyanoethylene +NaN_3+ $ZnCl_2$ Hydrothermal conditions at 130 °C	---	$[Na_2(bte)(H_2O)_5]_n$	31
2	H_2bte+MeOH +NaOH Layered on Toluene	$[Na(Hbte)(CH_3OH)_2]_n$	$[Na(Hbte)(H_2O)_3]_n$	30
3	H_2bte+MeOH +NaOH+NH_3(Aq) Layered on Toluene	$[Na_2(bte)(H_2O)_3]_n$	$[Na_2(bte)_2(H_2O)_3]_n{\cdot}2NH_4{\cdot}H_2O$	
4	H_2bte+MeOH +NaOH+CH_3CN Heating at 85 °C	$[Na(Hbte)(H_2O)]_n$	$[Na_2(Hbte)_2(H_2O)]_n$	
5	H_2bte+ NaOH Grinding and heating 85 °C	---	**$[Na_2(bte)(H_2O)_7]_n$**	This work

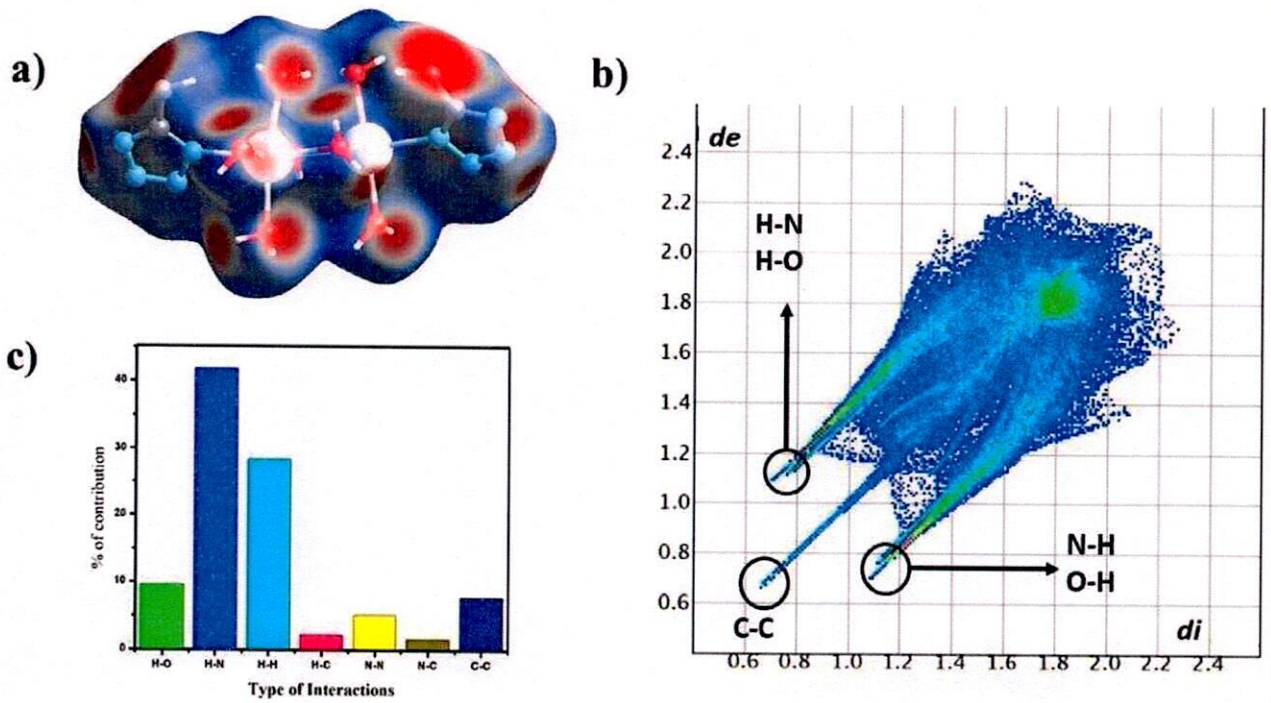

Fig. 5. (a) Hirshfeld surface of the title compound considering the asymmetric unit (b) 2D finger-plot diagram derived from the HSs representing the shortest possible interaction range of O–H and N–H interactions as spikes (c) percentage contribution of all interactions around the asymmetric unit (considering both the reciprocal contributions).

ter molecules hence the compounds represents the most stable supramolecular isomers unlike dense coordination sphere observed in Na-bte polymers reported in the literature [30]. The robust hydrogen bonding interactions present in these compounds achieves additional stability; hence the compounds with this type of entities have large energy land scape for self-assembly of metal ions and ligands to form homogeneous stable thermodynamic products.

3.1. Hirshfeld surface analysis

The dense non-covalent interactions around asymmetric unit containing two Na atoms and seven water molecules are further analyzed by the Hirshfeld surfaces (HSs) and 2D fingerprint plots (FPs), which are generated by using Crystal explorer 3.1 [39] based on the CIF file. These 3D Hirshfeld surfaces gave the information about long- and short-range interactions around the molecules and 2D finger plots obtained from the HSs reveal the type, nature, and the contribution of individual intermolecular interactions.

The HSs of the asymmetric unit have been mapped over d_{norm} which is a ratio encompassing the distance of any surface point to the nearest interior (d_i) and exterior (d_e) atom to the van der Waals radii (vdW) of the atoms (−0.6 to 1.0 Å) as shown in the Fig. 5a (The HSs mapped with the shape index and the curvedness are shown in supporting information) [40–42]. 2D finger plot indicates the contribution of different kinds of intermolecular interactions. To visualize the molecular structure the surfaces are displayed transparent. All the blue spots seen in the d_{norm} surface represent the area longer contacts than Van der Waal's radii and the large circular red spots are the closer contacts than Van der Waal's radii which represent the prominent hydrogen bonding interactions.

The large red circular depressions as shown in Fig. 5a represent the robust supramolecular interactions around the water molecules of the molecular structure. Nearly 48.5% among all the non-covalent interactions are contributed from the H···N/N···H (O–H··· N) interactions and 9.5% are from H···O/O···H (O–H···O) interactions. These two O–H···O interactions appeared as doublet on the left- and right-hand side in the 2D finger plot as shown in the Fig. 5b. These interactions appear in the region around 1.9 Å (d_e+d_i) representing the shortest distance between the atoms inside the molecular surface and outside of the surface. On the other hand, $\pi\cdots\pi$ interactions between the tetrazole rings recognized through C···C and N···N contacts contribute 7.8 and 5.1% respectively. The existence of $\pi\cdots\pi$ stacking is also evident from the relatively flat green region on the curvedness surface around tetrazole rings as shown in the figure S5, ESI. The relative contributions aroused due to different interactions are calculated and presented in the Fig. 5c and figure S6 ESI. The HSs and 2D finger plots clearly indicate the fidelity of O–H···O and O–H···N hydrogen bonding interactions in achieving additional stability for the compound.

4. PXRD and thermal analyses

To confirm the phase purity of the compound presented in this study, X-ray diffraction patterns of powder samples have been recorded. The reflections for the simulated data (calculated from single crystal data) are well matched with the observed data, which proves the bulk homogeneity of the crystalline solids (Fig. 6a). The intensities of few reflections were reduced, and some reflections are slightly broadened and shifted in the experimental patterns compared to those observed in the simulated pattern from the single-crystal data. The slight inconsistency observed in the PXRD pattern may be due to dehydration-rehydration of solvent water molecules coordinated to the Na(I) metal center which leads to reorganization of local structure with minimal difference. The PXRD pattern of the compound after one month was almost identical with the fresh as synthesized material clearly establishes the thermodynamic stability of the compound.

The thermal stability of the compound has been characterized by performing TGA and DSC experiments on a few milligram

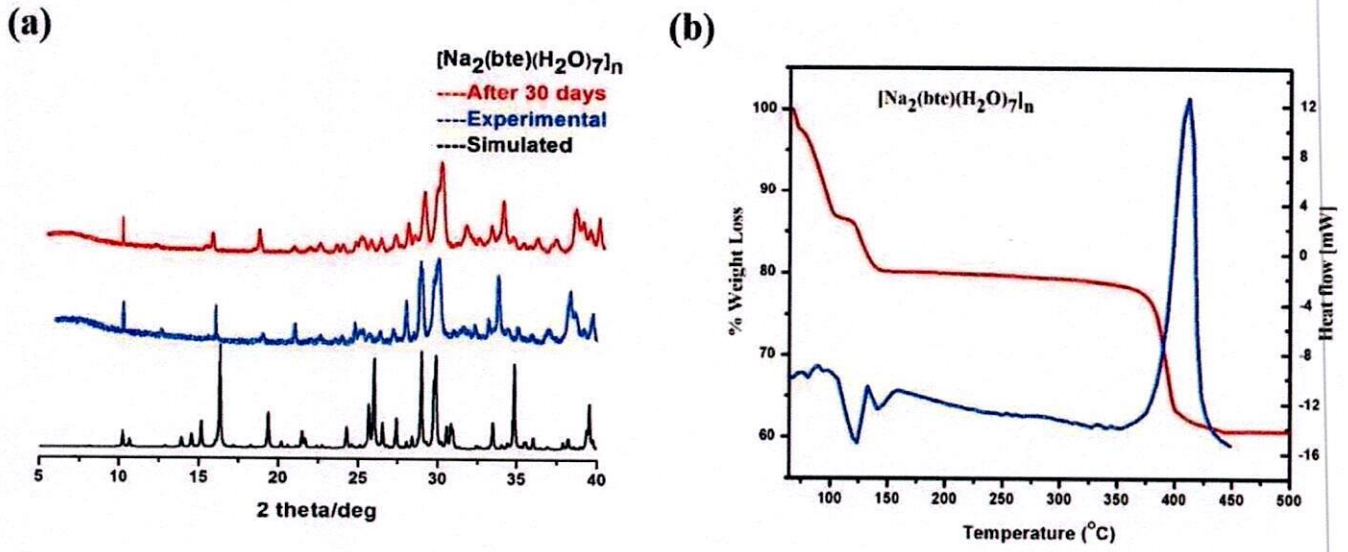

Fig. 6. (a) PXRD of the compound in comparison with the simulated pattern (b)TGA and DSC curves of the compound.

scales under inert atmosphere in the temperature range 50–450 °C (Fig. 6b). The compound shows continuous weight loss of 19.99% from room temperature to 150 °C which can be attributed to the loss four water molecules from the structure (Theoretical weight loss: 21.5%). The molecular structure contains four terminal water molecules hence it can be related that the dehydrated water molecules are terminal and bridging water molecules remain intact. The dehydrated framework remains stable in the region 150–376 °C with minimal weight loss. The compound then undergoes decomposition on further heating and shows sharp weight loss in the region 376–428 °C. The total weight loss observed in the region 150–428 °C is 19.2% which can be attributed to loss of carbon atoms from the linker (decomposition of linker) and one water molecule from the framework (Theoretical weight loss: 20.3%). The final residue accounts for nearly 60% of total mass which can be either metal oxides embedded in nitrogen matrix.

From the DSC curve it is observed that from room temperature to 150 °C the endothermic peaks detected are due to expulsion of water molecules from the framework which shows consistency with the TGA curve. A sharp exothermic peak was observed at 370 °C relates to the decomposition of the dehydrated framework.

5. Conclusion

In summary in this article we reported the 2D Na-bte coordination polymer $[Na_2(bte)(H_2O)_7]_n$ through grinding the reactants followed by crystallization. The reaction conditions result in the formation of homogeneous stable product unlike meta stable products reported in the literature. The 2D coordination polymer is constructed by 3-connected Na tetramer building blocks bridged by bte^{2-} linker protruding coordinated terminal water molecules in to void space. A strong hydrogen bonding interaction (O–H···O and O–H···N) exist between the coordinated water molecules of adjacent sheets to form a 3D supramolecular framework. The stability of the compound achieved through robust supramolecular interactions drives the formation of thermodynamic product by avoiding the meta stable products. Hirshfeld analyses reveal that nearly 60% interactions exhibited by the molecular structure are contributed by O–H···O and O–H···N interactions through water molecules clearly indicates the stability achieved by the compound through hydrogen bonding. The article highlights the role of supramolecular interactions in achieving the stable conformation of the molecule which ends up in the formation of thermodynamic products. Similar systematic studies are in progress to assess the cogent parameters underlying in the self-assembly process of coordination polymers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Bharat Kumar Tripuramallu: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Arvapalli Revathi:** Formal analysis, Software, Supervision. **Yoel Friedman:** Formal analysis, Investigation, Methodology. **Pilli V.V.N. Kishore:** Formal analysis. **Ravada Kishore:** Formal analysis.

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Supplementary data

Additional illustrations of crystal structure, Hirshfeld analyses, PXRD, Topological analyses, complete bond lengths and bond angle information's. CCDC 2019007 contain the supplementary crystallographic data title compound present in this article. These data can be obtained free of charge via HYPERLINK "http://www.ccdc.cam.ac.uk/"www.ccdc.cam.ac.uk/data_request/cif, by emailing HYPERLINK "mailto:data_request@ccdc.cam.ac.uk"data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223336033.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2020.129376.

References

[1] H. Zhang, X.M. Wang, K.C. Zhang, B.K. Teo, Molecular design and crystal engineering of a new series of inorganic polymers separated by organic spacers: structures of $[(18C6)K][Cd(SCN)_3]$ and $[(18C6)_2Na_2(H_2O)_2]_{1/2}[Cd(SCN)_3]$, J. Am. Chem. Soc. 118 (1996) 11813–11821.

[2] N.N. Adarsh, P. Dastidar, Coordination polymers: what has been achieved in going from *innocent*4,4′-bipyridine to bis-pyridyl ligands having a *non-innocent* backbone? Chem. Soc. Rev. 41 (2012) 3039–3060.

[3] S.R. Halper, L. Do, J.R. Stork, S.M. Cohen, Topological control in heterometallic metal–organic frameworks by anion templating and metalloligand design, J. Am. Chem. Soc. 128 (2006) 15255–15268.

[4] Y. Cui, B. Chen, G. Qian, Lanthanide metal-organic frameworks for luminescent sensing and light-emitting applications, Coord. Chem. Rev. 273 (2014) 76–86.

[5] L. Feng, K.-Y. Wang, J. Powell, H.-C. Zhou, Controllable synthesis of metal-organic frameworks and their hierarchical assemblies, Matter 1 (2019) 801–824.

[6] J.R. Li, J. Sculley, H.C. Zhou, Metal–organic frameworks for separations, Chem. Rev. 112 (2012) 869–932.

[7] Y.J. Colón, A.Z. Guo, L.W. Antony, K.Q. Hoffmann, J.J. De Pablo, Free energy of metal-organic framework self-assembly, J. Chem. Phys. 150 (2019) 104502-01-104502-12.

[8] G. Resnati, E. Boldyreva, P. Bombicz, M. Kawano, Supramolecular interactions in the solid state, IUCrJ. 2 (2015) 675–690.

[9] S. Li, M. Dharmarwardana, R.P. Welch, C.E. Benjamin, A.M. Shamir, S.O. Nielsen, J.J. Gassensmith, Investigation of controlled growth of metal–organic frameworks on anisotropic virus particles, ACS Appl. Mater. Interfaces 10 (2018) 18161–18169.

[10] K. Uzarevic, T.C. Wang, S.-Y. Moon, A.M. Fidelli, J.T. Hupp, O.K. Farha, T. Friscic, Mechanochemical and solvent-free assembly of zirconium-based metal–organic frameworks, Chem. Commun. 52 (2016) 2133–2136.

[11] J. Liu, X. Zou, C. Liu, K. Cai, N. Zhao, W. Zheng, G. Zhu, Ionothermal synthesis and proton-conductive properties of NH_2-MIL-53 MOF nanomaterials, Crystengcomm 18 (2016) 525–528.

[12] X. Wang, W-Y Gao, J. Luan, L. Wojtas, S. Ma, An effective strategy to boost the robustness of metal–organic frameworks *via* introduction of size-matching ligand braces, Chem. Commun. 52 (2016) 1971–1974.

[13] N. Stock, S. Biswas, Synthesis of metal-organic frameworks (MOFs): routes to various MOF topologies, morphologies, and composites, Chem. Rev. 112 (2011) 933–969.

[14] C. Duan, F. Li, H. Zhang, J. Li, X. Wang, H. Xi, Template synthesis of hierarchical porous metal–organic frameworks with tunable porosity, RSC. Adv. 7 (2017) 52245–52251.

[15] Z.Wei W.Lu, Z-Y Gu, T-F Liu, J. Park, J. Park, J. Tian, M. Zhang, Q. Zhang, T.G. III, M. Bosch, H-C. Zhou, Tuning the structure and function of metal–organic frameworks via linker design, Chem. Soc. Rev. 43 (2014) 5561–5593.

[16] P. Manna, B.K Tripuramallu, S.K. Das, Influential role of geometrical disparity of linker and metal ionic radii in elucidating the structural diversity of coordination polymers based on angular dicarboxylate and bis-pyridyl ligands, Cryst. Growth Des 14 (2014) 278–289.

[17] D. Wu, A. Navrotsky, Thermodynamics of metal-organic frameworks, J. Solid State Chem 223 (2015) 53–58.

[18] L. Applegarth, A.E. Goeta, J.W. Steed, Influence of hydrogen bonding on coordination polymer assembly, Chem. Commun. (2005) 2405–2406.

[19] B.K. Tripuramallu, S.K. Das, Influence of biphenyl spacer appended to the flexible phosphonate arms in modulating the dimensionality of the coordination polymers: synthesis, structural chemistry and magnetic properties, J. Solid State Chem 197 (2013) 499–507.

[20] M. Morshedi, M. Thomas, A. Tarzia, C.J. Doonan, N.G. White, Supramolecular anion recognition in water: synthesis of hydrogen-bonded supramolecular frameworks, Chem. Sci. 8 (2017) 3019–3025.

[21] C-C. Wang, G-B. Sheu, S-Y. Ke, C-Y. Shin, Y-J. Cheng, Y-T. Chen, C-H. Cho, M-L. Ho, W-T. Chen, R-H. Liao, G-H. Lee, H-S. Sheu, A 3D porous supramolecular architecture via π–π assembly of 2D metal–organic frameworks (MOFs): structure-versus-luminescence reversibility and gas adsorption properties, CrystEngComm 17 (2015) 1264–1272.

[22] R. Sun, Y-Z. Li, J. Bai, Y. Pan, Synthesis, structure, water-induced reversible crystal-to-amorphous transformation, and luminescence properties of novel cationic spacer-filled 3D transition metal supramolecular frameworks from N,N′,N″-Tris(carboxymethyl)-1,3,5-benzenetricarboxamide, Cryst. Growth Des. 7 (2007) 890–894.

[23] W. Yang, B. Li, H. Wang, O. Alduhaish, K. Alfooty, M.A. Zayed, P. Li, H.D. Arman, B. Chen, A microporous porphyrin-based hydrogen-bonded organic framework for gas separation, Cryst. Growth Des. 15 (2015) 2000–2004.

[24] B.K. Tripuramallu, P. Manna, S.K. Das, Supramolecular interactions mediated conformational modulation of flexible linker leading to the isolation of a metallo-macrocycle in a polyoxometalate matrix: Hirshfeld surfaces and 2D fingerprint plots, CrystEngComm 16 (2014) 10300–10308.

[25] C-D Si, D-C Hu, Y Fan, X-Y Dong, X-Q Yao, Y-X Yang, J-C Liu, Three-dimensional supramolecular architectures with co^{II} ions assembled from hydrogen bonding and $\pi\cdots\pi$ stacking interactions: crystal structures and antiferromagnetic properties, Cryst. Growth Des. 15 (2015) 5781–5793.

[26] S. Dey, P. Mondal, S.P. Rath, Aggregation-controlled excimer emission in an axial anthracene–Sn(iv)porphyrin–anthracene triad in the solid and solution phases, New J. Chem 39 (2015) 4100–4108.

[27] P. Kar, R. Biswas, M.G.B. Drew, A. Frontera, A. Ghosh, Host–guest supramolecular interactions in the coordination compounds of 4,4′-Azobis(pyridine) with MnX_2 ($X = NCS^-$, $NCNCN^-$, and PF_6^-): Structural analyses and theoretical study, Inorg. Chem. 51 (2012) 1837–1851.

[28] J.-P. Zhang, X.-C. Huang, X.-M. Chen, Supramolecular isomerism in coordination polymers, Chem. Soc. Rev. 38 (2009) 2385–2396.

[29] F. Gańdara, V.A. de la Pen˜ a-O'Shea, F. Illas, N. Snejko, D.M. Proserpio, E. Gutieírez-Puebla, M.A. Monge, Three lanthanum MOF polymorphs: insights into kinetically and thermodynamically controlled phases, Inorg. Chem. 48 (2009) 4707–4713.

[30] Y. Friedman, C. Mottillo, B.K. Tripuramallu, Influence of structural parameters on the formation of stable na-bte coordination polymers Cryst, Growth Des 18 (2018) 7708–7719.

[31] T. Hu, L. Liu, X. Lv, X. Chen, H. He, F. Dai, G. Zhang, D. Sun, Luminescent zinc and cadmium metal-organic frameworks based on tetrazole ligands, Polyhedron 29 (2010) 296–302.

[32] X.-F. Huang, Y.-M. Song, Q. Wu, Q. Ye, X.-B. Chen, R.-G. Xiong, X.-Z. You, 1D sodium ditetrazole coordination polymer obtained through in situ hydrothermal ligand synthesis, Inorg. Chem Commun. 8 (2005) 58–60.

[33] J.P. Demko, K.B. Sharpless, Preparation of 5-substituted 1-H tetrazoles from nitriles in water, J. Org. Chem. 66 (2001) 7945–7950.

[34] G.M. Sheldrick, Crystal structure refinement with SHELXL, Acta Crystallogr., Sect. A: Found. Adv. 71 (2015) 3–8.

[35] G.M. Sheldrick, Crystal structure refinement with SHELXL, Acta Crystallogr., Sect. C: Struct. Chem. 71 (2015) 3.

[36] V.A. Blatov, A.P Shevchenko, D.M. Proserpio, Applied topological analysis of crystal structures with the program package topospro, Cryst. Growth Des. 14 (2014) 3576–3586.

[37] S. Alvarez, P. Alemany, D. Casanova, J. Cirera, M. Llunell, D. Avnir, Shape maps and polyhedral inter conversion paths in transition metal chemistry, Coord. Chem. Rev. 249 (2005) 1693–1708.

[38] E.K. Brechin, L.M. Gilby, R.O. Gould, S.G. Harris, S. Parsons, R.E.P. Winpenny, J. Chem. Soc. Dalton Trans (1998) 265.

[39] **S.K. Wolff, D.J. Grimwood, J.J. McKinnon, M.J. Turner, D. Jayatilaka, M.A. Spackman, Crystal Explorer 3.1, in: University of Western Australia, Crawley, Western, Australia, 2013, pp. 2005–2013.** http://hirshfeldsurface.net/CrystalExplorer.

[40] S.K. Seth, D. Sarkar, A. Roy, T. Kar, Insight into supramolecular self-assembly directed by weak interactions in acetophenone derivatives: crystal structures and Hirshfeld surface analyses, CrystEngComm 13 (2011) 6728–6741.

[41] J.J. McKinnon, D. Jayatilaka, M.A. Spackman, Towards quantitative analysis of intermolecular interactions with Hirshfeld surfaces, Chem. Commun. (2007) 3814–3816.

[42] F.L. Hirshfeld, Bonded-atom fragments for describing molecular charge densities, Theor. Chim. Acta. 44 (1977) 129–138.

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Antioxidant, antimicrobial, DNA binding and cleavage studies of novel Co(II), Ni(II) and Cu(II) complexes of N, O donor Schiff bases: Synthesis and spectral characterization

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ABSTRACT

A series of bivalent metal complexes $M(L_1)_2$ and $M(L_2)_2$ where M = Cu(II), Ni(II), Co(II) and HL_1=2-((E)-(4-(trifluoromethoxy)phenylimino)methyl)-4-chlorophenol, HL_2=2-((E)-(4-(trifluoromethoxy)phenylimino)methyl)-4-nitrophenol were synthesized and characterized by elemental analysis, SEM, Mass, ^{1}H NMR, ^{13}C NMR, UV–Vis, FT-IR, ESR and magnetic susceptibility measurements. Based on the analytical and spectral data a square planar geometry has been assigned to all the metal complexes. DNA binding properties of these complexes have been explored using electronic absorption spectroscopy, fluorescence spectroscopy and viscosity measurements. The experimental evidence indicated that these binary complexes strongly bind to calf thymus DNA through an intercalation method. DNA cleavage efficacy of these metal(II) complexes have been investigated with super-coiled pBR322 DNA by gel electrophoresis in presence of H_2O_2 and UV light, and found that all complexes showed better nuclease activity. The in vitro antimicrobial activity results by paper disc method against few bacterial pathogens such as Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aureus as well as fungal species Aspergillus niger and Candida albicans showed that the complexes are more biocidal than free ligands. Further, the compounds were screened for antioxidant activity using 1,1-diphenyl-2-picryl hydrazyl (DPPH) which was compared with a standard drug ascorbic acid.

1. Introduction

According to National Institute of Cancer Prevention and Research (NICPR), one woman dies from cervical cancer every 8 minutes in India [1]. Every year new cancer patients are registering around 11,57,294 lakh. In India, in 2018 total deaths due to cancer is 7,84,821 [2]. Various research studies were conducted to eliminate cancer from the society. The interaction of transitional metal complexes with DNA is one of the vital concepts to produce effective DNA binding anticancer drugs, chemotherapeutic agents and best antineoplastic drugs [3]. For last few decades many researchers waged their efforts on synthesis and design of metal based therapeutics. Most of the researchers focused for the development of DNA targeted drugs [4]. DNA is a primary cellular target for most of the therapeutic drugs in clinical use [5]. Earlier platinum based drugs used as anticancer drugs. Later the platinum based drugs proved of undesirable side effects and other health problems. Now, researchers have been looking for suitable substitutes to replace the cis-platin and its derivatives. Many metal complexes were tested to know the anticancer activity of those metals. It is proved that the cationic characters, structural profiles, and other peculiar characters of Schiff base metal complexes are most credit worthy elements to behave as anticancer drugs [6–9]. Transition elements are the essential elements for human beings with their bio essential, oxidative nature and enzymatic activity. The complexes of copper, cobalt and nickel ions have gained enormous attention of inorganic chemists to focus on clinical applications [10–15]. Palaniandavar et al. pointed out among the transition metals Cu(II) is suitable alternative to cis-platin [16]. Cobalt complexes are found to be important antimicrobial, antiviral, antitumor, antioxidant and anticancer agents [17–19]. Nickel complexes have eminent role in bioinorganic chemistry, redox enzyme system, medicinal chemistry and recently they have been tested for inhibition of cancer cell proliferation [20,21].

Recently, our group have reported synthesis, characterization, DNA binding and nuclease efficacy, antimicrobial and antioxidant properties of Cu(II), Co(II) and Ni(II) complexes of various Schiff bases [22–25]. All the above facts stimulated our interest

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to focus on synthesizing the metal based drugs which can interact with DNA non-covalently with diminished side effects and various pronounced biological applications of complexes with 4-trifluoromethoxy aniline Schiff bases.

2. Experimental

2.1. Materials

All the required chemicals procured from Finar/Merck/ Hi Media Ltd. and Sigma-Aldrich. The solvents such as chloroform, ethanol, methanol and water were purified according to standard literature methods [26]. The CT-DNA (calf thymus) and supercoiled pBR322 DNA (Merck made) were purchased from Genei, Bangalore and stored at 4 °C. Buffer (Tris–HCl/NaCl), ethidium bromide (EB) purchased from Merck, Hyderabad, India.

2.2. Instrumentation

Microanalyses of C, H and N of ligands and metal complexes were accomplished by using Perkin Elmer 240C (USA) elemental analyzer. Metal content of the complexes was estimated by atomic absorption spectroscopy using GBC Avanta 1.0 AAS. Melting points were monitored on a Polmon instrument (model No. MP-96). NMR spectra of the ligands were recorded on Bruker 400 MHz NMR instrument using TMS (tetramethyl silane) as internal standard. Morphology and particle sizes of the ligands and metal complexes were investigated by SEM (scanning electron microscope, Zeiss evo18). Using the INCA EDX instrument, the surface elements of compounds were analyzed. Electronic spectral data were obtained from Shimadzu UV-Visible spectrophotometer (UV-2600). Fluorescence studies were performed on JASCO spectrofluorometer FP-8500. Magnetic moment values of all metal complexes were calculated on Gouy balance model 7550 with $Hg[Co(NCS)_4]$ used as the calibrant. Thermal studies of complexes were carried out on a Mettler Toledo Star system in the temperature range of 30-1000°C under dynamic nitrogen atmosphere (20 mL min^{-1}) with a heating rate of 10 °C min^{-1}. ESR spectra of metal complexes were recorded at liquid nitrogen temperature using JES-FA200 ESR spectrometer (JEOL-Japan).

2.3. Synthesis of Schiff bases and their binary metal complexes

2.3.1. Synthesis of Schiff bases HL_1 and HL_2

The methanolic solution (50 mL) of 4-(trifluoromethoxy)benzenamine (10 mM) was mixed with methanolic solution (50 mL) of 5-Chloro salicylaldehyde (10 mM) / 5-nitro salicylaldehyde (10 mM). The mixture was stirred for 2-4 h at 60-70°C temperature on an oil bath. The yellow colored precipitates obtained were isolated by filtration and recrystallized from methanol. The purity of the product and completion of reaction was monitored by TLC.

2.3.2. HL_1

($C_{14}H_9ClF_3NO_2$), Anal. Calc (%): C, 53.27; H, 2.87; N, 4.44. Found: C, 52.98; H, 2.68; N, 4.28. IR (KBr): $\nu_{(O-H)}$ 3450, $\nu_{(CH=N)}$ 1632, $\nu_{(C-O)}$ 1164. UV–vis; λ_{max}/nm (cm^{-1}) (DMSO): 253 (39525), 290 (34482). ^{1}H-NMR (400 MHz, $CDCl_3$) (δ): =14.13 (s, 1H), 8.68 (s, 1H), 7.57 (d, 1H), 7.47 (s, 1H), 7.29-7.27 (m, 2H), 7.10-7.09 (m, 3H) (Fig. S1). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.40, 151.60, 147.83, 147.75, 146.89, 123.98, 122.40, 122.06, 119.19, 119.04, 118.74, 116.64, 64.69, 14.87 (Fig. S2). MS (ESI): m/z 315 $[M+H]^+$ (Fig. S3). MP-167°C.

2.3.3. HL_2

($C_{14}H_9F_3N_2O_4$), Anal. Calc (%): C, 51.54; H, 2.78; N, 8.59. Found: C, 51.47; H, 2.72; N, 8.48. IR (KBr): $\nu_{(O-H)}$ 3435, $\nu_{(CH=N)}$ 1624, $\nu_{(C-O)}$ 1160. UV–vis; λ_{max}/nm (cm^{-1}) (DMSO): 273 (36630), 340 (29411). ^{1}H-NMR (400 MHz, $CDCl_3$) (δ): 14.05 (s, 1H); 8.72 (s, 1H); 8.43-8.29(m, 4H) 7.39-7.15 (m, 3H); (Fig. S4). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.25, 153.68, 152.46, 145.37, 135.22, 134.58, 133.38, 124.35, 121.70, 120.04, 119.67, 113.41, 114.08.35, 110.69 (Fig. S5). MS (ESI): m/z 326 $[M+H]^+$ (Fig. S6). MP-195°C.

2.4. Synthesis of binary metal complexes

Binary metal complexes were prepared using metal salts and ligands in 1:2 molar ratio. A hot methanolic solution of 10 mM of metal acetate [M=Cu(II)/Co(II)/Ni(II)] was added to a hot methanolic solution of Schiff base ligand [HL_1/HL_2] (20 mM) and the mixed solution was refluxed for 2-4 h at 60-70°C. The precipitate was kept aside for slow evaporation at room temperature. The solid formed was collected by suction filtration, washed thoroughly with methanol and then dried in desiccator over anhydrous $CaCl_2$. An outline of synthetic procedure of ligands and respective metal complexes are presented in Scheme 1.

2.4.1. $[Cu(L_1)_2]$

1a, ($C_{28}H_{16}F_6N_2O_4$ Cl_2Cu). Anal. Calc(%): C, 48.54; H, 2.33; N, 4.04; Cu, 9.17. Found: C, 48.43; H, 2.27; N, 3.97; Cu, 9.05. IR (KBr): $\nu_{(CH=N)}$ 1588, $\nu_{(C-O)}$ 1168, $\nu_{(M-O)}$ 529, $\nu_{(M-N)}$ 435. ESR: $g_{\parallel}$ = 2.138, $g_{\perp}$ = 2.088, G = 1.58, UV–vis; λ_{max}/nm (cm^{-1}) (DMSO): 260 (38461), 340 (29411), 420 (23809), 585 (17094). μ_{eff} (BM): 1.81. MS (ESI): m/z 690 $[M+H]^+$. MP-298 °C.

2.4.2. $[Co(L_1)_2]$

1b, ($C_{28}H_{16}F_6N_2O_4$ Cl_2Co) Anal. Calc(%): C, 48.86; H, 2.34; N, 4.07; Co, 8.56. Found: C, 48.66; H, 2.20; N, 3.95; Co, 8.32. IR (KBr): $\nu_{(CH=N)}$ 1601, $\nu_{(C-O)}$ 1166, $\nu_{(M-O)}$ 520, $\nu_{(M-N)}$ 440. UV–vis; λ_{max}/nm (cm^{-1}) (DMSO): 258 (38759), 351 (28490), 425 (23529), 568 (17605). μ_{eff} (BM): 2.21. MS (ESI): m/z 686 $[M+H]^+$. MP-302 °C.

2.4.3. $[Ni(L_1)_2]$

1c, ($C_{28}H_{16}F_6N_2O_4$ Cl_2 Ni) Anal. Calc(%): C, 48.88; H, 2.34; N, 4.07; Ni, 8.53. Found: C, 48.69; H, 2.29; N, 3.89; Ni, 8.37. IR (KBr): $\nu_{(CH=N)}$ 1580, $\nu_{(C-O)}$ 1172, $\nu_{(M-O)}$ 520, $\nu_{(M-N)}$ 471. UV–vis; λ_{max}/nm (cm^{-1}) (DMSO): 267 (37453), 373 (26809), 420 (23809), 568 (17605). μ_{eff} (BM): dia. MS (ESI): m/z 688 $[M]^+$. MP-275 °C.

2.4.4. $[Cu(L_2)_2]$

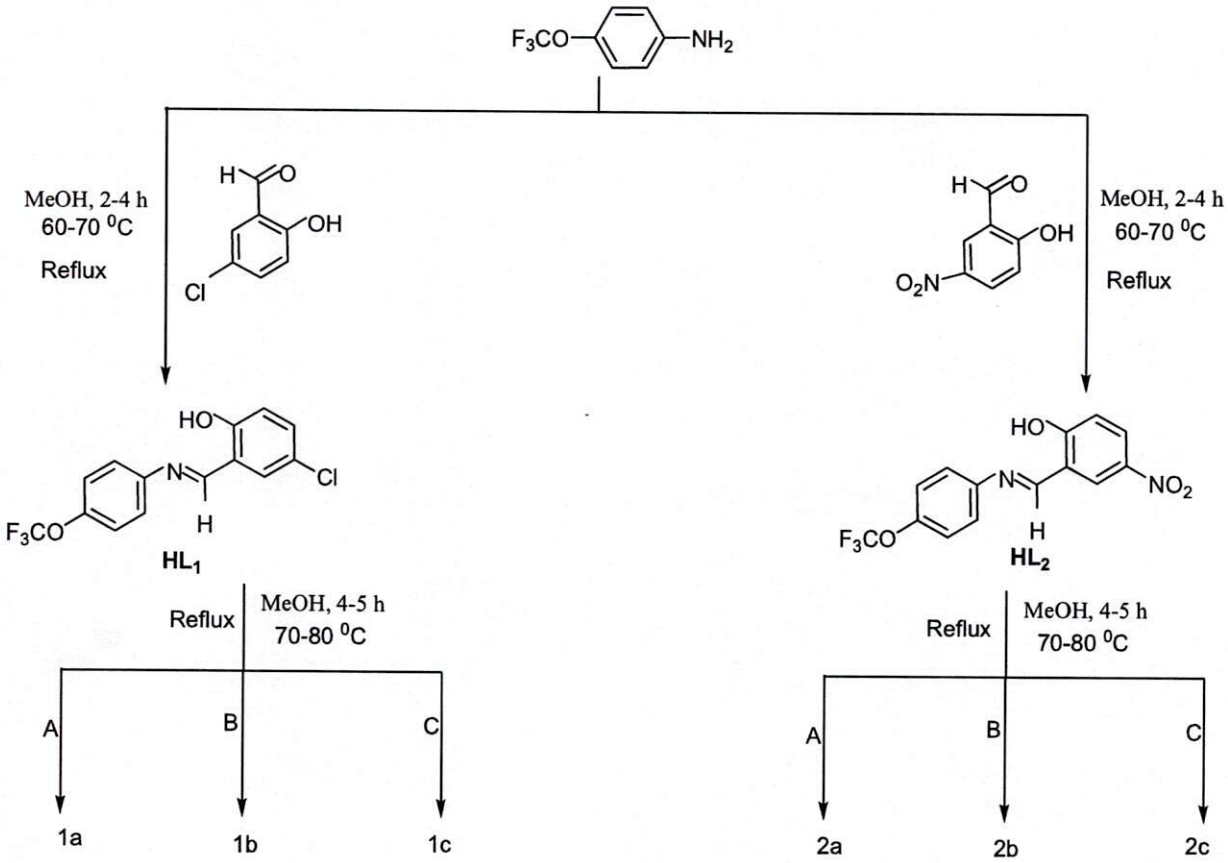
2a, ($C_{28}H_{16}F_6N_4O_8$ Cu) Anal. Calc(%): C, 47.10; H, 2.26; N,7.85; Cu, 8.90. Found: C, 47.01; H, 2.15; N, 7.67; Cu, 8.79. IR (KBr): $\nu_{(CH=N)}$ 1598, $\nu_{(C-O)}$ 1172, $\nu_{(M-O)}$ 531, $\nu_{(M-N)}$ 432. ESR: $g_{\parallel}$ = 2.140, $g_{\perp}$ = 2.075, G = 1.8. UV–vis; λ_{max}/nm (cm^{-1}) (DMSO): 271 (36900), 410 (24390), 567 (17636). μ_{eff} (BM): 1.74. MS (ESI): m/z 713 [M+1]. MP-310 °C.

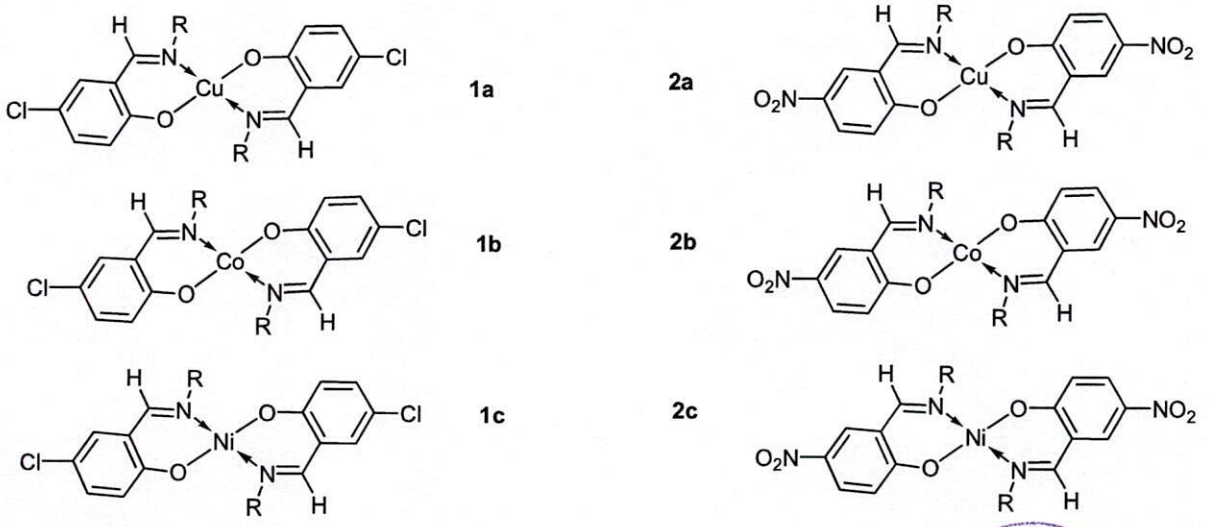
2.4.5. $[Co(L_2)_2]$

2b, ($C_{28}H_{16}F_6N_4O_8$ Co) Anal. Calc.(%): C, 47.41; H, 2.27; N, 7.90; Co, 8.31. Found: C, 47.29; H, 2.05; N, 7.78; Co, 8.09. IR (KBr) $\nu_{(CH=N)}$ 1602, $\nu_{(C-O)}$ 1175, $\nu_{(M-O)}$ 542, $\nu_{(M-N)}$ 425. UV–vis; λ_{max}/nm (cm^{-1}) (DMSO): 258 (38759), 391 (25575), 574 (17421). μ_{eff} (BM): 2.16. MS (ESI): m/z 709 $[M]^+$. MP-282 °C.

2.4.6. $[Ni(L_2)_2]$

2c, ($C_{28}H_{16}F_6N_4O_8$ Ni) Anal. Calc(%): C, 47.42; H, 2.27; N, 7.90; Ni, 8.28. Found: C, 47.28; H, 2.09; N, 7.78; Ni, 8.12. IR (KBr): $\nu_{(CH=N)}$ 1597, $\nu_{(C-O)}$ 1180, $\nu_{(M-O)}$ 529, $\nu_{(M-N)}$ 421. UV–vis; λ_{max}/nm (cm^{-1}) (DMSO): 263 (38022), 349 (28653), 415 (24096), 564 (17730). μ_{eff} (BM): dia. MS (ESI): m/z 708 [M+1]. MP-275 °C.





R = F_3CO–C$_6$H$_4$–

A = Copper(II) acetate mono hydrate
B = Cobalt(II) acetate tetra hydrate
C = Nickel(II) acetate tetra hydrate

Scheme 1. Synthesis of ligands and their Cu(II), Co(II) and Ni(II) complexes.

2.5. DNA binding studies

2.5.1. Electronic absorption study

Electron absorption study is an excellent technique to investigate the interaction of metal complexes with CT-DNA. The absorption spectra of complexes were recorded in TAE buffer (40 mM Tris base, 20 mM acetic acid and 1mM EDTA). Complexes stock solutions were prepared in buffer by taking the little amount of DMSO. UV absorbance of CT-DNA in buffer solution at 260 and 280 nm gave a ratio of 1.8-1.9/1, indicating the DNA being sufficiently free from protein [27]. Concentration of CT-DNA was determined with absorption spectroscopy by employing the known molar extinction coefficient of 6600 $M^{-1}cm^{-1}$ at 260 nm [28].

The intrinsic binding constants (K_b) were acquired by monitoring the changes in the absorbance of the complexes. Complex concentration is fixed as 10 μM and concentration of nucleotide is increased from 0–10 μM. Absorbance of CT-DNA is eliminated by adding equal amounts of CT-DNA to both the complex solution and the reference solution while measuring the absorption of complexes. Binding strength of the complexes against CT-DNA was compared quantitatively by substituting the data in the following equation.

$$[DNA]/ (\varepsilon_a-\varepsilon_f) = [DNA]/ (\varepsilon_b-\varepsilon_f) + 1/K_b (\varepsilon_b-\varepsilon_f)$$

where, [DNA] is the concentration of CT-DNA in base pairs. The apparent absorption coefficients ε_a, ε_f and ε_b correspond to $A_{obs}/[M]$, the extinction coefficients for the free and fully bound form of copper(II) complex, respectively [29]. K_b is given by the ratio of slope to the intercept.

2.5.2. Fluorescence quenching study

The emission quenching studies were also performed for additional support for relative binding of metal complexes to CT–DNA. Ethidium bromide (EB) is a renowned fluorescent probe for DNA moiety and used in evaluation of the binding mode between metal complex and CT–DNA [25]. In buffer solution, EB shows reduced emission intensity, whereas the fluorescence intensity dramatically increased by pre-treatment of EB (12.5 μM) with CT–DNA (125 μM) for 30 min in TAE buffer (pH 7.4) at 25 °C. Further the metal complexes in various amounts of concentrations (0-60 μM) were added to the pre-treated solution. The excitation wavelength of instrument fixed at 350 nm and the emission spectra of constant EB–DNA were recorded (λ = 520–680 nm) with changing the concentration of metal complex.

The quenching parameter can be calculated by Stern-Volmer equation.

$$I_o/I = 1 + K_{SV} [Q]$$

where, I_o and I are the emission intensities in the absence and presence of the quencher, respectively and [Q] is the quencher concentration (complex to that of DNA) and K_{SV} is the Stern-Volmer constant, the K_{SV} value is obtained as a slope from the plot of I_o/I versus [Complex].

2.5.3. Viscosity study

Further clarification of the interaction between metal complex and DNA was carried out by viscosity measurements by using an Ostwald capillary viscometer immersed in a thermostatic water bath maintained at 30 ± 1 °C. Keeping the CT–DNA (200 µM) concentration fixed and the metal complexes concentration was varied (0-200 µM). Flow time was recorded with a digital stop watch and every sample was measured at least thrice to get an average flow time. Data were presented as $(\eta/\eta_o)^{1/3}$ versus [complex]/[DNA], where as η is the viscosity of DNA solution in the presence of the complex, η_o is viscosity of the DNA alone.

2.6. DNA cleavage

Both, photolytic (under UV light) and oxidative (H_2O_2) cleavages of supercoiled pBR322 DNA by all the compounds were monitored by agarose gel electrophoresis technique at pH 7.2 in Tris–HCl buffer. The samples were incubated at 37°C for 2 h followed by mixing with loading dye 0.25% bromophenol blue to make a total volume of 16 μL. After proper incubation period, samples were loaded in the agarose gel and electrophoresis was performed at 70 V for 1 h. Finally, images were taken under UV–illuminator using Bio-Rad Gel documentation system.

2.7. Antioxidant activity

The antioxidant activity of metal complexes against the stable free radical DPPH (1,1-Diphenyl-2-picryl-hydrazyl) was evaluated. The scavenging capacity of DPPH was determined by using UV-visible spectrophotometer. In this scavenging assay, DPPH acts as a stable radical due to the paramagnetic nature arises from its odd electron. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolorized as the color changes from deep violet to light yellow. Briefly, 500 µM stock solutions of the complexes in DMSO (25 mL) were prepared. From the stock solution, different (20, 40, 60, 80 and 100 mM) concentrations of complexes were prepared by serial dilutions. 1 mL of each complex solution having different concentrations was taken in different test tubes and 4 mL of 0.1 mmol DMSO DPPH solution was added and shaken vigorously for about 2–3 min, then incubated in a dark room for 30 min at room temperature. A blank DPPH solution was utilized for the baseline correction because the odd electron in the DPPH gave a strong absorption maximum at 517 nm. After incubation, the absorbance values of each solution were measured at 517 nm and there was a change (decrease) in the absorbance values indicating that the complexes showed moderate free radical scavenging activity. Ascorbic acid (AA) was used as the reference or positive control and the percentage of the free radical scavenging activity of the tested samples expressed as percentage inhibition of DPPH, was calculated according to the formula,

$$\text{DPPH Scavenging Activity (\%)} = (A_o\text{-}A_t)/A_o \times 100$$

where A_t is the absorbance of sample solution and A_o is the absorbance value of control solutions or standards for positive control. Percent inihibition was plotted against log concentration and the equation for the line was used to obtain IC_{50} value. A lower IC_{50} value indicated greater antioxidant activity.

Calculation of IC_{50} values: A linear regression fitting between the % inhibition and log concentration was done and the concentration corresponding to 50% inhibition was expressed as IC_{50} value.

2.8. Antibacterial activity

All the synthesized complexes of respective ligands were screened for in vitro antibacterial activity against two Gram-negative bacteria such as Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853) and two Gram-positive bacteria such as Staphylococcus aureus (ATCC 25923) and Bacillus subtilis (MTCC 121). The agar well diffusion method, which was described earlier by Balouiri et al [30], was adopted for measuring the antibacterial assays. Briefly, all cultures were routinely maintained on

NA (nutrient agar) and incubated at 37 °C for overnight. The culture was centrifuged at 1000 rpm and pellets were re-suspended and volume of 0.1 mL diluted bacterial culture suspension was spread uniformly with the help of spreader on NA plates. Wells of 6 mm diameter were punched into the agar medium and loaded with ligands and their different metal complexes. Antibiotic disc, Ampicillin (100 mg/disc) was used as a positive and negative control. The plates were then incubated for 24 h at 37 °C and the resulting zone of inhibitions were measured in mm.

2.9. Antifungal activity

All the compounds were screened for in vitro antifungal activity against the inoculums of non-sporing fungi, A. niger and C. albicans, by growing the culture in SDA broth at 37 °C for overnight. All cultures were routinely maintained on sabouraud dextrose agar (SDA) and incubated at 28 °C. Volume of 0.1 mL of diluted fungal culture suspension was spread with the help of spreader on SDA plates uniformly. Sterile 6 mm discs were impregnated with the test complexes. Wells of 6 mm size were cut and loaded with different concentrations of the complexes. Ketoconazole (100 mg/disc) was used as a positive control. A. niger and C. albicans plates were incubated at 37 °C for 24–48 h and antifungal activity was determined by measuring the diameters of the inhibition zone (mm).

3. Results and discussion

The complexes were prepared in good yields (70–79%) by the reaction of free ligands ($\mathbf{HL_1}$ & $\mathbf{HL_2}$) with Cu(II), Co(II) and Ni(II) salts in methanol medium, as indicated in Scheme 1. All the complexes are stable to air and moisture for extended periods. The ligands are soluble in organic solvents like methanol, ethanol, acetonitrile, chloroform, DCM, DMF and DMSO and the complexes are soluble in DMF and DMSO only, whereas both are insoluble in H_2O. The analytical data of all the synthesized complexes are in good agreement with calculated values for 1:2 stoichiometric ratios of metal to ligand.

3.1. Fourier-transform infrared spectroscopy

The FT-IR analysis was carried out in the range of 4000–250 cm^{-1} using KBr pellets on a Perkin Elmer Frontier FT-IR/FIR spectrometer. The coordination modes were evaluated by comparing some important infrared spectral bands of free ligands with their corresponding metal complexes shown in Table 1 and Fig. S7. The Schiff bases showed a characteristic strong band around 1632–1624 cm^{-1} due to the υ(C=N). This band is shifted by 20–44 cm^{-1} to a lower frequency region in complexes indicating coordination of azomethine nitrogen atom of Schiff bases to metal ion. A broad band appeared in the range of 3450–3435 cm^{-1} attributed to υ(OH) in the Schiff bases, is disappeared in complexes indicating deprotonation of the phenolic proton prior to coordination.

Table 1
IR data (cm−1) of Schiff bases and their binary metal(II) complexes (1a-2c).

Compound	$\upsilon_{(OH)}$	$\upsilon_{(HC=N)}$	$\upsilon_{(C-O)}$	$\upsilon_{(M-O)}$	$\upsilon_{(M-N)}$
$\mathbf{HL_1}$	3450	1632	1164	-	-
1a	–	1588	1168	529	435
1b	–	1601	1166	520	440
1c	–	1580	1161	520	471
$\mathbf{HL_2}$	3435	1624	1160	-	-
2a	–	1598	1159	531	432
2b	–	1602	1170	542	425
2c	–	1597	1160	529	421

Table 2
Electronic spectral data of Schiff bases and their metal complexes (1c-2c) in nm (cm^{-1}).

Complex	π-π^* (aromatic)	n-π^* (azomethine)	CT transition	d-d transition
$\mathbf{HL_1}$	253 (39525)	290 (34482)	-	-
1a	260 (38461)	340 (29411)	420 (23809)	585 (17094)
1b	258(38759)	351(28490)	425(23529)	568 (17605)
1c	267 (37453)	373 (26809)	420 (23809)	568 (17605)
$\mathbf{HL_2}$	273 (36630)	340 (29411)	-	-
2a	271 (36900)	-	410 (24390)	567 (17636)
2b	258 (38759)	391(25575)	-	574 (17421)
2c	263 (38022)	349 (28653)	415 (24096)	564 (17730)

This is further confirmed by strong intense band of phenolic C–O stretching observed at 1160–1164 cm^{-1} in the ligands is shifted to higher frequency of 1180–1172 cm^{-1} in complexes. In addition, the new non-ligand bands are observed at lower frequency regions 471–421 cm^{-1} and 542–520 cm^{-1} due to stretching frequency of υM–O and υM–N respectively [31,32].

3.2. Electronic absorption analysis

UV–Vis absorption spectra, given in Table 2 and Fig. S8, of the six metal complexes were recorded in DMSO solution. Compounds shown a broad band at 564-585 nm corresponding to $^2B_{1g}$-^2E_g for Cu(II) complexes [33], $^1A_{1g}$-$^1B_{1g}$ for Co(II) complexes, $^1A_{1g}$-$^1A_{2g}$ for Ni(II) complexes [34,35] which is consistent with a square planar geometry. The ligands shown absorption spectral bands in the range of 253–274 (π-π^*) nm and 290–340 (n-π^*) nm, and upon complexation, all bands shifted to 258–271 nm and 340-391 nm [36]. The effective magnetic moments of Cu(II) and Co(II) complexes are found to be 1.81 (1a), 2.21 (1b), 1.74 (2a), and 2.16 (2b) respectively. Ni(II) complexes are found to be diamagnetic [37]. The electronic transitions and magnetic moment results are consistent with a square planar geometry for all the complexes.

3.3. Powder X-ray diffraction analysis

The X-ray diffractograms of Cu(II), Co(II) and Ni(II) complexes of $\mathbf{HL_1}$ and $\mathbf{HL_2}$ were scanned in the range of 2θ = 4–80° at a wavelength of 1.543 A° and are shown in Fig. 1. The single crystals of complexes could not be isolated because of their insolubility in most of organic solvents. The powder XRD of complexes showed sharp crystalline peaks indicating its crystalline phase. The diffractograms and associated data depicted the 2θ value for each peak, the relative intensity and inter-planar spacing (d-values). Hence, the powder-XRD pattern of compounds has been studied in order to test the degree of crystallinity of the compounds. The inter-planar spacing (d) has been calculated by using Bragg's equation, $n\lambda = 2d \sin \theta$. To evaluate the crystallite sizes of the synthesized compounds, D was determined using Debye-Scherrer formula given by

$$D = 0.94\lambda / \beta \cos \theta$$

where β is the full width at half maximum of the predominant peak and θ is the diffraction angle and λ is the wavelength of X-ray. The sizes of the crystallites of the ligands $\mathbf{HL_1}$ for 92.61, $\mathbf{HL_2}$ for 84.26 and their complexes are found to be 21.24 for complex **1a**, 34.58 for complex **1c**, 38.54 for complex **1b**, 29.21 for complex **2a**, 26.27 for complex **2c**, 34.85 nm for complex **2b**.

3.4. Scanning electron microscope analysis

The particle size, purity, morphology and the surface structure of the compounds can be predicted from the scanning electron mi-

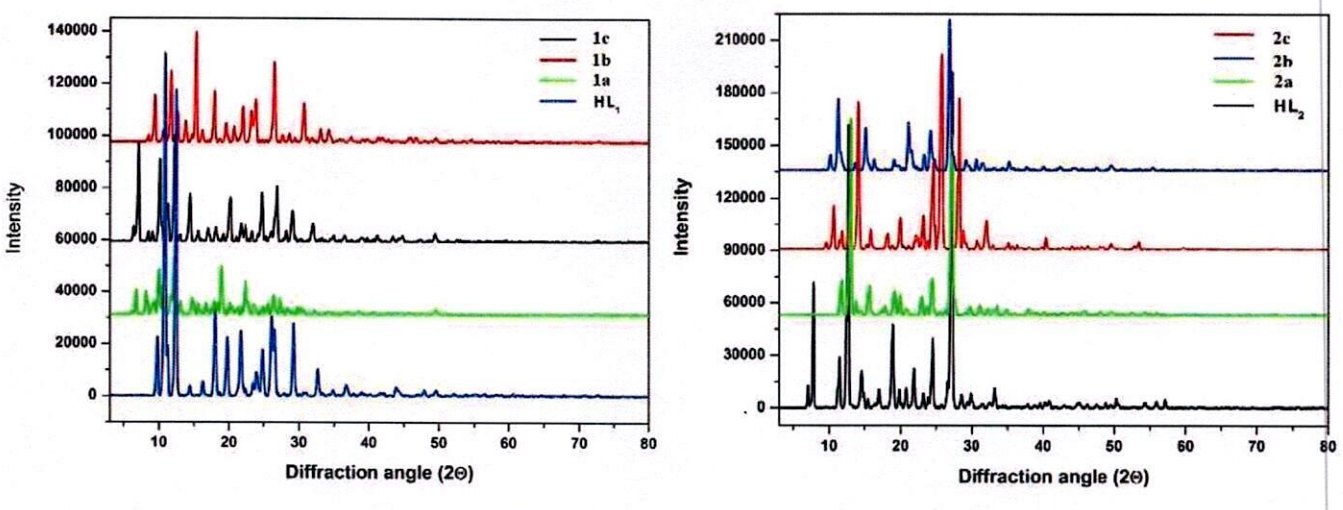


Fig. 1. The powder XRD spectra of synthesized ligands and their metal complexes.

crograph (SEM). SEM pictographs of free ligands and corresponding complexes are shown in Fig. 2. The SEM images revealed that the metal complexes having particles are agglomerated with the controlled morphological structure and the presence of irregular spherical like structures. However, particles of the size less than 100 nm were also observed, which group to form an agglomerate of larger size. From the SEM images, the complexes have homogeneous matrix with even interface having ideal shape of uniform phase material. The elemental composition of corresponding ligands and complexes were shown in EDX spectra. The EDX spectra are demonstrated the exact constituent elements present in the ligands and complexes. The average crystalline sizes of these complexes obtained from XRD are poly crystalline with nano-sized grains.

3.5. Mass spectra

Mass spectra provide a preliminary clue for structure elucidation of compounds. The ESI mass spectra of ligands exhibited $[M+H]^+$ peaks at m/z 315 and 326 for (**HL_1**) and (**HL_2**) respectively. The molecular ion peaks at m/z 690 [M+1] for (**1a**), 713 [M+1] of (**1b**). 686, [M+1] for (**2a**), 790 [M+1] for (**2b**), 688 $[M]^+$ for (**1c**) and 709 [M+1] for (**2c**) complexes show in Fig.S9–S14. The elemental analysis and mass spectral data are in good agreement with 1:2 stoichiometric ratios for the formation of complexes.

3.6. Thermogravimetric analysis

TGA is a prominent tool to investigate the thermal behavior and the decomposition pattern of all the synthesized complexes (**1a-2c**). The thermal analysis of Cu(II), Co(II) and Ni(II) complexes was monitored at a heating rate suitably controlled at 10 °C/min under nitrogen atmosphere over an ambient temperature range of 30-1000 °C. The thermograms of complexes are depicted in Fig. 3 and Fig. S15. Each thermogram shows two decomposition steps. All the complexes exhibit high thermal stability up to 200 °C evidencing the absence of water molecules within/out of the coordination sphere. In first step, the complexes showed a sudden weight loss around 240 °C. In second degradation step, a gradual weight loss is observed in the temperature range of 240–470 °C may be corresponding to the complete decomposition of the organic part around the metal ion and the further horizontal line may be due to metal oxide residue as final part.

3.7. Electron spin resonance spectra

ESR spectral studies provide information about the delocalization of unpaired electrons and bonding nature between the metal and ligand. ESR spectra of Cu(II) metal complexes were recorded in DMSO at liquid nitrogen temperature (LNT), shown in Fig. 4. The values of ESR parameters $g_{\parallel}$, $g_{\perp}$ and G values have been calculated. The order of g values are found to be, $g_{\parallel} > g_{\perp} > g_e$ (2.0023) suggesting the unpaired electron is localized in $d_{x^2-y^2}$ orbital with $^2B_{1g}$ ground state which is an indicative of square planar geometry for Cu(II) complex [38]. The G [$G = (g_{\parallel}-2.0023) / (g_{\perp}-2.0023)$] values found to be 1.58 for **1a** and 1.8 for **2a** suggesting considerable exchange interactions in Cu(II) complexes. As $g_{\parallel} < 2.3$, these complexes are covalent in nature [25].

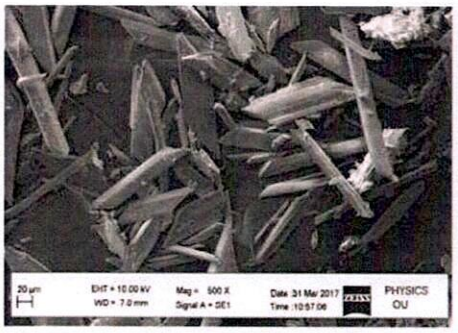
3.8. DNA binding studies

3.8.1. Electronic absorption study

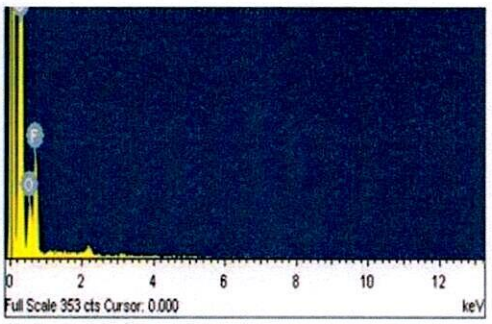
Electronic absorption spectroscopy is one of the most powerful experimental methods for probing metal complex-DNA interactions. In general, the binding nature of the metal complexes towards DNA helix can be investigated by observing the changes in wavelength and absorbance. Complex binding with DNA via intercalation usually results in the reduction in absorbance (hypochromism) and the shift towards higher wavelength (red shift/bathochromic shift) due to a strong π-π^* stacking interaction between the aromatic chromophore and the base pairs of DNA [39]. The extent of the hypochromism and bathochromism is commonly related to the intercalative binding strength. The electronic absorption spectra of **1a** (279 nm), **2a** (293 nm), **1b** (258 nm), **2b** (296 nm), **1c** (265 nm) and **2c** (279 nm) showed intensive absorption bands Fig. 5. These bands are attributed to the intraligand π-π^* transitions. Upon each addition of DNA, the intensity of the π-π^*transition band reduced by 9-27% (hypochromism) with a slight red shift of 3-8 nm. The intrinsic binding constant K_b is calculated from a plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ versus [DNA] using the equation:

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_a - \varepsilon_f) + 1/K_b\ (\varepsilon_b - \varepsilon_f)$$

where, [DNA] is the concentration of DNA in base pairs, and ε_a, ε_f and ε_b are the apparent absorption coefficients of the compound in the presence of DNA, free compound and the fully DNA-bound compound, respectively. K_b is calculated from the slope to intercept ratio. The K_b values for ligands and complexes are $1.43\pm0.02 \times 10^5\ M^{-1}$ (**1a**), $1.31\pm0.02 \times 10^5\ M^{-1}$

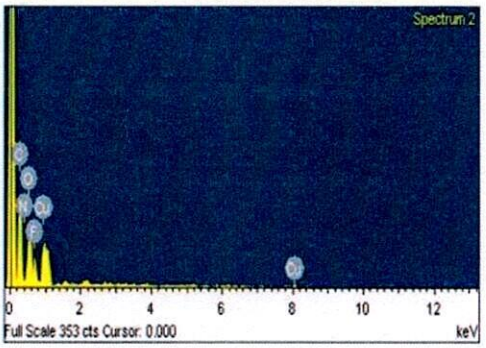


SEM photograph of HL_1

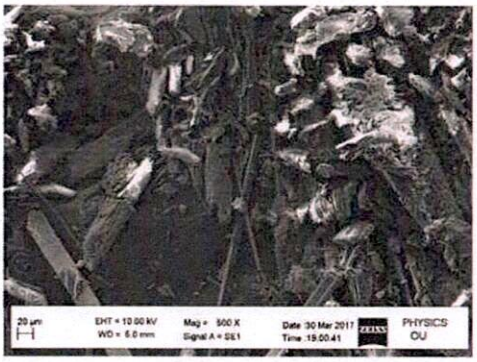


EDX graph of HL_1

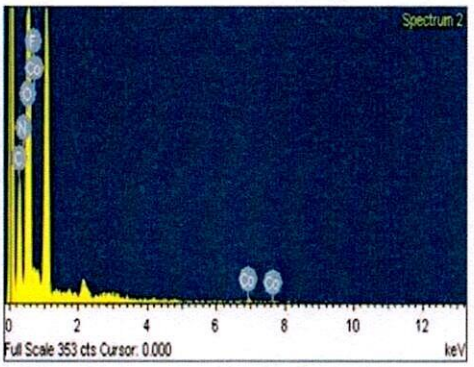
SEM photograph of **1a**



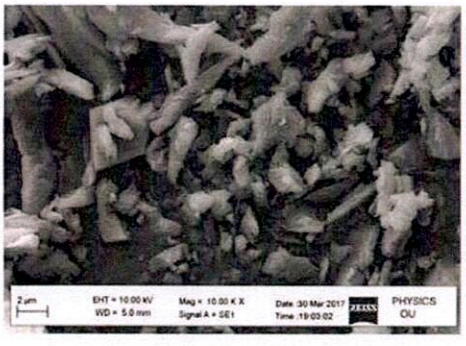
EDX graph of **1a**



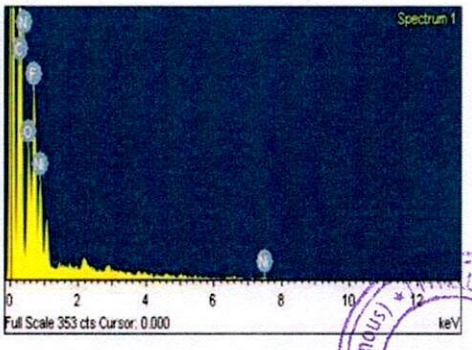
SEM photograph of **1b**



EDX graph of **1b**



SEM photograph of **1c**



EDX graph of **1c**

Fig. 2. SEM and EDX images of HL_1 and its metal complexes (**1a-1c**).

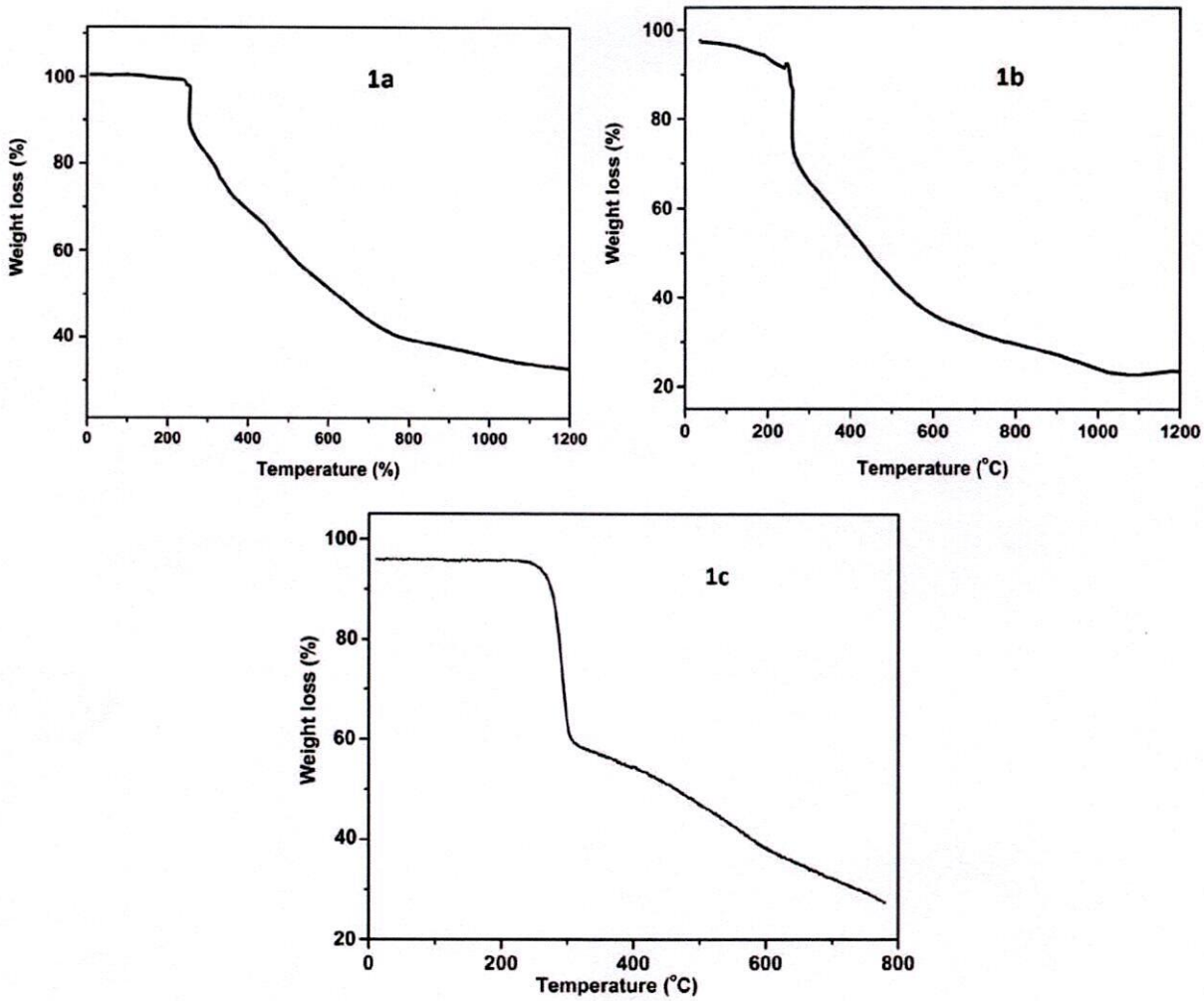


Fig. 3. The TGA curves of metal complexes (**1a-1c**).

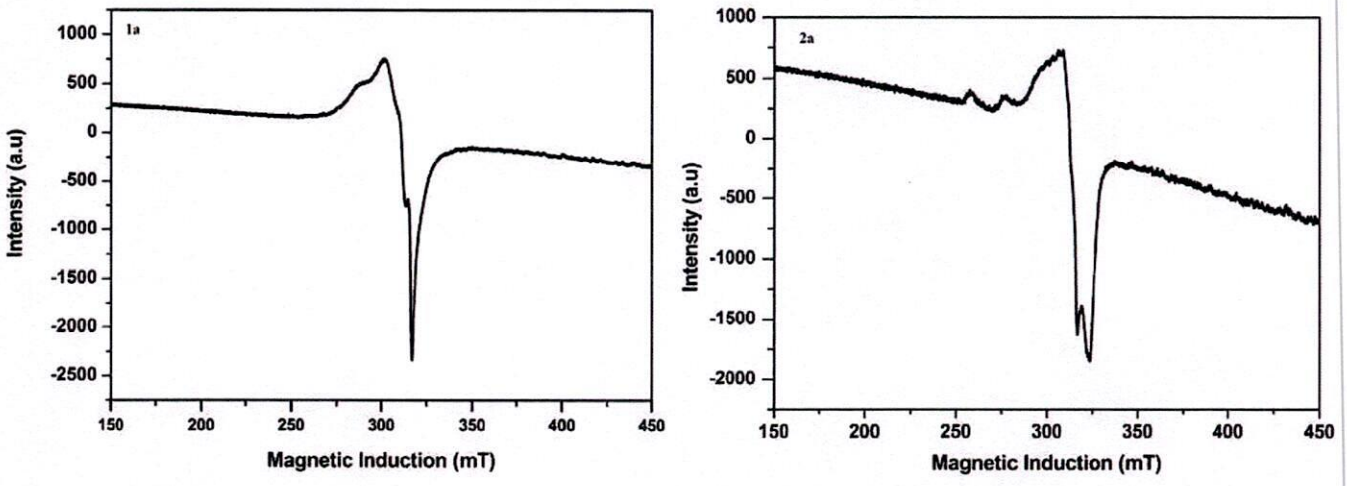


Fig. 4. ESR spectra of complexes, **1a** and **2a.**

(**2a**), 1.15±0.02 × 10^5 M^{-1} (**1b**), 1.09±0.02 × 10^5 M^{-1} (**2b**), 0.92±0.02 × 10^5 M^{-1} (**1c**) and 0.63±0.02 × 10^5 M^{-1} (**2c**) respectively. These K_b values revealed the Cu(II) complex showed stronger binding affinity. However, the binding affinity between the compounds and CT-DNA is less than that of potential intercalator like ethidium bromide (EB) (K_b = 7 × 10^7 M^{-1}) [40]. Among all, the Cu(II) complexes exhibit more binding strength than the set of Co(II) and NI(II) complexes.

3.8.2. Fluorescence quenching study

Fluorescence experiments were executed to get support for the mode of binding of ligands and complexes with CT-DNA. The ca-

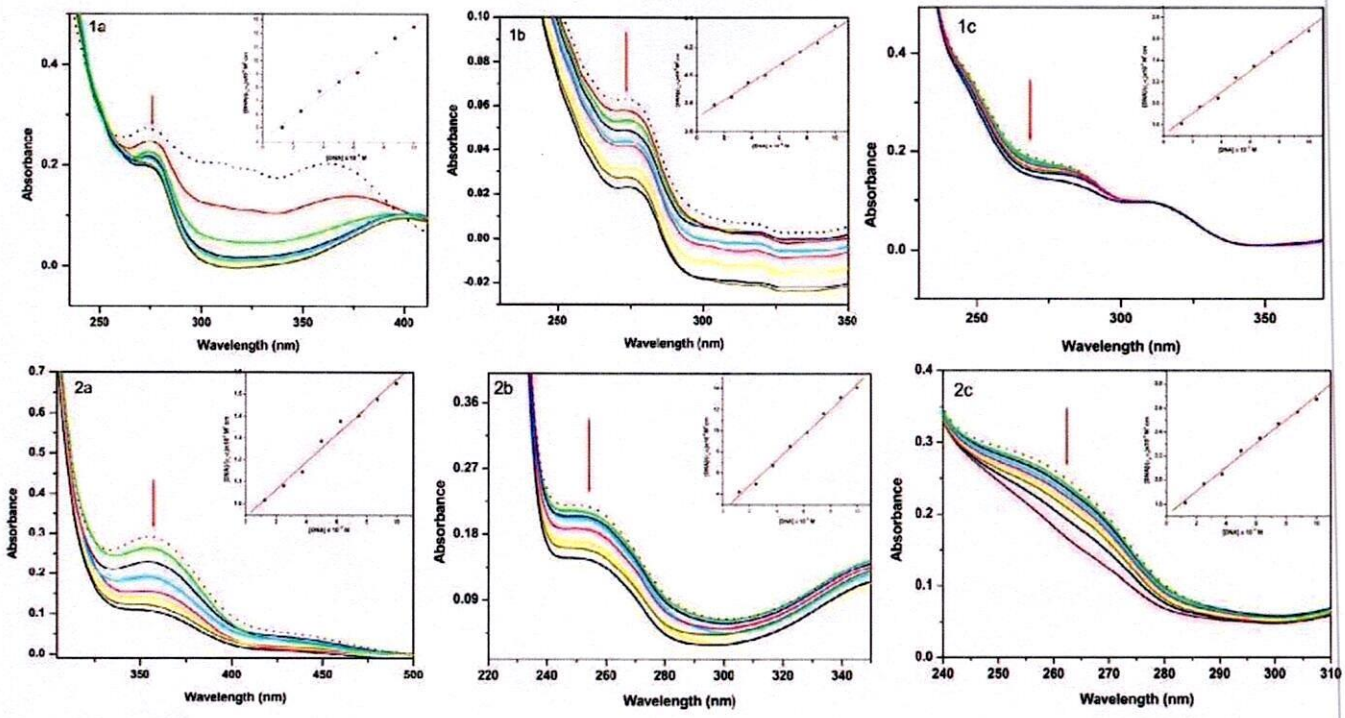


Fig. 5. UV–vis absorption spectra of complexes **1a-2c** in the absence (dashed line) and presence (solid lines) of increasing concentrations of CT-DNA in TrisHCl/Nacl buffer (pH 7.2). Conditions: [Ligand] or [Complex] =10μM, [DNA] = 0 - 10μM.

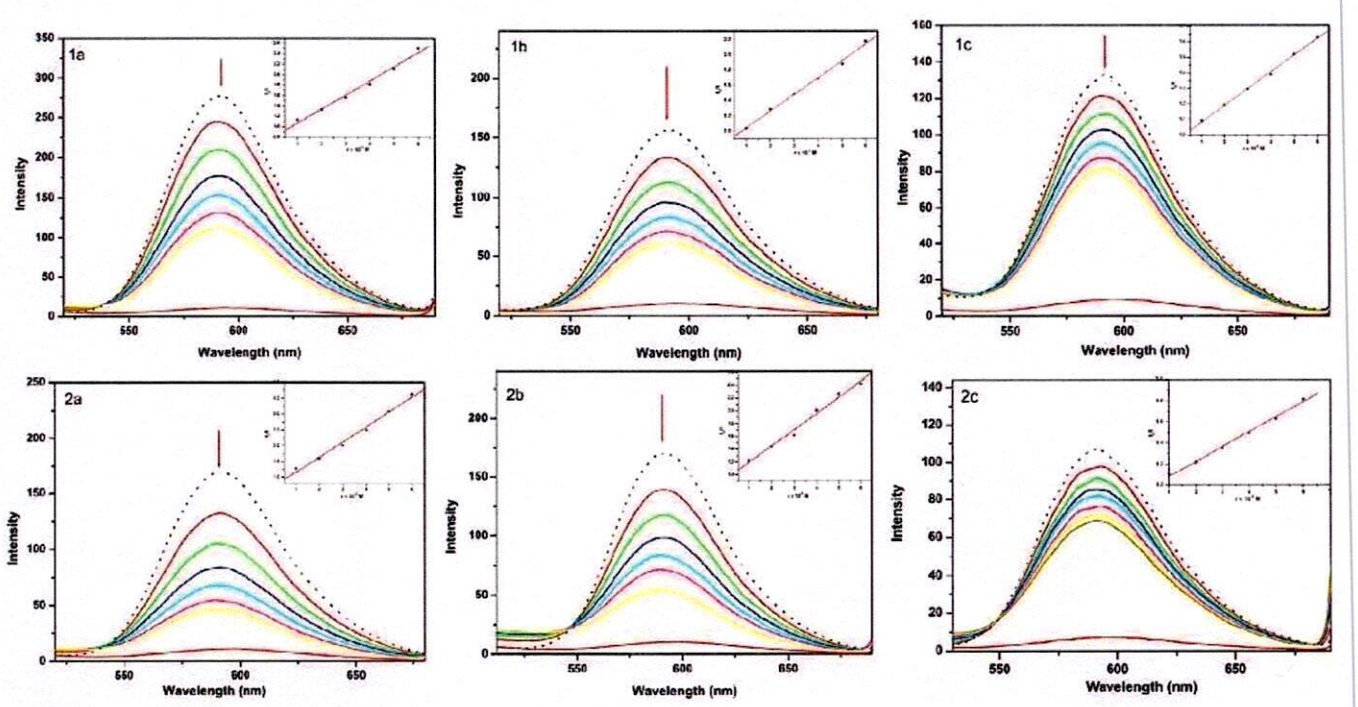


Fig. 6. Fluorescence emission spectra of EB-DNA system in the absence and presence of increasing concentration of complexes **1a-2c** in Tris HCl buffer (pH 7.2) at 25 °C. Conditions: [Complex] = 0-60 μM, [DNA] = 125 μM, [EB] = 12.5 μM.

pability of binding of the compounds with DNA has been investigated by displacement of ethidium bromide (EB). EB is a poor fluorescent compound whose intensity is enhanced in presence of CT-DNA as it intercalates between the DNA base pairs. Upon addition of metal complex to EB-DNA adduct, there is a sudden decrease in the intensity of EB-DNA adduct at 590 nm, and further increase in the concentration of complexes decreases the further intensity more, shown in Fig. 6. Such degradation of fluorescence intensity is due to the displacement of the EB by the compounds [41]. The results confirmed that all the compounds bind with DNA by an intercalation mode. The extent of fluorescence quenching expressed in terms of K_{SV} values calculated from the slope of plot I_o/I versus Q. The relative binding constants of complexes are found to be 6.43-3.25 × 10^4 M^{-1}.

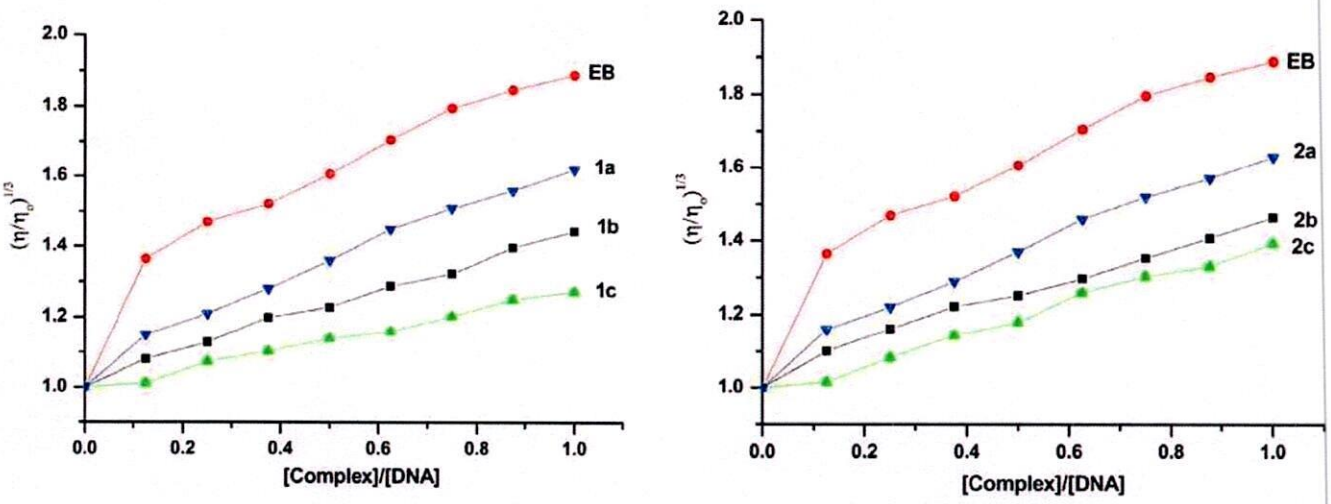


Fig. 7. Effect of increasing amounts of EB and set of complexes **1a-1c, 2a-2c** on the relative viscosity of CT-DNA at 30 ± 0.1 °C.

3.8.3. Viscosity study

The viscosity studies were also carried out to get further evidence of binding mode of metal complexes with CT-DNA. Generally, in absence of any crystallographic structural data, the hydrodynamic measurements (such as viscosity, sedimentation, etc) are the most sensitive techniques to determine the DNA binding [42]. A molecule binds to DNA, in a classical intercalation, can cause an increase in the viscosity of DNA since it increases the separation between base pairs at intercalation site, hence an increase in overall length of the double helix. However, a partial and non-classical intercalation of the complex results in bending of the DNA helix, which reduces the effective length of DNA, with a concomitant decrease in its viscosity [43]. The experimental results, shown in Fig. 7, revealed that the relative viscosity of CT-DNA increased gradually on successive addition of increasing concentration of complexes which is an indicative of intercalative binding mode. All the complexes intercalated between base pairs of DNA duplex causing an increase in the viscosity of DNA solution.

3.9. DNA cleavage activity

The DNA cleavage activity of ligands $\mathbf{HL_1}$, $\mathbf{HL_2}$ and their metal complexes against super-coiled pBR322 plasmid DNA was carried out by agarose gel electrophoresis method, in presence of H_2O_2 (oxidative cleavage) as well as under UV light (photolytic cleavage) [44–48]. The cleavage ability of complexes is measured by comparing the band patterns obtained from untreated and treated (without and with complexes) plasmid DNA by photolytic and oxidative cleavage methods. The efficiency of the complexes in DNA cleavage is estimated by the conversion of DNA from Form I (circular supercoiled) to Form II (nicked) and Form III (linear form). When circular plasmid DNA is subjected to electrophoresis, the fast migration will be obtained for the circular form. If one strand is cleaved, a relaxed nicked form will be generated which migrates slower than Form I [49].

The cleavage patterns of Cu(II), Co(II) and Ni(II) complexes are shown in Figs. 8 and 9. In oxidative method, no DNA cleavage was observed in lane 1 (DNA control), lane 2 (DNA+H_2O_2) and lane 3 ($\mathbf{HL_1}$) but in lane 4 **1a**, lane 5 **1b** and lane 6 **1c** complexes efficiently cleaved supercoiled DNA into nicked form. In photolytic method, no DNA cleavage is observed in lane 1 (DNA control), lane 2 ($\mathbf{HL_2}$) but in lane 3 **2a**, lane 4 **2b** and lane 5 **2c** complexes efficiently cleaved DNA into nicked form. All complexes cleaved the super-coiled pBR322 DNA more efficiently.

Table 3
IC_{50} values of Ascorbic acid (**AA**) and complexes **1a-2c**.

Compound	IC_{50} (μM)
1a	3.68
1b	4.39
1c	7.05
2a	4.25
2b	5.72
2c	8.16
AA	0.42

4. Antioxidant activity

A systematic investigation was conducted to explore the antioxidant potency of the compounds by DPPH method employing ascorbic acid as a reference. All the analyses were done in three replicates and the representative graph is shown in Fig. 10. And IC_{50} values are presented in Table 3. Reduction capability of complexes on free radical (DPPH) is determined by the decrease in its absorbance value at 517 nm (blank) which can be induced by antioxidant activity. In the present investigation, the antioxidant activity of metal complexes was expressed as IC_{50} values. The IC_{50} values were calculated by plotting the percentage scavenging effects on the y-axis and concentration (μM) on the x-axis. It is seen from the results that all the complexes exhibited moderate activity compared to the standard ascorbic acid [50–52]. Statistically, the scavenging effect of the compounds with DPPH radical is in the order **(1a)** > **(1b)** > **(1c)** and **(2a)** > **(2b)** > **(2c)**. From the results, it is clear that the Cu(II) complexes have higher activities than the Co(II) and Ni(II) complexes.

4.1. Antimicrobial activity

The synthesized ligands and their complexes were screened for in vitro antibacterial activity against Gram-positive bacteria B. subtilis, S. aureus and Gram-negative bacteria E. coli, P. aeruginosa and antifungal activity against A. niger and C. albicans. The relevant data and plots are shown in Table 4 and Fig. S1. Ampicillin and Ketoconazole are the standard drugs respectively. The zone of inhibition of diameter was measured and compared with the standard drugs. The experimental results revealed that the complexes show better activity than respective ligands. Further, the complexes exhibited varying degree of inhibitory effects on the growth of

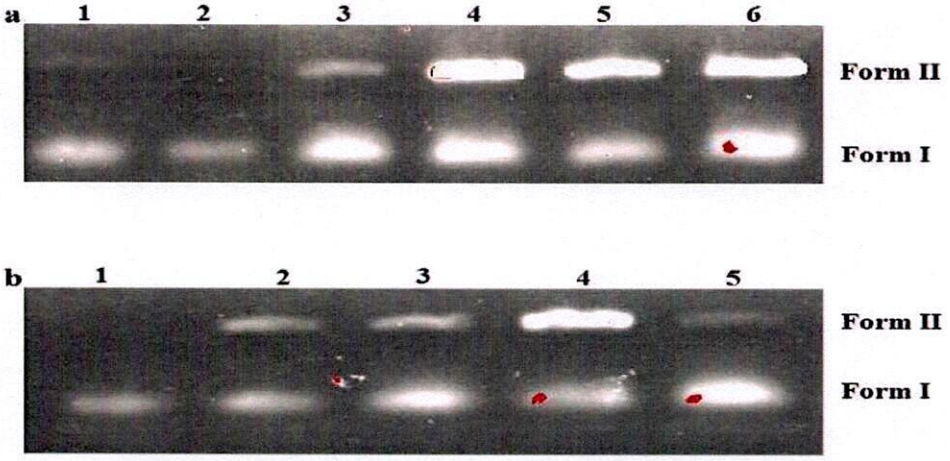


Fig. 8. (a) Oxidative cleavage of supercoiled pBR322 DNA (0.2 μg, 33.3 μM) at 37°C in 5mM Tris HCl/5 mM NaCl buffer by the metal complexes. Lane 1, DNA control; Lane 2, DNA +H_2O_2 (1mM); Lane 3, DNA + H_2O_2 (1mM) + **HL_1**; Lane 4, DNA + H_2O_2 (1mM) + **1a** (20 μM); Lane 5, DNA + H_2O_2 (1mM) + **1b** (20 μM); Lane 6, DNA + H_2O_2 (1mM) + **1c** (20 μM). **(b)** Photoactivated cleavage of supercoiled pBR322 DNA (0.2 μg, 33.3 μM) at 37°C in 5mM Tris HCl/5 mM NaCl buffer by the complexes. Lane 1, DNA control; Lane 2, DNA + **HL_1** (20 μM); Lane 3, DNA + **1a** (20 μM); Lane 4, DNA + **1b** (20 μM); Lane 5, DNA + **1c** (20 μM).

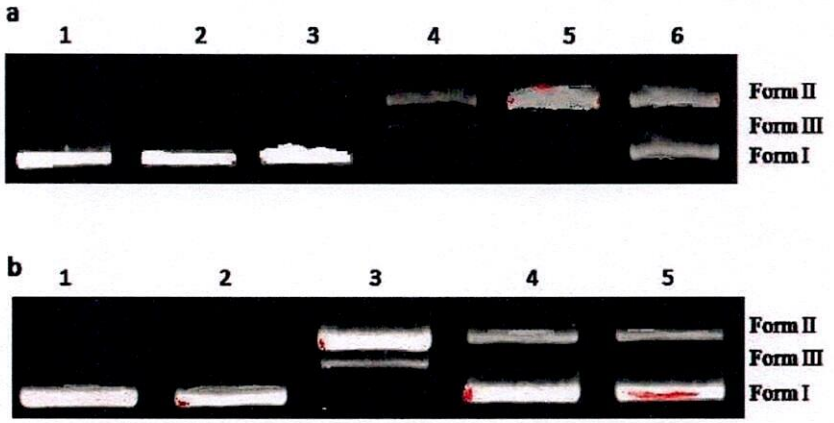


Fig. 9. (a) Oxidative cleavage of supercoiled pBR322 DNA (0.2 μg, 33.3 μM) at 37°C in 5mM Tris HCl/5 mM NaCl buffer by the metal complexes. Lane 1, DNA control; Lane 2, DNA +H_2O_2 (1mM); Lane 3, DNA + H_2O_2 (1mM) + **HL_2**; Lane 4, DNA + H_2O_2 (1mM) + **2a** (20 μM); Lane 5, DNA + H_2O_2 (1mM) + **2b** (20 μM); Lane 6, DNA + H_2O_2 (1mM) + **2c** (20 μM). **(b)** Photoactivated cleavage of supercoiled pBR322 DNA (0.2 μg, 33.3 μM) at 37°C in 5mM Tris HCl/5 mM NaCl buffer by the complexes. Lane 1, DNA control; Lane 2, DNA + **HL_2** (20 μM); Lane 3, DNA + **2a** (20 μM); Lane 4, DNA + **2b** (20 μM); Lane 5, DNA + **2c** (20 μM).

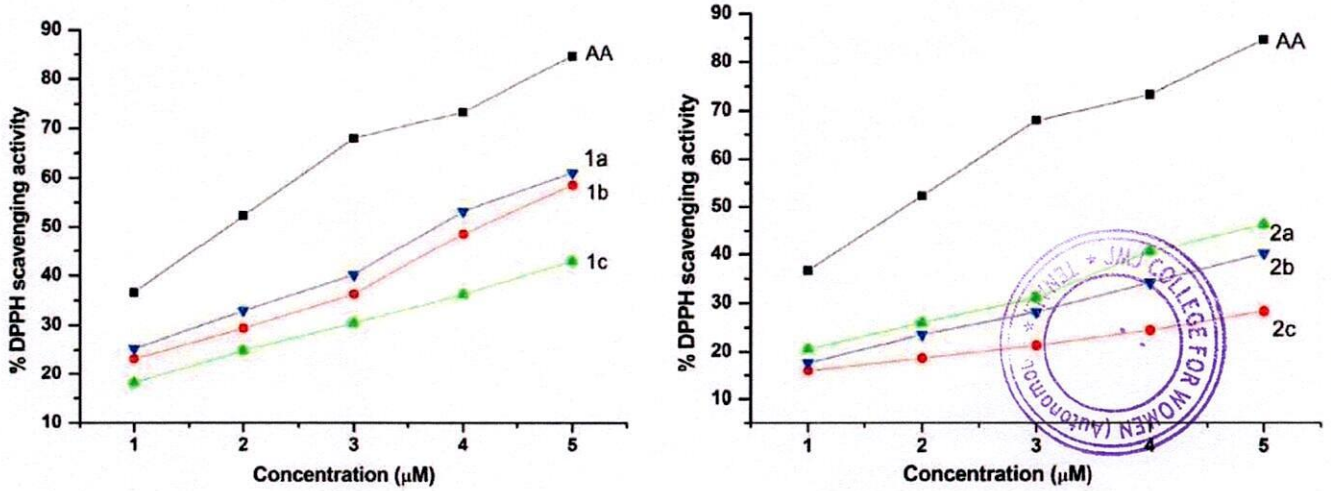


Fig. 10. DPPH radical scavenging activity of metal complexes at different concentrations (20, 40, 60, 80 and 100 μM).

Table 4
The antibacterial and antifungal activity data of synthesized ligands and their metal complexes.

Antibacterial					Antifungal	
Gram-positive		Gram-negative				
Sample	B. subtilis	S. aureus	E. coli	P. aeruginosa	A. niger	C. albicans
HL_1	3	2	4	3	4	5
1a	9	10	8	9	11	12
1b	8	9	8	7	8	9
1c	9	8	9	8	7	8
Ampicillin	15	16	15	15	-	-
Ketoconazole	-	-	-	-	16	15
HL_2	4	3	4	3	5	4
2a	9	12	11	12	12	11
2b	10	9	8	9	8	7
2c	9	8	9	8	7	8
Ampicillin	15	16	15	15	-	-
Ketoconazole	-	-	-	-	15	16

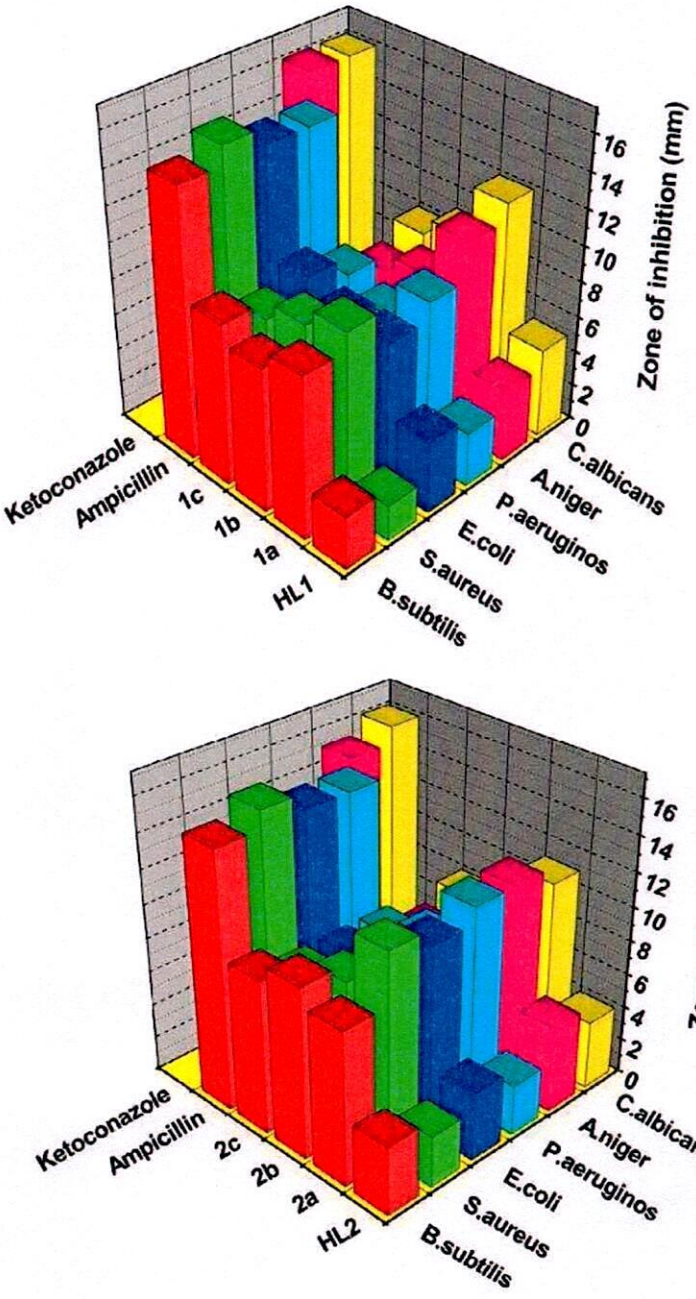


Fig. 11. The bar diagrams of the antibacterial and antifungal activities of HL_1, HL_2 and their metal complexes.

bacterial and fungal strains may be due to varying effect of the metal ion on the cell metabolism. The **1a** compound showed remarkably good antibacterial activity against B. subtilis and E. coli while moderate activity against S. aureus and P. aeruginosa. The **2a** complex exhibited pronounced activity against bacteria S. aureus, B. subtilis and Gram-negative bacterium P. aeruginosa. All the metal complexes exhibited prominent antifungal activity against all fungi with significant inhibition zone of diameter. Such highness of activity of the complexes can be attributed to the fact that the lipid membranes that surround the cell favors the passage of only the lipid-soluble materials which make liposolubility as an important factor that controls the microbial activity. On chelation with metal ions, the polarity of metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Furthermore, the chelation increases the delocalization of electrons over the whole chelate ring and also enhances the lipophilicity of the metal complexes [53–61].

5. Conclusion

In the present investigation, two Schiff bases and corresponding mononuclear binary Cu(II), Ni(II) and Co(II) complexes have been synthesized and well characterized. From the data, it is clear that all metal complexes have adopted a square planar geometry. The binding mode of complexes with CT-DNA was evaluated and found an intercalative mode of binding. The DNA cleavage studies proved that these complexes efficiently cleaved supercoiled pBR322 DNA both in the presence of H_2O_2 and UV light. The antioxidant activity studies have shown better free radical scavenging activity. The extent of scavenging activity is expressed in terms of IC_{50} values and the order of activity found to be **1a > 2a > 1c > 2c > 1b > 2b**. All the compounds are screened for antimicrobial activity and it is found that complexes have shown better inhibitory potential towards the examined pathogenic microorganisms and the results denoted that the complexes are more potent than their free Schiff base ligands. Among all, the Cu(II) complexes exhibited better DNA binding, cleavage, antioxidant and antimicrobial activity compared to Ni(II) and Co(II) complexes.

Authors' statement

Manuscript title: Antioxidant, antimicrobial, DNA binding and cleavage studies of novel Co(II), Ni(II) and Cu(II) complexes of N, O donor Schiff bases: synthesis and spectral characterization. **Conception and design of study:** Prof. Shivaraj, V. Sumalatha, Mr. Sreenu Daravath; **Acquisition of data:** Ms. V. Sumalatha, Mr. Sreenu Daravath; **Analysis and/or interpretation of data:** Ms. V. Sumalatha, Mr. Sreenu Daravath, Dr. Gali Ramesh; **Drafting the manuscript:** Prof. Shivaraj, Ms.V. Sumalatha, Mr. Sreenu Daravath, Dr. Aveli Rambabu; **Revising the manuscript critically for important intellectual content:** Ms.V. Sumalatha, Mr. Sreenu Daravath, Dr. Aveli Rambabu; All persons who have made substantial contributions to the work reported in the manuscript are Ms. V. Sumalatha, Mr. Sreenu Daravath, Dr. Aveli Rambabu, Dr. Gali Ramesh.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2020.129606.

References

[1] WHO Summary report on HPV & cervical cancer statistics in India (18/03/2008).
[2] J. Ferlay, I. Soerjomataram, M. Ervik, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.; Sept 2018.
[3] Q. Zhang, J. Liu, H. Chao, G. Xue, L. Ji, J. Inorg. Biochem. 83 (2001) 49–55.
[4] A. Bolhuis, J.R. Aldrich-Wright, Bioorg. Chem. 55 (2014) 51.
[5] S.K. Boda, S. Pandit, A. Garai, D. Pal, B. Basu, RSC Adv. 6 (2016) 39245–39260.
[6] R.P. Lange, H.H. Locher, P.C. Wyss, R.L. Then, Curr. Pharm. Des 13 (2007) 3140–3145.
[7] M. Zaki, F. Arjmand, S. Tabassum, Inorg. Chim. Acta 444 (2016) 1–22.
[8] H.K. Liu, P.J. Sadler, Acc. Chem. Res. 44 (2011) 349–359.
[9] K.E. Erkkila, D.T. Odom, J.K. Barton, Chem. Rev. 99 (1999) 2777–2795.
[10] E.R. Jamieson, S.J. Lippard, Chem. Rev. 99 (1999) 2467–2498.
[11] H.T. Chifotides, K.R. Dunbar, Acc. Chem. Res. 38 (2005) 146–156.
[12] D.R. Green, J.C. Reed, Science 281 (1998) 1309–1312.
[13] Y. Ma, L. Cao, T. Kawabata, T. Yoshino, B.B. Yang, S. Okada, Free Radic. Biol. Med. 25 (1998) 568–575.
[14] F. Liang, C. Wu, H. Lin, T. Li, D. Gao, Z. Li, J. Wei, C. Zheng, M. Sun, Bioorg. Med. Chem. Lett. 13 (2003) 2469–2472.
[15] J. Easmon, G. Pürstinger, G. Heinisch, T. Roth, H.H. Fiebig, W. Holzer, W. Jäger, M. Jenny, J. Hofmann, J. Med. Chem. 44 (2001) 2164–2171.
[16] S. Tabassum, S. Amir, F. Arjmand, C. Pettinari, F. Marchetti, N. Masciocchi, G. Lupidi, R. Pettinari, Eur. J. Med. Chem. 60 (2013) 216–232.
[17] V.M. Manikandamathavan, V. Rajapandian, A.J. Freddy, T. Weyhermuller, V. Subramanian, B.U. Nair, Eur. J. Med. Chem. 57 (2012) 449–458.
[18] S. Ramakrishnan, D. Shakthipriya, E. Suresh, V.S. Periasamy, M.A. Akbarsha, M. Palaniandavar, Inorg. Chem. 50 (2011) 6458–6471.
[19] A. Bottcher, T. Takeuchi, K.I. Hardcastle, T.J. Meade, H.B. Gray, Inorg. Chem. 36 (1997) 2498–2504.
[20] T. Takeuchi, A. Bottcher, C.M. Quezada, T.J. Meade, H.B. Gray, Bioorg. Med. Chem. 7 (1999) 815–819.
[21] R.B. Manikandan, P. Viswanathamurthi, K. Velmurugan, R. Nandhakumar, T. Hashimoto, A. Endo, J. Photochem. Photobiol. B. 130 (2014) 205–216.
[22] M.C. Rodrìguez-Arguelles, M.B. Ferrari, F. Bisceglie, et al., J. Inorg. Biochem. 98 (2004) 313–321.
[23] F. Liang, P. Wang, X. Zhou, et al., Bioorg. Med. Chem. Lett. 14 (2004) 1901–1904.
[24] R. Aveli, K. Marri Pradeep, S. Tejaswi, V.K. Narendrula, Shivaraj, J. Photochem. Photobiol. B. 165 (2016) 147–156.
[25] N. Jyothi, N. Ganji, S. Daravath, Shivaraj, J. Mol. Struct. 1207 (2020) 127799.
[26] D. Sreenu, K. Marri Pradeep, R. Aveli, V.K. Narendrula, G. Nirmala, Shivaraj, J Mol. Struct. 1144 (2017) 147–158.
[27] V.K. Narendrula, D. Sreenu, G. Nirmala, N. Pasha, N. Pasha, Shivaraj, Inorg. Chem. Commun. 113 (2020) 107767.
[28] J. Mendham, R.C. Denney, J.D. Barnes, M.J.K. Thomas, Vogel's Quantitative Chemical Analysis, sixth ed., 2000 New York.
[29] J. Marmur, J. Mol. Biol. 3 (1961) 208–218.
[30] M.E. Reichmann, S.A. Rice, C.A Thomas, P. Doty, J. Am. Chem. Soc. 76 (1954) 3047–3053.
[31] A. Wolfe, G.H Shimer, T. Meehan, Biochemistry 26 (1987) 6392–6396.
[32] M. Balouiri, M. Sadiki, S.K. Ibnsouda, J. Pharm. Anal. 6 (2016) 71–79.
[33] S. Saydam, E. Yilmaz, Spectrochim. Acta Part A 63 (2006) 506–510.
[34] K. Nakamoto, Infrared and Raman Spectra of Inorganic and CoordinationCompounds, fifth ed., Wiley-Interscience, New York, 1997.
[35] B.J. Hathaway, D.E. Billing, Coord. Chem. Rev. 5 (1970) 143–207.
[36] D. Kivelson, R. Neiman, J. Chem. Phys. 35 (1961) 149–155.
[37] J.K. Barton, A.T. Danishefsky, J.M. Goldberg, J. Am. Chem. Soc. 106 (1984) 2172–2176.
[38] S.A. Tysoe, R.J. Morgan, A.D. Baker, T.C. Strekas, J. Phys. Chem. 97 (1993) 1707–1711.
[39] S. Parveen, F. Arjmand, Spectrochim. Acta A 85 (2012) 53–60.
[40] R. Aveli, G. Nirmala, D. Sreenu, V. Kadiyala, N. Rangan, K. Rangan, Shivaraj, J. Mol. Struct. 1199 (2020) 127006.
[41] R. Aveli, G. Nirmala, D. Sreenu, R. Gali, Shivaraj, J. Fluoresc. (2020), doi:10.1007/s10895-020-02614-y.
[42] M.J. Waring, J. Mol. Biol. 13 (1965) 269–282.

[43] B.C. Baguley, M.L. Bret, Biochemistry 23 (1984) 937–943.
[44] V.G. Vaidyanathan, B.U. Nair, J. Inorg. Biochem. 93 (2003) 271–276.
[45] S. Satyanarayana, J.C. Dabrowiak, J.B. Chaires, Biochemistry 31 (1992) 9319–9324.
[46] X. Li, C.F. Bi, Y.H. Fan, X. Zhang, X.D. Wei, X.M. Meng, Transit. Met. Chem. 39 (2014) 577–584.
[47] B.K. Santra, P.A.N. Reddy, G. Neelakanta, S. Mahadevan, M. Nethaji, A.R. Chakravarty, J. Inorg, Biochem. 89 (2002) 191–196.
[48] S.Y. Tsang, S.C. Tam, I. Bremner, M.J. Burkitt, Biochem. J. 317 (1996) 13–16.
[49] J. Bernadou, G. Pratviel, F. Bennis, M. Girardet, B. Meunier, Biochemistry 28 (1989) 7268–7275.
[50] Z.K. Khoramdareh, S.A. Hosseini-Yazdi, B. Spingler, A.A. Khandar, Inorg. Chim. Acta 415 (2014) 7–13.
[51] D.S. Sigman, Acc. Chem. Res. 19 (1986) 180–186.
[52] S.B. Bukhari, S. Memon, M. Mahroof-Tahir, M.I. Bhanger, Spectrochim. Acta A 71 (2009) 1901–1906.
[53] V. Kadtala, K. Marri Pradeep, R. Aveli, V.K. Narendrula, D. Sreenu, K. Rangan, Shivaraj, J. Mol. Struct. 1160 (2018) 198–207.
[54] E. Ramachandran, S.P. Thomas, P. Poornima, P. Kalaivani, R. Prabhakaran, V.V. Padma, K. Natarajan, Eur. J. Med. Chem. 50 (2012) 405–415.
[55] R. Gali, D. Sreenu, M. Swathi, V. Sumalatha, S.S. Dasari, Shivaraj, Chem. Data Collect. 28 (2020) 100434.
[56] S.S. Dasari, G. Nirmala, D. Sreenu, V. Kadtala, Shivaraj, Chem. Data Collect. 28 (2020) 100439.
[57] R. Gali, D. Sreenu, G. Nirmala, R. Aveli, V. Kadtala, Shivaraj, J. Mol. Struct. 1202 (2020) 12733.
[58] D. Sreenu, R. Aveli, S.S. Dasari, Shivaraj, Chem. Data Collect. 24 (2019) 100293.
[59] V. Kadtala, G. Nirmala, D. Sreenu, K. Kanneboina, N. Ganji, S. Daravath, K. Kanneboina, K. Rangan, Shivaraj, Polyhedron 171 (2019) 86–97.
[60] D. Sreenu, R. Aveli, V.K. Narendrula, G. Nirmala, Shivaraj, J. Coord. Chem. 72 (2019) 1973–1993.
[61] V. Sumalatha, R. Aveli, V.K. Narendrula, G. Nirmala, D. Sreenu, Shivaraj, Chem. Data Collect. 20 (2019) 100213.

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SUSTAINABLE, ECO-FRIENDLY POLYMER: APPLICATIONS AND FUTURE SCOPE

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ABSTRACT

Bio-polymers are produced from renewable resources, which are classified into two types are named as Bio-based polymers and biodegradable polymer with huge difference. Both the physical and chemical properties of the biodegradable polymers tend to deteriorate and degrade when exposed to all microbial fermentation includes aerobic, anaerobic process with water, carbon dioxide and methane. Bio-based polymers are biodegradable but not all. Polymer conjugates are complex molecules with major societal implications. Many advances in the fields of medicine, biotechnology and nanotechnology. In this review focused on various manufacturing methods used, to prepare conjugate-polymer substance could reveal the structural features of the polymer.

Keywords: Polymer, Conjugate, Biodegradable Polymers, Medicinal Application, Metals, Macromolecules, Conjugate, Polymerization Antibodies, Immune System, In Vivo.

1.0. Introduction

1.1. Super Absorbing Polymers (SAPs)

The function of the superabsorbent polymer is a cross linked voluminous polymer could absorb large volumes of water and retain them in it (Lejcus*et al.*, (2018)). It is been industrialized for the production of improved water holding capacity and for the application of agricultural farming fields USDA (1970) Daniel Hellerstein *et al.*, (2019). The product also used for the manufacturing of towels, surgery, diapers and female hygiene products (Anita (2020)). SAPs classified into many types based on their functional group, charge and affinity (RoselenaFaez*et al.*, (2017)).

SAPs based on ionic nature they are categorized into two types they are nonionic and ionic charge (cationic and anionic SAPs) classified into hydrophilic and hydrophobic based on affinity (Anand and Mohapatra Mamata (2020)). The water absorbing property of SAP comes from osmatic imbalance between interior and exterior of the polymer and electrostatic repulsion between charges on the polymer chain, which also forms hydrogen bonds with water molecules (Wu et al., (2020)). The polymer cross-linking increases, which makes the polymer insoluble, form (Monika and Vinay (2019). The SAP prepared in two ways known as bulk polymerization and suspension polymerization (Enas M. Ahmed (2019). Methodology used to the quantification of SAPs follows are water absorption capacity, swelling rate, swollen gel strength, wicking capacity, sol fraction, residual monomer and ionic sensitivity (Wang et al., (2019)). The

swelling ability of the polymer can be controlled by either cross-linking between polymeric chains or morphology of the SAP includes (porosity and particle size) (Misiewicz, Lejcus and Dabrowska (2019)). Longer polymer chains with increased absorbing capacity but smaller polymer chains do not support the water absorption due to its polymer ends (Chen *et al.,* (2019)). The smaller size of SAP, could absorb larger quantity of water. The environmental factors effects the change of SAPs volume (De meyst et al., (2019)).

1.2. Uses of Super Absorbent Polymers in Agriculture

The water-absorbing rate of SAPs is in huge volumes and retain them for many applications in agriculture cultivation. Physical and chemical properties of the SAP includes soil-SAP mixing ratio and swelling increases, which effects and decrease the hydraulic conductivity of soil. Porosity of the sandy soil significantly. The hydraulic conductivity of soil initially increases and then decreases. Soil saturated and residual water content, water holding capacity and available water content increases by the application of SAPs stated (Megan *et al.,* (2020)). The soil holds more moisture for longer periods by the application of SAPs. Infiltration rates, bulk density, soil structure, compaction, soil texture, aggregate stability, crust hardness and evaporation rates of the soil properties effected by the application of SAPs (Salim, Saifuldeen (2015), (2019)). When the bulk density of soil decreases which effects the application rates and reduces soil infiltration that avoids potential loss to deep percolation of SAPs (Al-Omran et al., (2018)). The process of Expansion and contraction of SAP in the soil during the water absorption and evaporation, which helps to improve the air in the soil, especially in clayey soils (Yuansong Xiao et al., (2018)). SAPs controls the irrigation-induced erosion and soil water seepage and uniformity of furrow water applications. SAP is that it greatly reduces the irrigation frequency and water management practices in arid and semi-arid regions discussed. SAPs are also biodegradable and further their products do not harm the microbial community present in the soil. States that SAP increases yield and water use efficiency of plants that is increase in plant biomass (Georgios Nikolaou et al., 2020). SAP induce faster growth of plants by availing plant water and prolong survival of plants under water stress and drought conditions (Suresh Rahul et al., (2018)).

1.3. Limitations of Super Absorbent Polymers in Agriculture

Though the SAP amendment in soil has many beneficial uses, there are some limitations to its applications in soil. SAPs are quite fragile and tend to break apart easily thereby losing their water retention property. Further SAPs can also dehydrate rapidly in a matter of hours thus losing their absorbed water (Olayemi et al., (2020)). The water absorption of SAPs greatly reduces in the soils as SAPs are under pressure and unable to swell and take in water. The water absorption of SAPs in soils further decreases due to formation of additional crosslinks with certain ions like Ca^{2+} and Al^{3+} present in the soil (Chenhao Zhao et al., (2019)). The water absorption of the SAP also decreases with increase in salinity of irrigation water (Summy Yadav et al., (2020). The SAPs in soils releases water with increase in temperature and this water could be potentially lost to deep percolation. It could be inferred that the effectiveness of SAP

decreased on rewetting and can affect the hydraulic properties of soil only if applied in higher application rates. In addition, the efficacy of the SAP decreases over a period time and to compensate for these loses; higher application rates are required Wenju Zhao et al., (2019). This factor affects the economic value of crops grown on fields amended with SAP (TandziNgoune Liliane and Mutengwa Shelton Charles (2020)). SAP amendment also has negative effect on plant growth (Yongbin Li et al., (2019)). The height of wheat plant grown in SAP amended soil is shorter than on control. In addition, the height of plant decreased with increase in SAP amendment percentage in soil. In certain cases, the wilting point of the plants was not affected significantly (Abrisham, et al., (2018)). The higher application rate of SAPs may lead to plant mortality.

For water-soluble fertilizer, quantity of potassium leached increases with increase in water absorption capacity of polymers. The presence of hydrogels increased dissolution of controlled-release fertilizer (Abobatta (2018)). SAPs modification increases water content and increases dissolution of fertilizers, which potentially percolated to deeper depths (Krzysztof Lejcus et al., (2018)). The potential benefit of SAP alteration may be realize only in early stages of plant production and little or no benefit later in production and in post-production (Thiago Zanguetin (2020)). Mixed response of applications of SAPs in agriculture and physical properties are to compare the results. It emphasizes that lack of knowledge on SAPs and its application in soils (Shah Fahad et al., (2017)), (Behera, Sabyasachi and Mahanwar, Prakash. (2019)). A detailed cross-disciplinary approach is required.

1.4. Biopolymers

1.5.

1.5.1. International status of the Biopolymers

Bio-based polymers still hold a tiny fraction of the total global plastic market (Sza, Aata, Kamila and Gumi, Tania (2017) and Papermaking (2019)). Currently, biopolymers share less than 1% of the total market. At the current growth rate, it is expected that biopolymers will account for just over 1% of polymers, (Babu, Connor and Seeram (2013)). Worldwide status of Bio-based polymer has occupied worldwide demand based on the need for the non-fossil fuel-based polymers. According to the ISI Web of Sciences and Thomas Innovations, there is an incredible increase on bio-based polymers and its applications at present (Dublin, (2020)).

Carbon dioxide emission reduction in the environment takes place by the usage of bio-based polymers and reduces the dependency of fossil fuels. Which causes the replacement of petroleum based derived raw materials with renewable resource based raw materials for the production of polymers leads to the worldwide demand for biopolymers according to the EUROPEAN UNION and USA market (Dahiya et al., 2020).

1.6. Generations of Biopolymers

1.6.1. The first generation: Bio-based polymers derived from corn, potatoes, and other carbohydrate agricultural bi-products. However, the food-based resources and

significant breakthroughs in biotechnology were not encourageble to produce bio-based polymers.

1.7. Conventional bio-based polymers

Bio-based polymers are produced using renewable resources by bacterial fermentation are known as conventional polymers. Example starch, cellulose, fatty acid, lingo-cellulosic biomass and organic waste (Yin Lia (2019)).

1.7.1. Natural bio-based polymers

Naturally, derived polymers (proteins, nucleic acids, and polysaccharides) are known as Natural Bio-based polymers. At present these polymers have been used widely in the technology development and its applications. Example Polysaccharides, Proteins, Nucleic acids and their derivatives (Nurul Asmak Md Lazim et al., (2019) and Zahra Shariatinia (2019)).

1.7.2. Starch

The starch is chemical substance, which is a sole carbohydrate source and naturally derived from photosynthesis, it is granular in structure. The main source of starch, which is naturally derived from plants and is abundantly occurred in wheat, rice, corn and potato. Starch is the lineage polysaccharide of both amylose and highly branched amylopectin and thermoplastic in nature. The temperature is the influencing factor of the starch resistance and presence of plasticizer (SpaseStojanov and Ales Berlec (2020)). Commercial production of starch plastics is facing problems with ductility due to complexity and partly non-linear nature of starch. Thermo plasticity of starch agonize outstanding retrogradation, causes brittleness and crystallinity. Plasticizer, a substance added to plastics to make them more pliable are the chemical substances, used to fabricate starch plastics. By chemical modification, could able to overcome problems arising in the process of retrogradation to produce the biodegradable polymers with mechanical strength, flexibility and water holding properties to reduce the cost of production (Vieira, et al., 2011).

1.7.3. Commercial bio-based natural polymers

Novamont is one of the leading companies in processing starch-based products. The company produces various types of starch-based products using proprietary blend formulations. There are other companies around the world producing starch-based products in a similar scale for various applications (Aleksandra Nesic et al., 2020). Starch and modified thermoplastic starch polymers have wide range of applications in the food and non-food sectors, such as for shopping, bread, fishing bait bags, overwraps, flushable sanitary product, packing materials, pharmaceuticals, films, foam loose-fill packaging and special molded products such as readymade food package containers (Halley and Averous (2014)). The estimated consumption of starch based polymers was approximately used 54% of food applications and 46% of non-food applications (is about 7.9 million tons) and largest consumers of (30%) starch was used for the production or manufacturing the papers, cardboard, corrugating industries in the European Union, (Ramesh et al., 2013). Some other starch applications are textiles,

cosmetics, pharmaceuticals paints and constructions. Moreover, the starch will play an increasing role in the area of production of packaging materials, renewable raw materials, molded products and biodegradable plastics (Vibhore Kumar Rastogi and Pieter Samyn (2015)).

1.7.4. Cellulose

Cellulose is a complex polysaccharide with crystalline morphology abundantly present in the plant cell wall. Structure of the cellulose varies from starch by the linkages of cellulose β-1,4-glycosidic bonds, whereas the bonds in starch are predominantly α-1,4 linkages (Abhilash, M and Thomas, D (2017)). Cotton fibers and wood are the key source for the production of cellulosic plastic (Diego et al., 2019). Cellulose derivatives are namely cellulose acetate, cellulose esters (molding, extrusion, and films), and regenerated cellulose for fibers were industrially produced (Mostafaa et al., 2019). Cellulose is a hard polymer and has a high tensile strength of 62 to 500 MPa and elongation of 4% and the Tg of cellulosic derivatives ranged between 53°C and 180°C (Garcia Ibarra (2016) James (2019). While blending the cellulose with other plasticizers the mechanical and thermal properties differ from blend to blend. The chemical composition also effect the cellulosic plasticizers formation processing (Hiba Shaghaleh et al., 2018), (Kumar Ramamoorthy et al., 2019). Eastman Chemical is a major producer of cellulosic polymers. FKuR launched a biopolymer business in the year 2000 and has a capacity of 2,800 metric ton/year of various cellulosic compounds for different applications (Frank Hermanutz et al., 2018) (Babu et al., 2013).

Cellulosic polymers are classified into three types based on chemical modification. It has various applications like in the production of cellulose esters into cellulose nitrate and cellulose acetate film and fibers (John et al., 2020). Cellulose ether derivatives of cellulose, such as carboxymethyl cellulose polymers and hydroxyethyl cellulose polymers, are generally applied for construction, food, personal care, pharmaceuticals, paint, and pharmaceutical applications (Fijul Kabir et al., 2018). Cellulosic bio-based polymers applied to produce for fiber and film globally were used textiles, hygienic disposables and home furnishing fabrics because of its thermal stability and modulus discussed (Abhilash and Thomas (2017)) (Keshipour and Maleki (2018)). Cellulose was produced by bacterial fermentation and is characterized by its purity and strength and its biomedical applications were limited because of high production cost. Large-scale production at industrial level gives low yields and high costs of bacterial cellulose applications include acoustic diaphragms, mining, paints, oil gas recovery, and adhesives (Jing Wang et al., 2019).

Chitin and chitosan is an amino polysaccharide, which is a natural bio-based polymer extracted by chemical extraction from shells of prawns and crabs and demineralization by acid and deproteination by the action of alkali followed by deacetylated into chitosan (Oyatogun et al., (2020), Suneeta Kumari and Rupak Kishor (2020)). Microbial fermentation and enzymatic hydrolysis also used to produce chitin and these are not industrially economical. Chitin and chitosan are industrially produced worldwide in the USA, Canada, Scandinavia, and Asia.

1.7.5. Chitosan

Chitosan has been applied in varied applications with different characteristics features shown as biodegradability, biocompatibility, chemical inertness, high mechanical strength, good film-forming properties, chemical pharmaceutical, cosmetic products to water treatment, plant protection and low cost production. Chitosan is a friendly chemical substance, is used for the cosmetic product production with many biologically active components. Chitosan is applied in the manufacturing of bio-medical devices with its low toxicity, biocompatibility and bioactivity became as an attractive bio-based polymer (Soleimani, Khadijeh et al., 2018). And chitosan has been used in the different human daily deeds such as in shampoos, rinses, and permanent hair-coloring agents and skin care conditioner because of low cost it's a good substitute to the hyaluronic acid (Biotechnology in Personal Care, Cosmetic Science: (2006).

1.7.6. Pullulan

It is a linear polysaccharide contains maltotriose component with α-1,6 glycosidic linkage and soluble in water obtained by the fermentation using *Aureobasidiumpullulans* (Mahmoud Nasrollahzadeh, Zahra Issaabadi, (2019)). Production of pullulans by simple fermentation medium with minimal nutritional factors containing sugars (Sheng, Long., Tong, Qunyi and Ma, Meihu. (2016), Khvostov, Tolstikova, and Borisov, (2019)). Chemically modification of pullulan to produce a bio-based polymer that is either less soluble or completely insoluble in water. Which has distinctive properties due to its characteristic glycosidic linkage. It is easily modified to develop pH sensitivity by introducing functional reactive group. Pullulan has several viable applications includes as a food additive, a flocculant, a blood plasma substitute, an adhesive, and a film. The pullulan is used to produce decoration articles are looks like polystyrene. It is a tasteless, odourless, food ingredient with low-calorie gives bulk and texture to the macromolecule. The pullulan is used widely in the food industry due to its preservation property; it possesses oxygen barrier and excellent moisture retention property inhibits the growth of fungi. At present it has been used in the biomedical applications to deliver the drug into targeted area, tissue engineering, wound healing and imaging studies. Pullulan also for the production of oral care products and capsule formulation in the dietary supplements and pharmaceutical leading to increased demand for this irreplaceable biopolymer revealed.

1.7.7. Proteins

Collagen is the major insoluble fibrous protein in the extracellular matrix and in connective tissue. In fact, it is the single most abundant protein in the animal kingdom. There are at least 27 types of collagens, and the structures all serve the same purpose, to help tissues withstand stretching (Babu, O'Connor and Seeram (2013)). The most abundant sources of collagen are pigskin, bovine hide, and pork and cattle bones. However, the industrial use of collagen is obtained from non-mammalian species. Gelatin is obtained through the hydrolysis of collagen. The degree of conversion of collagen into gelatin depends on the pretreatment, function of temperature, pH, and extraction time.

Collagen is one of the most useful biomaterials due to its biocompatibility, biodegradability, and weak antigenicity, Alvin Bacero Bello et al., (2020). The main application of collagen films in ophthalmology is as drug delivery systems for slow release of incorporated drugs. It was also used for tissue engineering including skin replacement, bone substitutes, and artificial blood vessels and valves. The classical food, photographic, cosmetic, and pharmaceutical applications of gelatin is based mainly on its gel-forming properties. Recently in the food industry, an increasing number of new applications have been found for gelatin in products in line with the growing trend to replace synthetic agents with more natural ones. These include emulsifiers, foaming agents, colloid stabilizers, biodegradable film-forming materials, and microencapsulating agents.

1.7.8. Alginates

Alginate is a linear polysaccharide that is abundant in nature as it is synthesized by brown seaweeds and by soil bacteria (Yuefei Zhu, Zhiqing Pang, (2019)). Sodium alginate is the most commonly used alginate form in the industry since it is the first by-product of algal purification (Siddhesh and Pawar (2017)). Sodium alginate consists of α-l-guluronic acid residues (G blocks) and β-d-mannuronic acid residues (M blocks), as well as segments of alternating guluronic and mannuronic acids.

Although alginates are a heterogeneous family of polymers with varying content of G and M blocks depending on the source of extraction, alginates with high G content have far more industrial importance (Roya et al., 2018). The acid or alkali treatment processes used to make sodium alginate from brown seaweeds are relatively simple. The difficulties in processing arise mainly from the separation of sodium alginate from slimy residues (Ramesh et al., 2013). It is estimated that the annual production of alginates is approximately 38,000 tons worldwide (Jaya chandran et al., 2014). Alginates have various industrial uses as viscosifiers, stabilizers, and gel-forming, film-forming, or water-binding agents (Ramesh et al., 2014). These applications range from textile printing and manufacturing of ceramics to production of welding rods and water treatment (Abhilash and Thomas, (2017)). The polymer is soluble in cold water and forms thermostable gels. These properties are utilized in the food industry in products such as custard creams and restructured food. The polymer is also used as a stabilizer and thickener in a variety of beverages, ice creams, emulsions, and sauces.

Alginates are widely used as a gelling agent in pharmaceutical and food applications. Studies into their positive effects on human health have broadened recently with the recognition that they have a number of potentially beneficial physiological effects in the gastrointestinal tract (Gheorghe Adrian et al., 2019). Alginate-containing wound dressings are commonly used, especially in making hydrophilic gels over wounds, which can produce comfortable, localized hydrophilic environments in healing wounds (SaghiSaghazadeh et al., 2018). Alginates are used in controlled drug delivery, where the rate of drug release depends on the type and molecular weight of alginates used (Amit Kumar et al., 2020). Additionally, dental impressions made with alginates

are easy to handle for both dentist and patient as they fast set at room temperature and are cost-effective (Onsoyen 1996). Recent studies show that alginates can be effective in treating obesity, and currently, various functional alginates are being evaluated in human clinical trials (Saquib Hasnain et al., 2020).

1.7.0. Production of bio-based polymers

There are three principal ways to produce bio-based polymers using renewable resources:

1.7.1.Partial modification of natural bio-base polymers (e.g., starch):

Blending the biopolymers with rich in on centration of proteins meat and bone meal (MBM) and describing the properties of plastics. Theoretical information concerning biodegradable materials.

1.7.2.Producing bio-based polymers directly by bacteria (e.g., polyhydroxyalkanoates)

1.7.2.1. Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHAs) are a family of polyesters produced by variety of organisms; even though prefer bacterial fermentation having ability to replace conventional hydrocarbon-based polymers. In 1923, Lemoigne carried out depicted by using *Bacillus megaterium* Polyhydroxybutyrate (PHB), is the derivatives of simplest PHA, and was discovered in 1926 by Maurice Lemoigne (Yusof, et al., 2018). PHA, has been produced by using a large fermentation vessel is filled with mineral medium along with feed stock includes cellulosics, vegetable oils, organic waste, municipal solid waste, and fatty acids depending on the specific PHA required and inoculated with a seed culture supplemented with suitable bacterial strains and incubated for 48 hrs (Dyson et al., 2019). PHA, cells are isolated and purifying step followed by concentration, dried and extraction with acetone or chloroform. Solid-liquid separation process been used to remove the cell debris followed with precipitation with methanol (Xuan-Thanh *et al.,* 2019). According to the Steinbuchel and Valentin, more than 150 PHA monomers were identified in 1995 and having diversified applications along with bio-based polymers with a wide range of properties (Salem *et al.,* 1970). Poly-3-hydroxybutyrate was the first bacterial PHA and has greatest attention in characterization and industrial-scale production. Has similar thermal and mechanical properties to those of polystyrene and polypropylene.

Even though it has many drawbacks, which includes slow crystallization, narrow processing temperature range, and tendency to 'creep' (Reis *et al.,* 2008). Several companies have developed PHA copolymers with typically 80% to 95% (R)-3-hydroxybutyric acid monomer and 5% to 20% of a second monomer in order to improve the properties of PHAs. Poly(3HB): Poly(3-hydroxybutyrate)

Poly(3HB-co-3HV) : Poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV

Poly(3-HB-co-4HB): Poly(3-hydroxybutyrate-co-4-hydroxybutyrate)

Poly(3HB-co-3HH) : Poly(3-hydroxyoctanoate-co-hydroxyhexanoate)

Poly(3HO-co-3HH) : Poly(3-hydroxyoctanoate-co-hydroxyhexanoate)
Poly(4-HB) : Poly(4-hydroxybutyrate).

The copolymer poly (3HB-co-3HV) has a much lower crystallinity, decreased stiffness and brittleness, and increased tensile strength and toughness compared to poly (3HB) while remaining biodegradable. It also has a higher melt viscosity, which is a desirable property for extrusion and blow molding (Hanggi, 1995). The first commercial plant for PHBV was built in the USA in a joint venture between Metabolix and Archer Daniels Midland. However, the joint venture between these two companies ended (Ramesh et al., 2013). Tianan Biologic Material Co. in China is the largest producer of PHB and PHB copolymers. Tianan's PHBV contains about 5% valerate, which improves the flexibility of the polymer. Tainjin Green Biosciences, China, invested along with DSM to build a production plant with 10-kton/year capacity to produce PHAs for packing and biomedical applications. PHA polymers are thermoplastic, and their thermal and mechanical properties depend on their composition. The Tg of the polymers varies from −40°C to 5°C, and the melting temperatures range from 50°C to 180°C, depending on their chemical composition. PHB is similar in its material properties to polypropylene, with a good resistance to moisture and aroma barrier properties. Polyhydroxybutyric acid synthesized from pure PHB is relatively brittle and stiff. PHB copolymers, which may include other fatty acids such as beta-hydroxyvaleric acid, may be elastic.

PHAs can be processed in existing polymer-processing equipment and can be converted into injection-molded components: film and sheet, fibers, laminates, and coated articles; nonwoven fabrics, synthetic paper products, disposable items, feminine hygiene products, adhesives, waxes, paints, binders, and foams. Metabolix has received FDA clearance for use of PHAs in food contact applications. These materials are suitable for a wide range of food packing applications including caps and closures, disposable items such as forks, spoons, knives, tubs, trays, and hot cup lids, and products such as housewares, cosmetics, and medical packaging (Yield Bioscience, (2016).

PHA and its copolymers are widely used as biomedical implant materials. Various applications of PHA and their polymer blends. These include sutures, suture fasteners, meniscus repair devices, rivets, bone plates, surgical mesh, repair patches, cardiovascular patches, tissue repair patches, and stem cell growth (Alejandra Rodríguez-Contreras et al., 2019). Changing the PHA composition allows the manufacturer to tune the properties such as biocompatibility and polymer degradation time within desirable periods under specific conditions. PHAs can also be used in drug delivery due to their biocompatibility and controlled degradability. Only a few examples of PHAs have been evaluated for this type of applications, and it remains an important area for exploitation (Vibhore Kumar Rastogi [and]Pieter Samyn (2015).

1.7.2.2. Polybutylene succinate

Polybutylene succinate (PBS) is an aliphatic polyester with similar properties to those of PET. PBS is produced by condensation of succinic acid and 1, 4-butanediol.

PBS can be produced by either monomers derived from petroleum-based systems or the bacterial fermentation route (Satti and Shah (2020)). There are several processes for producing succinic acid from fossil fuels. Among them, electrochemical synthesis is a common process with high yield and low cost. However, the fermentation production of succinic acid has numerous advantages compared to the chemical process. Fermentation process uses renewable resources and consumes less energy compared to chemical process (Paulo et al., 2019). Several companies (solely or in partnership) are now scaling bio-succinate production processes, which have traditionally suffered from poor productivity and high downstream processing costs. Mitsubishi Chemical (Japan) has developed biomass-derived succinic acid in collaboration with Ajinomoto to commercialize bio-based PBS (Oscar Rosales-Calderon and ValdeirArantes (2019)). DSM and Roquette are developing a commercially feasible fermentation process for the production of succinic acid 1,4-butanediol and subsequent production of PBS. Myriant and Bioamber have developed a fermentation technology to produce monomers. There are several companies around the world developing technologies for the production of PBS, including North America and China (Nathalie Gontard et al., 2018). Conventional processes for the production of 1,4-butanediol use fossil fuel feedstocks such as acetylene and formaldehyde (Pratima Bajpai et al., 2019). The bio-based process involves the use of glucose from renewable resources to produce succinic acid followed by a chemical reduction to produce butanediol (Pratima Bajpai, (2019)). PBS is produced by transesterification, direct polymerization, and condensation polymerization reactions. PBS copolymers can be produced by adding a third monomer such as sebacic acid, adipic acid, and succinic acid, which is also produced by renewable resources (Angélica Díaz et al., 2014).

PBS is a semi-crystalline polyester with a melting point higher than that of PLA. Its mechanical and thermal properties depend on the crystal structure and the degree of crystallinity. PBS displays similar crystallization behavior and mechanical properties to those of polyolefin such as polyethylene. It has a good tensile and impact strength with moderate rigidity and hardness. The Tg is approximately −32°C, and the melting temperature is approximately 115°C (Asim Kumar Roy Choudhury (2017)). In comparison with PLA, PBS is tougher in nature but with a lower rigidity and Young's modulus. By changing the monomer composition, mechanical properties can be tuned to suit the required application. PBS and their blends have found commercial applications in agriculture, fishery, forestry, construction, and other industrial fields, which includes mulch film, packaging, and flushable hygiene products and used as a non-migrant plasticizer for polyvinyl chloride (PVC). In addition, it is used in foaming and food packaging application. The relatively poor mechanical flexibility of PBS limits the applications of 100% PBS-based products (YajieZhonga et al., 2020). However, this can be overcome by blending PBS with PLA or starch to improve the mechanical properties significantly, providing properties similar to that of polyolefin (Formela, et al., 2017).

1.7.2.3.Polylactic acid

Polylactic acid (PLA) has been known since 1845 but not commercialized until early 1990. PLA belongs to the family of aliphatic polyesters with the basic

constitutional unit lactic acid. The monomer lactic acid is the hydroxyl carboxylic acid, produced by bacterial fermentation using starch or sugars as a carbon source. Even though, corn has the advantage of providing a high-quality feedstock for fermentation, results in a high-purity lactic acid leads to efficient synthetic process. Depending on the microbial seed culture, dictates the production of l-lactic acid or d-lactic acid (HayatiSamsudin and NorziahM.Hani (2020)).

PLA can be synthesized from lactic acid by direct polycondensation reaction or ring-opening polymerization of lactide monomer. However, it is difficult to obtain high molecular weight PLA via polycondensation reaction because of water formation during the reaction. Nature Works LLC (previously Cargill Dow LLC) has developed a low-cost continuous process for the production of PLA (Binay Bhushan and Rakesh Kumar (2019)). In this process, low molecular weight pre-polymer lactide dimers are formed during a condensation process. In the second step, the pre-polymers are converted into high molecular weight PLA via ring-opening polymerization with selected catalysts. Depending on the ratio and stereo-chemical nature of the monomer (l or d), various types of PLA and PLA copolymers had been produced.

PLA is a commercially interesting polymer as it shares some similarities with hydrocarbon polymers such as polyethylene terephthalate (PET). It has many unique characteristics, including good transparency, glossy appearance, high rigidity, and ability to tolerate various types of processing conditions. PLA is potential to replace traditional polymers namely PET, PS, and PC, with its thermoplastic nature. Which has been used for packaging to electronic and automotive applications (Sanjay Kumar Sharma, and AckmezMudhoo, (2010)). While PLA has similar mechanical properties to traditional polymers, but the thermal properties are not attractive due to low Tg of 60°C. Nature Works LLC, USA, is the major supplier of PLA sold under the brand name Ingeo, with a production capacity of 100,000 ton/year. There are other manufactures of PLA based in the USA, Europe, China, and Japan developing various grades of PLA suitable for different industrial sectors such as automobile, electronics, medical devices, and commodity applications (Aleksandra Nesic et al., 2020). PLA is widely been used in food packing (including food trays, tableware such as plates and cutlery, water bottles, candy wraps, cups, etc.). Generally, PLA has highest heat resistances and mechanical strengths of all bio-based polymers. Even though it is still not suitable for use in electronic devices and other engineering applications. NEC Corporation (Japan) recently produced a PLA with carbon and kenaf fibers with improved thermal and flame retardancy properties (Yousefi, Hossein et al., 2018). Fujitsu (Japan) developed a polycarbonate blend with PLA to make computer housings. PLA also been used as a membrane material in chemical industry.

The ease of melt processing has led to the production of PLA fibers, which are a wide variety of textiles from dresses to sportswear, furnishing to drapes, and soft nonwoven baby wipes to tough landscape textiles. These textiles can outperform traditional textiles made from synthetic counterparts (Ramesh et al., 2013). Bioresorbable scaffolds produced with PLA and various PLA blends used in implants

for growing living cells. The US Food and Drug Administration (FDA) has approved the use of PLA for certain human clinical applications. In addition, PLA-based materials been used for bone support splints.

1.7.2.4. Bio-polyethylene

Polyethylene (PE) is an important engineering polymer traditionally produced from fossil resources. PE produced by polymerization of ethylene under pressure, temperature, in the presence of a catalyst. Traditionally, ethylene produced through steam cracking of naphtha or heavy oils or ethanol dehydration (Xiue Han and Xiaodong Li (2020)). With increases in oil prices, microbial PE or green PE being manufactured from dehydration of ethanol produced by microbial fermentation. The concept of producing PE from bioethanol is not a particularly new one. In the 1980s, Braskem made bio-PE and bio-PVC from bioethanol. However, low oil prices and the limitations of the biotechnology processes made the technology unattractive at that time. Bio-PE is a by-product derived from bioethanol at industrial scale using sugarcane and other bio-renewable feed-stocks including sugar, beet, starch crops such as maize, wood, wheat, corn, and other plant wastes through fermentation (Investopedia (2020). Bio-based polyethylene has exactly the same chemical, physical, and mechanical properties as petrochemical polyethylene. Braskem (Brazil) is the largest producer of bio-PE with 52% market share, and this is the first certified bio-PE in the world. Similarly, Braskem is developing other bio-based polymers such as bio-polyvinyl chloride, bio-polypropylene, and their copolymers with similar industrial technologies. The current Braskem bio-based PE grades are towards food packing, cosmetics, personal care, automotive parts, and toys (Daniel Loeschen (2019)). Dow Chemical (USA) in cooperation with Crystalsev is the second largest producer of bio-PE with 12% market share. Solvay (Belgium), another producer of bio-PE, has 10% share in the current market. However, Solvay is a leader in the production of bio-PVC with similar industrial technologies. China Petrochemical Corporation also plans to set up production facilities in China to produce bio-PE from bioethanol (Haung*et al.*, 2008). Bio-PE has advantages than fossil-based PE and is been widely used in engineering, agriculture, packaging applications because of its low price and good performance.

A rich source and plenty of available Natural fatty acids conversion into long-chain aliphatic polymers was recent past developments. Different kind of monomers were developed and further converted into aliphatic greener long chains of polymers but not having similar physicochemical properties like commercial. Moreover, there is a need to develop new stratagies to develop highly purified and economically cost effective, which is more feasible to the customer reliable monomers, along with said qualities it must possess high molecular weight long chain aliphatic polymers must bring into or convert in microstructures and controlled for specific application.

2.0. Conclusions and future Scope

Renewable biomass have playing vital role in the generation of newly derived with more compatibility and viable polymers to replace the existing polymers. Lignin-

derived poly-meric materials, biobased polyolefins, and long-chain aliphatic polymers are the new era of polymers occupies the sustainable polymers role at commercial point of view. Among the listed one PA11, commercialized in the market with small occupancy. Due to the ever-increased rate of demand of commercial polymers, there is a need to supply in a feasible or low economic to the consumer and to overcome the present challenges. Henceforth there is need to implement to attain the sustainable economy in the production of renewable biomass based polymers, different physicochemical properties change has to be done to compete with commercial polymers. The cost of biobased polymers less economical than commercial polymers. Polyolefins are the alternative for renewable biobased polymers to replace the market or commercial polymers. Novel polymeric materials with efficiency in its function has to be synthesized. Biomass such as lignin, cellulose, and fatty acid are cheap and could modify easily. Care must be taken while designing the greener polymers to achieve the best derivatives of monomers and polymers. In the coming era of chemical synthesis of bio-based polymers.

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References

1. Abbass A. Hashim (2012). The Delivery of Nanoparticles, InTech.
2. Abhilash M and Thomas D (2017) 15-Biopolymers for Biocomposites and Chemical Sensor Applications; Biopolymer Composites in Electronics :405-435.
3. Abhilash M and Thomas, D (2017). 15 - Biopolymers for Biocomposites and Chemical Sensor Applications, Biopolymer Composites in Electronics, Pg: 405-435.
4. Abhilash, M and Thomas, D (2017). 15-Biopolymers for Biocomposites and Chemical Sensor Applications; Biopolymer Composites in Electronics:405-435.
5. Abobatta W. (2018). Impact of hydrogel polymer in agricultural sector. Adv Agr Environ Sci. Vol (1)2: 59–64.
6. Abrisham, Elham and Jafari, Mohammad and Ali, Tavili and Rabii, Ahmad and ZareChahouki, Mohammad Ali and Salman, Zare and Egan, Todd and Yazdanshenas, Habib and Ghasemian, Davood and Tahmoures, Mohammad. (2018). Effects of a super absorbent polymer on soil properties and plant growth for use in land reclamation. Arid Land Research and Management:1-14.
7. Alain Dufresne (2014). Crystalline starch based nanoparticles, Current Opinion in Colloid & Interface Science, Vol (19)5:397-408.
8. Alejandra Rodríguez-Contreras., Elisa Rupérez., María Soledad Marqués-Calvo., José María Manero (2019). Chapter 7 - PHAs as matrices for drug delivery, Materials for Biomedical Engineering, Nanomaterials-Based Drug Delivery: 183-213.
9. Aleksandra Nesic., Catalina Castillo., Patricia Castaño., Gustavo Cabrera-Barjas and Jesus Serrano (2020).Chapter-8-Bio-based packaging materials, Biobased Products and Industries: 279-309.
10. Alvin Bacero Bello, Deogil Kim, Dohyun Kim, Hansoo Park, and Soo-Hong Lee (2020).Engineering and Functionalization of Gelatin Biomaterials: From Cell Culture to Medical Applications, Tissue Engineering Part B: Reviews Vol. (26)2.
11. Amin Shavandi, PouyaSaeedi, M. Azam Ali, and EsmatJalalvandi (2019). Green synthesis of polysaccharide-based inorganic nanoparticles and biomedical aspects, Functional Polysaccharides for Biomedical Applications. 2019 : 267–304.

12. Amit Kumar Nayak, Bibhash Chandra Mohanta Md Saqui Hasnain, Mohammad Niyaz Hoda, Garima, Tripathi (2020). Chapter 14 - Alginate-based scaffolds for drug delivery in tissue engineering, Alginates in Drug Delivery: 359-386.
13. Angélica Díaz, RamazKatsarava and Jordi Puiggali (2014). Synthesis, Properties and Applications of Biodegradable Polymers Derived from Diols and Dicarboxylic Acids: From Polyesters to Poly (ester amides), Int J Mol Sci. 15(5): 7064–7123.
14. Anita A .Desai (2020). Medical Textiles: Healthcare And Hygiene Products.
15. Arafat Alkhasha., Abdulrasoul Al-Omran and Anwar Aly (2018).Effects of Biochar and Synthetic Polymer on the Hydro-Physical Properties of Sandy Soils; sustainability In MDPI.
16. Asim Kumar Roy Choudhury (2017). Biopolymers in textile industry, Biopolymers in Textile Industry.
17. Babu, R.P., O Connor, K. and Seeram, R. (2013). Current progress on bio-based polymers and their future trends. Prog Biomater Vol (2) 8.
18. Behera, Sabyasachi and Mahanwar, Prakash. (2019). Superabsorbent polymers in agriculture and other applications: a review. Polymer-Plastics Technology and Materials:1-16.
19. Binay Bhushan and Rakesh Kumar (2019). Chapter-11-Plasma treated and untreated thermoplastic biopolymers/biocomposites in tissue engineering and biodegradable implants, Materials for Biomedical Engineering Hydrogels and Polymer-Based Scaffolds :339-369.
20. Biotechnology in Personal Care, Cosmetic Science: (2006).
21. Chen, R., Xu, X., Yu, D. et al. (2019). Temperature-regulated flexibility of polymer chains in rapidly self-healing hydrogels. NPG Asia Mater, Vol (11) 22.
22. Chenhao Zhao, Min Zhang, Zhiguang Liu, Yanle Guo, and Qiang Zhang (2019).Salt-Tolerant Superabsorbent Polymer with High Capacity of Water-Nutrient Retention Derived from Sulfamic Acid-Modified Starch; ACS Omega. Vol 4(3): 5923–5930.
23. Chunhua Zhao., Yanping Zhang and Yin Lia (2019). Production of fuels and chemicals from renewable resources using engineered Escherichia coli, Biotechnology Advances; Vol(37) 7:
24. Dandekar, Prajakta & Jain, Ratnesh&Stauner, Thomas &Loretz, Brigitta& Koch, Marcus & Wenz, G. & Lehr, Claus-Michael. (2012). A Hydrophobic Starch Polymer for Nanoparticle-Mediated Delivery of Docetaxel. Macromolecular bioscience.
25. Daniel Hellerstein, Dennis Vilorio, and Marc Ribaudo (editors) (2019). United States Department of Agriculture Economic Research Service Agricultural Resources and Environmental Indicators, Economic Information Bulletin Number 208.
26. Daniel Loeschen (2019).10-Products of agricultural biotechnology.
27. De Meyst, Laurence and Mannekens, Els and Araújo, Maria and Snoeck, Didier and Tittelboom, Kim and Van Vlierberghe, Sandra and Belie, Nele. (2019). Parameter Study of Superabsorbent Polymers (SAPs) for Use in Durable Concrete Structures. Materials. Vol (12).
28. Diego M. D. L. Navarro, Jerubella J. Abelilla and Hans H. Stein (2019). Structures and characteristics of carbohydrates in diets fed to pigs: Journal of Animal Science and Biotechnology: Vol (10)39.
29. Dou, Hongjing& Tang, Minhua& Yang, Weihai & Sun, Kang. (2007). One-pot synthesis, characterization, and drug loading of polysaccharide-based nanoparticles with carboxy functional groups. Colloid Polym. Sci., Vol (285): 1043-1047.
30. Dublin, (2020). (globe newswire),The Global Market for Bioplastics (2nd Edition): Research And Markets.com's offering.
31. Dyson, Lisa, Reed, John, Geller, Jil, Hande, Sonali (2019). Microbial Conversion of CO2 and Other C Substrates to Vegan Nutrients, Fertilizers, Biostimulants, and Systems for Accelerated Soil Carbon Sequestration United States Patent Application. International Classes: Fisher Broyles LLP-Kiverdi.
32. Enas M. Ahmed (2019). Hydrogel: Preparation, characterization, and applications: A review Journal of Advanced Research, Vol (6)2:105-121.
33. Extraction and characterization of an alginate from the Iranian brown seaweed Nizimuddiniazanardini, International Journal of Biological Macromolecules, Vol (118) Part-A: Pg: 1073-1081.
34. Fijul Kabir, S. M., Partha P. Sikdar, B. Haque, M. A., Rahman Bhuiyan, A., Ali and Islam M. N. (2018). Cellulose-based hydrogel materials: chemistry, properties and their prospective applications, Progress in Biomaterials, Vol (7):153–174.
35. Formela, K., Hejna, A., Haponiuk, J and Tercjak A (2017). In situ processing of biocomposites via reactive extrusion, Biocomposites for High-Performance Applications, Current Barriers and Future Needs Towards Industrial Development :95-246.
36. Frank Hermanutz., Marc Philip Vocht., Nicole Panzier and Michael Buchmeiser, R (2018). Processing of Cellulose Using Ionic Liquids; Macromolecular Materials and Engineering.

37. Garcia Ibarra, V and Rodriguez-Bernaldo de Quiros, A (2016). Antimicrobial Food Packaging Based on Biodegradable Materials in Antimicrobial Food Packaging.
38. Georgios Nikolaou, DamianosNeocleous, Anastasis Christou, EvangeliniKitta and Nikolaos Katsoulas (2020). Implementing Sustainable Irrigation in Water-Scarce Regions under the Impact of Climate Change, agronomy; MDPI journals.
39. Gheorghe Adrian, Martau and Mihai, Mihaela and Vodnar, Dan. (2019). The Use of Chitosan, Alginate, and Pectin in the Biomedical and Food Sector—Biocompatibility, Bioadhesiveness, and Biodegradability. Polymers. 11: 1837.
40. Goodarzi, Navid&Varshochian, Reyhaneh&Kamalinia, Golnaz&Atyabi, Fatemeh &Dinarvand, Rassoul. (2013). A review of polysaccharide cytotoxic drug conjugates for cancer therapy. Carbohydrate polymers; Vol(92): 1280-93.
41. Halley, P., and Averous. L (2014) Starch Polymers ebook :484.
42. HayatiSamsudin and NorziahM.Hani (2020). Chapter 8 - Use of Starch in Food Packaging, Starch-Based Materials in Food Packaging Processing, Characterization and Applications: 229-256.
43. Hiba Shaghaleh ab, Xu Xu ORCID logo*abc and Shifa Wang abc (2018). Current progress in production of biopolymeric materials based on cellulose, cellulose nanofibers, and cellulose derivatives, RSC Adv., Vol (8):825-842.
44. Huanli Sun., Yifan Zhang and Zhiyuan Zhong (2018). Reduction-sensitive polymeric nanomedicines: An emerging multifunctional platform for targeted cancer therapy, Advanced Drug Delivery Reviews
45. Ida Idayu Muhamad., Nurul Asmak Md Lazim and Suguna Selvakumaran (2019). Chapter-18-Natural polysaccharide-based composites for drug delivery and biomedical applications; Natural Polysaccharides in Drug Delivery and Biomedical Applications:419-440.
46. Investopedia (2020). How Do Supply and Demand Affect the Oil Industry, guide to investigate oil market.
47. James M. MacDonald, Penni Korb, and Robert A. Hoppe (2013). Farm Size and the Organization of U.S. Crop Farming United States Department of Agriculture, Economic Research Report Number 152,
48. James N. BeMiller (2019). in Carbohydrate Chemistry for Food Scientists (Third Edition), Cellulose and Cellulose-Based Hydrocolloids.
49. Jaya chandran Venkatesan R.Nithya Prasad N.Sudha Se-KwonKim (2014). Chapter Four - Role of Alginate in Bone Tissue Engineering, Advances in Food and Nutrition Research, Vol (73) Pg:45-57.
50. Jing Wang., JavadTavakoli and You hong Tang (2019).Bacterial cellulose production, properties and applications with different culture methods, Carbohydrate Polymers; Vol (219):63-76.
51. John A. Hangasky, Michael A. Marletta, (2020). Glycosidic Bond Oxidation: The Structure, Function, and Mechanism of Polysaccharide Monooxygenases; In Reference Module in Chemistry, Molecular Sciences and Chemical Engineering,
52. Keshipour S., and Maleki A. (2018) Modification of Cellulose. In: Mondal M. (eds) Cellulose-Based Superabsorbent Hydrogels. Polymers and Polymeric Composites: A Reference Series. Springer, Cham: 17-1.
53. Khvostov, M.V., Tolstikova, T.G., Borisov, S.A. (2019) Application of Natural Polysaccharides in Pharmaceutics. Russ J Bioorg Chem 45, 438–450.
54. Krzysztof Lejcus, MichalSpitalniak and Jolanta D abrowska (2018). Swelling Behaviour of Superabsorbent Polymers for Soil Amendment under Different Loads; polymers ; MDPI.
55. Krzysztof Lejcus, Michal Spitalniak and Jolanta Dabrowska (2018). Swelling Behaviour of Superabsorbent Polymers for Soil Amendment under Different Loads, Polymers Vol, 10(3): 271.
56. Kumar Ramamoorthy., Dan Akesson., RathishRajan., Aravin Prince Periyasamy and Mikael Skrifvars (2019).Mechanical performance of biofibers and their corresponding composites, Mechanical and Physical Testing of Biocomposites, Fibre-Reinforced Composites and Hybrid Composites, Wood head Publishing Series in Composites Science and Engineering: 259-292.
57. Li Li., Jun Ki Kim., Kang Moo Huh., Yong-kyu Lee and So Yeon Kimc (2012). Targeted delivery of paclitaxel using folate-conjugated heparin-poly(β-benzyl-l-aspartate) self-assembled nanoparticles, Carbohydrate Polymers, Vol (87) 3:2120-2128.
58. Liming Yuan, Bu Guo, Wu Zhong, Yu Nie, Xiaoyan Yao, Xiaofeng Peng, Rong Wang, Hongyuan Yu, Shanyi Yang, Chunlian He, Xiaojun Tao and Qiufang Zhang (2019). Interaction of Mitoxantrone-Loaded Cholesterol Modified Pullulan Nanoparticles with Human Serum Albumin and Effect on Drug Release, Journal of Nanomaterials.
59. Luo, Juntao, Shi, Changying and Guo, Dandan (2020) "Functional segregated Telodendrimers and nanocarriers and methods of making and using same, https://www.freepatentsonline.com/y2020/0254012.html.

60. Majidian, Parastoo., Tabatabaei, Meisam., Zeinalabedini, Mehrshad., Naghshbandi., mohammadpooya and Chisti, Yusuf. (2017). Metabolic engineering of microorganisms for biofuel production. Renewable and Sustainable Energy Reviews.
61. Martin Gericke, Peter Schulze and Thomas Heinze (2020). Nanoparticles Based on Hydrophobic Polysaccharide Derivatives—Formation Principles, Characterization Techniques, and Biomedical Applications, Macromolecular Bioscience, Vol (20)4.
62. Megan S. Ostrand Thomas M. DeSutter Aaron L. M. Daigh Ryan F. Limb Dean D. Steele (2020). Superabsorbent polymer characteristics, properties, and applications; Agrosystems, Geosciences & Environment, Vol (3)1.
63. Mingchang Zhang, Shaodi Zhang, Zhuoran Chen, Mingzhi Wang Jinzhen Cao and Ruoshui Wang (2019). Preparation and Characterization of Superabsorbent Polymers Based on Sawdust, Polymers (Basel). Vol1 (1)11: 1891.
64. Misiewicz, J. Lejcus, K., Dabrowska, J. (2019). The Characteristics of Absorbency Under Load (AUL) for Superabsorbent and Soil Mixtures. Sci Rep Vol (9).
65. Mohapatra, Mamata and Anand S (2010). Synthesis and applications of nano-structured iron oxides/hydroxides - A review. International Journal of Engineering, Science and Technology. Vol (2).
66. Mona Alibolandi and Mohammad Ramezani (2018). Active Targeted Nanoscale Delivery Systems for Brain Tumor Therapeutics, In Nanotechnology-Based Targeted Drug Delivery Systems for Brain Tumors.
67. Monica Puri Sikka, Vinay Kumar Midha, (2019). The role of biopolymers and biodegradable polymeric dressings in managing chronic wounds, in Advanced Textiles for Wound Care (Second Edition) 2019.
68. Mosaiab, Tamim, Farr, Dylan, Kiefel, Milton J, Houston, Todd A (2019). Carbohydrate-based nanocarriers and their application to target macrophages and deliver antimicrobial agents, Advanced Drug Delivery.
69. MostafaaAwatef N.A., FaragbHala A., Abo-diefadAghareed M. and Tayebc M (2019). Production of biodegradable plastic from agricultural wastes, Arabian Journal of Chemistr,Vol (11)4:546-553.
70. Nathalie Gontard, Ulf Sonesson ORCID Icon, Morten Birkved ORCID Icon, Mauro Majone ORCID Icon, David Bolzonella ORCID Icon, Annamaria Celli ORCID Icon (2018). A research challenge vision regarding management of agricultural waste in a circular bio-based economy, Journal Critical Reviews in Environmental Science and Technology, Vol (48)6: 614-654.
71. National Service Center for Environmental Publications (NSCEP), (2007). Bioengineering for Pollution Prevention Through Development of Biobased Energy and Materials State of the Science Report 2007.
72. National Service Center for Environmental Publications (NSCEP), (2007)).
73. Natural Polysaccharides in Drug Delivery and Biomedical Applications (2019).
74. Nitta SK and Numata K (2013). Biopolymer-based nanoparticles for drug/gene delivery and tissue engineering.International Journal of Molecular Sciences; Vol14(1):1629-1654.
75. Oladapo P. Olayemi, Cynthia M. Kallenbach, Joel P. Schneekloth, Francisco J. Calderón, Merle F. Vigil and Matthew D. Wallenstein. (2020). From Factory to Field: Effects of a Novel Soil Amendment Derived From Cheese Production on Wheat and Corn Production, Front. Sustain. Food Syst.,
76. Oscar Rosales-Calderon and ValdeirArantes (2019). A review on commercial-scale high-value products that can be produced alongside cellulosic ethanol, Biotechnology for Biofuels Vol (12)240.
77. Oyatogun., G.M., Esan., T.A., Akpan., E.I., Adeosun., S.O., Popoola., A.P.I., Imasogie., B.I., Soboyejo., W.O., Afonja., A.A., bitoye., S.A., Abere., V.D., Oyatogun., A.O., Oluwasegun., K.M., Akinwole., E., and Akinluwade, K.J. (2020). Chapter-11-Chitin, chitosan, marine to market; Handbook of Chitin and Chitosan, Chitin and Chitosan based Polymer Materials for Various Applications, Vol (3):341-381.
78. Papermaking (2019). From The Publishers Of Paper Technology; paper industry technical association, Vol (5)2:
79. Paripurnanda Loganathan, Michael Gradzielski, Heriberto Bustamante and Saravanamuthu Vigneswaran (2020). Progress, challenges, and opportunities inenhancing NOM flocculation using chemically modified chitosan: a review towards future development, Environmental Science Water Research & Technology.
80. Paulo C.Torres-Mayanga., Daniel Lachos-Perez., AckmezMudhoo., Sunil Kumar Avery B.Brown., Maksim Tyufekchievd Giuliano Dragone., Solange I.Mussatto., Mauricio A.Rostagno Michael Timko., T.Forster-Carneiro (2019).Production of biofuel precursors and value-added chemicals from hydrolysates resulting from hydrothermal processing of biomass: Biomass and Bioenergy, Vol (130).
81. Peipei Zhang, Yu-Chieh Chiu, Lisa H. Tostanoski, and Christopher M. Jewell (2015). Polyelectrolyte Multilayers Assembled Entirely from Immune Signals on Gold Nanoparticle Templates Promote Antigen-Specific T Cell Response, ACS Nano; Vol(9)6:6465–6477.

82. Platt, Virginia &Szoka, Francis. (2008). Anticancer Therapeutics: Targeting Macromolecules and Nanocarriers to Hyaluronan or CD44, a Hyaluronan Receptor. Molecular pharmaceutics, Vol(5):474-86.
83. Pratima Bajpai, in Biobased Polymers, (2019). Properties of biobased packaging material, Polybutene.Pratima Bajpai, (2019). Properties of biobased packaging material, in Biobased Polymers,
84. Preparation and Properties: 1-33.MahmoudNasrollahzadeh, Zahra Issaabadi, (2019). In Interface Science and Technology; An Introduction to Green Nanotechnology.
85. Priscila Milani, Debora Franca, Aline Gambaro Balieiro and RoselenaFaez (2017). Polymers and its applications in agriculture, Polimeros: Vol.(27)3.
86. Qingjie Sun (2018).Chapter-18-Starch Nanoparticles, Starch in Food (Second Edition),Structure, Function and Applications, Wood head Publishing Series in Food Science, Technology and Nutrition: 691-745.
87. Ramesh P Babu, Kevin O'Connor and Ramakrishna Seeram (2013). Current progress on bio-based polymers and their future trends, Progress in Biomaterials, Vol (2) 8.
88. Roya AbkaKhajoueia, JavadKeramat, Nasser Hamdami, Alina-Violet, Ursu, Cedric Delattre, Céline Laroche, Christine Gardarin, Didier Lecerf, Jacques Desbrières, GholamrezDjelveh, Philippe Michaud (2018).
89. Sachiko Kaihara Nitta and KeijiNumata (2013). Biopolymer-Based Nanoparticles for Drug/Gene Delivery and Tissue Engineering, Int. J. Mol. Sci.,Vol 14(1):1629-1654.
90. SaghiSaghazadeh, Chiara Rinoldi, MaikSchot, Sara Sahe, Kashaf, Fatemeh Sharifi, Elmir, Jalilian, KristoNuutila, Giorgio Giatsidis, Pooria, Mostafalu, Hossein Derakhshandehh Kan Yueab Wojciech Swieszkowskic Adnan Memici Ali Tamayolabh Ali Khademhosseiniabijkln (2018). Drug delivery systems and materials for wound healing applications, Advanced Drug Delivery Reviews, Vol (127):138-166.
91. Salem, M.A. Scrivener James E. R. Couper Richard Erdlac Pradip Khaladkar Norman Lieberman W. Kent Muhlbauer S. A. Sherif (1970). Handbook of Bioplastics and Biocomposites Engineering Applications. Scrivener Publishing, Winter Street, Suite.,
92. Salim, Saifuldeen (2015). Effect Of Water-Retaining Agent(Sky Gel) On Soil Physical Properities, Growth, Yield And Water Use Efficiency Of Wheat (Triticum aestivum L.) Plant, j.Biol.Chem.Environ.Sci. Vol 6(1).
93. Salim, Saifuldeen (2019). Effect Of Water-Retaining Agent(Sky Gel) On Soil Physical Properities, Growth, Yield And Water Use Efficiency Of Wheat (Triticum aestivum L.) Plant. : https://wj.Biol.Chem.Environ.Sci. 6(1) 2015.
94. Sanjay Kumar Sharma, and AckmezMudhoo, (2010). Microwave Heating as a Tool for Sustainable Chemistry, Green Chemistry for Environmental Sustainability.
95. Saquib Hasnain Md, Amit Kumar Nayak, Mallesh Kurakula and Mohammad NiyazHoda (2020). Chapter 6 - Alginate nanoparticles in drug delivery, Alginates in Drug Delivery :129-152.
96. Satti, S.M. and Shah A.A. (2020). Polyester-based biodegradable plastics: an approach towards sustainable development. Letters in applied Microbiology, Separation and Purification Technology, Vol (239)15.
97. Shah Fahad, Ali A. Bajwa, Usman Nazir, Shakeel A. Anjum, Ayesha Farooq, Ali Zohaib, Sehrish Sadia, Wajid Nasim, Steve Adkins, Shah Saud, Muhammad Z. Ihsan, Hesham Alharby, Chao Wu, Depeng Wang, and Jianliang Huang. (2017). Crop Production under Drought and Heat Stress: Plant Responses and Management Options; Front Plant Sci., Vol (8): 1147.
98. Sheng, Long., Tong, Qunyi and Ma, Meihu. (2016). Why sucrose is the most suitable substrate for pullulan fermentation by Aureobasidium pullulans CGMCC1234. Enzyme and Microbial Technology.
99. Shikha Dahiya, RanaprathapKatakojwala, Seeram Ramakrishna, S. Venkata Mohan (2020). Biobased Products and Life Cycle Assessment in the Context of Circular Economy and Sustainability; Materials Circular Economy, vol (2)7.
100. Siddhesh and N.Pawar (2017). Chapter 8 - Chemical Modification of Alginate, Seaweed Polysaccharides,Isolation, Biological and Biomedical Applications: 111-155.
101. Soleimani, Khadijeh., Dadkhah Tehrani, Abbas., and Adeli, Mohsen (2018). Preparation of new GO-based slide ring hydrogel through a convenient one-pot approach as methylene blue absorbent, PubMed, your gate to U.S, federal scienc, science .Gov.
102. SpaseStojanov and Ales Berlec (2020). Cartilage Extracellular Matrix Scaffold with Kartogenin-Encapsulated PLGA Microspheres for Cartilage Regeneration; frontiers, bioengineering and Biotechnology.
103. Stefano Rimondo., Giuseppe Perale and Filippo Rossi (2019). 6-Polysaccharide-based scaffold for tissue-regeneration, Functional Polysaccharides for Biomedical Applications:189-212.

104.Steinman, Noam & Campos, Luis & Feng, Yakai&Domb, Abraham &Hosseinkhani, H.. (2020). Cyclopropenium Nanoparticles and Gene Transfection in Cells. Pharmaceutics; Vol (12):768.
105.Suhail Ahmad., Kaiser Manzoor., Mudasir Ahmad., RohiPurwar and Saiqa Ikram (2020). Chapter-7, Starch-based Bionanocomposites, Bionanocomposites, Green Synthesis and Applications, Micro and Nano Technologies:157-171.
106.Summy Yadav, Payal Modi, Akanksha Dave, AkdasbanuVijapura, Disha Patel and Mohini Patel (2020). Effect of Abiotic Stress on Crops, Home > Books > Sustainable Crop Production; intechopen.88434.
107.Suneeta Kumari and Rupak Kishor (2020). Chapter-1-Chitin and chitosan: origin, properties, and applications; Handbook of Chitin and Chitosa; Vol(1):
108.Suresh, Rahul and Prasher, Shiv and Patel, Ramanbhai and Qi, ZhimingElsayed, Eman and Schwinghamer, Timothy and Ehsan, Abdul. (2018). Super Absorbent Polymer and Irrigation Regime Effects on Growth and Water Use Efficiency of Container-Grown Cherry Tomatoes. Transactions of the ASABE. (61). 523-531.
109.Swierczewska, M. & Han, Hwa Seung & Kim, K. & Park, J.H. & Lee, S.. (2015).Polysaccharide-based Nanoparticles for Theranostic Nanomedicine. Advanced Drug Delivery Reviews, Vol(99).
110.Sza A., ata, Kamila; Gumi, Tania (2017). NASA Astrophysics Data System (ADS); BioArtificial polymers: Science.gov; your gateway to U.S federal science.
111.TandziNgoune Liliane and Mutengwa Shelton Charles (2020). Factors Affecting Yield of Crops, Agronomy-Climate Change & Food Security; Intechopen.90672.
112.Thiago Zanguetin (2020) SAP S/4HANA 1909 System Conversion Steps & Details – How to be prepared; Technical Articles.
113.Tianxin Miao, Junqing Wang, Yun Zeng, Gang Liu and Xiaoyuan Chen (2018). Polysaccharide-Based Controlled Release Systems for Therapeutics Delivery and Tissue Engineering: From Bench to Bedside, Adv Sci (Weinh). 2018 Apr; 5(4):
114.Torres-Ginera S., Hilliou L., Melendez-Rodrigueza B., Figueroa-Lopeza, B., Madalenac D., Cabedod, L., Covas J.M., Vicentec, A.A., Lagarona, J.M (2020). Food Packaging and Shelf Life, Vol (17):39-49.
115.Vibhore Kumar Rastogi and Pieter Samyn (2015). Bio-Based Coatings for Paper Applications, Coatings, 5(4), 887-930.
116.Vieira, M. and Silva, Mariana and Santos, Lucielen and Beppu, Marisa. (2011). Natural-based plasticizers and biopolymer films: A review. European Polymer Journal. 47. 254-263.
117.Wenju Zhao, Taohong Cao, Pinxin Dou, Jie Sheng, and Minqiang Luo (2019). Effect of various concentrations of superabsorbent polymers on soil particle-size distribution and evaporation with sand mulching, Sci Rep. Vol (9): 3511.
118.Xiue Han and Xiaodong Li (2020). Chapter-7-Processes and characterization of bioethanol and biopolyethylene, Processing and Development of Polysaccharide-Based Biopolymers for Packaging Applications:171-183
119.Xuan-Thanh Bui Chart Chiemchaisri Takahiro Fujioka Sunita Varjani (2019). Melt processability, characterization, and antibacterial activity of compression-molded green composite sheets made of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) reinforced with coconut fibers impregnated with oregano essential oil. Water and Wastewater Treatment Technologies.
120.Xunyi Wu Xiao Huang Yan zhe Zhu Jing Li Michael R. Hoffmann (2020). Synthesis and application of superabsorbent polymer microspheres for rapid concentration and quantification of microbial pathogens in ambient water.
121.YajieZhonga., Patrick Godwin., YongcanJin and Huining Xiao (2020). Biodegradable polymers and green-based antimicrobial packaging materials: A mini-review, Advanced Industrial and Engineering Polymer Research, Vol(3) 1: 27-35.
122.Yield Bioscience, (2016). Inc. (YTEN) form Annual Report, About: Yield10 Bioscience, Inc. (YTEN. Annual report pursuant to section 13 or 15(d) of the securities exchange act of 1934.
123.Yongbin Li, Haowen Shi, Haowei Zhang, and Sanfeng Chen (2019). Amelioration of drought effects in wheat and cucumber by the combined application of super absorbent polymer and potential biofertilizer; PeerJ; Vol (7): 6073.
124.Yousefi, Hossein., Azad, Sona., Mashkour, Mahdi and Khazaeian, Abolghasem (2018). Cellulose nanofiber board.Science.Gov; your gateway to U.S. federal Science.
125.Yuansong Xiao, Yan Peng, Futian Peng, Yafei Zhang, Wen Yu, Maoxiang Sun and Xiaolan Gao (2018). Effects of concentrated application of soil conditioners on soil–air permeability and absorption of nitrogen by young peach trees, Journal Soil Science and Plant Nutrition; Vol (64)3: Rhizo P special section.

126. Yuefei Zhu, Zhiqing Pang, (2019). In Polysaccharide Carriers for Drug Delivery, Polysaccharide nanoparticles for cancer drug targeting.
127. Yujie Zhang., Tao Sun and Chen Jiang (2018). Biomacromolecules as carriers in drug delivery and tissue engineering, Acta PharmaceuticaSinica B, Vol (8)1:34-50.
128. Yusof, N. Z.; Samsuddin, N. S.; Hanif, M. F.; Syed Osman, S. B. (2018). Peat soils stabilization using Effective Microorganisms (EM) NASA Astrophysics Data System (ADS), science.gov, your gateway to U.S. federal science.
129. Zahra Shariatinia (2019). Chapter-2-Pharmaceutical applications of natural polysaccharides; Natural Polysaccharides in Drug Delivery and Biomedical Applications: 15-57.

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Digital Marketing – An Intermediary To Commerce And Digital Technologies

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ABSTRACT

Most recent twenty years are considered as the improvement period in the field of Digital Technologies. The advancement of computerized innovation reflects in pretty much every space. Academics, Finance, Agriculture, Banking, Transport, and wellbeing areas promptly acknowledged the execution of Digital innovation. Contrasted with all different spaces advanced innovation encouraged the commerce sector to its fullest. The improvement of Digital Marketing during the 1990s and 2000s changed the route brands to advance their items and their advertising arrangements. Online business organizations like Paytm, Mobikwik, Amazon, Flipkart, and so forth out of nowhere turned into an essential piece of our everyday life. Advanced Marketing is the mix of trade and computerized innovation proposed to develop the business and increment business results. It likewise focuses to give simple reach to clients who favor possibly one of the two methods of on the web and window shopping. Digital Marketing is the ideal blend of Computer Science and Commerce to arrive at the most extreme number of clients in next to no time. The showcasing and client reach quickens manifolds due to ever-developing advancements in the field of social media and internet culture. Every one of these improvements in Digital Marketing causes us to think about the numerous irrefutable realities. It is clear that with the ceaseless development of digital showcasing, someplace our own space and security are undermined. Our paper principally centers around the definite examination of two boundaries, understanding the genuine capability of Digital Marketing according to the current situation and to distinguish distinctive advanced promoting procedures to support up the commerce sector. We additionally investigate whether Digital Marketing exploits our data and what strategies should be actualized to keep up client information respectability. The case studies and contextual investigations are additionally considered to validate our perceptions and results on campaign marketing, e-commerce marketing, social media optimization marketing, e-mail direct marketing, etc.

Keywords

Campaign marketing, e-commerce marketing, social media optimization marketing, e-mail direct marketing, etc.

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Introduction

There was a time when marketing meant newspaper, television, radio, magazine ads but with the growth in the digital sector and internet, today's digital technology is at its peak. Everyone relies on the digital form of marketing as it is easy to reach, responsive, and cheap. Digital Marketing is one of the most recent domain which equally utilizes the technology of Computer Science and business strategies of Commerce sectors. This perfect partnership makes it one of the most eminent and Sureshot techniques for successful business and capital sustainability. Reaching the client, understanding their needs, landing them on genuine and original products are the three major roles of digital marketing. The sudden improvements in the hirings of Digital Marketing Experts in the corporate sector display the true caliber of digital marketing.

The Google marketing team identified that 33% of the customers directly visit the product websites to purchase their required products. It is also identified that 48% of customers like to visit search engine sites to find the suitable and desired products they would potentially be purchasing. Mobile apps are also one of the major digital marketing accelerators by acquiring 26% of the product search. [1]

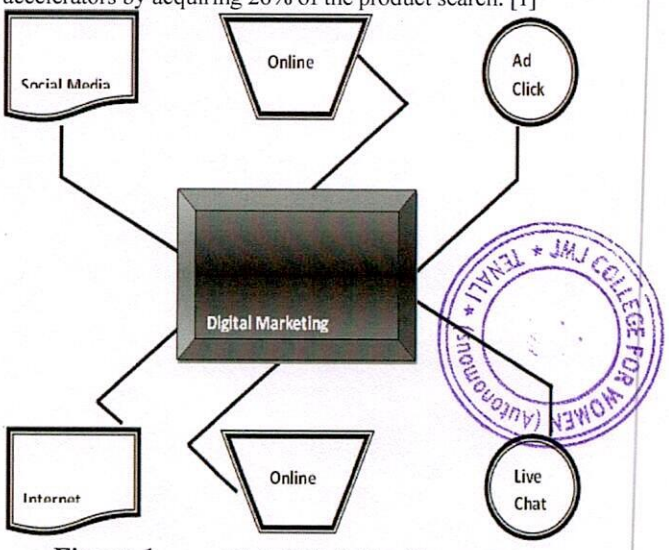

Figure 1. Digital Marketing Components.

Online marketing is a must for companies to sell their products through internet applications and E-Commerce websites. The popularity of E-Commerce due to digital marketing is so high that many companies don't even open their outlets but sell through online mode only. This makes them save a huge sum of money on office rentals and staff hiring. To promote their products to the highest possible customers, companies always research over different popular platforms. Some of the major platforms popular now to promote products through digital marketing are Facebook, Google, Twitter, Instagram, etc. Digital Marketing always relies on one of its main strategies called engagement marketing. The principle of engagement marketing uses the collected information on potential and returning customers based on their interest, budget, requirement, etc. The targeted audience is one of the sure-shot customers. Old couples with good financial stability will always look for health insurance and the young couple with good financial stability will look for cars, tours, and holidays. The audience based on their most required needed products is considered as the targeted audience. They are the most prominent customers, with whom the sales are much easier. This complete information can be segregated and targeted using digital marketing much efficiently.

Literature Survey

[1]. Laura Lake explained the strategies to promote business online in her article published in the *(BSB) Balance Small Business.* It is briefly explained about the steps to be taken to promote the business. The overall strategies are categorized into 10 processes. Some of the major suggestions are to provide a better website so that users would be engaged with the contents, to utilize *(SEO) Search Engine Optimization* so that users will land onto the company page easily, to hire professionals for digital marketing, to use E-Mail marketing, to maintain a blog and so on. The strategies are innovative and have sure-shot implementational benefits. The website is one of the most basic and landing spaces for the strategic customer planning of digital marketing which is well explained to be interesting and informative. SEO is very important to get more customers on the website. The more number of customers landing on the company page reflects better sales and business. Professionals of digital marketing can use their specialized skills to promote the company's business to its ultimate heights. E-Mail marketing directly reaches the customers who need to purchase the required products, it enables the companies to provide all information about their product to a customer who did not even reach their website. It is a personalized marketing strategy for digital marketing. Overall all concerned matters are covered and possible to implement in a real-time digital marketing scenario. [2]

[2]. Neil Patel explained the tactics of digital marketing. Most of the promotional tactics are almost similar to Laura Leke's but many additional points are there to consider. Some of the major highlighting points are to join or create a Facebook page, participate in different forums, comment on relevant content, start an eBay store, try in the foreign market, and so on. If the above said tactics are considered it could seriously affect the business positively and profitably. Joining or creating the Facebook page can attract a large number of customers due to its largest database and maximum customer reach. Facebook ads can be launched with a minimum sum by targeting different audiences based on marketing needs. Different forums joining and discussing the products lead to a large number of audience attraction who are most potential customers. Starting an eBay store is always a good idea to sell the product online. The marginal portion of the amount given to eBay as the sales margin provides worldwide access to several online selling facilities. He also explained a key factor that is generally not considered. He said, just if you are a small business, it doesn't mean that you should not try in the international market. The listing and sales in the international market can provide the business with its ultimate possibilities and promotion. [3]

[3]. Md Azharuddin explained the future scope of Digital Marketing in India. He explained that 59% of the world's population uses the internet. Just before 25 years, the total percentage of internet users worldwide was just 1%. In this 59% population also maximum percentage is for youth and the highest percentage of youth is in India. These statistics are evident enough to justify the calculation of google's report of India's potential to reach 100 Billion $ in E-Commerce. For centuries India is one of the biggest markets and after the promotion of Digital India, its E-Commerce strength has increase manifolds. India already surpassed the USA in the number of Internet users with the launch of Reliance Jio which further strengthened E-Commerce through Digital Marketing in India. He further extended that Digital marketing has increased job opportunities in India in Junior, Mid Level, and Higher level career options. Starting with the Digital Marketing intern to the Digital Branding head there are many positions available in the Digital Marketing field. Every organization welcomes someone who can increase their clientele. Digital Marketing has huge scope in India both in terms of job opportunities as well as for business extension and profitability. [4]

Digital Marketing & Traditional Marketing: An Overview

Digital Marketing utilizes the digital channels of marketing like web-based media, sites, and informing to advance business and items. At the point when we visit any site and the promotion ads pop up, that is an illustration of Digital Marketing.

Traditional marketing is the general type of marketing system which was being utilized for quite a long time like paper advertisements, announcements, hoardings, and so on. Till the 1990s everywhere in the world the main method of marketing present was traditional marketing. [5]

A. Digital Marketing vs. Traditional Marketing

TABLE I. DIGITAL MARKETING VS. TRADITIONAL MARKETING

Traditional Marketing	Digital Marketing
The Audiences conventionally encounter marketing messages like	The audience encounters marketing messages in digital forms like

newspapers. Hoardings. billboards. etc.	marketing ads popups on websites and social media.
Traditional marketing is expensive. hard to calculate the reach to customers. direct interaction is not present.	Digital marketing is comparatively cheaper. easy to calculate the reach to customers. direct interaction is present in the form of live chat.
It is less annoying but engaging. validity is reliable. does not keep changing all the time.	It is annoying sometimes. keeps changing all the time. validity is not guaranteed.
As per the current scenario. it's less popular.	As per the current scenario. it's more popular.

a. Comparison table of Traditional and Digital Marketing

Types of Digital Marketing

There are several types of Digital Marketing but mainly they are categorized into seven major types.

A. *Search Engine Optimization (SEO)*
B. *Social Media Marketing*
C. *Pay Per Click (PPC)*
D. *Content Marketing*
E. *Email Marketing*
F. *Marketing Analytics*
G. *Mobile Marketing*

A. Search Engine Optimization (SEO)

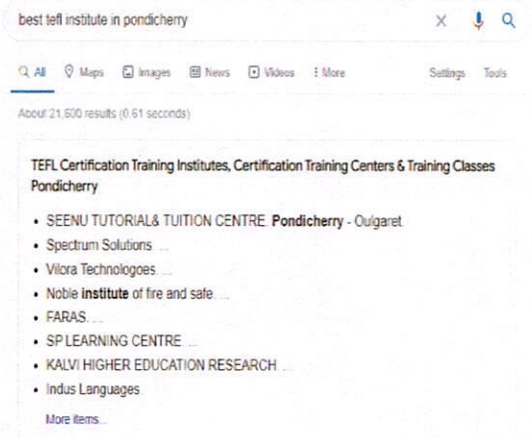

Figure 2. Google Keyword Search.

Most of the search for the products start from search engines. SEO deals with the correct ranking of the seller's website on the topmost listing. If the SEO is managed well on the website then users land immediately after their search to the seller's website. The contents of the website should be readable, links and hyperlinks should be active, and search terms should be relevant to queries. These steps make the website much visible in the internet galaxy of websites. [6]

B. Social Media Marketing

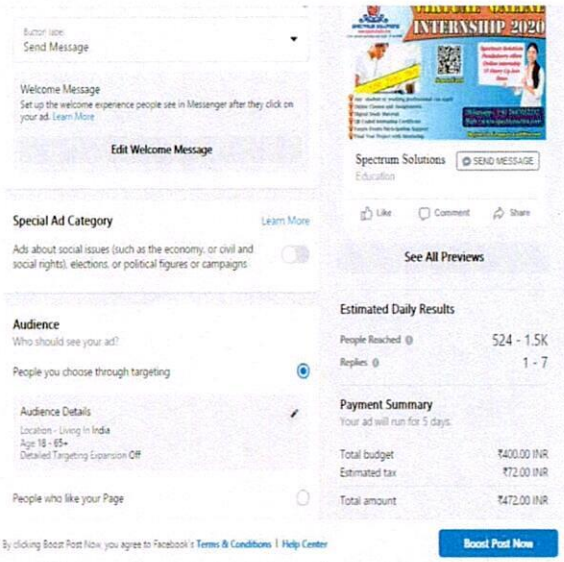

Figure 3. Social Media Product Promotion.

Social Media Marketing is a strategy to attract the maximum possible number of customers online. Most of the people around the globe are available on social media and almost active throughout time. Any ad which appears on social media platforms is highly appealing and has the maximum reach to potential customers. Several social media platforms like Facebook, Instagram, Twitter, etc. are available to the companies for their product promotion. Facebook allows the companies and sellers to target their audience based on the number, age group, and region with variable price tags. This enables the sellers to reach the customers of their product choice. [6]

C. Pay Per Click (PPC)

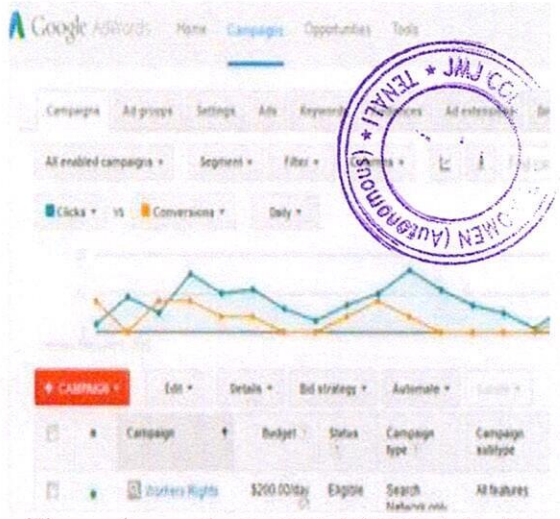

Figure 4. Pay Per Click (PPC) Statistics. [7]

Pay Per Click (PPC) is a Digital Marketing type in which a seller or company needs to pay to the ad service host only when the visitor clicks on their ad and lands on the advertising entity's website. YouTube and many ads appearing on several websites on corners are examples of Pay Per Click (PPC) ads. Many companies pay millions of dollars for these PPC ads. The cost of advertising depends on the value of the keywords. The more people searching for the same or similar keyword means the costlier will be the keyword to acquire. PPC also allows selecting whether the people searching the keywords will receive the same result worldwide or in a particular geographical location. If the companies are aware of these they can save a huge sum of money by targeting the ads on their potential customers' locations only. [6]

D. Content Marketing

Content marketing deals with making the brand trust to make bulk sales. It is the process of initially making a good relationship with the customers by trusting them and understanding their needs. It is generally not designed for a single sale but series of sales with trusted marketing. It uses the technique of brand explanation to increase the understanding of the product so that customer gets attracted toward the products. It also performs crucial processes like Mail Signup, Newsletter and to get more information about the customers. Several E-Books, videos, and animations are generally used to provide brand understanding to win the customer's trust. [6]

E. Email Marketing

Email Marketing is still an important strategy even after the development of social media to the large extent. Email marketing directly reaches the customers for their exact needs. The marketing team should make Email Marketing many interesting contents to attract the customers and to provide them the outlook of the products. [6]

F. Marketing Analytics

The Marketing analytics deal with the customer's behavioral analysis. Digital marketing has a unique feature that is trackable and transparent. The Marketing analytics allow the seller to track a lot of customer behaviors like the number of times the coupon codes are used, the emails are opened, the newsletter is read, links are clicked, and how many times the webpage is executed, etc. This makes the marketer target their ads to potential clients. [6]

G. Mobile Marketing

Most people use the internet on their mobile phones. It is much handier to catch people on their smartphone ads. This mode of marketing concentrates on social media, app links, websites, and text messages. Receiving an advertisement and promotional text message usually comes under this category. Only in the United States customers spend five hours a day on their smartphones. More or less all over the world maximum time spent on any digital medium is the smartphone, which makes it the most suitable channel for marketing. Proper strategic planning of Mobile marketing can boost the brand value and purchase to its maximum. [6]

Cyber Security Concerns in Digital Marketing

The Digital Marketing process passes through the gateway of the huge amount of personal data obtained from the customers. The personal data exposure to an illegitimate entity can lead to disastrous consequence. The sales information, card details, purchased products are sufficient to bring heavy consequences in one's life. It is the prime duty of digital marketers to guarantee the security of personal data acquired from the end-users. Certain Guidelines should be provided to the customers and even the marketing service provider should also keep their security features intact to the most priority slab. [8]

The major considerations and suggestions are as follows.

- Password Security Should be on top priority to avoid any unauthorized access by 2 step verification.
- Hardware and network should be monitored properly for hacking chances and security breaches.
- Employees' policies should be strict enough so that they should not leak any user's data.
- Click Frauds, Man in the Middle attacks should be continuously checked by the professionals.

Conclusion

Thus we analyzed the current scenario of Digital Marketing in India. Digital Marketing has the potential to easily bring a brand to its maximum sales target due to its reach and popularity through several digital media. There are several types of digital marketing and based on our requirements we need to choose them wisely. We also discussed the possibilities of data breaches and the chance of their misuse. It is further discussed that how we can maintain the user's data security using certain key points..

References

[1] Marketo Engage, "Digital Marketing", https://www.marketo.com/digital-marketing.

[2] Laura Lake "10 Strategies for Marketing Your Business Online", The Balance Small Business.

[3] Neil Patel, "23 Amazing Online Marketing Tactics for Small Businesses With Low Budgets "https://neilpatel.com/blog/low-budget-online-marketing-strategies/.

[4] Md Azharuddin, "Future Scope of Digital Marketing in India 2020-2021 & Career Opportunities", 2020, https://www.webtrainings.in/what-is-scope-of-digital-marketing-in-india.

[5] Jennifer Bailey, " The Vocational Implementation and Analysis of Computing Software Digital marketing vs. traditional marketing: what's the difference?" 2020, https://99designs.com/blog/marketing-advertising/digital-marketing-vs-traditional-marketing/.

[6] Susan Bogle, "What are the 7 Types of Digital Marketing?", Southern New Hampshire Univerity, Feb 2020.

[7] Consultwebs "Effective Law Firm PPC Can Deliver Results For Your Firm", 2020.

[8] Shane Pollard "Privacy and Security Concerns in Digital Marketing", 2020

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REVIEW ON INDIAN ENGLISH LANGUAGE AND LITERATURE

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Abstract: *The purpose of this observe become to perceive the elements associated with the trainer's attitude involved in oral communique of ESL college students. The investigation changed into executed at Bahawalpur, Pakistan. This research changed into pronounced on a survey study and used a questionnaire for the students and interview format for the teachers as tools to investigate the issue. The interview become taken from 40 particularly skilled ESL/EFL teachers; and for questionnaire almost one hundred students from each college were selected, total 498 ESL college students participated on this research. The information supplied thru interview from instructor was analyzed qualitatively and the records from the students turned into analyzed thru SPSS and Microsoft Excel. the subsequent conclusion changed into drawn from the records gathered from this research: instructor's mind-set motivates debilitating speakme factors. This investigation has proved explanatory implications and moreover this look at additionally examined the attitudes and opinions of language teachers approximately second language studying and teaching. moreover, the research advised an expansion of techniques for beginners to cope with second language tension.*

Keywords: *Oral communication snooping; Communicative skills; Teacher's demotivating attitude; Teacher's essential motivation; Hesitation due to interruption; Anxiety in oral competency.*

I. Introduction

In step with Hewitt and Stephenson (2012) teachers and researchers are well known for many years that language studying method, no doubt, may be very traumatic enjoy for lots students. Examiners in general have been concerned in exploring, recounting, and sufficiently measuring debilitating factors, in foreign languages specially, simply to help the students to deal with anxieties and to enlighten their language presentation. nerve-racking overseas language learners consider the reality that even though they are properly prepared for language elegance they get annoying. extra warfare lead them to tenser because they continually feel that other students will perform higher than them (Gregerse and Hurwitz, 2002). moreover, constant disturbance or anxiousness has opposed affects on language acquisition. So there's want to tackle the troubles associated with oral competency. this will be helpful to recognize what interventions want to lower adversarial anxiety, substituted by using facilitating kind tension which may encourage the students in mastering method (Hewitt and Stephenson, 2012).

there may be serious want to recognition on existence of talking factors and its function in language mastering before it receives worst. Macintyre and Gardner (1991) state that, the difference among language anxieties and other form of anxiety is understood and now could be called the principle problem to recognize the position of anxiety in language learning. college students are experiencing uneasiness in language studying for decades. in the records of language anxiety, speak me tension is counted as maximum tension generating (Zhang and Zhong, 2012). moreover, younger (1991) found that peers revel in superb fear in speak me the target language before, others in evaluation of writing and studying. proposes that to address with the language problems first there's want to apprehend the speak me anxieties, terrible assessment and different elements which generate overseas language studying anxieties.

II. Statement of Problem

In Pakistan English has end up repute representation in social life. The globalization of English in every discipline specially in schooling device of Pakistan is compelling students to talk in English but sometime due to instructor's attitude, the scholars sense strain in oral verbal exchange. So there is unique want to attention on 2d language mastering anxiety prompted due to instructor's manners.

III. Aims and Objectives

The purposes of this investigation were:

- to spotlight the most important stressors and debilitating factors arose due to trainer"s mind-set amongst ESL students at graduate stage, worried in talking English.
- To become aware of the debilitating elements

in classroom at graduate level instructions because of the instructor"s attitude.

- To help the teachers to recognize the pupil"s tension perfectly because of their own attitude.

IV. Research Questions

1. whether factors associated with teacher's mindset bog down learners" verbal exchange talents and put off ESL studying procedure and learner's development?
2. Is there any courting between ESL oral competency skills and teacher"s mindset closer to inexperienced persons.

V. Hypotheses

1. Teacher's attitude motivates debilitating speaking factors.

Significance of the Study

The basic purpose of this studies turned into to analyze the lifestyles and the extent of English language speaking issues among ESL graduate degree college students due to instructor's attitude. The final results changed into legitimate for educators to check their coaching strategies and college students facing tension. The examine was supportive for teachers to apprehend their scholar's problems deeply with a higher guidance in resolving their apprehensions through heading off their demanding coaching style. furthermore, studies changed into beneficial to have a look at talking anxiety issues related to the teacher's attitude notably in college surroundings of Pakistan.

Delimitations of the Study

- This studies handiest explored the outcomes of trainer's attitudes on talking competencies.
- information became accumulated handiest from graduate degree government colleges of Bahawalpur, with suggest age eighteen to twenty years.
- The look at changed into delimited to research the function of debilitating factors on learners" talking skills most effective.

VI. Literature Review

This chapter provides complete assessment about stressful elements arose from trainer's mind-set concerned in oral interaction

Anxiety and Language Learning

Anxiety tension may be described as the sensation of burden and nervousness in particular associated with the second language gaining knowledge of environments; might be in speaking, listening and in writing (Onwuegbuzie et al., 1999). it's far nicely defined that learner is suffering from anxiety when he isn't learning nicely, bunking from elegance, not able to reply or even no longer doing assignments; teachers and educators are atypical that what taking place. young (1991) indexed six resources of classroom language anxiety given below: (1)"personal and interpersonal anxieties, (2) learner beliefs about language mastering, (3) instructor beliefs about language mastering, (4) teacher-learner interactions, (5) classroom methods,(6) language trying out"(Pr i , 2 thirteen).

VII. Foreign Language Anxiety

There t are numerous styles of tension however whilst anxiety buddies with foreign language then it's miles called FLA. no question it's vary complex and multidimensional enjoy (Tanveer, 2007). FLA can be described as an man or woman's feeling of tension, uneasiness, anxiety, and burden related with a stimulation of the automatic apprehensive gadget (MacIntyre and Gardner, 1991; Tanveer, 2007). in step with Al-Saraj (2011) Oxford at some point of the procedure of studying students revel in anxiety and it's far foreign language anxiety (FLA). in keeping with a ramification of researchers (Horwitz et al., 1986; MacIntyre and Gardner, 1991) FLA can break the process of fulfillment in mastering the second language. FLA occurs due to "self-assumed" critiques, emotions and performances associated with lecture room of 2nd language getting to know for the duration of language learning procedure (Horwitz et al., 1986). FLA is also unique from trait tension, which is a long lasting. in step with Horwitz et al. (1986) in truth FLA is situation particular tension and unique from all other anxiety. This kind of anxiety occurs in positive conditions like public speaking or contributing in magnificence (Ellis, 2008). FLA is unique in feel that it occurs particularly within the precise foreign language getting to know environment. Many investigators (Horwitz et al. (1986) mentioned in price (1991)) said that in overseas language learning first tension felt by means of novices, in listening and speaking.

Anxiety Induced by Instructor's Attitude

According in keeping with behaviorist learning technique that teacher's negative reactions to learner's mistakes can promote learner's fear and preclude future verbal exchange. commonly teachers initiate nervousness due their antique ideas in language coaching or because of way of errors improvement. sometime instructors acts like "drill sergeant" rather than a "facilitator" (younger (1991) stated in Tanveer (2007)). common correction on errors is furnished in the classroom leads rookies to frustration and awkwardness and they end up acutely aware of their

lacks (Tanveer, 2007).
college students found out that during school room their errors and deficiencies are mentioned due to strict and reserved lecture room surroundings that initiate their pressure (Tanveer, 2007).
Harmer in 2011 condemns the idea that students get stressed in a state of affairs being pressured to speak English and to grow to be unbiased and stated that the reality is that in the phrases of an vintage English proverb, you may lead a horse to water but you could make it drink. And if it does now not want or want to drink, you have to not make it achieve this anyway. a few college students, like horses at water's part, simply don"t get it; for them the instructor is the one who is chargeable for their learning, and that they anticipate the teacher to do their task. confronted with the reluctance of at least a number of the students in a collection to assume employer, we should recall what we will do both for the ones college students and for others in the organization who're keener at the idea of taking learner responsibility. (P.23)
The commanding, embarrassing and humiliating conduct of the educators toward students is debilitating element gambling essential function in oral competency (Tanveer, 2007). Many instructors take lecture room time as simplest overall performance time for college kids, no longer studying duration. The maximum not unusual grievance approximately teachers turned into determined that lots of them made lecture room time a performance rather than a studying time (Tanveer, 2007). Learner's bond with their instructors also causes anxieties (Na, 2007).

IX. ESL Learning and Teaching in Educational Perspective of Pakistan

it's far vital to evaluate the connection among English language, its development, studying English as a overseas or 2nd language, and pedagogical implication of English language. English is now a lingua franca or a „international language" identified on global level. In Pakistan, at each the person and national levels English language is innovative language (Shamim, 2011).
No hesitation, the linguistic map of Pakistan is complex because of many languages as all provinces have their personal dominant languages and some of minority languages. So Pakistani societies are multilingual and multicultural (Shame, 2011). Its somewhat difficult to command over all languages flawlessly but as English is world language so humans need to gather English flawlessly.
according to (Shamir, 2011) in Pakistan current authorities coverage „schooling for All" centered on ESL coaching and gaining knowledge of, beautify the use of English within the global level. perhaps its complicated policy troubles each for aid and achievement nice in English language in Pakistan. furthermore, English in Pakistan is successful, repute image and additionally a key to national progress for both rich and negative for destiny lifestyles of their kids.

ESL Learning

ESL learning in Pakistan isn't always as an awful lot a success due to many factors. nearly greater than half of of learner's ESL mastering is demanding because of mental and sociocultural elements as this take a look at become additionally research of debilitating factors involved in English verbal exchange. In Pakistan, training systems are of different typed, totally on bases of medium of instruction as diagnosed English medium and Urdu medium. by and large for higher class is personal English medium faculties and the Urdu medium colleges are specially for poorer (Shamim, 2011).
English medium non-public faculties absolute confidence deliberate high exceptional training and evolved school rooms via the medium of education in English, resulting rich college students in full command and higher degrees of expertise in English (Shamim, 2011).

ESL Teaching

According consistent with Pr i (2 13) that the objectives of foreign language teaching are to make students fluent in goal language verbal exchange. To analyze second language correctly, wellknown know-how is used in speaking and writing referred to Communicative Language coaching (CLT). in step with Larsen-Freeman (2000) that Communicative Language coaching (CLT) became hooked up in Nineteen Eighties while it have become clear that to make beginners fluent in verbal exchange desired no longer handiest linguistic competence, but also in other wider communicative competence. moreover, in Pakistani groups ESL coaching isn't always as a whole lot a hit as became anticipated because of instructor"s mindset as mentioned above in this bankruptcy. as a consequence, for every succeeding government the coaching of English mentioned as serious difficulty (Shamim, 2008).

X. Research Methodology

Research Design

Questionnaire's questions were drawn from the literature overview. initially, all authorities graduate colleges were decided on for statistics series.

Sampling changed into two staged sampling. before everything stage one of a kind arts and technological know-how instructions had been selected through cluster random sampling. At 2nd stage actual sample from the ones clusters have been decided on through random sampling. instructors pattern became decided on via convenient sampling from the same colleges, on the idea of suitability with humans most convenient to method in restrict of time.

The survey held throughout the ordinary magnificence hours. Questionnaires had been given to the inexperienced persons in published form to fill them. The researcher first in brief brought the subject of the research and administered and translated the questions in Urdu to keep away from misunderstandings. students have been free to present their own and authentic answers. students have been allowed now not to mention their names to maintain their secrecy in the event that they feel uncomfortable. moreover it changed into non-compulsory to present their answers in Urdu in the event that they couldn't explicit in English to recognize the real reason of speaking troubles. A face to face direct semi dependent direct interview in English became desired for the lecturers.

Research Population

The target populace become the male and girl students from different disciplines of technology and humanities organization. The approximate population of college students at graduate level changed into about 4000 analyzing in different faculties of Bahawalpur town.

Sample Size

From the above given populace 498 college students were decided on. The imply age of sample became from eighteen to 20 years. For this research surprisingly skilled 40 English language teachers had been decided on from the equal faculties. said age of teachers become from thirty to fifty years and their coaching enjoy ranged from eight to twenty 5 years.

Data Collection Tools

For this evaluation, the statistics changed into gathered thru open and closed ended questionnaire from students and from language instructors with the help of interview. The questionnaire consists of fourteen closed ended questions. Semi structured interview become based totally on 4 questions covered all possible debilitating socio cultural elements.

Tools Validity

The specialists and experienced personals with extra than ten years of teaching reports of the relevant situation have analyzed the device validity; ten commandants (Ph D) have been taken to validate the questionnaire.

3.6. Data Analysis

This changed into blended studies. Qualitative information became taken and research questions have been glad via the quantitative analysis of by way of using SSPS, Microsoft Excel, Tables and charts. in addition, both quantitative and qualitative strategies have been followed in the course of studying data and given interpretations consequently.

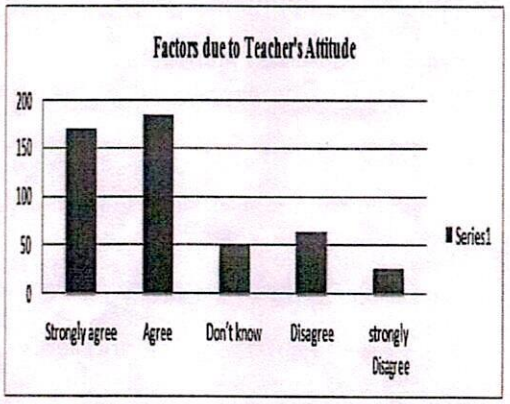
XI. Findings and Discussion

Findings

The The look at investigated that (84.1%) students have been within the want of the statement that much less fluent speaker turned into made more irritating by using the lecturers, in contrast to fluent speaker. the general suggest score was 4.04.information revealed that (ninety.6%) college students had been within the choose of the statement that facilitating and nice mindset of teachers preserve ESL newbie's at ease and stimulated learner's oral overall performance and the overall suggest rating changed into four.35.facts uncovered that (seventy five.3%) college students were in the choose of the assertion that scholars get confused in a state of affairs of being forced to talk in English. the overall suggest rating changed into four.01.moreover, (sixty six.7%) college students had been agreed that Formal & reserved elegance setting is major cause of strain and barrier in fluency. the general imply score turned into three.57.statistics confirmed that (70.5%) college students have been within the choose of the announcement that their English teachers did now not opt to deliver lectures in English. the overall mean rating became 3.eighty three. data uncovered that (seventy two. three%) college students had been within the desire of the statement that scholars felt lack of confidence whilst they're requested to compare their very own oral competency with their classmate. the overall imply rating became three. ninety three. facts found out that (64.7%) college students have been within the prefer of the announcement that teachers used humiliating behavior to snub oral performance. the general imply score turned into 3.71.information found out that (sixty two%) college students have been in the desire of the declaration that because of authoritative and humiliating behavior to oral performance, rookies felt hesitations even communicating earlier than their teachers. the overall imply score was three.60.records uncovered that (77.1%) students have been inside the desire of the assertion that wonderful attitude of instructors, facilitated learner's oral performance. the overall mean rating changed into 3.96.statistics found out that (sixty seven. Nine%) students have been within the favor of the statement that frequent comments on pupil's error even as speaking; acted as

condemning conduct to speak in addition. the general suggest score changed into three. sixty five.In interview evaluation, the most repeated remark turned into that instructors themselves stated that the teacher's mindset turned into also responsible to shatter the learner's motivations. additionally, loss of professional and educated teachers in substandard faculty was additionally nourishing tense learners. often in Pakistani cultural teachers act like a commander and never hesitate to humiliate learner's admire on mistakes. because of loss of skilled and educated instructors, sub trendy college schooling, inadequate self assurance and practice; college students aren't obtaining oral competency flawlessly and sense it as tough to accumulate.

Graph Showing Affecting factors due to teacher's attitudes



The gathered and brand new evaluation indicated that there is combined opinion as proven in graph.
Strongly agreed and agreed graph"s moves confirmed that scholars fantastically authorized the statements.

Discussion

Analysis of statistics correctly proved hypotheses. As hypothesized, this studies correctly portrait the all probable elements related to the teacher"s attitude. research analyzed that teacher"s commanding; embarrassing attitude and frequent remarks on mistakes had been superb elements, aroused due to instructors and become additionally discussed in studies of (Tanveer, 2007) and (young, 1991). Others researchers also explored from their studies like Hilleson (1996), Tanveer (2007) and Harmer (2011) that sometimes simply due to teachers (due to many motives) students suffered. college students experience pressure while compelled to offer oral performances, or hesitate to make contributions in crowd activities, leading to frustration and annoyance. In Pakistan the language teachers aren't professional in oral competency in particular at early college and that they themselves sense uncomfortable, now not being fully professional in grammar, pronunciation, accessory and the rules related to the spoken English. The linguistic English particularly is neither fully taught nor tested even via training academies. The running shoes even hardly ever have any concept that their schooling can also help the freshmen in developing their communicative proficiencies because of lacking records in regard to education and teaching strategies, leading to worst future with complete of anxieties (Mahmood and Ghani, 2012).moreover novices explored the various demanding attitudes of the teachers which affects burden on novices.

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XII. Recommendations and Conclusion

Recommendations

Continued endured education must be mandatory for a train and be observed by using an expert panel to be aware any tension in writing and spoken English before one"s selection. otherwise one may deliver the identical tension and pressure among the language newcomers and couldn't stability with the ones destructive feelings and the pupil's bright future (Hashemi, 2011). As, 2nd language mastering on the whole relies upon on instructors and each language trainer can undertake unique behaviors to lessen pressure. So first of all teachers need to effectively recognize and talk the language tension after which assist disturbing students. 2nd language studying is difficult but this process does now not require unique management. It simplest wishes suspicious conduct of the language teachers so that it will recognize anxieties (Tseng, 2012). The herbal and excellent behavior of teachers toward their scholar can be extra relaxing as opposed to authoritative mind-set and is probably greater obedient. instructors have to assess their coaching standards and keep away from the anxiety upsetting state of affairs. teachers should lessen the opposition among students in the event that they sense that in place of advantages, competitions create uneasiness and sense of insecurity among stressful learners (Hilleson, 1996). instructors have to apprehend learner's pressure and need to adopt techniques from cognitive, affective, and behavioral techniques to reduce their tension (Tanveer, 2007) and avoid speaking greater honestly that would contribute and provoke tight lipped however inflammation in the elegance (Tsui, 1996). destiny studies can be undertaken in the path for similarly identity of debilitating factor involved in oral competency, which must be extra affordable, as learning a second language is complete of hysteria. unique study is likewise mandatory on elements removing debilitation in oral competency, considering the societies of Pakistan.

Conclusions

Researcher concluded that embarrassing conduct of a supervisor towards student turned into a debilitating component due to the fact students can't talk with teacher with out hesitation and even can not ask questions without problems due to their authoritative and humiliating attitude. The formal, reserved, authoritative and forcing conduct of supervisors is excessive purpose of pressure among novices, which make the students disinterested and non stimulated. college students get careworn in a scenario of being pressured to talk. in the main English instructors do not prefer to supply lectures in English as a substitute in local language and help widespread dialect handiest in written English. furthermore, studies analyzed that trainer's commanding; embarrassing mind-set and frequent comments on mistakes were ideally suited factors. The study investigated that typically instructor encouraged fluent rookies and examine them with much less fluent speaker, which created lack of confidence in demanding college students. furthermore researcher concluded that formal & reserved magnificence setting is main reason of pressure and barrier in fluency. additionally it is revealed that teachers used humiliating conduct to snub oral overall performance so due to authoritative and humiliating conduct to oral overall performance. In reality instructor's attitude become accountable to shatter the learner's motivations. teachers in no way hesitated to overwhelm scholar's admire on errors. additionally, lack of professional and educated instructors in substandard faculty became additionally nourishing worrying rookies. it's miles concluded that facilitating and advantageous attitude of teachers keep ESL learners cozy and prompted learner's oral performance.

References

1.Al-Saraj, T. (2011).'Foreign language anxiety: what is it?'.Paper presented at 4th Bloomsbury Student Conference in Applied Linguistics, Birkbeck College, University of London, and The UK.

2.Ellis, R. (2008). The study of second language acquisition 2nd edn: Oxford University Press: Oxford.

3.Gregerse, T. and Horwitz, E. (2002). Language Learning and Perfectionism: Anxious and Non-Anxious Language Learners" Reactions to Their Own Oral Performance. The Modern Language Journal, 86(8): 562-70.

4.Harmer, J. (2011). The practice of English language teaching. 4th edn: Edinburgh Gate: Pearson Longman. Hashemi, M. (2011). Language stress and anxiety among the English language learners. Procedia – Social and Behavioral Sciences, 30(38): 1811–16.

5.Hewitt, E. and Stephenson, J. (2012). Foreign Language Anxiety and Oral Exam Performance: A Replication of Phillips"s MLJ Study. The Modern Language Journal, 11(3): 170–89.

6.Hilleson, M. (1996). Reticence and anxiety in second language learning. In K. M. Bailey & D. Nunan (Eds.), Voice from the language classroom. Cambridge University Press: New York. 248-77.

7.Horwitz, E. K., Horwitz, M. B. and Cope, J. (1986). Foreign language classroom anxiety. Modern Language Journal, 70(4): 125-32.

8.Larsen-Freeman, D. (2000). Techniques and principles in language teaching. Oxford University Press:

9.MacIntyre, P. D. and Gardner, R. C. (1991). Investigating Language Class Anxiety Using The Focused Essay Technique. The Modern Language Journal, 75(3): 296-304.

10.Mahmood, A. and Ghani, M. (2012). Communicative Skills of Student Teachers in Pakistan. International Journal of Research in Linguistics and Lexicography, 1(8): 1724-2227.

11.Na, Z. (2 7). A study of high school students" English learning anxiety. The Asian EFL Journal, 9(3): 22-34. Onwuegbuzie, A., J., Bailey, P. and Daley, C., E. (1999). Factors Associated With Foreign Language Anxiety.Applied Psycholinguistics, 202(2): 217-39.

12.Price, M. L. (1991). The subjective experience of foreign language anxiety: Interviews with highly anxious students. In E. K. Horwitz & D. J. Young (Eds.), Language anxiety: From theory and research to classroom implications. Prentice Hall: Englewood Cliffs, NJ. 101-08.

13.Pr i , . (2 13). Speaking anxiety among high school EFL students: effects and possible remedies, Master thesis, (English department. University of Ni).

14.Shamim, F. (2008). Trends, issues and challenges in English language education in Pakistan. Asia Pacific Journal of Education, 28(3): 235-49.

15.Shamim, F. (2011). Dream English as the language for development in Pakistan: Issues, challenges and possible solutions. s and Realities: Developing Countries and the English Language. British Council.

16.Tanveer, M. (2007). Investigation of the factors that cause language anxiety for ESL/EFL learners in learning speaking skills and the influence it casts on communication in the target language. Unpublished Thesis, (Faculty of Education, University of Glasgow).

17.Tseng, S.-F. (2012). The Factors Cause Language Anxiety for ESL/EFL Learners in Learning Speaking. WHAMPOA - An Interdisciplinary Journal, 63(2): 75-90.

18.Tsui, A. B. M. (1996). Reticence and anxiety in second language learning. In K. M. Bailey & D. Nunan (Eds.), Voice from the language classroom. Cambridge University Press: New York. 145-67.

19.Young, D. J. (1991). Creating a Low-Anxiety Classroom Environment: What Does Language Anxiety Research Suggest? The Modern Language Journal, 75(4): 426-39.
20.Zhang, R. and Zhong, J. (2012). The Hindrance of Doubt: Causes of Language Anxiety. International Journal of English Linguistics, 2(3): 27-33.

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Biodegradable Super Absorbent Nano Polymer: Properties and its Applications

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Abstract: *Polymers from naturally derived ones, such as polysaccharides, proteins, cellulose, and rubber, among the listed starch (polysaccharides) has found various applications due to their abundant presence in nature and their biocompatibility and elastic physicochemical properties. Polysaccharides and starch derivatives contribute a key role to the nanoparticles, Nano clay, Nano polymers, and nanocomposites preparation. Metallic nanoparticles have been prepared; aside from them, silver nanoparticles are widely used and considered a metal nanoparticle with low toxicity—naturally available and strong antimicrobial. The present study focuses on the most frequently used polysaccharides in different applications with silver or silver nanoparticles. The work also provides a detailed overview of the synthesis, physicochemical properties, toxicity, and agriculture farming.*

Keywords: *Biodegradable Nanoparticles, Silver Nanoparticles, Toxicity, Properties, Applications, and Polysaccharides.*

INTRODUCTION

Daniel and Astrum depicted and summarized the Nanoparticles' historical perspective (Harish Kumar et al. 2018) (Gitashree Darbdhara et al., 2019). Metallic Nanoparticles are uniform in size with sharp distribution in Nanoscale (David et al., 2015; Jorg Polte (2015)). These particles have been synthesized with a specific function and could be mimicked according to bind the ligand (Prerna Khanna et al., 2019). It falls at the size of 10-100nm. Metallic Nanoparticles possess unique surface Plasmon resonance and optical properties (Hardik Khandelwalin Chemi (2020)). In the gold solution in golden yellow color but 20nm gold Nanospheres in red ruby color whereas 200nm Nanospheres look like bluish color. Various research fields revealed that the silver and gold metals are noble metals in Nanoparticle preparation for different applications ((2019): Nanoscale Materials in Water Purification). Faraday documented the metallic nanoparticles in 1908, and Mie gave the quantitative clarification of the metallic Nanoparticle color (Tiwari et al., 2015).

Metallic nanoparticles are being used to decorate the cathedral in the primitive era (Shivarama Krishnan., Gurumurthy and Balasubramanian (2017)). A predictable feature of Nanoparticles is the ratio of surface area to volume, which allows them to interact and diffuse directly to treat the damaged tissues or cells at low temperatures. Raman spectroscopy with fluorescence enhances the surface and refractive index. Sensing nanoparticles have application in the field sensitive optical process (Avantes (2020)). The visible region of wavelength resonance is influenced by the localized surface Plasmon directed by metal nanoparticles' optical properties. The growth of gram-positive and gram-negative bacteria growth was inhibited by the silver and gold nanoparticles (Anes Al-Sharqi et al., 2019). Living organisms acting as a production system for Nano devise but have difficulty in synthesis due to the formation of toxic metallic nanoparticles (Manoj et al., 2016). Fungi, Bacteria, and Plants are alternatives to the production of metallic Nanoparticles.

Stabilizing agents, reducing sugars, metal ion kinetic interactions, size, stability, physical, chemical properties, morphology, etc., are the factors that influence the metallic nanoparticles' characteristics (Ajay Vasudeo Rane et al., 2018). Physical and chemical properties metal nanoparticles have many industrial applications, including mechanical strengths, high surface area, low melting point, optical properties, and magnetic properties (Chavali and Nikolova (2019)). Gold Nanorods used as storage enhanced more than 100 times than the common disk with optical properties (Nikalje, Anna. (2015)). The optical properties of gold, silver, lead, platinum nanoparticle arise from the resonant oscillation of their free electrons in the presence of light, also known as Localized surface Plasmon resonance (LSPR) (Organic Electronics. (2014)). Silver is a valuable property, and pure compare to gold. Silver has many medicinal properties, anti-bacterial and

antiseptic properties (Josh Axe, DC, DMN, CNS (2019) Vijilvania, et al., 2020). Cisplatin (platinum) has antitumor activity (Heyam Saad Ali et al., 2020). Gold nanoparticles are used to treat fever and syphilis (Baker and Perianova (2019)).

BIODEGRADABLE/BIO-BASED POLYMERS CLASSIFICATION

Depending on the synthesis, process biopolymers are classified into three categories, and these include (Wound Healing Biomaterials 2016). Polymers from biomass; Polymers from microbial synthesis and chemically and conventionally synthesized from biomass monomers.

BIODEGRADABILITY OF POLYMERS

Biodegradable plastic is defined as those that degrade from the action of under the action of microorganisms. Biodegradable polymers occupy a tremendous role in agricultural products and waste disposal problems and bioplastics resources' sustainability (Trivedi et al., 2016). Alone natural polymers have drawbacks and not that effective in the action or functioning of polymer (Khalid Mahmood et al., 2017). Hence, there is a demand for blending synthetic and natural polymers to become popular with vital applications, such as cutlery, flowerpots, and food trays (Brittter Horst et al., 2019). Bioplastics are manufactured in two forms: wet and dry (Luzi et al., 2019). In the wet process, biopolymers dispersion in a film-forming solution and is used edible film coatings but has the drawback is not eco-friendly (Ilyas et al., 2020). In case dry, melt process, thermoplastic nature of the biopolymer used in edible coatings. Biopolymers degrade through the enzymatic action of bacteria, fungi, algae, and other living organisms. The degradation products are carbon dioxide, new biomass, and water (Nair et al., 2017). Degradation comparison is difficult to establish due to different composting conditions such as humidity and temperature (Siracusa, Valentina. (2019)). Some general rules are applicable in estimating the evolution of biodegradability, and these include an increase in hydrophobic character, molecular weight, and crystalline nature of the size of spherulites decrease biodegradability, and on the contrary, the presence of polysaccharides favors degradation (Özçimen, Didem, et al., 2017; Ambrish Singh (2011)).

Principally biodegradable compounds must have suitable chemical nature and other factors, which contribute to biodegradability, namely polymer morphology, radiation and chemical treatments, and molecular weight, which tend to cleanse to degrade the environment (Naba Kumar et al., 2020). Take care and must-see while synthesizing the polymer; chains must be flexible enough to fit into the enzyme's active site (Yajaman et al., 2006). Therefore, that degradation using the enzyme, catalysis would be comfortable. Proteins are natural polymers, which differ from synthetic polymers in the composition, but proteins do not have equivalent repeating units present in the entire peptide chain (James, Leo, and Tawfik, Dina. (2002)). So due to its irregular nature, it has biodegradable. The effect of molecular weight plays a vital role in the biodegradability of a polymer by conducting the experiments with microbial enzymes, namely Exo and endoenzymes, which could not degrade the polymer instantly, while molecular weight increases (Koutny, Marek, et al., 2006). Current status and future trends: Bio-based feedstocks are not a novel concept in the chemical sector, and are industrially feasible and are avail more than a decade ago (Ndolo Obonyo et al., 2019; Kiran and Patil, Kiran. (, 2014).

The bio-based polymer industry easily spread with the fossil fuel-based chemical industry in the last 20 years. It leads to a foundation for the advancement of white biotechnology in the real world and bio-based polymers and other chemicals from renewable resources (Bikash Kumar and Pradeep Verma (2020)). In this connection, the first-generation used food resources such as corn, starch, rice, etc., to produce bio-based polymers (Deepak Kumar and Vijay Singh (2019)). Further focused on cellulose-based feedstocks ascended on waste from food, wood, paper industries, plant waste, solid waste, municipal waste, etc., were screened. The final stage of this full-pledged chemicals took more than 20 years to span to give the shape (Peter Kalmus (2017)). In the coming years, challenges that we have to face to solve include managing raw materials, the performance of bio-based materials, and their cost for production (Lidija Runko and Luttenberger (2020)). To produce economical bio-based monomers and polymers from renewable sources is another challenge ahead. New technologies' experience and survival rate, implementation, and functioning of technology and its supply and demand matter to balance (Majidian, Parastoo 2017; Lin Lina Zhou 2011). Bio-based industry effort to make bio-versions of existing monomers and polymers. The functioning of these products is actively known.

It is very easy to replace the existing product with similar bio-versions (Hamad, Kotiba. (2015)). All the polymers mentioned above often display similar properties to current fossil-based polymers. Many efforts were towards introducing new bio-based polymers with higher performance and value (Raghvendra Kumar Mishra et al., 2018). Several modifications were, made to develop various polyamides, polyesters, polyhydroxyalkanoates, etc., with a high differentiation in their final properties in automotive, electronics, and biomedical applications (Aitor Larrañaga and Erlantz Lizundia (2019)). New bio-based polymers could not fit in the current processing equipment is the main drawback (Storz, Henning. (2014);). This may be overcome by additive-based chemistry development that improves the bio-based polymers' fitment and function (Andrea Sorrentino., Giuliana Gorrasi, and Vittoria Vittoria (2007)). Bio-based polymers like PLA and PHA, additives, industrialized to improve their performance by blending with other polymers, or making new copolymers (Lee et al., 2020). Nanoparticles are being used as an additive to improve the polymer functioning for petroleum-based polymers (Dangge Gao et al., 2015).

Renewable feedstocks used for manufacturing bio-based monomers and polymers often compete with requirements for food-based products (Selorm Torgbo and Prakit Sukyai (2020)). The expansion of first-generation bio-based fuel production was not favorable and causes a threat to the viability of biochemical and biopolymer production as it is to food production (Shaoqing Cuia et al., 2019). The European Commission has declared that the survey tells about first-generation biofuels downfall market, and preference given for non-food sources of sugar for biofuel production (Eleni Stylianou et al., 2020). May trail and errors were initiated to produce the sugars as a feedstock for biofuels, biochemicals, and biopolymers (Oscar Rosales-Calderon and Valdeir Arantes (2019)).

NATURAL FIBERS AS REINFORCING FILLERS FOR COMPOSITES

Biodegradability uses natural fibers as plasters due to environmental and disposal concerns for non-biodegradable materials (Seema Agarwal (2020)). One of the Natural fibers, cellulose, comes under the main vegetable fiber used in composites (Layla Filiciotto and Gadi Rothenberg (2020)). Natural fibers are widely used in polymeric materials to improve mechanical properties (Khubab Shaker Yasir Nawab and Madeha Jabbar (2020)). These fibers can be classified as bast, leaf, or see-hair fibers (Murugesh Babu, (2018)). Depending on the natural fiber properties, the origin, quality of the plant's locations, the plant's age, and the preconditioning (Mohau Moshoeshoe 2017). Natural fibers have many drawbacks: poor wettability, incompatibility with some polymer matrices, moisture absorption and low processing temperatures due to fiber degradation, or the possibility of volatile emission that could affect the composite performance (Soo-Ling Bee et al., 2018). According to the main demerit to supplement, the natural fibers were manipulated to prepare composites (Alan Kintak Lau and Karen Hoi Yan Cheung (2017)). The natural fiber is the lack of strong plaster nature to the matrix adhesion, which reduces the composite activity overcome by the physical treatments, including cold plasma treatment and corona treatment. Chemical treatment includes maleic anhydride, organosilanes, isocynates, sodium hydroxide, permanganate, and peroxide (Yinji Wan et al., 2019).

BIOPOLYMERS FOR RESTORATIVE RELEVANCE IN NANOTECHNOLOGY

Nanotechnology was used in various engineering, electronics, mechanical, biomedical, and space engineering (Trepti Singh et al., 2017). The biomedical field applied much more in different aspects, including controlled drug/gene delivery, tissue engineering, imaging of specific sites, and DNA structure probing (Ibrahim Khan et al., 2019). Therapies using nanoparticle application are widely used to treat cancer, diabetes, allergy, infection, and inflammation (Mona Elsayed and Ayman Norredin (2019)). The fact behind nanoparticle application in therapies is that the particle exists in the same size domain as proteins, and large surface areas can allow a number of ligands (Jagpreet Singh et al., 2018). In addition, biopolymers have a rapid absorption with high diffusion and volume change (Physiologic Factors Related to Drug Absorption (2017)). According to the requirement, the particle size and surface characteristics can be tailored or controlled (Ruslan Melentieva and Fengzhou Fanga (2020. Organic and inorganic combination mixture has been used to produce nanoparticles. Polymeric nanoparticles have also been used in therapeutic applications. Biomaterials are a delivery carrier of therapeutic molecules such as drugs and genes and tissue engineering scaffolds (Yongda Sun (2016)).

Even though polymeric nanoparticles were having difficulty scaling up and low drug-loading capacity (Carina et al., 2017), compared to ceramic or metal nanoparticles, polymeric nanoparticles have wide sustainability to the local drug therapeutic agents up to weeks (Carina I.C. Crucho and Maria Teresa Barros (2017)). Both naturally derived and synthetic biomaterials have advantageous features (Mojtaba Abbasian, 2019). Synthetic polymers give well-defined and fine-tunable degradation kinetic and mechanical properties (Fa-Ming Chen and Xiaohu Liu (2016)). Proteins offer many advantages when compared to natural, synthetic. Peptides are easily digestible by metabolizable by digestive enzymes and tixic degradation products (Alejandra Acevedo-Fani 2020). It is more potent to the site-specific target drug delivery (Yaghoub Safdari et al., 2016); as cited above, polysaccharides are also digested by the specific enzyme (Sagar Aryal (2019)). Polysaccharides have advantages over synthetic polymers, for example, PEG (Thomas, Barclaya, et al., 2019).

NANOPARTICLES FOR DRUG/GENE ACCOUCHEMENT

Polymeric drug/gene-loaded nanoparticles were injected into bodies, passed through epithelial barriers, and circulate in the blood vessels before reaching the target site (Thomas Malachowski and Austin Hassel (2020)). Escape of nanoparticles from the vascular circulation occurs in either continuous or fenestrated tissues (Raquel Ferreira and Liliana Bernardino (2020)). Therefore, the drug nanoparticle particle penetration enhanced and accumulates drugs in tumor sites called enhanced permeation and retention (Ting Jiang et al., 2017). Tumor growth induces neovasculature development characterized by discontinuous endothelium with large gaps (200-700 nm), allowing nanoparticle passage (Lakshmi Pallavi Ganipineni et al., 2018). While passing into the tissue, interactions may cause toxicity will happen (fluids, cells, and tissues), which drives the possible direction of entry pathway into the target organ (Amanda Lautieri and Sophie Stein, (2019)). Nanoparticles trigger the mediators at the target organ site to activate the inflammatory or immunological responses due to maintaining the specific size (Richard Nho (2020)). Particle sizes, solubility, biodegradability, and surface properties play a vital role in the site-specific delivery or controlled drug delivery, and the internalized mechanism triggers endocytosis (Sarita Rani et al., 2017).

In encapsulated drugs, released by diffuse or degrade controlled by stimuli, namely temperature, pH, or ionic strengths (Sarita Rani et al., 2017). Effective drug delivery application of nanoparticles depends on pH sensitivity range for normal tissues (7.2-7.4) but in solid tumors (6.2-6.9) (Carmen Alvarez-Lorenzo et al., 2013). Gene therapy is also done in particular diseases to cure, namely cancer, AIDS, and cardiovascular diseases, by replacing mimicked or mutated genes into specific patients' specific cells (Iftikhar et al., 2015). While transferring the genes at the site of the mimicked gene in the cell, care must be taken to avoid the nucleases and endocytosis enzymes until they reach the target (Yi Li et al., 2016). In gene therapy, both viral and non-viral vectors have been used, and the most suitable and convenient one is non-viral vectors because of low immunogenicity (Lesca M. Holdt et al., 2018). The nanoparticles of genes and the cationic polymers could be modified with proteins (knob, transferrin, or antibodies/antigens) to allow for cell-specific targeting and enhanced gene transfer (BAlicia Rodri'guez Gasco'n 2012). Nitric oxide-releasing materials (nanoparticles) potential therapeutics in wound healing antimicrobial actions and it acts as biocompatible nanomaterial matrices (chitasone and dextrose are used as matrices) (Zeenat Mirza and Sajjad Karim (2019)).

NANOPARTICLES FOR TISSUE ENGINEERING

It is the one kind of drug delivery system with controlled release, enhancing tissue engineering's efficacy (Yasuhiko Tabata (2005). Therapeutic genes could enhance the absorption of the tissue construct, growth, and digestion with neighboring tissues (Yang et al., 2020). Biopolymer gene function as a DNA complexing agents and structural scaffolds involves cell growth and maintenance in the tissue engineering application, leading to foundation treatment in regeneration medicine (Narmatha Christya et al., 2020). GAM (gene-activated matrix) blends both the DNA complexing agents and structural scaffold strategies and serves as a local bioreactor (Hasan Uludag et al., 2019).

PROTEIN-BASED NANOPARTICLES FOR DRUG DELIVERY

Natural proteins called collagen, elastin, and fibronectin have been used extensively as biomaterials (Hasan Uludag et al., 2019). They are cheap polymers with low toxicity, no antigenicity, high nutritional value, high stability, and binding capacity of various drugs such as paclitaxel and ibuprofen with biodegradability (Showkat Ahmad Bhawani et al., 2019). Moreover, nanoparticles have the capability of emulsification, gelation, and water-binding (Ahmed O.Elzoghbya et al., 2012). Genetically engineered proteins and peptides tools have been used to mimic the polymer properties such as degradation rate, biocompatibility, and cell penetration ability to generate new protein sequences, including bioactive domains or protein motifs Elastin-like polypeptides (ELP) (Wensi Zhanga et al., 2018). Stable and precise biodegradable nanopolymers with determined or known size polymers can utilize various proteins such as silk, albumin, collagen, and elastin. Protein-based nanoparticles are easily self-assembled to form particles, fibers, sheets, etc. (Malgorzata. Et al. (2020)

SILK-BASED NANOPARTICLES

Recombinant silks particles are synthesized by the elucidation of silk genetics, structures, and biophysics. Silk fibroins are stable, spherical, negatively charged, and low toxic silk nanoparticles (150–170 nm) procured from Bombyxmori and tropical Tasar silkworm Antheraea mylitta (Chandra Mohan Srivastava et al., 2019). Silk fibrin nanoparticles have been used in cancer treatment. They show good recovery, and sustained growth factors were found within 3 weeks of treatment (cytosol of murine squamous cell carcinoma cells) (Mhd Anas Tomeh et al., 2019). Conjugated Silk fibroin and chitosan polymers were blended non-covalently to form nanoparticles (<100 nm) for local and sustained therapeutic curcumin delivery to cancer cells (Raluca Ioana Teleanu et al., 2019). The crystalline silk protein nanoparticles (40–120 nm) have been conjugated with insulin via covalent cross-linking (Fatemeh Mottaghital et al., 2015). Silk fibroin was also bio-conjugated with L-asparaginase to form crystalline nanoparticles with 50–120 nm in diameter (Shuangquan Gou et al., 2019). Nanoparticles were composed of DNA and recombinant silks, which contained cell-penetrating peptide, tumor-homing peptide, Arg-Gly-Asp (RGD) motifs cationic sequences, have been designed for gene therapy (Laura Chambre et al., 2020).

Collagen and Gelatin-Based Nanoparticles:

Collagen is an extracellular matrix widely used as biomaterials with promising biocompatibility, low antigenicity, and biodegradability (Socrates Radha Krishnan et al., 2019). It forms hydrogels without chemical cross-linking, but it needs chemical treatments due to weak mechanical strength (Parinaz Nezhad-Mokhtari et al., 2019). Controlling the particle sizes, a large surface area, high adsorption capacity, and dispersion ability in the water, collagen nanoparticles exhibited sustained releasing of various drugs (Raj Kumar et al., 2018). The acid-alkaline hydrolysis has separated gelatin from collagen, consisting of glycine, proline, and 4-hydroxyproline residues with a typical structure of-Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro (Showkat Ahmad Bhawani et al., 2019). Gelatin solution undergoes coil-helix shift followed by aggregation of the helices through the formation of collagen-like triple-helix, enabling the formation of nanoparticles (Maria Helminger et al., 2014). Polymer functional groups chemically modified ex: cross-links, ligands (Rajiv M.Desai et al., 2015). Insulin-loaded gelatin nanoparticles were prepared for diabetes therapy, by a novel water-in-water emulsion technique with gelation by glyceraldehyde (Momoh A.Mumuni et al., 2020). Blood glucose level curves showed obvious decreases in the first 4hour in rats indicating their fast and stable hypoglycemic effect (Lin-Lin Pan et al., 2019).

ß-CASEIN-BASED NANOPARTICLES

ß-Casein is the major milk protein used as delivery carriers and self-assembled into the micellar structure by intermolecular hydrophobic interactions due to its amphiphilic nature (Tomasz Konrad Glab and Janusz Boratynski (2017)). 15–60 ß-casein molecules together form ß-casein micelles with a radius of 7–14nm (MoLiac Remco et al., 2019). Alterations in the properties like temperature, pH, ionic strength, water activity, and high hydrostatic pressure treatment arrange the size distribution according to the micelles requirement, leading to self-developing the rigid three-dimensional tertiary structure. ß-casein micelles have demerit critical to stabilizing the micelles by cross-linking (Lehninger Principles of Biochemistry (2011)). Crosslinking of lysine residues in casein by glutamine residues of transglutaminase (T Gase) increased the intra-micellar stability of casein micelles (Min Yang, Ying Shi, and Qi Liang (2016)). These are all edible or oral delivery systems used in cancer and gastric cancer (Bingren Tian et al., 2020).

ZEIN-BASED NANOPARTICLES

Zein is a protein derived from corn kernels, soluble in both water and alcohol (Shukla, Rishi, and Cheryan, Munir (2001)). Hydrophilic and hydrophobic amino acid residues are promising carriers for encapsulation and controlled release of hydrophobic compounds (Solmaz Maleki Dizaj et al., 2014). In vivo studies revealed that the particles were mostly accumulated in the liver and adequately remained in the blood for at least 24h due to tis relatively higher molecular weight and smaller particle size (34th Annual Meeting 2019).

ALBUMIN-BASED NANOPARTICLES

Albumin is a plasma protein's main protein with a molecular weight of 66.5 kDa (Shijie Li et al., 2015). Human serum albumin (HSA) is one of the smallest and the most abundant proteins present in blood plasma, indicating many metabolic compounds and therapeutic drugs, transported by HAS (Yujie Zhang., Tao Sun and Chen Jiang (2018)). Unisized albumin particles with controlled desolvation, thermal gelation, emulsion formation, and self-assembly come under the preparation of albumin-based nanoparticles (Elmira Karami et al., 2020). Albumin nanoparticles are separated by continuous dropwise addition of ethanol to an aqueous solution and continuous stirring by phase separation (Showkat Ahmad et al., 2019). Additional treatments such as cross-linking are often required to stabilize the nanoparticle morphology (Evangelos Georgilis et al., 2020). HSA-based nanoparticles are prepared by desolvation method and stabilized by cross-linking with glutaraldehyde or heat denaturation(Weber et al.,2000). Sulfhydryl groups were added by covalent linkage to the HSA-based nanoparticles to increase the reactive sites (Mohamad et al., 2017).

Nanoparticle albumin-bound (nab)-technology is a new technology for anti-cancer drug delivery system that has been developed by American Bioscience, Inc. Albumin particles with paclitaxel (nab-paclitaxel, 100–200 nm) was approved in 2006 for use in patients with metastatic breast cancer due to their superior antitumor efficacy over paclitaxel (Ahmed., Elzoghby Wael et al., 2015). Ibuprofen encapsulated BSA-dextran nanoparticles (70nm) were prepared by heat treatment method. Conjugation of dextran and BSA stabilization of nanoparticles in aqueous solution (Krishnendu Chatterjee et al., 2014). Self-assembly of albumin to form nanoparticles by adding lipophilic drugs and diminishment of primary amino groups on protein surfaces (Hasan Kouchakzadeh et al., 2015).

POLYPEPTIDE NANOPARTICLES

Proteins are mimicked into polypeptide-based nanoparticles are synthesized to produce the desirable characteristic proteins (Evangelos Georgilisa Mona et al., 2020. ELP can be produced recombinant and is composed of the repeating amino acid sequence (Val-Pro-Gly-Xaa-Gly) m. Xaa is the hydrophobic domain that facilitates self-aggregation and elastomeric functions (Nasim Annabi et al., 2013). Nanoparticles were produced by self-assembly of ELP with a sequence of VPAVG and showed a sustained release of loaded dexamethasone phosphate for about 30 days (Ahmed O.Elzoghbya Wael et al., (2012). Well-designed ELP block copolymers are often produced to control their phase separation behavior, add stimuli-responsivity, and introduce the cross-linking domain into ELP (Machado, Raul, et al., 2012). Temperature-triggered micelle assembly of ELP was achieved by the modulation of the local density of arginine (Arg) residues of diblock ELP (Sarah, Mac Ewan, and Ashutosh Chilkoti (2014)). ELP-based nanoparticles (~40 nm) were further formed from the diblock ELP decorated with the knob domain of adenovirus serotype 5 fibrous proteins for drug and gene delivery (Iraklis, Kourtis, et al., 2013). Thus, they have been widely used in drug delivery and tissue engineering fields (Xiao-Xia Xia et al., 2011). Other polypeptides are also produced recombinantly to form nanoparticles (Oyarzun-Ampuero, Felipe, et al., 2014).

Nanoparticles (100–200 nm) formed from cationic polyarginine and anionic hyaluronic acid is one of the examples (Lee, Mihyun., Zenobi-wong, Marcy and Chang, Jin (2019)). Another example is the zwitterionic diblock copolymer consisting of poly (L-glutamic acid)-b-poly (L-lysine) (PGA-b-PLys) (María Gabriela Villamizar-Sarmiento et al., 2019). This block copolymer self-assembled into schizophrenic vesicles that can reversibly be produced in moderate acidic or basic aqueous solutions. Polysaccharides are highly stable, biocompatible, and biodegradable (Aja Aravamudhan et al., 2014). Thus, polysaccharides and their derivatives are commonly used for applications in food, biomedical, and environmental fields (Tinesha Selvaraj Veeradasan et al., 2020). Their native charges classify polysaccharides that have been used for the preparation of nanoparticles: cationic (chitosan), anionic (alginate, heparin, hyaluronic acid), and nonionic (pullulan, dextran) (Ida Idayu Muhamad et al., 2019). The most commonly used polysaccharide for

nanoparticle fabrication is chitosan. Chitosan is a linear cationic heteropolymer of N-acetyl-d-glucosamine and D-glucosamine linked by beta-(1–4) glycosidic bonds. It is obtained by the partial deacetylation of naturally derived chitin (Einallah Khademian et al., 2020).

In particular, due to its mucoadhesive property, chitosan-based gene delivery systems have been successfully applied to oral and nasal route gene therapy systems, which will be discussed below (Chandra, Dinesh, et al., 2014). Alginate is a linear anionic polysaccharide composed of alternating blocks of 1,4-linked ß-D-mannuronic acid (M) and a-l-guluronic acid (G) residues (Rajalekshmy G.P.Lekshmi et al., 2019). Alginate has some advantages in its high mucoadhesiveness, aqueous solubility, and a tendency for gelation in proper condition, biocompatibility, and non-toxicity (Gheorghe Adrian et al., 2019). Insulin-loaded nanoparticles were prepared from calcium cross-linking, alginate-chitosan, or –alginate complexes with sufficient insulin loading capacity (Jayanta Kumar Patra et al., 2018). Even delivery of genes loaded in alginate-based nanoparticles has been successful (Yuefei Zhu et al., 2019). In this study, alginate-chitosan nanoparticles showed high transfection ability while maintaining biocompatibility and low toxicity (Dileep Janagam et al., 2017).

POLYSACCHARIDE NANOPARTICLES BY CROSSLINKING

Preparation of polysaccharide nanoparticles by cross-linking can be achieved by either ionic cross-linking (physical cross-linking) or covalent cross-linking (chemical cross-linking) (Patil, Sachinkumar, and Jadge, (2008)). Covalently cross-linked polysaccharide nanoparticles enable the network structure to be permanent since irreversible chemical links are formed unless biodegradable or stimuli-responsive crosslinkers are employed (Nimish Shah et al., 2013). The rigid network allows absorption of water and bioactive compounds without dissolution of the nanoparticles even when the pH drastically changes (Amir Sheikhi et al., 2020). An oil in water (o/w) emulsion polymer cross-linking method was employed to prepare tamoxifen citrate (a non-steroidal antiestrogenic drug)-loaded guar gum nanoparticles cross-linked with glutaraldehyde. (Jianyu Xu et al., 2018). This method gives nanoparticles reversibility and is considered biocompatible due to the lack of harsh preparation or toxic crosslinkers (Daniel Klinger Katharin and Land fester (2012)).

Ionically-crosslinked nanoparticles are generally pH-sensitive, a suitable feature for stimuli-sensitive controlled release (Lei Xing et al., 2019). Tripolyphosphate (TPP), a non-toxic anionic molecule, has been widely used to prepare cross-linked chitosan nanoparticles, and several drugs have been encapsulated within these nanoparticles (Ai Wu Pan Bei et al., 2019). TPP cross-linked chitosan nanoparticles have been used for protein, oligonucleotides, and plasmid DNA deliveries due to their high physical stability and encapsulation efficiencies (Loïc Bugnicourt and Catherine Ladavière (2016)). CS/TPP nanoparticles (300 nm) showed high encapsulation efficiencies both for plasmid DNA and dsDNA oligomers (20-meters), high physical stability, and high gene expression levels in HEK 293 cells (Maria Abdul Ghafoor et al., 2015). Chitosan/TPP nanoparticles were also used to improve cyclosporine A's delivery to the ocular mucosa (Dileep R.Janagam., Linfeng Wu, and Tao L.Lowe (2017)). Cyclosporine A was specifically concentrated in external ocular tissues (i.e., cornea and conjunctiva) during at least 48 h with maintaining negligible or undetectable CyA levels in inner ocular structures, blood, and plasma (Basaran, Ebru et al., 2011). Other examples of ionic cross-linking of polysaccharides are using of inorganic ions such as Fe(CN)64-, Fe(CN)63- citrate, and calcium ions as crosslinkers (Carmen Alvarez-Lorenzo et al., 2013). For example, alginate's carboxylic acids were cross-linked by calcium ions to form nanoparticles (80 nm), exhibiting a high transfection rate of plasmid DNA into non-phagocytic cells via endocytosis pathway (Julieta C.Imperiale et al., 2018).

POLYSACCHARIDE NANOPARTICLES BY POLYION-COMPLEX

Polysaccharide nanoparticles are also prepared by direct electrostatic interactions of oppositely charged polysaccharides in solution (Swierczewska et al., 2013). Chitosan is the most commonly used cationic polysaccharide for polyion-complex. In contrast, carboxymethyl cellulose (CMC), dextran sulfate, carrageenan, heparin, hyaluronic acid, alginate, and carboxymethyl pachyman are used as anionic polysaccharides (Leena Kumar and Hemant Ramachandra Badwaik (2019)). Chitosan-CMC was subsequently coated with plasmid DNA for genetic immunization. Both chitosan and a chitosan oligomer could complex CMC to form stable cationic nanoparticles for subsequent plasmid DNA coating. Corporation of polyelectrolyte complexation and ionic gelation prepared Chitosan/carrageenan/TPP nanoparticles (150–300 nm)(Fatemeh Farjadiana et al., 2019). Sufficient loading capacity of insulin in the nanoparticles showed their application as an oral insulin delivery (Gonçalves, Nádia, et al., 2012). A pH-sensitive polyion complexes (average particle size <200 nm) were formed from trimethyl chitosan and a-galactosidase A through self-assembly (Statements of Significance, (2019). These nanoparticles were able to release the enzyme at acidic pH and were efficiently internalized by human endothelial cells and mostly accumulated in lysosomal compartments. ?-Poly (glutamic acid) (PGA) was combined with chitosan to form nanoparticles for transdermal delivery of DNA.

POLYSACCHARIDE NANOPARTICLES BY SELF-ASSEMBLY

The introduction of hydrophobic segments into hydrophilic polysaccharide backbones enables the forming of self-assembled structures such as micelles, particles, and hydrogels (Tianxin Miao et al., 2018). Deoxycholic acid

cholesterol, carboxylic acids, and hydrophobic polymers are examples of such hydrophobic segments. By manipulating the introduction condition such as polysaccharide/hydrophobic segments molar ratios and the lengths of polysaccharides and hydrophobic segments, nanoparticles are formed to minimize interfacial free energy. Grafting hydrophobic groups from hydroxyl, amino, or carboxyl groups of the main polysaccharide chains (Mosaiab et al., 2019) introduces hydrophobic segments polysaccharides. These chemically modified amphiphilic macromolecules can self-associate in an aqueous solution by intra- and inter-molecular hydrophobic interaction, forming nanoparticles (Martin Gericke, Peter Schulze, and Thomas Heinze (2020). Water-insoluble drugs are solubilized and encapsulated within the hydrophobic core and become soluble in water due to the hydrophilic shell. The drugs are then released from the nanoparticles' inner core via outer stimuli changes such as pH, temperature, and ionic strength by Luo et al., 2020). Recently, there have been a number of studies on the syntheses of polysaccharide-based self-aggregated nanoparticles for drug delivery systems (Amin Shavandi et al., 2019). Chitosan has been chemically modified by grafting hydrophobic groups from amino groups of the main chains. The amine groups' proportion depends on the acetylation degree of the polymer (Paripurnanda Loganathan et al., 2020).

Doxorubicin, paclitaxel, ibuprofen, and the amphiphilic adriamycin have been loaded in chitosan-based nanoparticles. Plasmid DNA was introduced into nanoparticles (160 nm) aggregated from deoxycholic acid-grafted chitosan, and their efficient transfection of COS-1 cells was detected (Nitta and Numata, 2013). Dextran was chemically modified by grafting bile acids, a natural product consisting of a facially amphiphilic steroid nucleus with a hydrophobic ß-side and a hydrophilic aside lauryl chains (Sachiko Kaihara Nitta and Keiji Numata (2013)). Self-assembly assisted graft copolymerization of acrylic acid from dextran under the presence of crosslinker produced pH-sensitive nanoparticles (40–140 nm) (Huanli Sun et al., 2018). Dextran could form interpolymer complexes with poly(acrylic acid) (PAA) in acid medium owing to hydrogen bond interaction of carboxyl groups and proton-acceptors in glucose units. (Peipei Zhang et al., 2015). Plasmid DNA was loaded into complex nanoparticles (100–150 nm), which had high gene transfection yield, efficient gene delivery ability in different cancer cell lines, especially in MCF-7cells (Steinman, Noam, et al., 2020). Similarly to chitosan and dextran, hyaluronic acid (HA) was chemically modified with the 5ß-cholanic acid to form self-assembled nanoparticle (200–400 nm) that combine both passive tumor targeting based on the EPR effect and a more specific or active targeting exploiting the affinity of HA towards CD44(Goodarzi et al., (2013), Yujie Zhang et al., 2018). Amphiphilic block copolymers were also synthesized to form nanoparticles via self-assembly.

Doxorubicin loaded nanoparticles based on poly (?-benzyl L-glutamate)-block-hyaluronan were produced by self-assembly. These particles could be used as a self-targeting drug delivery cargo in over-expressed CD44 glycoprotein cells of breast cancer (Platt et al., (2008), Swierczewska et al., 2015). Nonionic pullulan is also modified by hydrophobic molecules such as cholesterol and cancer drugs. The cholesterol-bearing pullulans with different molecular weights and degrees of substitution have been synthesized to form self-assembled nanoparticles (Liming Yuan et al., 2019). A recent study demonstrated paclitaxel-incorporated nanoparticles prepared from pullulan hydrophobically modified by acetic anhydride to evaluate their antitumor activity in vitro and in vivo (Abbass and Hashim (2012)). These nanoparticles showed lower antitumor activity in vitro against HCT116 human colon carcinoma cells and reduced tumor growth in vivo using HCT116 human colon carcinoma-bearing mice (Suhail Ahmad et al., 2020).

Propyl-starch nanoparticles were reported to increase the polymer's solubility in low hazardous organic solvents and high encapsulation efficiency for model drugs (Alain Dufresne (2014)). Effective controlled release of doxorubicin was shown from drug-conjugated dialdehyde starch nanoparticles. Docetaxel, an anti-cancer agent, was loaded in nanoparticles prepared from a hydrophobic propyl starch with a controlled degree of substitution via the solvent emulsification/diffusion technique (Qingjie Sun (2018)). It was confirmed that nanoparticle enhanced internalization by the cancerous cells (Caco-2 and NHDF-p cells), and their peri-nuclear localization was detected (Dandekar et al., 2012). Heparin, a negatively-charged polysaccharide used as an anticoagulant, is often applied for the preparation of self-assembled nanoparticles (Stefano Rimondo et al., 2019). A self-assembled nanoparticulate system (140–190 nm) composed of a folate-conjugated heparin-poly (ß-benzyl-L-aspartate) (HP) amphiphilic copolymer was proposed for targeted delivery of the antineoplastic drug paclitaxel (Li Li et al., 2012). The presence of folate enhanced intracellular uptake via endocytosis, and these nanoparticles exhibited great cytotoxicity in KB cells. Deoxycholic acid bearing heparin amphiphilic conjugates (120–200 nm) with different degrees of substitution were also synthesized (Mona Alibolandi and Mohammad Ramezani (2018)).

AN OPINION ARRIVED AT THROUGH A PROCESS OF REASONING

Biodegradable nanocomposites and nanoparticle preparation, manufacturing, and production still have many demerits and are accompanied by the consumer economically and not affordable. Among the discussed metallic nanoparticles, silver nanoparticles have versatile utility. Remained practiced metallic nanoparticles have narrow application and are restricted to the special constraint or for a single application but not versatile and cost-effective: platinum, gold, and other metallic nanoparticles rather than silver nanoparticles. Genetically mimicking metal tolerance and increasing application

activity and the functional feature will compete with commercial polymers and biopolymers. Henceforth green or natural polymers have more compatibility to enhance the rate of demand and produce a novel Nano metallic compound for feature use.

SELF-REVELATION

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References

[1] Abbass A. Hashim. The Delivery of Nanoparticles, InTech. (2012).

[2] Aderemi T. Adeleyea Chuks Kenneth Odoha Obieze Christian Enudib Oluwakemi Oluwabunmi Banjokoc Osigbeminiyi Oludare Osiboyed Emmanuel Toluwalope Odedirane Hitler Louis . Sustainable synthesis and applications of polyhydroxyalkanoates (PHAs) from biomass, Process Biochemistry, (96): (2020). 174-193.

[3] Ahmed O. Elzoghby., Mayada M. Elgohary and Nayra M.Kamel.Chapter Six - Implications of Protein- and Peptide-Based Nanoparticles as Potential Vehicles for Anticancer Drugs, Advances in Protein Chemistry and Structural Biology, (98): (2015) 169-221.

[4] Ahmed O.Elzoghbya Wael., M.Samy Nazik A.Elgindya (2012).Protein-based nanocarriers as promising drug and gene delivery systems, Journal of Controlled Release, (161)1:38-49.

[5] Ahmed O.Elzoghbya., Wael M.Samy Nazik and A.Elgindya (2012).Protein-based nanocarriers as promising drug and gene delivery systems, Journal of Controlled Release, (161)1:38-49.

[6] Ai Wu Pan Bei., Bei Wua Jian and Min Wua Chitosan nanoparticles cross-linked by glycidoxy propyl trimethoxy silane for pH triggered the release of protein. Chinese Chemical Letters, 20(1) (2019) 79-83.

[7] Aitor Larrañaga and Erlantz Lizundia (2019).A review of polyesters' thermomechanical properties and biodegradation behavior, European Polymer Journal, 121:109296.

[8] Aja Aravamudhan., Daisy M.Ramos., Ahmed A.Nada., Sangamesh G.Kumbar Chapter 4-Natural Polymers: Polysaccharides and Their Derivatives for Biomedical Applications, Natural, and Synthetic Biomedical Polymers (2014). 67-89.

[9] Ajay Vasudeo Rane., Krishnan Kanny., Abitha, V.K., and Sabu Thomas. Chapter 5 - Methods for Synthesis of Nanoparticles and Fabrication of Nano composites, Synthesis of Inorganic Nano materials, Advances and Key Technologies, Micro, and Nano Technologies: (2018) 121-139.

[10] Alain Dufresne. Crystalline starch-based nanoparticles, Current Opinion in Colloid & Interface Science, (19)5 (2014) 397-408.

[11] Alan Kintak Lau and Karen Hoi Yan Cheung 1 - Natural fiber-reinforced polymer-based composites, Natural Fiber-Reinforced Biodegradable and Bioresorbable Polymer Composites: (2017)1-18.

[12] Alejandra Acevedo-Fani., Anant Dave, and Harjinder Singh.Nature-Assembled Structures for Delivery of Bioactive Compounds and Their Potential in Functional Foods, Front. Chem., (24). (2020).

[13] Amanda Lautieri and Sophie Stein (2019). The Path Drugs Take Through the Body.

[14] Ambrish Singh, Biodegradable, Biotechnology, and Biopolymers. (2011).

[15] Amin Shavandi, Pouya Saeedi, M. Azam Ali, and Esmat Jalalvandi. Green synthesis of polysaccharide-based inorganic nanoparticles and biomedical aspects, Functional Polysaccharides for Biomedical Applications. (2019): 267–304.

[16] Andrea Sorrentino., Giuliana Gorrasi, and Vittoria Vittoria. Potential perspectives of bio-nano composites for food packaging applications, Trends in Food Science & Technology, Vol (18)2 (2007) 84-95.

[17] Anes Al-Sharqi., Kasing Apun., Micky Vincent., Devagi Kanakaraju, and Lesley Maurice Bilung. Enhancement of the Anti-bacterial Efficiency of Silver Nanoparticles against Gram-Positive and Gram-Negative Bacteria Using Blue Laser Light, International Journal of Photoenergy. (2019).

[18] Avantes B.V. Applications of Surface Plasmon Resonance Spectroscopy (SPR), Azo Materials. (2020).

[19] Baker, S., Perianova, O.V. Bio-nanobactericides: an emanating class of nanoparticles towards combating multi-drug resistant pathogens. SN Appl. Sci. (1):699 (2019).

[20] BAlicia Rodríguez Gascón., Ana del Pozo-Rodríguez and María Ángeles Solinís (2012). Non-Viral Delivery Systems in Gene Therapy.

[21] Basaran, Ebru & Demirel, Muzeyyen & Sirmagül, Başar & Yazan, Yasemin. (2011). Polymeric Cyclosporine-A Nanoparticles for Ocular Application. Journal of biomedical nanotechnology. (7):714-23.

[22] Bikash Kumar and Pradeep Verma (2020). Enzyme mediated multi-product process: A concept of the bio-based refinery, Industrial Crops, and Products, (154).

[23] Bingren Tian., Shiyao Huab, and Jiayue Liu. Cyclodextrin-based delivery systems for chemotherapeutic anti-cancer drugs: Carbohydrate Polymers, (232). (2020).

[24] Biopolymers are polymers produced from natural sources, either chemically synthesized from biological material or entirely biosynthesized by living organisms. Wound Healing Biomaterials. (2016).

[25] Biotechnology for Biofuels, (12): 240.

[26] Brittter Horst., Naiem S.Moiemen and Liam M.Grover. 6 - Natural polymers: biomaterials for skin scaffolds, Biomaterials for Skin Repair and Regeneration: 151-192. (2019).

[27] Carina I.C. Crucho., and Maria Teresa Barros.Polymeric nanoparticles: A study on the preparation variables and characterization methods, Materials Science and Engineering: C, 80(1) (2017) 771-784.

[28] Carina I.C.Crucho., Maria Teresa Barros.Polymeric nanoparticles: A study on the preparation variables and characterization methods, Materials Science and Engineering: C, 80(1) (2017) 771-784.

[29] Carmen Alvarez-Lorenzo., Barbara Blanco-Fernandez., Ana M.Puga, and Angel Concheiro. Cross-linked ionic polysaccharides for stimuli-sensitive drug delivery, Advanced Drug Delivery Reviews, Vol (65)9: 1148-1171.

[30] Carmen Alvarez-Lorenzo., Barbara Blanco-Fernandez., Ana M.Puga, and Angel Concheiro. Cross-linked ionic polysaccharides for stimuli-sensitive drug delivery, Advanced Drug Delivery Reviews, Vol (65) (2013) 1148-1171.

[31] Chandra Mohan Srivastava., Roli Purwara, and Anek Pal Gupt. Enhanced potential of biomimetic, silver nanoparticles functionalized Antheraea mylitta (tasar) silk fibroin nanofibrous mats for skin tissue engineering, International Journal of Biological Macromolecules, Vol(130) (2019)437-453.

[32] Chandra, Dinesh & Chaurasia, Shashi & Singh, Vijay & Yadav, Kamlesh.. Chitosan-based mucoadhesive microsphere: versatile carrier for nasal drug delivery system. Wjpr. (3) (2014).

[33] Chapter 8 - Properties and Characterization of PLA, PHA, and Other Types of Biopolymer Composites

[34] Chavali, M.S., and Nikolova, M.P. (2019). Metal oxide nanoparticles and their applications in nanotechnology. SN Appl. Sci. (1): 607.

[35] Dandekar, Prajakta & Jain, Ratnesh & Stauner, Thomas & Loretz, Brigitta & Koch, Marcus & Wenz, G. & Lehr, Claus-Michael. . A Hydrophobic Starch Polymer for Nanoparticle-Mediated Delivery of Docetaxel. Macromolecular bioscience. (2012).

[36] Dangge Gao., Rui Lia., Bin Lv., Jian zhong Ma., Fen Tiana and Jing Zhang. Flammability, thermal and physical-mechanical properties of cationic polymer/montmorillonite composite on cotton fabric Composites Part B: Engineering, (77) (2015) 329-337.

[37] Daniela Pamfil and CorneliaVasile.Chapter 2 - Responsive Polymeric Nanotherapeutics, Polymeric Nanomaterials in Nanotherapeutics, Micro and Nano Technologies. (2019) 67-121.

[38] David D. Evanoff Jr. George Chumanov Prof. Synthesis and Optical Properties of Silver Nanoparticles and Arrays, Journal of chemphyschem 6(7) (2005) 1221-1231.

[39] Deepak Kumar and Vijay Singh. Chapter 22 - Bioethanol Production From Corn, Corn (Third Edition), Chemistry and Technology: (2019) 615-631

[40] Dileep Janagam R., Linfeng Wu Tao, and Lowe L..Nanoparticles for drug delivery to the eye's anterior segment, Advanced Drug Delivery Reviews, (122) (2017) 31-64.

[41] Dileep R.Janagam., Linfeng Wu and Tao L.Lowe.Nanoparticles for drug delivery to the eye's anterior segment, Advanced Drug Delivery Reviews, (122) (2017) 31-64.

[42] Einallah Khademian., Ehsan Salehi., Hamidrez., Sanaeepur., Francesco Galianoc Alberto Figolic .A systematic review on carbohydrate biopolymers for adsorptive remediation of copper ions from aqueous environments-part A: Classification and modification strategies, Science of The Total Environment, (738) (2020).

[43] Eleni Stylianou., Chrysanthi Pateraki., Dimitrios Ladakis., María Cruz-Fernández., Marcos Latorre-Sánchez., Caterina Coll, and Apostolis Koutinas. Evaluation of organic fractions of municipal solid waste as a renewable feedstock for succinic acid production, Biotechnology for Biofuels, (2020) 13(72).

[44] Elmira Karami Mahdi., Behdani Fatemeh, and Kazemi-Lomedasht. Albumin nanoparticles as nanocarriers for drug delivery: Focusing on antibody and nanobody delivery and albumin-based drugs, Journal of Drug Delivery Science and Technology, (2020) (55).

[45] Elmira Karami., Mahdi Behdani., Fatemeh and Kazemi-Lomedasht.Albumin nanoparticles as nanocarriers for drug delivery: Focusing on antibody and nanobody delivery and albumin-based drugs, Journal of Drug Delivery Science and Technology, (2020) (55).

[46] Evangelos Georgilis., Mona Abdelghani., Jan Pille., Esra Aydinlioglua., Jan C.M.van., Hest., Sébastien., Lecommandouxa., Elisabeth and Garangera .Nanoparticles based on natural, engineered, or synthetic proteins and polypeptides for drug delivery applications, International Journal of Pharmaceutics, 586(30) (2020).

[47] Evangelos Georgilisa Mona., Abdelghani., JanPille., Esra Aydinlioglu., Jan C.M.Van Hest., Sébastien Lecommandouxa Elisabeth Garangera. Nanoparticles based on natural, engineered, or synthetic proteins and polypeptides for drug delivery applications, International Journal of Pharmaceutics, (2020) (586).

[48] Fa-Ming Chen and Xiaohu Liu .Advancing biomaterials of human origin for tissue engineering, Progress in Polymer Science, (53) (2016) 86-168.

[49] Fatemeh Farjadiana Amir Roointan., Soliman., Mohammadi-Samani., Majid Hosseinide. Mesoporous silica nanoparticles: Synthesis, pharmaceutical applications, biodistribution, and biosafety assessment, Chemical Engineering Journal, (359) (2019) 684-705.

[50] Fatemeh Mottaghital., Mehdi Farokhi., Mohammad Ali Shokrgozar., Fatemeh Atyabi., Hossein Hosseinkhani. Silk fibroin nanoparticle as a novel drug delivery system, Journal of Controlled Release, (206) (2015)161-176

[51] Ganjali Mansoureh Vahdatkhah Parisa Chapter 19 - Synthesis of metal nanoparticles using laser ablation technique, Emerging Applications of Nanoparticles and Architecture Nanostructures Current Prospects and Future Trends, Micro and Nano Technologies, (2018) 575-596.

[52] Gheorghe Adrian., Martau, Mihai., Mihaela, Vodnar, Dan.. The Use of Chitosan, Alginate, and Pectin in the Biomedical and Food Sector—Biocompatibility, Bioadhesiveness, and Biodegradability. Polymers, (11) (2019) 1837.

[53] Gitashree Darbdhara., Manash R.Das., Surya P.Singh., Aravind K.Rengan., Sabine Szunerits., Rabah Boukherroub. Ag and Au nanoparticles/reduced graphene oxide composite materials: Synthesis and application in diagnostics and therapeutics, Advances in Colloid and Interface Science, (271)2019.

[54] Gonçalves, Nádia., Oliveira, Hugo., Pego, Ana Paula and Saraiva, Maria.. A novel nanoparticle delivery system for in vivo targeting of the sciatic nerve: Impact on regeneration. Nanomedicine (London, England), Vol (7) (2012)1167-80.

[55] Goodarzi, Navid & Varshochian, Reyhaneh & Kamalinia, Golnaz & Atyabi, Fatemeh & Dinarvand, Rassoul.. A review of polysaccharide cytotoxic drug conjugates for cancer therapy. Carbohydrate polymers; (92) (2013) 1280-93.

[56] Hamad, Kotiba.. Properties and Medical Applications of Polylactic Acid: A Review. express Polymer Letters, (9) (2015).

[57] Hardik Khandelwalin Chemi. Nanomaterials, Health, Nanotechnology, Research. (2020).

[58] Harish Kumar K., Nagasamy Venkatesh., Himangshu Bhowmik and Anuttam Kuila, Metallic Nanoparticle, Biomed J Sci & Tech Res. (2018).

[59] Hasan Kouchakzadeh Maryam Sadat Safavi Seyed Abbas Shojaosadati. Chapter Four-Efficient Delivery of Therapeutic Agents Using Targeted Albumin Nanoparticles, Advances in Protein Chemistry and Structural Biology, (98) (2015) 121-143.

[60] Hasan Kouchakzadeh., Maryam Sadat., Safavi Seyed., Abbas Shojaosadati. Chapter Four - Efficient Delivery of Therapeutic Agents Using Targeted Albumin Nanoparticles, Advances in Protein Chemistry and Structural Biology, (98) (2015) 121-143.

[61] Hasan Uludag., Anyeld Ubeda and Aysha Ansari. At the Intersection of Biomaterials and Gene Therapy: Progress in Non-viral Delivery of Nucleic Acids, Front Bioeng Biotechnol, (7) (2019) 131.

[62] Hasan Uludag., Anyeld Ubeda and Aysha Ansari. At the Intersection of Biomaterials and Gene Therapy: Progress in Non-viral Delivery of Nucleic Acids.Front Bioeng Biotechnol, Vol(7) (2019) 131.

[63] Heyam Saad Ali., Babiker M.El-Haj., Salim Saifullah., Muhammad Kawish. Chapter 4 - Gold nanoparticles in cancer diagnosis and therapy, Metal Nanoparticles for Drug Delivery and Diagnostic Applications, Micro and Nano Technologies: (2020) 43-58.

[64] Huanli Sun., Yifan Zhang, and Zhiyuan Zhong. Reduction-sensitive polymeric nanomedicines: An emerging multifunctional platform for targeted cancer therapy, Advanced Drug Delivery Reviews (2018).

[65] Ibrahim Khan., Khalid Saeed, and IdreesKhan.Nanoparticles: Properties, applications, and toxicities, Arabian Journal of Chemistry (2019).

[66] Ida Idayu Muhamad., Nurul Asmak Md Lazim and Suguna Selvakumaran.Chapter-18-Natural polysaccharide-based composites for drug delivery and biomedical applications, Natural Polysaccharides in Drug Delivery and Biomedical Application (2019) 419-440.

[67] Ilyas R.A. Sapuan S.M. Abudu keremu., Kadier Mohd Sahaid Kalil., Rushdan Ibrahim., Atikah M.S.N., Mohd Nurazzi N., Nazrin A., Lee Mohd Nor C.H., Faiz Norrrahim., Nasmi Herlina., Sari Edi Syafri., Hairul Abral., Latifah Jasmani and Ibrahim M.I.J.. Advanced Processing, Properties, and Applications of Starch and Other Bio-Based Polymers (2020) 111-138.

[68] International Journal of Biological Macromolecules

[69] Iraklis C. Kourtis., Sachiko Hirosue., Alexandre Titta., Stephan Kontos, Toon Stegmann, Jeffrey A. Hubbell., Melody A. Swartz. Peripherally Administered Nanoparticles Target Monocytic Myeloid Cells, Secondary Lymphoid Organs and Tumors in Mice, Plos one. (2013).

[70] Jagpreet Singh., Tanushree Dutta., Ki-Hyun Kim., Mohit Rawat., Pallabi Samddar and Pawan Kumar. 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation Journal of Nanobiotechnology 16(84) (2018).

[71] James, Leo & Tawfik, Dina.. Catalytic and binding poly-reactivities shared by two unrelated proteins: The potential role of promiscuity in enzyme evolution. Protein science: a publication of the Protein Society. (2002).

[72] Jayanta Kumar Patra, Gitishree Das, Leonardo Fernandes Fraceto, Estefania Vangelie Ramos Campos, Maria del Pilar Rodriguez-Torres, Laura Susana Acosta-Torres, Luis Armando Diaz-Torres, Renato Grillo, Mallappa Kumara Swamy, Shivesh Sharma, Solomon Habtemariam & Han-Seung Shin. Nano based drug delivery systems: recent developments and prospects, Journal of Nanobiotechnology 16 (71) (2018).

[73] Jianyu Xu., Xin Liu., Xiuyan Ren and Guanghui Gao. The role of chemical and physical cross-linking in different deformation

stages of hybrid hydrogels, European Polymer Journal, (100) (2018)86-95.
[74] Jorg Polte. Fundamental growth principles of colloidal metal nanoparticles – a new perspective, crystEngComm, Vol (17) (2015) 6809-6830.
[75] Josh Axe, DC, DMN, CNS. Proven Colloidal Silver Benefits Or An Unsafe Hoax. (2019).
[76] Julieta C.Imperiale., Gabriela., B.Acosta and Alejandro Sosnik.Polymer-based carriers for ophthalmic drug delivery, Journal of Controlled Release, (285) (2018)106-141.
[77] Juozas Baltusnikasa., Andrej Fokina., Johannes Winkler and Julius Liobikas.Long-term regulation of gene expression in muscle cells by systemically delivered siRNA, Journal of Controlled Release, 256(28) (2017) 101-113.
[78] Kaihara, Sachiko., Suzuki, Yoichi, and Fujimoto, Keiji.. In situ synthesis of polysaccharide nanoparticles via polyion complex of carboxymethyl cellulose and chitosan. Colloids and surfaces. B, Biointerfaces. (85) (2011) 343-8.
[79] Kaustubh A.Gawdea Samaresh Saua Katyayani Tatipartia Sushil K.Kashaw., Mohammad Mehrmohammadi., Asfar S.Azmief and Arun K.Iyerad. Paclitaxel and di-fluorinated curcumin loaded in albumin nanoparticles for targeted synergistic combination therapy of ovarian and cervical cancers, Colloids and Surfaces B: Biointerfaces, 167(1) (2018) 8-19.
[80] Khalid Mahmood Zia Shazia Tabasum Muhammad Nasif Neelam Sultan Nosheen Aslam Aqdas Noreen Mohammad Zuber . A review on synthesis, properties, and applications of natural polymer-based carrageenan blends and composites, International Journal of Biological Macromolecules (96)2017:282-301.
[81] Khubab Shaker Yasir Nawab Madeha Jabbar, Bio-composites: Eco-friendly Substitute of Glass Fiber Composites, Handbook of Nanomaterials and Nanocomposites for Energy and Environmental Applications:1-25. (2020).
[82] Kiran, D, and Patil, Kiran.. Review of Green Chemical Technologies for Sustainable Developments in Chemical Process Industries. Trends in Chemical Engineering. 2. (2014)12-19.
[83] Koutny, Marek., Sancelme, Martine., Dabin, Catherine., Pichon, Nicolas., Delort, Anne-Marie and Lemaire, Jacques.. Acquired biodegradability of polyethylene containing pro-oxidant additives. Polymer Degradation and Stability - Polym Degrad Stabil, (91) (2006)1495-1503.
[84] Krishnendu Chatterjee., Sreerupa Sarkar., Jagajjanani, K. and Rao Santanu Paria.Core/shell nanoparticles in biomedical applications, Advances in Colloid and Interface Science, (209) (2014) 8-39.
[85] Lakshmi Pallavi Ganipineni., Fabienne Danhier, and Véronique Préat. Drug delivery challenges and future of chemotherapeutic nanomedicine for glioblastoma treatment, Journal of Controlled Release, (281) (2018) 42-57.
[86] Laura Chambre., ZairaMartín-Moldes., Rachael N.Parker and David L. Kaplan. Bioengineered elastin- and silk-biomaterials for drug and gene delivery, Advanced Drug Delivery Reviews, (160) (2020)186-198
[87] Layla Filiciotto and Gadi Rothenberg. Biodegradable Plastics: Standards, Policies, and Impacts, chemistry-sustainability-energy-materials(2020).
[88] Lee C.H., Sapuan S.M., Ilyas R.A., Lee S.H., and Khalina A. (2020).Chapter 5 - Development and Processing of PLA, PHA, and Other Biopolymers, Advanced Processing, Properties, and Applications of Starch and Other Bio-Based Polymers:47-63.
[89] Lee, Mihyun., Zenobi-wong, Marcy, and Chang, Jin. Nanocomposite Materials Comprising Cationic Nanoparticles and Anionic polymers useful in methods for 3D printing thereof, United States Patent (2019) Application 20190010288Kind Code:
[90] Leena Kumar and Hemant Ramachandra Badwaik (2019).18 - polysaccharide-based nanogels for drug and gene delivery ().Polysaccharide Carriers for Drug Delivery:497-557.
[91] Lehninger Principles of Biochemistry. Lehninger Principles of Biochemistry, 5th Edition. (2011).
[92] Lei Xing., Ya-TongFan., Li-Jun Shen., Chen-XiYang., Xiao-Ying Liua Yan-NiMa., Lian-Yu QiaKi-Hyun Choe Chong-SuChof Hu-Lin Jiangabcd .pH-sensitive and specific ligand-conjugated chitosan nanogels for efficient drug delivery, International Journal of Biological Macromolecules, 141(1) (2019) 85-97.
[93] Lei Zhao., Dongdong Bi., Xiaoyu Qi., Yifei Guo., Feng Yue., Xiangtao Wang and Meihua Han. Polydopamine-based surface modification of paclitaxel nanoparticles for osteosarcoma targeted therapy, Nanotechnology, 30(25) (2019).
[94] Lesca M. Holdt., Alexander Kohlmaier, and Daniel Teupser.Molecular functions and specific roles of circRNAs in the cardiovascular system, Noncoding RNA Res., 3(2) (2018) 75–98.
[95] Li Li., Jun Ki Kim., Kang Moo Huh., Yong-kyu Lee, and So Yeon Kimc. Targeted paclitaxel delivery using folate-conjugated heparin-poly(β-benzyl-l-aspartate) self-assembled nanoparticles, Carbohydrate Polymers, Vol 87 (3)(2012)2120-2128.
[96] Lidija Runko and Luttenberger. Waste management challenges transition to the circular economy – Case of Croatia, Journal of Cleaner Production, (256) (2020) 120495.
[97] Liming Yuan, Bu Guo, Wu Zhong, Yu Nie, Xiaoyan Yao, Xiaofeng Peng, Rong Wang, Hongyuan Yu, Shanyi Yang, Chunlian He, Xiaojun Tao and Qiufang Zhang. Interaction of Mitoxantrone-Loaded Cholesterol Modified Pullulan Nanoparticles with Human Serum Albumin and Effect on Drug Release, Journal of Nanomaterials. (2019).
[98] Lin-Lin Pan., Qi-Hui Sun., Gui-Rong Liu, and Jia-Yin Guo. Urinary Metabolomics Study of the Intervention Effect of Hypoglycemic Decoction on Type 2 Diabetes Mellitus Rats Model, Evidence-Based Complementary and Alternative Medicine. (2019).
[99] Localized Surface plasmon resonance (SPR) is an attractive characteristic of metal nanoparticles SPR is a collective oscillation of conduction band electrons in metal nanoparticles excited by the incident light's electromagnetic Organic Electronics, (2014).
[100] Loïc Bugnicourt and Catherine Ladavière. Interests of chitosan nanoparticles ionically cross-linked with tripolyphosphate for biomedical applications, Progress in Polymer Science, Vol (60) (2016) 1-17.
[101] Luo, Juntao, Shi, Changying and Guo, Dandan Functional segregated Telodendrimers and nanocarriers and methods of making and using same, https://www.freepatentsonline.com/y2020/0254012.html, (2020)
[102] Luzi F., Torre L., Kenny J.M., and Puglia D. Bio- and Fossil-Based Polymeric Blends and Nanocomposites for Packaging: Structure-Property Relationship. Materials (Basel, Switzerland), (2019) 12(3).
[103] Machado, Raul., Bessa, Paulo., Reis, Rui L., Rodríguez-Cabello, José and Casal, Margarida. (2012). Elastin-Based Nanoparticles for Delivery of Bone Morphogenetic Proteins. Methods in molecular biology (Clifton, N.J.). Vol (906): 353-63.
[104] Magician, Parastoo., Tabatabaei, Meisam., Zeinalabedini., Mehrshad and Naghshbandi., Mohammad Pooya and Chisti, Yusuf. (, 2017). Metabolic engineering of microorganisms for biofuel production. Renewable and Sustainable Energy Reviews. 82.
[105] Małgorzata., Stanisz., Łukasz., Klapiszewski and Teofil Jesionowski (2020).Recent advances in the fabrication and application of biopolymer-based micro-and nanostructures: A comprehensive review, Chemical Engineering Journal, Vol (397).
[106] Manoj B. Gawande, Anandarup Goswami, François-Xavier Felpin, Tewodros Asefa, Xiaoxi Huang, Rafael Silva, Xiaoxin Zou, Radek Zboril and Rajender S. Varma (2016). Cu and Cu-Based Nanoparticles: Synthesis and Applications in Catalysis, Chem. Rev., (116) 6:3722–3811.
[107] Maria Abdul Ghafoor., Raja, Haliza Katas, and Thum Jing Wen (2015). Stability, Intracellular Delivery, and Release of siRNA from Chitosan Nanoparticles Using Different Cross-Linkers, Plos one.
[108] María Gabriela Villamizar-Sarmiento., Ignacio Moreno-Villoslada., Samuel Martínez., Annesi Gracaman., Victor Miranda., Alejandra Vidal., Sandra E. Orellana., Miguel Concha., Francisca Pavicic., Judit G. Losoni., Lisette Leyton and Felipe A. Oyarzun-Ampuero (2019).Ionic Nanocomplexes of Hyaluronic Acid and Polyarginine to Form Solid Materials: A

Green Methodology to Obtain Sponges with Biomedical Potential, Nanomaterials (Basel), Vol(9)7: 944.

[109] Maria Helminger., Baohu Wu., Tina Kollmann., Dominik Benke., Dietmar Schwahn., Vitaliy Pipich., Damien Faivre., Dirk Zahn, and Helmut Cölfen (2014). Synthesis and Characterization of Gelatin-Based Magnetic Hydrogels, Adv Funct Mater, Vol(24)21: 3187–3196.

[110] Martin Gericke, Peter Schulze, and Thomas Heinze (2020). Nanoparticles Based on Hydrophobic Polysaccharide Derivatives—Formation Principles, Characterization Techniques, Biomedical Applications, Macromolecular Bioscience, Vol (20)4.

[111] Mhd Anas Tomeh., Roja Hadianamrei, and Xiubo Zhao (2019). Silk Fibroin as a Functional Biomaterial for Drug and Gene Delivery, Pharmaceutics. Vol (11)10: 494.

[112] Min Yang, Ying Shi, and Qi Liang (2016). Effect of microbial transglutaminase cross-linking on yak caseins' functional properties: a comparison with cow caseins, Dairy Science & Technology, Vol (96):39–51.

[113] Mohamad., Tarhini., Hélène Greige-Gerges and Abdelhamid Elaissaria (2017).Protein-based nanoparticles: From preparation to encapsulation of active molecules, International Journal of Pharmaceutics, Vol (522)1–2:172-197.

[114] Mojtaba Abbasian., Bakhshali Massoumi., Rahim Mohammad-Rezaei., Hadi Samadian, and Mehdi Jaymand (2019). Scaffolding polymeric biomaterials: Are naturally occurring biological macromolecules more appropriate for tissue engineering, International Journal of Biological Macromolecules, 134(1) (2019) 673-694.

[115] MoLiac Remco., Fokkink., Yuanying Niac and Mieke Kleijn J.Bovine beta-casein micelles as delivery systems for hydrophobic flavonoids, Food Hydrocolloids, (96) (2019) 653-662.

[116] Momoh A.Mumuni., Franklin C.Kenechukwua., Kenneth C.Ofokansia., Anthony A.Attama and David DíazDíaz.Insulin-loaded mucoadhesive nanoparticles based on mucin-chitosan complexes for oral delivery and diabetes treatment, Carbohydrate Polymers, (229) (2020).

[117] Mona A. Elsayed and Ayman Norredin. The Potential Contribution of Nanoparticles in the Treatment of Inflammatory Diseases. (2019).

[118] Mona Alibolandi and Mohammad Ramezani. Active Targeted Nanoscale Delivery Systems for Brain Tumor Therapeutics, In Nanotechnology-Based Targeted Drug Delivery Systems for Brain Tumors. (2018).

[119] Mosaiab, Tamim, Farr, Dylan, Kiefel, Milton J, Houston, Todd A. Carbohydrate-based nanocarriers and their application to target macrophages and deliver antimicrobial agents, Advanced Drug Delivery. (2019).

[120] Naba Kumar., Kalita Siddharth., Mohan Bhasney., Ajay Kalamdhad, and Vimal Katiyara. Biodegradable kinetics and behavior of bio-based polyblends under simulated aerobic composting conditions, Journal of Environmental Management, (261): (2020) 110211.

[121] Nair N.R., Sekhar V.C., Nampoothiri K.M. and Pandey A..32 - Biodegradation of Biopolymers, Current Developments in Biotechnology and Bioengineering Production, Isolation and Purification of Industrial Products (2017) 739-755.

[122] Narmatha Christya P., Khaleel Basha S., Sugantha Kumaria V., Bashired A.K.H., Maazaed M., Kaviyarasued K., Mariadhas Valan Arasuef Naif Abdullah Al-Dhabie Savarimuthu Ignacimuthuf.Biopolymeric nanocomposite scaffolds for bone tissue engineering applications – A review, Journal of Drug Delivery Science and Technology, (55) (2020).

[123] Nasim Annabi., Suzanne M. Mithieux., Gulden Camci-Unal., Mehmet R. Dokmeci., Anthony S. Weiss and Ali Khademhosseini. Elastomeric Recombinant Protein-based Biomaterials, Biochem Eng J, (77) (2013) 110–118.

[124] Ndolo Obonyo., Dennis, Njuguna., Elizabeth, Adetunji., Charles Harbor., Chioma, Rowe., Arielle, Den Breeyen., Alana, Sangeetha., Jeyabalan, Singh., Gurpreet, Szewczyk., Boguslaw, Anjorin., Toba, Thangadurai., Devarajan, Hospet, and Ravichandra.. Research and Development of Biopesticides: Challenges and Prospects. Outlooks on Pest Management. 30. (2019) 267-276.

[125] Nikalje, Anna.. Nanotechnology and its Applications in Medicine. Medicinal Chemistry. Vol 5(5) (2015) 081-089.

[126] Nimish Shah., Rajubhai K. Mewada, and Tejal Mehta. Nano-Polysaccharides at Drug Delivery Systems, International Conference on Multidisciplinary Research & Practice, Vol 1(8) (2013) 490.

[127] Nitta, Sachiko, and Numata, Keiji.. Biopolymer-Based Nanoparticles for Drug/Gene Delivery and Tissue Engineering. International journal of molecular sciences. (14) (2013)1629-54.

[128] Noble metal nanoparticles (NPs) have been deposited onto the TiO2 surface as a way to develop highly efficient photocatalysts under visible and solar light: Nanoscale Materials in Water Purification. (2019).

[129] Oscar Rosales-Calderon and Valdeir Arantes (2019). A review on commercial-scale high-value products that can be produced alongside cellulosic ethanol,

[130] Oyarzun-Ampuero, Felipe., Goycoolea, Francisco., Torres, Dolores and Alonso, Maria.. A new drug nanocarrier consisting of polyarginine and hyaluronic acid. European journal of pharmaceutics and biopharmaceutics:official journal of Arbeitsgemeinschaft für Pharmazeutische Verfahrenstechnik, (79) (2011) 54-7.

[131] Özçimen, Didem., İnan., Benan, morkoç., Ogün and Efe, Aybüke.. A Review on Algal Biopolymers. Journal of Chemical Engineering Research Updates, (4) (2017) 7-14.

[132] Parinaz Nezhad-Mokhtari., Marjan Ghorbanic., Leila Roshangar., Jafar Soleimani Rad.Chemical gelling of hydrogels-based biological macromolecules for tissue engineering: Photo- and enzymatic-crosslinking methods, International Journal of Biological Macromolecules, (139) (2019) 760-772.

[133] Paripurnanda Loganathan, Michael Gradzielski, Heriberto Bustamante, and Saravanamuthu Vigneswaran. Progress, challenges, and opportunities inenhancing NOM flocculation using chemically modified chitosan: a review towards future development, Environmental Science Water Research & Technology. (2020).

[134] Patil, Sachinkumar, and Jadge, D.. Crosslinking Of Polysaccharides: Methods And Applications. Pharmaceutical Reviews, (6) (2008).

[135] Peipei Zhang, Yu-Chieh Chiu, Lisa H. Tostanoski, and Christopher M. Jewell. Polyelectrolyte Multilayers Assembled Entirely from Immune Signals on Gold Nanoparticle Templates Promote Antigen-Specific T Cell Response, ACS Nano; 9(6) (2015) 6465–6477.

[136] Peter Kalmus. Highlights from Being the Change: Live Well and Spark a Climate Revolution. (2017).

[137] Physiologic Factors Related to Drug Absorption. Patient-Related Factors Affecting Drug Absorption(2017).

[138] Platt, Virginia & Szoka, Francis.. Anti-cancer Therapeutics: Targeting Macromolecules and Nanocarriers to Hyaluronan or CD44, a Hyaluronan Receptor. Molecular pharmaceutics, Vol(5):474-86.

[139] Polymer Degradation and Stability, Vol (179):109232.

[140] Prerna Khanna., Amrit Kaur, and Dinesh Goyal. Algae-based metallic nanoparticles: Synthesis, characterization and applications, Journal of Microbiological Methods, (2019) (163).

[141] Qingjie Sun (2018).Chapter-18-Starch Nanoparticles, Starch in Food (Second Edition), Structure, Function and Applications, Wood head Publishing Series in Food Science, Technology and Nutrition: 691-745.

[142] Raghvendra Kumar Mishra., Sung Kyu Hab., Kartikey Verma and Santosh Tiwaribd K.. Recent progress in selected bio-nano materials and their engineering applications: An overview, Journal of Science: Advanced Materials and Devices, 3(3) (2018) 263-288.

[143] Raj Kumar., Ashutosh Singh., Neha Garg (2018). Solid lipid nanoparticles for the controlled delivery of poorly water-soluble non-steroidal anti-inflammatory drugs. Ultrasonics Sonochemistry, (40), Part A: 686-696.

[144] Rajalekshmy G.P.Lekshmi., Devi L. Jasmin and Joseph Rekha M.R. (2019).2-An overview on the potential biomedical applications of polysaccharides, Functional Polysaccharides for Biomedical Applications: 33-94.

[145] Rajiv M.Desai., Sandeep T.Koshy., Scott A.Hilderbrand., David J.Mooney and Neel S.Joshiab (2015). Versatile click alginate hydrogels cross-linked via tetrazine–norbornene chemistry, Biomaterials, Vol (50):30-37.
[146] Raluca Ioana Teleanu., Oana Gherasim., Tudor George Gherasim., Valentina Grumezescu., Alexandru Mihai Grumezescu and Daniel Mihai Teleanu (2019). Nanomaterial-Based Approaches for Neural Regeneration, Pharmaceutics. Vol (11)6: 266.
[147] Randal Shogren., Delilah Wood., William Orts and Gregory Glenn.Plant-based materials and transitioning to a circular economy, Sustainable Production and Consumption, (19) (2019)194-215
[148] Raquel Ferreira and Liliana Bernardino. 27-Nanotechnology for intracellular delivery and targeting, Nanoengineered Biomaterials for Advanced Drug Delivery, Wood head Publishing Series in Biomaterials: (2020) 683-696.
[149] Research Letters., (9): 252.
[150] Richard Nho. Pathological effects of nano-sized particles on the respiratory system, Nanomedicine: Nanotechnology, Biology, and Medicine. (2020).
[151] Ruslan Melentieva and Fengzhou Fanga. Tailoring of surface topography for tribological purposes by controlled solid particle impacts. Wear, (2020) 444–445.
[152] Sachiko Kaihara Nitta and Keiji Numata. Biopolymer-Based Nanoparticles for Drug/Gene Delivery and Tissue Engineering, Int. J. Mol. Sci., (14) (2013) 1629-1654.
[153] Sachiko Kaihara Nitta and Keiji Numata (2013). Biopolymer-Based Nanoparticles for Drug/Gene Delivery and Tissue Engineering, Enzyme Research Team, Riken Biomass Engineering Program, Riken, Saitama 351-0198, Int. J. Mol. Sci. 14(1) (2013) 1629-1654.
[154] Sagar Aryal. Digestion and Absorption of Carbohydrates, Proteins, and Fats (2019).
[155] Sarah R. Mac Ewan and Ashutosh Chilkoti. Digital Switching of Local Arginine Density in a Genetically Encoded Self-Assembled Polypeptide Nanoparticle Controls Cellular Uptake, Nano Lett., 12(6) (2012) 3322–3328.
[156] Sarita Rani., Ashok K.Sharma., Iliyas Khan., Avinash Gothwal., Sonam Chaudhary and Umesh Gupta.Chapter7-Polymeric Nanoparticles in Targeting and Delivery of Drugs, Nanotechnology-Based Approaches for Targeting and Delivery of Drugs and Genes, (2017) 223-255.
[157] Seema Agarwal. Biodegradable Polymers: Present Opportunities and Challenges in Providing a Micro plastic-Free Environment, molecular chemistry, and Physics. (2020).
[158] Selorm Torgbo and Prakit Sukyai. Biodegradation and thermal stability of bacterial cellulose as a biomaterial: The relevance in biomedical applications. (2020).
[159] Shaoqing Cuia, Joshua Borgemenke, Yusheng Qin., Zhe Liu, and Yebo Lic.Chapter Five-Bio-based polycarbonates from renewable feed stocks and carbon dioxide, Advances in Bioenergy, (4) (2019) 183-208.
[160] Shijie Li., Linlin Li., Zhuo Chena., Guangpu Xue., Longguang Jiang., KeZheng Jincan., Chena Rui., Lia Cai and Yuand Mingdong Huangac.A novel purification procedure for recombinant human serum albumin expressed in Pichia pastoris, Protein Expression and Purification, (149) (2015) 37-42.
[161] Shivarama Krishnan, B & Gurumurthy, B and Balasubramanian, A.. Potential Biomedical Applications of metallic nanobiomaterials: A review. International Journal of Pharmaceutical Sciences and Research. (8): (2017) 985-1000.
[162] Showkat Ahmad Bhawani., Abdul Moheman., Amr A.Yakout., Mohamad Nasir and Mohamad Ibrahim. Chapter 10 - Nanostructured Biopolymers for Application as Drug-Delivery Vehicles, Nanostructured Polymer Composites for Biomedical Applications, Micro and Nano Technologies (2019)189-210.
[163] Showkat Ahmad Bhawani., Abdul Moheman., Amr A.Yakout., Mohamad Nasir and Mohamad Ibrahim. Chapter-10-Nanostructured Biopolymers for Application as Drug-Delivery Vehicles, Mater Biol Appl. (2010).
[164] Showkat Ahmad, Bhawani., Abdul Moheman., Amr A.Yakout., Mohamad Nasir and Mohamad Ibrahim.Chapter 10 - Nanostructured Biopolymers for Application as Drug-Delivery Vehicles, Nanostructured Polymer Composites for Biomedical Applications, Micro and Nano Technologies: (2019) 189-210.
[165] Showkat Ahmad, Bhawani., Abdul Moheman., Amr A.Yakout., Mohamad Nasir and Mohamad Ibrahim. Chapter 10-Nanostructured Biopolymers for Application as Drug-Delivery Vehicles, Nanostructured Polymer Composites for Biomedical Applications, Micro and Nano Technologies: (2019) 189-210.
[166] Shuangquan Gou., Yamei Huang., Ying Wand., Ya Mab., Xin Zhou., Xiaoling Tong., Jin Huange., Yuejun Kang., Guoqing Pana., Fangyin Daia., Bo Xiaoa.Multi-bioresponsive silk fibroin-based nanoparticles with on-demand cytoplasmic drug release capacity for CD44-targeted alleviation of ulcerative colitis, Biomaterials, (212) (2019) 39-54.
[167] Shukla, Rishi, and Cheryan, Munir. Zein: The industrial protein from corn. Industrial Crops and Products. (13) (2001) 171-192.
[168] Siracusa, Valentina.. Microbial Degradation of Synthetic Biopolymers Waste. Polymers, (11) (2019)1066.
[169] Socrates Radha Krishnan., Sakthivel Nagarajan., Mikhael Bechelany and Narayana Kalkura S. (2019). Collagen Based Biomaterials for Tissue Engineering Applications: A Review, Processes, and Phenomena on the Boundary Between Biogenic and Abiogenic Nature, 3-22.
[170] Solmaz Maleki Dizaj., Samira Jafari, and Ahmad Yari Khosroushahi. A sight on the current nanoparticle-based gene delivery vectors, Nanoscale. (2014).
[171] Soo-Ling Bee., Abdullaha M.A.A., Soo-Tueen Beec Lee TinSinc Rahmat A.R.. Polymer nanocomposites based on silylated-montmorillonite: A review, Progress in Polymer Science, (85) (2018) 57-82.
[172] Stefano Rimondo., Giuseppe Perale, and Filippo Rossi. 6-Polysaccharide-based scaffold for tissue-regeneration, Functional Polysaccharides for Biomedical Applications (2019) 189-212.
[173] Steinman, Noam & Campos, Luis & Feng, Yakai & Domb, Abraham & Hosseinkhani, H... Cyclopropenium Nanoparticles and Gene Transfection in Cells. Pharmaceutics; (12) (2020) 768.
[174] Storz, Henning.. Bio-based plastics: status, challenges and trends. Land baufor schung (63) (2014) 321-332.
[175] Substrate stiffness- and topography-dependent differentiation of annulus fibrosus-derived stem cells is regulated by Yes-associated protein. Statements of Significance, Acta Biomaterials. (2019).
[176] Suhail Ahmad., Kaiser Manzoor., Mudasir Ahmad., Roli Purwar, and Saiqa Ikram. Chapter-7, Starch-based Bionanocomposites, Bionanocomposites, Green Synthesis and Applications, Micro and Nano Technologies(2020)157-171.
[177] Swierczewska, M., Han H.S., Kim K., Park J.H., and Lee S.Polysaccharide-based nanoparticles for the Ranostic Nanomedicine, Advanced Drug Delivery Reviews, (99) Part A: 70-84.
[178] Swierczewska, M. & Han, Hwa Seung & Kim, K. & Park, J.H. & Lee, S...Polysaccharide-based Nanoparticles for Theranostic Nanomedicine. Advanced Drug Delivery Reviews, (2015) (99).
[179] Thomas G.Barclaya., Candace Minhthu Day., Nikolai Petrovsky and Sanjay Garga.Review of polysaccharide particle-based functional drug delivery, Carbohydrate Polymers, (221) (2019) 94-112.
[180] Thomas Malachowski and Austin Hassel.Engineering nanoparticles to overcome immunological barriers for enhanced drug delivery, Engineered Regeneration, (1) (2020) 35-50.
[181] Tianxin Miao, Junqing Wang, Yun Zeng, Gang Liu, and Xiaoyuan Chen. Polysaccharide-Based Controlled Release Systems for Therapeutics Delivery and Tissue Engineering: From Bench to Bedside, Adv Sci (Weinh). 5(4) 2018.
[182] Tinesha Selvaraj Veeradasan., Perumal Shing., Fhan Khor., Leonard Sean., Anthony, Subash., Gopinathef C.B., Norani and Muti Mohamedbd.The recent development of polysaccharides biomaterials and their supercapacitor applications' performance, Materials Research Bulletin, (126) (2020).
[183] Ting Jiang., Kai Jin., Xianpping Liu and Zhiqing Pang. 8-Nanoparticles for tumor targeting, Biopolymer-Based Composites Drug Delivery, and Biomedical Applications: (2017) 221-267.
[184] Iftikhar., Ali Khawara., Jung HoKima., Hyo-Jeong Kuhab.Improving drug delivery to solid tumors: Priming the

tumor microenvironment, Journal of Controlled Release, 201(10) (2015) 78-89.

[185] Tiwari, P.M., Bawage, S.S. and Singh S.R. (2015). 13 - Gold nanoparticles and their applications in photomedicine, diagnosis and therapy, Applications of Nanoscience in Photomedicine, 249-266.

[186] Tomasz Konrad Głąb and Janusz Boratyński (2017). Potential of Casein as a Carrier for Biologically Active Agents, Topics in Current Chemistry, Vol (375)71.

[187] Trepti Singh., Shruti Shukla., Pradeep Kumar., Verinder Wahla., Vivek K. Bajpai, and Irfan A. Rather (2017).Application of Nanotechnology in Food Science: Perception and Overview, Front. Microbiol., Vol (7).

[188] Trivedi, P. & Hasan, Adria & Akhtar, Salman & Siddiqui, Mohammad & Sayeed, Usman and Khan, Mohd Kalim Ahmad. (2016). Role of microbes in the degradation of synthetic plastics and the manufacture of bioplastics. 8. 211-216.

[189] Vijilvania, C., Bindhu M.R., Frincy F.C., Mohamad., AlSalhic S., Sabitha S., Saravanakumar K., Sandhanasamy Devanesan., Umadevie M., Mamduh Aljaafreh J. and Muhammad Atif (2020). Antimicrobial and catalytic activities of biosynthesized gold, silver, and palladium nanoparticles from Solanum nigurum leaves, Journal of Photochemistry and Photobiology B: Biology, (202).

[190] Weber, C., Coester, C, Kreuter, J., Langer, K.Desolvation process and surface characterization of protein nanoparticles, International Journal of Pharmaceutics, 194(1), (2000) 20:91-102.

[191] Wensi Zhanga., Xiaoqing Yua., YangLia Zhiqiang Sua Klaus and D. Jandtbcd Gang Weie.Protein-mimetic peptide nanofibers: Motif design, self-assembly synthesis, and sequence-specific biomedical applications, Progress in Polymer Science, (80) (2018) 94-124.

[192] Xiao-Xia Xia., Qiaobing Xu., Xiao Hu., Guokui Qin and David Kaplan L.. Tunable Self-Assembly of Genetically Engineered Silk–Elastin-like Protein Polymers, Biomacromolecules, 12(11) (2011) 3844–3850.

[193] Yaghoub Safdari., Vahideh Ahmadzadeh., Masoumeh Khalili., Hossein Zarei Jaliani., Vahid Zarei Vahid Erfani-Moghadam. Use of Single-Chain Antibody Derivatives for Targeted Drug Delivery, Mol Med (22) (2016) 258–270.

[194] Yajaman Sudhakar Ketousetuo Kuotsu and Bandyopadhyay A.K..Buccal bioadhesive drug delivery — A promising option for orally less efficient drugs, Journal of Controlled Release, 114(1) (2006)15-40.

[195] Yang, D., He, Y., Wu, B.. Integrated bioinformatics analysis for the screening of hub genes and therapeutic drugs in ovarian cancer. J Ovarian Res 13(10) (2020).

[196] Yasuhiko Tabata.Significance of release technology in tissue engineering, Drug Discovery Today, (10)23–24 (2005) 1639-1646.

[197] Yi Li., Feihu Wang, and Honggang.Peptide-based supramolecular hydrogels for delivery of biologics, Bioeng Transl Med. 2016 Sep; 1(3) (2016) 306–322.

[198] Yinji Wan, Yifei Fan, Jianming Dan, Chenglin Hong, Shengchao Yang, and Feng Yu.A review of recent advances in two-dimensional natural clay vermiculite-based nanomaterials, 6(10) (2019).

[199] Yi-Yan Yang., Yong Wang., Ross Powell and Peggy Chan.Polymeric core-Shell Nanoparticles for Therapeutics, Clinical, and Experimental, Pharmacology, and Physiology. (2006).

[200] Yongda Sun.Carrier free inhaled dry powder of budesonide tailored by supercritical fluid particle design, Powder Technology, (304) (2016) 248-260.

[201] Yuefei Zhu., Yiyang Liu., Kai Jin and Zhiqing Pang . 13 - Polysaccharide nanoparticles for cancer drug targeting, Polysaccharide Carriers for Drug Delivery: (2019) 365-396.

[202] Yujie Zhang., Tao Sun, and Chen Jiang. Biomacromolecules as carriers in drug delivery and tissue engineering, Acta Pharmaceutica Sinica B, 8(1) (2018) 34-50.

[203] Yujie Zhang., Tao Sun, and Chen Jiang .Biomacromolecules as carriers in drug delivery and tissue engineering, Acta Pharmaceutica Sinica B, 8(1) (2018) 34-50.

[204] Zeenat Mirza and Sajjad Karim.Seminars in Cancer Biology, Nanoparticles-based drug delivery, and gene therapy for breast cancer: Recent advancements and future challenges.

[205] 34th Annual Meeting & Pre-Conference Programs of the Society for Immunotherapy of Cancer (SITC 2019): Part 1-National Harbor, MD, USA. 6-10 (2019). Journal for ImmunoTherapy of Cancer, (2019) (7): 282.

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కర్షకుని సౌశీల్యము

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శ్రమ లేఖయే పలములు దుముకబోవు

పిండి కొలదియే రొట్టె యోపిన విధాన

కష్టపడుము కృషివలా క్లుగు సుఖము

ఉత్తియాసాలు కన్నా మేలుద్యమంబు

అని కర్షకుని గురించి చెప్పినవాడు దువ్వురి .ఆదునిక ఆంధ్ర సాహిత్యములో కృషి వలా కవిత్వానికి పదులు తీసిన కవిగా ప్రసిద్ధికెక్కిన ఉత్తమ కవీంద్రులుదువ్వురి రామి రెడ్డి ఆంగ్ల సాహిత్యంలో పోస్ట్రాల్ పోఎట్రీ ప్రభావము ఈయన మీద ఉన్నది గ్రామీణ సౌభాగ్యాన్ని కర్ష్కుని దైనందిన జీవితాన్ని వర్ణిస్తూ కృషి వలుడు కావ్యాన్ని రచించాడు

ఓ రైతన్న నీ జీవితాన్ని వర్ణించాలి అని నేను అనుకున్నప్పుడు వెంటనే సెలయేరు ప్రవాహములుగా వాక్కులు అనేది ప్రవాహము పరేగేట్టుతుంది .అది చూసిన కొంతమంది ఈర్ష్య పరులు నన్ను కర్షక పక్షపాతి అంటున్నారు అని వాపోయాడు దీనిని బట్టి ఆయనకు రైతు జేవితము మీద అతని దినచర్య మీద ఎంత పట్టు ఉన్నదో మనము అర్థము చేసుకోవచ్చు. పంజరములోని చిలుక ఎలా స్వాతంత్యాన్ని కోరుకున్నుడో నేను కూడా ఆదునిక కవిత్వము పట్ల స్వాతంత్యాన్ని కోరుకుంటున్నానని ధైర్యముగా చెప్పాడు దువ్వురి ఎవరు ఏమనుకున్న ఈ కాలములో మార్పు గలగటం సహజం .ఈ మార్పుతో మన ఆలోచనలను వెల్లడించటానికి భయపడవలిసిన పనిలేదు అని అంటాడు

కృషివలా : నువ్వు భారత భూమండలంలోని వీరులలో శ్రేష్టు డువు . రాజు పాలనా దండము కన్నా నీ హలము గొప్పది నీ కోర్కెలు నిత్యసరాలకు మించి వుండవు నీ ఆలోచన ఉహా , నేఇపున్యం పచ్చని పైరు పొలాల మద్యనే

ఉంటుంది.సూర్యోదయం నుంచు సూర్యాస్తమయం వరకు పొలములో కష్టపడి పనిచేస్తావే కానీ ఇరుగు పొరుగు వారి సంపద చూసి అసూయా చెందవు నీ హృదయం అనే మొగ్గ ఎంత స్వచ్ఛ మైనది అని కర్ష కుని పొగుడుతాడు దీనిని బట్టి చుస్తే దువ్వూరిని కర్షక పక్షపాతి అని నిజముగా అనవచ్చు. కర్శకుని స్వచ్ఛమైన మనస్సుని తెలియ జేస్తున్నాడు.ఈనాడు ప్రపంచీకరణ అని మనము అంటున్నాము కానీ కవి ఏనాడో గుర్తించాడు పల్లి ఒకటే నీ సర్వ ప్రపంచం, నీ భార్య యే నీ రమణీయ విగ్రహంటు బంగారం పండించే పంట పొలలే నీ భాగ్య నిధులు ప్రతీరోజు స్రమించుతయేనీ మతము నీ సంసరములోనే లోట్లు, పాట్లునుముడువ కంటికి తెయనియ్యవు పరిమేతమైన కోరకైలతో జీవితాన్ని సాగిస్తావు నీకు తినటానికి తిండి ఉన్న లేకపోవేన పరిల సొమ్ముకు ఆశపడవు అని మనస్తత్వాన్ని తెలియ జేస్తున్నాడు ఆకలితో ఇంటికి వచ్చిన అతిధి తినక తరగక పోయన దినము లేదునీకు ఉన్న లేఖ పోయన పట్టించుకోవు అంతటి సంస్కారమున్నవ్యక్తివి నీవు అని గిప్పగా చిత్రికరిస్తున్నాడు

వ్యవసయమే సకల ప్రపంచము పరిశ్రమలకు మూలం సమాజంలోనిసౌభ్యగ్యకు కారణం నీవు నీవు కష్టపడి పని చేస్తే పంచ భక్ష్య పరమాన్నాలు తింటున్నవారు నీ గురించి ఆలోచించరు నీవు ప్రపంచనికి భోజనము పెడతావు నీకు భోజనము లేదు నీకు తినటానికి తిండి కట్టుకోవడానికి బట్ట దొరకదు పలాలు పండిస్తు దాన్ని అనుభవిస్తూ ఆనందానుభూతి పొందుతరుగని నీ గురించి పట్టించుకోరు ఎప్పుడు చెట్టును పెంచిపోపించిన రైతు గురించి ఆలోచించరు అల ఆలోచించక పోయన నీవు మాత్రమూ నిస్వర్థముగా వారికి మంచి పలాలు అందిస్తావు రైతు వ్యవసాయము చేసి సమయములో తన శరీరము ఎముకల గూడుగా ఉన్న అతివృష్టి వచ్చిన కరువు వచ్చిన సారీరక బలమే ఆదారం చేసుకుని జీవనం సాగిస్తున్నావు కర్శ్కుని సేవలు ఎవరు గుర్తించరు వెలుగుకు ముందు చీకటి వర్శనుకి ముందు మేఘాలు అడంభారాన్ని ప్రదర్శిస్తాయీ ఇది ప్రకృతి ధర్మం చక్రనికున్న ఆకులూ క్రిడివి పైకి లేచినట్టి జీవితం కూడా ఎగుడు దుగుడుగ ఉంటుంది అన్న నగన్ సత్యాన్ని తెలియ జేశాడు నిరంతరం శ్రమిస్తూ కష్టాలలో ఉన్న ఉన్న రైతు మీద పెత్తనం సగించావారు వారు ఎవ్వరు లేరు కవిని ఆత్మ జ్ఞానంతో నిడిన వ్యక్తిగా కవి భావిస్తాడు దారిద్ర్య అనే పెద్ద ఓడను దాటటానికి రైతుకు స్వేచ్ఛ అనే తెడ్డు అవసరం అంటాడు ఈ తెడ్డు ద్వార రాయి జీవితంలోని లోటుపాట్లను సరిచేసుకోవచ్చు ఒత్తికి

లోగకుండా కోరికలని తొలగించుకొని కష్టాల్ని సాహిస్తి ముందుకు సాగాలి . రైతు ధైర్యం కోల్పోవద్దు అని హితవు చెప్పుతాడు ధైర్యంతో ప్రారంభిస్తే మంచి ఫలితాలు ఉంటాయని చెప్పాడు

రైతుల కష్టాన్ని వివరిస్తూ వారికి హితబోద చేసాడు వారు చేసి వ్యవసాయం పై దేశ భవిష్యత్తు ఆధారపడి ఉంటుంది అని స్పష్టం చేసాడు రైతు జీవిత వర్ణనతోపాటు అతని మనసు స్వాతంత్ర్య0 పై పయనిస్తుంది అని చెప్పటం దువ్వూరి రైతు స్వాత్రంత్ర్య అభిలాషను కోరుకున్నట్లు తెలియజేసాడు ఈ విధంగా కృషీవలుడు కావ్యం సాహిత్య ప్రపంచములో ఉన్నత స్థానము దక్కింది

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Development and validation of stability indicating rp-hplc method for quantitative estimation of levofloxacin injection 5mg/ml dosage form

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Abstract

A new analytical "reverse phase high performance liquid Chromatography (RP-HPLC) assay method has been developed for estimation of" Levofloxacin in injection phase. The separation was achieved by using column Inertsil ODS-3V (250 x 4.6mm, 5µm) mobile phase consisted of 0.05 M solution of citric acid monohydrate and 10 ml of 1.0 M ammonium acetate buffer and acetonitrile in the ratio of (85:15 v/v) . The flow rate was 1.0mL.min-1. Levofloxacin was detected using UV detector at the wavelength of 293 nm. The retention time of Levofloxacin was noted to be 11.20 min respectively. The method was evaluated as per ICH guidelines. The proposed method was found to be advantageous than the existing methods towards accuracy, reproducibility, and consistent.

Keywords: RP-HPLC, Levofloxacin, Forced degradation and Validation.

Introduction

Levofloxacin hemihydrate (Figure 1) is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class and is used to treat severe life-threatening bacterial infection or bacterial infection that have failed to respond to other antibiotic classes. IUPAC name is (S)-9-fluoro-2,3- dihydro- 3-methyl -10 - (4-methylpiperazin-1-yl) -7-oxo- 7H- pyrido[1,2,3-de]-1,4-benzoxazine – 6 -carboxylic acid.

Levofloxacin hemihydrate is highly water and organic solvents like glacial acetic acid and chloroform, sparingly soluble in methanol, slightly soluble in ethanol, and practically insoluble in ether. Levofloxacin hemihydrate is odourless drug. Methods for quantitative analysis of Levofloxacin by HPLC [1–7], by UV [8–10] spectroscopy in single as well as in combination, are available in the literature. The method was developed and validated as per ICH [11–13] and USP [14] guideline.

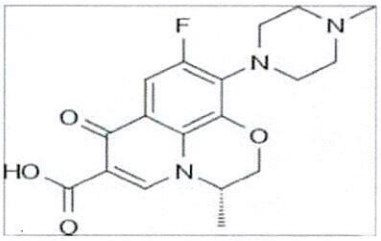

Fig.1.1. Structure of Levofloxacin

Experimental

Chemicals and reagents: Analytical-grade Ammonium acetate, Citric acid monohydrate and Hydrochloric acid was from Merck chemicals Mumbai, India. Methanol, Acetonitrile and water both HPLC-grades, were from Merck chemicals. Mumbai, India. Millex syringe filters (0.45 µm) were from Millex-HN, Millipore Mumbai, India.

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Instrumentation: Agilent HPLC model:1260 with DAD, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were used.

Preparation of 0.05 M solution of citric acid monohydrate: Accurately weighed and dissolved 10.5 g of citric acid monohydrate in 1000 ml of water and sonicated to dissolved and mixed well.

Preparation of 1.0 M Ammonium acetate solution: Accurately weighed and dissolved 7.71 g of ammonium acetate in 100 ml of water and sonicated to dissolved and mixed well.

Preparation of buffer solution: Mixed accurately 840 ml of a 0.05 M solution of citric acid monohydrate and 10 ml of 1.0 M ammonium acetate solution.

Mobile phase: Mixed buffer solution and acetonitrile in the ratio of 85:15 v/v, filtered and degassed.

Blank preparation: Use Milli-Q water.

Standard preparation: Accurately weighed 50.0 mg of Levofloxacin working standard or reference standard was transferred into a 50 ml volumetric flask. Added 7.5ml of 0.1M Hydrochloric acid solution stirred well and diluted to the volume with 0.1M Hydrochloric acid solution. Transferred 5.0 ml of resulting solution into a 25 ml volumetric flask and diluted to volume with water and mixed well. The solution was diluted to volume with diluent and mixed well. (Concentration of Levofloxacin is about 0.2mg/ml).

Placebo solution: Transferred 10 ml of the placebo solution , added 7.5 mL of 0.1 M solution of hydrochloric acid, and diluted to volume with mobile phase and mixed well in 50 mL volumetric flask,. Further transferred 5.0 ml of the resulting solution into a 25 mL volumetric flask and diluted to volume with water and mixed well.

Sample preparation: Transferred 10 ml of the sample solution into a 50 mL volumetric flask, added 7.5 mL of 0.1 M solution of hydrochloric acid, and diluted to volume with mobile phase and mixed well.

Further transferred 5.0 ml of the resulting solution into a 25 mL volumetric flask and diluted to volume with water and mixed well.

Chromatographic conditions: Chromatographic analysis was performed on 250x4.6mm, 5µm column. The mobile phase consisted of 0.05 M solution of citric acid monohydrate and 10 ml of 1.0 M ammonium acetate buffer and acetonitrile in the ratio of (85:15 v/v). The flow rate was 1.0mL/min, column oven temperature 25°C, the injection volume was 10iL, and detection was performed at 293 nm using a photodiode array detector (PDA).

Results & Discussion

Method development: The Spectral data of compound Levofloxacin showed that maximum UV absorbance (ëmax) at 293 nm. To develop a suitable and robust LC method for the determination of Levofloxacin, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Inertsil ODS-3V, 250x4.6mm, 5µm with the following different mobile phase compositions of 0.05 M solution of citric acid monohydrate buffer and acetonitrile in the ratio of 85:15 v/v. It was observed that when Levofloxacin was injected, higher retention time, Peak Tailing are not satisfactory. For next trial the mobile phase consisted of 0.05 M solution of citric acid monohydrate and 10 ml of 1.0 M ammonium acetate buffer and acetonitrile in the ratio of 85:15 v/v was employed at the flow rate of 1.0 mL/min. UV detection as performed at 293nm. The retention time of Levofloxacin is 11.20 minutes and the peak shape was good. The chromatogram of Levofloxacin standard using the proposed method is shown in **Figure: 1.2** system suitability results of the method are presented in **Table:1.2.**

Method validation:

The developed RP-HPLC method extensively validated for assay of Levofloxacin using the following parameters.

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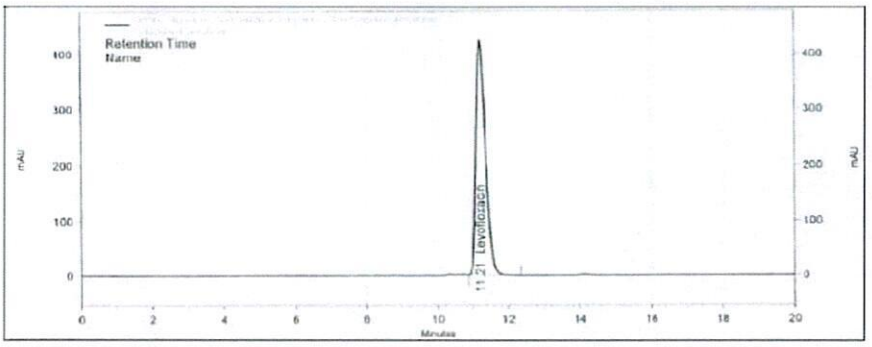

Figure 1.2. Chromatogram peak of Levofloxacin

Specificity & System suitability:
Preparation of blank solution: Used Milli-Q water as a blank solution.

Preparation of Placebo solution: Transferred 10 ml of the placebo solution into a 50 mL volumetric flask, added 7.5 mL of 0.1 M solution of hydrochloric acid, and diluted to volume with mobile phase and mixed well. Further transferred 5.0 ml of the resulting solution into a 25 mL volumetric flask and diluted to volume with water and mixed well.

Blank and Placebo interference: A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **Figure:1.3** showed no peak at the retention time of Levofloxacin peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Levofloxacin in Levofloxacin injection. Similarly chromatogram of placebo solution **Figure: 1.4** showed no peaks at the retention time of Levofloxacin peak. This indicates that the placebo used in sample preparation do not interfere in estimation of Levofloxacin in Levofloxacin injection.

Table 1.1: Specificity results for Levofloxacin

S.No	Name	Retention Time (min)
1	Blank	ND
2	Placebo solution	ND
3	Standard solution	11.24
4	Sample solution	11.22

The chromatogram of blank and placebo are not showing any peak at the retention time of Levofloxacin.

Table 1.2: System suitability parameters for Levofloxacin

No.of injections	Tailing factor	Tailing plates	Theoretical Area of Levofloxacin
Inj-1	1.4	7622	1088768955
Inj-2	1.4	7580	1086835821
Inj-3	1.4	7590	1088318629
Inj-4	1.4	7527	1089977609
Inj-5	1.4	7598	1086265129
		Average %RSD	1088033229 0.14

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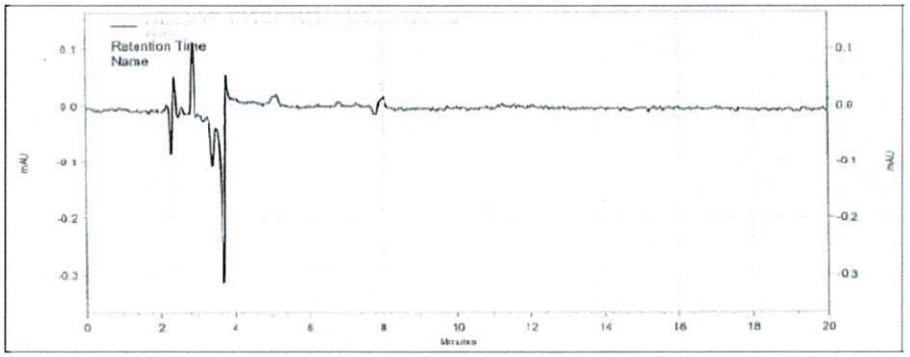

Figure: 1.3. Chromatogram showing the no interference of diluent for Levofloxacin.

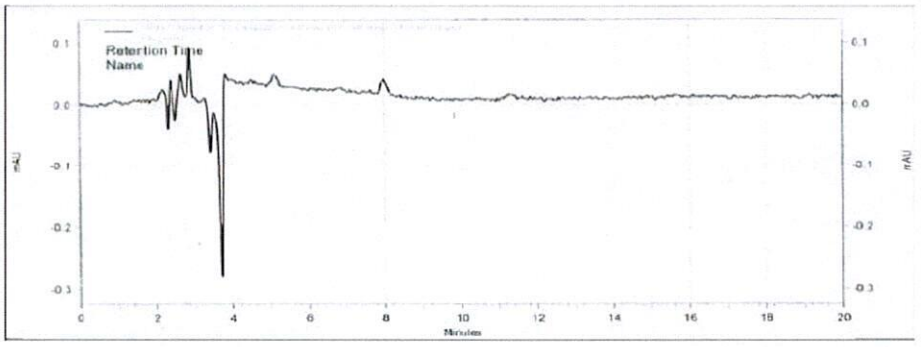

Figure: 1.4 Chromatogram showing the no interference of placebo for Levofloxacin.

Forced Degradation study: The study involves assessing the effect of acid (0.1N HCl, 2 hrs at 60°C temperature), base (0.1N NaOH, 2 hrs at 60°C temperature), hydrogen peroxide (3%, 2 hrs at 60°C temperature), Thermal (105°C for 48 hours) and UV light (7days) on Levofloxacin injection samples. The chromatograms obtained from various stress conditions are shown in **Figure:1.5**. The percent assay, percent degradation and peak purity of Levofloxacin and retention time of degradants produced in all stress conditions are determined and summarized in **Table:1.3**. Levofloxacin was found to be more stable in applied acid, base, thermal and photolytic stress conditions. Levofloxacin was sensitive to adopted stress condition like oxidation. The results proved that the developed assay method has good selectivity and specificity, and is suitable for assay of Levofloxacin in the presence of stress degradation products.

Method precision: The precision of test method was evaluated by doing assay for six samples of Levofloxacin injection as per test method. The content in mg and % label claim for Levofloxacin for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The chromatogram was shown in **Figure: 1.6** and data were shown in **Table: 1.4**.

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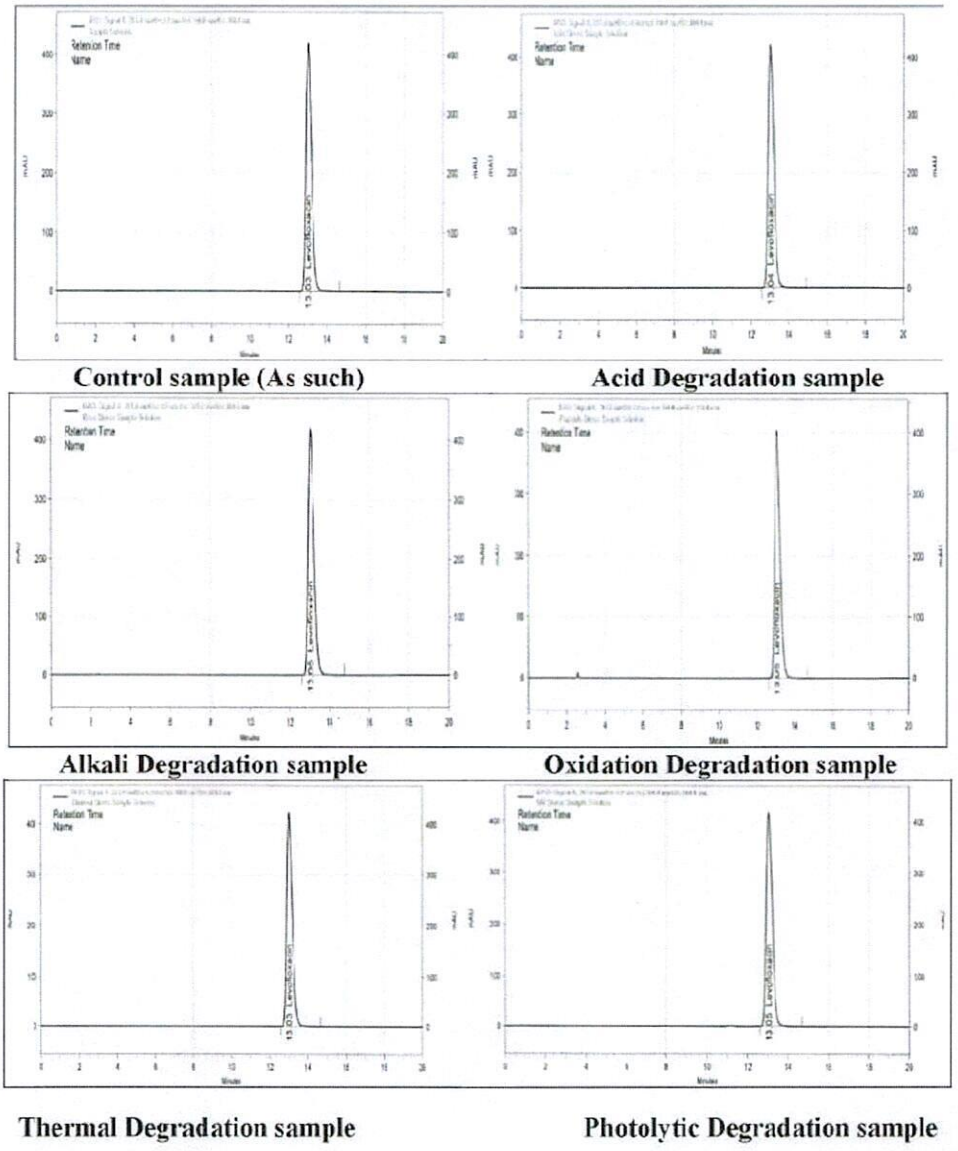

Figure: 1.5. Degradation chromatograms for Levofloxacin.

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Table: 1.3. Forced degradation results of Levofloxacin

Degradation condition	Levofloxacin % Assay	% Degradation	Peak Purity
Unstressed Sample	105.16	NA	1
0.1N HCl/60°C for 2 hours	106.47	No degradation observed	1
0.1N NaOH/ 60°C for 2 hours	105.59	No degradation observed	1
3% H_2O_2/60°C for 30 min	101.26	3.90	1
Thermal 105°C for 48 hours	106.56	No degradation observed	1
UV Light at 254nm for 7 days	104.98	0.18	

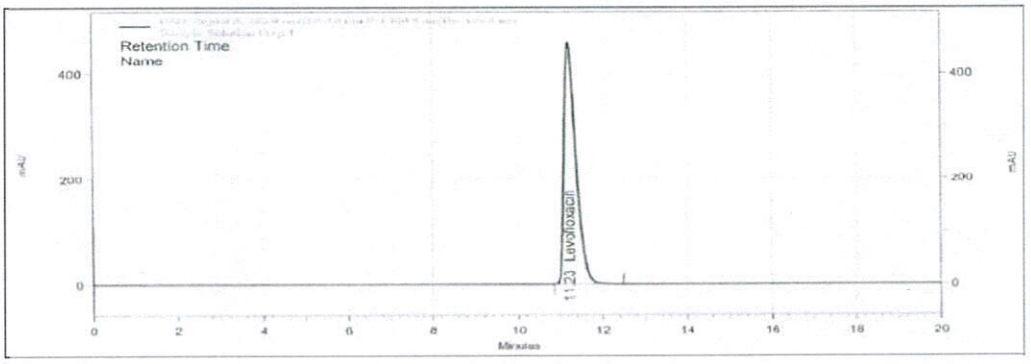

Figure: 1.6. Method precision sample chromatogram

Table: 1.4 Method precision data for Levofloxacin

No. of injections	Levofloxacin % assay
1	106.2
2	105.7
3	105.3
4	105.1
5	104.5
6	105.4
Average	**105.4**
SD	**0.57**
% RSD	**0.54**

4.3 Intermediate Precision : The intermediate precision of test method was demonstrated by carrying out method precision study in six samples, representing a single batch by two different analysts on two different days, different column, different HPLC system and by different analyst. These samples were prepared as per the test method. The % assay was calculated for each of these samples. The precision of the method was evaluated by computing the % Relative standard deviation of % assay of Levofloxacin.

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Table: 1.5 Intermediate precision data for Levofloxacin

S.No.	Area of Levofloxacin	Assay of Levofloxacin
1.	186778849	104.4
2.	187067790	104.6
3.	187523508	104.9
4.	187333783	104.8
5.	187033298	104.6
6.	186616560	104.4
	Average	**104.6**
	%RSD	**0.19**

- Overall and individual % of Assay are complies as per test method specification.
- The relative standard deviation of six assay preparations is **0.19.**
- The overall relative standard deviation of six assay preparations of precision study and six assay preparations of intermediate precision study is **0.54.**

4.4 Linearity of detector response: The standard curve was obtained in the concentration range of 100.07-300.21ig/ml for Levofloxacin. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r] of standard curve were calculated and given in **Figure: 1.7** to demonstrate the linearity of the proposed method. From the data obtained which given in **Table: 1.6** the method was found to be linear within the proposed range.

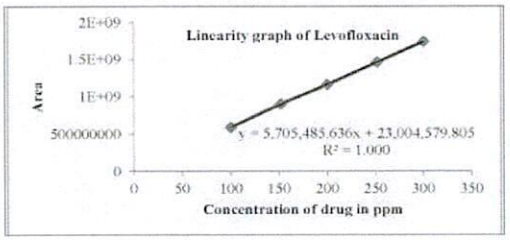

Fig. 1.7. Calibration curve for Levofloxacin

Table: 1.6 Linearity studies for Levofloxacin by proposed method

% Level	Concentration (ppm)	Levofloxacin Area
50	100.0713	590667579
75	152.1083	900530185
100	200.1425	1157439141
125	252.1796	1461276205
150	300.2138	1737499643
	correlation coefficient	0.9999
	Slope	5705485.636
	Intercept	23004636.17
	%Y-intercept	1.99

4.4 Accuracy:

The accuracy of the test method was demonstrated by preparing recovery samples of Levofloxacin at 50%, 75%, 100%, 125% and 150% of the target concentration level. The recovery samples were prepared in triplicate for each concentration level except 50% and 150 % (50% and 150% are six preparations). The above samples were chromatographed and the percentage recovery of each sample was calculated for the amount added. Evaluated the precision of the recovery at each level by computing the Relative Standard Deviation of six preparations for 50% and 150% level recovery samples results. The percentage recoveries with found in the range of 99.0 to 99.9 for Levofloxacin. The chromatogram was shown in **Figure: 1.8 to 2.2** the data obtained which given in **Table: 1.7** the method was found to be accurate.

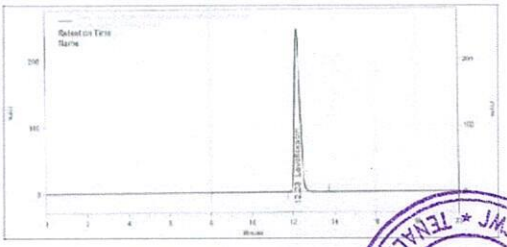

Fig. 1.8. Accuracy (Spike level 50%) chromatogram

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Table: 1.7 Recovery studies for Levofloxacin by proposed method
Recovery of Levofloxacin

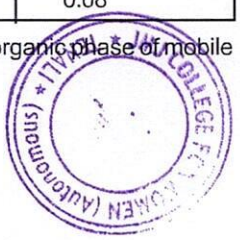
Sample	% Recovery	Mean % Recovery	%RSD
50% sample-1	100.9	101.0	0.27
50% sample-2	100.8		
50% sample-3	101.2		
50% sample-4	101.3		
50% sample-5	101.2		
50% sample-6	100.6		
75% sample-1	101.0	101.3	0.3
75% sample-2	101.3		
75% sample-3	101.6		
100% sample-1	101.8	101.8	0.25
100% sample-2	102.0		
100% sample-3	101.5		
125% sample-1	100.9	100.7	0.25
125% sample-2	100.7		
125% sample-3	100.4		
150% sample-1	101.5	101.4	0.28
150% Sample-2	101.7		
150% Sampe-3	101.1		
150% sample-4	101.2		
150% sample-5	101.7		
150% sample-6	101.1		

4.6 Robustness studies: To validate the method robustness the chromatographic performance at changed conditions was evaluated compared to nominal conditions of the method. Standard solution was injected at each of the following changed conditions:

Table: 1.8. Robustness studies Results

Parameter		Theoretical plates	Tailing factor	%RSD of peak area
Flow variation ±10%	10%	8599	1.4	0.07
	-10%	9820	1.5	0.12
Temperature variation ±5°C	+5°C	8467	1.3	0.16
	-5°C	8126	1.3	0.06
Mobile phase Variation± 10%	10	7622	1.4	0.10
	-10	8064	1.4	0.08

- Method is robust for changes like column oven temperature, flow rate and organic phase of mobile phase.



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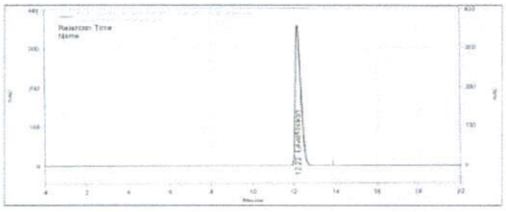

Fig. 1.9. Accuacy (Spike level 75%) chromatogram

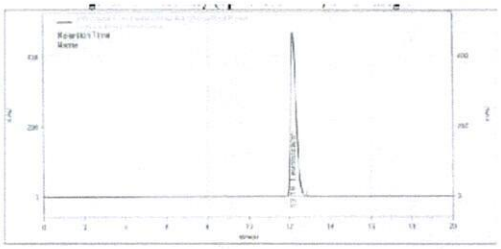

Fig. 2.0. Accuracy (Spike level 100%) chromatogram

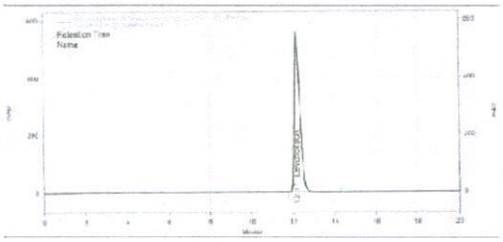

Fig. 2.1. Accuracy (Spike level 125%) chromatogram

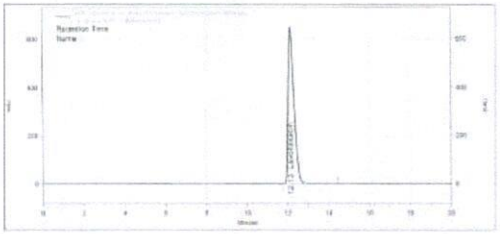

Fig. 2.2. Accuracy (Spike level 150%) chromatogram

4.7 Solution stability of analytical solutions: Levofloxacin standard and sample solutions were kept for about 48 hrs at room temperature in transparent bottles in auto sampler and in refrigerator 2-8°C. The response of these was compared with respect Initial standard solution and sample solution.

Table: 1.9. Results for solution stability of standard at room temperature

Time Interval	similarity factor
Initial	-
24hrs	1
48hrs	1

Table: 2.0 Results for solution stability of standard in Refrigerator

Time Interval	similarity factor
Initial	-
24hrs	1
48hrs	1

Table: 2.1 Results for solution stability of standard at room temperature

Time Interval	% Assay	% of Assay difference
Initial	106.2	NA
24hrs	107.52	1.32
48hrs	107.07	0.87

Table: 2.2 Results for solution stability of standard in Refrigerator

Time Interval	% Assay	% of Assay difference
Initial	106.2	NA
24hrs	107.85	1.65
48hrs	107.77	1.57

- Standard and sample solutions are stable for 48 hours when stored at room temperature (RT) and 2-8°C in refrigerator.

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5.0 Conclusion

An RP-HPLC method for estimation of Levofloxacin was developed and validated as per ICH guidelines. A simple, accurate and reproducible reverse phase HPLC method was developed for the estimation of Levofloxacin in bulk drugs and formulations. The optimized method consists of mobile phase 0.05 M solution of citric acid monohydrate and 10 ml of 1.0 M ammonium acetate buffer and acetonitrile in the ratio of (85:15 v/v) with Inertsil ODS-3V(250 x 4.6mm, 5µm) column. The retention time of Levofloxacin was found to be 11.20 minutes. The developed method was validated as per ICH Q2A (R1) guidelines. The proposed HPLC method was linear over the range of 100.07-300.21ppm, the correlation coefficient was found to be 0.9999. The percentage recoveries (accuracy) with found in the range of 99.0 to 99.9 for Levofloxacin. Relative standard deviation (%RSD) for method precision and intermediate precision was found to be 0.54 and 0.19. Solution stability of the Standard and sample solutions are stable for 48 hours when stored at room temperature (RT) and 2-8°C in refrigerator. Our developed method to be considered as fast, simple and reliable analytical method for determination of Levofloxacin in pharmaceutical preparation using RP-HPLC. As there is no interference of blank and placebo at the retention time of Levofloxacin, It is very fast with good reproducibility and response. Validation of this method was accomplished and getting results to meet all the requirements. The method is simple, reproducible, with a good accuracy and Linearity. It allows reliably the analysis of Levofloxacin in its different pharmaceutical dosage forms.

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Conflict of interests : The authors claim that there is no conflict of interest.

References

1. T. Kumar, A. Chitra, V. Amrithraj, and N. Kumar, "New RPHPLC method development and validation for estimation of Levofloxacin in tablet dosage form," Journal of Global Trends in Pharmaceutical Sciences, vol. 2, no. 3, pp. 264–276, 2011.
2. K. Kothekar, J. Balasundaram, A. Khandhar, and R. Mishra, "Quantitative determination of Levofloxacin and Ambroxol hydrochloride in pharmaceutical dosage form by reversed phase high performance liquid chromatography," Eurasian Journal of Analytical Chemistry, vol. 2, no. 1, 2007.
3. T. Santhoshi, K. Kumar, V. Rao, and A. Ravipati, "Development and validation of RP-HPLC method for simultaneous estimation of Levofloxacin and Ornidazole in pharmaceutical dosage form," Journal of Pharmacy Research, vol. 4, no. 11, p. 3864, 2011.
4. N. S. Lakka and N. Goswami, "A novel isocratic RP-HPLC method development and validation for estimation of 5HMF in Levofloxacin Hemihydrate intravenous infusion," International Journal of Research in Pharmaceutical Sciences, vol. 2, no. 1, pp. 45–51, 2011.
5. U. Neckel, C. Joukhadar, M. Frossard, W. J¨ager, M. Muller, and B. X. Mayer, "Simultaneous determination of Levofloxacin and ciprofloxacin in microdialysates and plasma by high performance liquid chromatography," Analytica Chimica Acta, vol. 463, no. 2, pp. 199–206, 2002.
6. J. Mehta, Y. Pancholi, V. Patel, N. Kshatri, and N. Vyas, "Development and validation of a sensitive stability indicating method for quantification of Levofloxacin related substances and degradation products in pharmaceutical dosage form," International Journal of PharmTech Research, vol. 2, no. 3, pp. 1932–1942, 2010.

Vol. 15 (1) 52-62 January 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.1.11

7. N. W. Sheikh, A. S. Tripathi, V. Chitra, A. Choudhury, and A. P. Dewani, "Development and validation of RP-HPLC assay for Levofloxacin in rat plasma and saliva: application to pharmacokinetic studies," African Journal of Pharmacy and Pharmacology, vol. 5, no. 13, pp. 1612–1618, 2011.

8. S. Rahar, S. Dogra, D. Panchru, P. Singh, and G. Shah, "Development and validation of UV-visible spectroscopic method for the estimation of Levofloxacin hemihydrate in bulk and marketed formulation," International Journal of Institutional Pharmacy and Life Sciences, vol. 1, no. 2, 2011.

9. A. A. Shirkhedkar and S. J. Surana, "Quantitative determination of Levofloxacin hemihydrate in bulk and tablets by UV-Spectrophotometry and first order derivative methods," Pakistan Journal of Pharmaceutical Sciences, vol. 22, no. 3, pp. 301–302, 2009.

10. V.N.Desai,O. E.Afieroho, B.O.Dagunduro, T. J.Okonkwo, and C. C. Ndu, "A simple UV spectrophotometric method for the determination of Levofloxacin in dosage formulations," Tropical Journal of Pharmaceutical Research, vol. 10,no. 1, pp. 75–79, 2011.

11. FDA, ICH-Q1A (R2): Stability Testing of New Drug Substances and Products, vol. 68, US Food and Drug Administration, Washington, DC, USA, 2nd edition, 2003.

12. FDA, ICH-Q1B: Photo-Stability Testing of New Drug Sub-Stances and Products, vol. 62, US Food and Drug Administration, Washington, DC, USA, 1997.

13. FDA, ICH-Q2 (R1): Validation of Analytical Procedures: Text and Methodology, vol. 60, US Food and Drug Administration, Washington, DC, USA, 1995.

14. United State Pharmacopoeia, The U.S. Pharmacopeia Convention, United State Pharmacopoeia, Rockville, Md, USA, 30th edition, 2007.

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FUNGAL ALKALINE PROTEASES: APPLICATIONS AND PROPERTIES

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ABSTRACT

Synthetic chemical usage enhanced day by day at both industrial level and medical fields, together causing vulnerability in the ecological pyramids and leads to loss of ecological equilibrium around the globe. In addition, the enormous growth of industries, affecting the health of both environment and community health. For the betterment of life to achieve in the community, step has taken to develop the different processes for eco-friendly products. Instead of chemical usage, enzymatic processes are the alternatives to attain the healthy environment. Take a glance on the Alkaline proteases derived from fungi having various industrial applications and eco-friendly in nature, includes textile, detergent, leather, feed, waste, water recycling, bioremediation, tannery effluent, effluent treatment and medical applications. The present discussion focused on alkaline proteases applications and properties. Type of production with various influencing parameters.
Interpreting the results and depicting the multifunctional properties used to promote fungal alkaline proteases became versatile in the fields of eco-friendly and medicinal applications, which has gained prominent usage of stable properties.

KEYWORDS: Fungal proteases, proteolytic enzymes, alkaline enzymes, physicochemical properties of enzymes, substrate-specific, applications of proteases.

INTRODUCTION:

Enzymes are highly specific and act under mild conditions than traditional chemicals (Elaine, Luo (2018)). Readily biodegradable and usually no toxic to the environment, dumped from the industries (Layla Filiciotto and Gadi Rothenberg 2020).

Industries produces many types of enzymes by using the various production processes, such as pulp and paper production (Industrial Uses Of Enzymes, Infinita Biotech, (2019); Jiménez et al., 1999; Nguyen et al., 2008), leather production (Dayanandan et al., 2003; Saravanabhavan et al., 2004; Valeika et al., 2009; Kandasamy et al., 2012; Vishnuvardhan Reddy and Sultanpuram, 2016), textile production (Aly et al., 2004; Vankar et al., 2007; Chen et al., 2007; Zhou et al., 2008), detergent production (Hemachander and Puvanakrishnan, 2000; Saeki et al., 2007), food production (Minussi et al., 2002; Ramos and Malcata, 2011), beverage production (Grassin and Fauquembergue, 1996; Okamura-Matsui et al., 2003), animal feed production (Gado et al., 2009; Zhu et al., 2011; Dalel Daassi et al., 2016), pharmaceuticals production (Bonrath et al., 2002; Woodley, 2008),fine chemicals production (Panke et al., 2004; Gavrilescu and Chisti, 2005), cosmetics production (Sim et al., 2000; Lods et al., 2001) and biodiesel production (Kumari et al., 2007; Hernandez-Martín and Otero, 2008).

Enzyme-catalysed processes are gradually replacing chemical processes in many areas of industry (Peter, Robinson (2015)). Enzymes have all the properties of true catalysts (Iva Colter (2020)). In the presence of an appropriate enzyme, a chemical reaction occurs at a much higher rate but the enzyme is not consumed by the reaction (Ganapati, Yadav and Deepali Magadum (2017)).

Recently, a greater awareness of conservation issues have forced industries with a history of polluting to consider alternative, cleaner methods so there is now significant growth of biotechnology outside of the pharmaceutical and food industries (Robert et al., 2020). Serine proteases are used in various industrial sectors such as food, pharmaceutical, leather, diagnostics and waste management (Gupta et al., 2002; Maurer 2004; Razzaq, Abdul et al., 2019).

At present, only about 20 enzymes are produced on a truly industrial scale. 35 producers make these enzymes with 4 major companies holding 75% of the market (Mayuri Sharma et al., 2019).

In 1992, the market value of these enzymes was approximately US$800 million with a predicted yearly increase of around 10- 15%. The market is highly competitive, suffers from over-capacity, has small profit margins and is technologically intensive. Matching an enzyme with a process is the greatest challenge to a research and its development cost in an industrial plant can be modified to accommodate the limitations of an enzyme but this is costly and a better approach is to find an enzyme more suited to the existing process (Gallezot, Pierre. (2008)).

Worldwide Status of Proteases:

Proteases termed as Industrial masters occupy the third largest position in enzyme production .Real Hernandez and Elvira Gonzalez de Mejia (2019); Swapna Vadlamani et al., 2012).

Proteases are the most important industrial enzymes accounting for approximately 40% of the total industrial enzyme market (Gupta et al., 2002; Alya Sellami-Kamoun et al., 2006; Anderson F. Santos et al., (2013)) Proteases and alpha amylases are the two most important industrial enzymes, together representing more than 70% of the total worldwide enzyme market (Ramachandran, et al., 2004; Hamid Mukhtar and Ikram-ul-Haq, 2008; Aline Machado de Castro et al., 2018).

It is reported that in the year 2005, the global proteolytic enzyme demand for protease was increased dramatically to 1–1.2 billion dollars (Neetu, Jabalia., Mishra, and Chaudhary, Nidhee. P.C. (2014);Godfrey and West, 1996; Vida Maghsoodi et al., 2014).

Microbial protease (Proteolytic enzymes):

Constitutes one of the most valuable groups of industrial enzymes that cater to the requirements of nearly 60% of the world enzyme market (Rao et al., 1998; Woods et al., 2001; Ramnani et al., 2005; Hamid Mukhtar and Ikram-Ul-Haq, 2009; Nirmal et al., 2011; Zanphorlina, et al.,2011; Nilgun Tekin1 et al., 2012; Jayasree et al., 2013; Nai-Wan Hsiao et al., 2014; Vida Maghsoodi et al., 2014); Vida Maghsoodi et al., 2014).

Among the various proteases, bacterial proteases are the most significant (Gupta et al., 2002; Shivasharana et al., 2012) and accounts for nearly 60% of the total worldwide enzyme sales (Adinarayana et al., 2003; Beg et al., 2003; Shivasharana et al., 2012).

According to Johnvesly et al., 2002, 2/3 of the total amount of the enzymes sale in the world is of microbial origin (Johnvesly et al., 2002). 5 billion in 2000 (Kirket al., 2002). As per the forecast, the global demand for enzymes will rise 7% per annum through 2006 to $6 billion in 2011 (McCoy, 2000).

Proteases, also referred to as peptidases or proteinases, are one of the most useful, large category of degradative enzymes that catalyze the hydrolysis of peptide bonds into peptides and amino acids (Lopez- Otin and Bond, 2008; Jayasree et al., 2013; Lakshmi, et al., 2013; Nai-Wan Hsiao et al., 2014). Proteases hydrolyze protein by adding water across peptide bonds and break them in to smaller peptides in organic solvents (Al-Shehri, 2004; Charu Lata et al., 2014).

Proteases are ubiquitous, being found in a wide diversity of sources such as plants, animals, and microorganisms (Alnahdi, 2012; Charu Lata et al., 2014). Proteolytic enzymes are high temperature resistant with high specific activities, execute a wide variety of functions and superior physical and chemical characteristics which seem to be good for future biotechnological applications, that is why they have wide applications in a large number of industrial processes and are produced in large quantities by microorganisms through the fermentation process (Rao et al., 1998; Gupta et al.,2002; Mohan et al., 2005; Temiz et al., 2008; Hamid Mukhtar and Ikram-ul-Haq, 2008; Hamid Mukhtar et al., 2009; Zanphorlina et al., 2011; Mukesh Kumar et al., 2012).

They have diverse applications in a wide variety of industries (Kumar, Takagi 1999; De Coninck et al., 2000; Gupta et al., 2002a; Gupta et al.,2002b; Mohan et al., 2005; Rai and Mukherjee, 2009; Swapna Vadlamani et al., 2012ani et al., 2012) such as detergent, food, baking, brewing, meat tenderization, cosmetics, peptide synthesis, waste treatment (Gupta et al., 2002; Padmavathi, 2013; Charu Lata et al., 2014), pharmaceutical, leather, silk, medicine diagnostics and therapeutic applications (Ward, 1985) the recovery of silver from used X-ray films (Cowan,1996; Kumar and Takagi, 1999; Alya Sellami-Kamoun et al., (2006) and bioremediation processes (Horikoshi (1999); Nilegaonkar et al., 2002; Kanekar et al., 2002; Denizci et al., 2004; Anwar and Saleemuddin, 1998; Gupta et al., 2002; Padmavathi, 2013).

Sources of Proteases:
Proteases are ubiquitous and physiologically one of the most important classes of enzymes that are expressed as essential constituents of all living organisms, on earth in a wide diversity of sources such as plants, animals, and microorganisms (Alnahdi, 2012) including prokaryotes (bacteria, archea), viruses, fungi (Gupta et al., 2002; Joo et al., 2003; Dubey et al., 2007; Shivakumar Srividya; Majumdar Mala, 2009; Shivasharana et al., 2012; Padmavathi, 2013).

The main sources of the enzymes were from animals (e.g. calf stomach), plants (e.g. pineapple, fig and papaya), microbes (e.g. Bacillus spp., Pseudomonas spp.) (Puri et al., 2002; Swapna Vadlamani et al., 2012; Shafee et al., 2005; Rao et al., 1998).

At least a quarter of the microorganisms such as bacteria, fungi, yeast, plant and mammalian tissues are known to produce alkaline proteases (Ellaiah et al., 2002; Prakasham et al., 2005; Alya Sellami-Kamoun et al., 2006; Mukesh Kumar et al., 2012).

Alkaline proteases: (EC.3.4.21–24, 99; EC 3.4.21-24)
The alkaline proteases are defined as the proteases that are active or work optimally in a neutral to alkaline pH range (Barett, 1994; Gupta et al., 2002; Lakshmi, et al., 2013) generally between 9.0 and 11.0, with the exception of a few higher pH values of about 12.0 and 13.0 and are used widely in washing powders and to dehair hides (Kumar and Takagi, 1999; Gupta et al., 2002; Ibrahim et al., 2007; Shampa Sen et al., 2009; Nilgun Tekin et al., 2012; Gupta et al., 2012; Sangeeta Saxena et al., 2014).

They either have a serine center (serine protease) or are of metallotype (metalloprotease) and the alkaline serine proteases are the most important group of enzymes exploited commercially (Ainsworth,1994; Fujiwara et al.,1993; Rao et al.,1998; Sangeeta Saxena et al., 2014;

In recent years, the use of alkaline proteases in a variety of industrial processes like detergents, food, leather and silk has increased remarkably (Kembhavi et al., 1993; Gessesse, 1997; Shampa Sen et al., 2009; Ponnuswamy Vijayaraghavan et al., 2013).

Enzymatic depilation has been widely accepted as a sound alternative to the chemical process (Taylor et al., 1987; Roja Rani et al., 2012; Gupta, 2017).

The vast biochemical diversity of alkaline protease of microbial origin with elevated activity and stability in the high alkaline range especially those from bacteria and fungi (Madan et al., 2002; Kalpana Devi et al., 2008; Jellouli et al., 2009; Wang et al., 2011) and the specificity of their action at alkaline pH (Ganesh Kumara and Hiroshi Takagib, 1999), emerging novel properties and their exotic catalytic nature have attracted worldwide scientific community of protein chemistry and protein engineering and applied fields (Lakshmi et al., 2013; Jayasree, et al., 2013) to exploit their physiological, bioengineering and biotechnological applications (Poldermans, 1990; Fox et al., 1991; Rao et al.,1998; Kumar and Takagi, 1999; Agarwal et al., 2004; Devi et al., 2008; Asokan and Jayanthi, 2010; Nirmal et al., 2011; Shivasharana et al., 2012; Lakshmi et al.,2013; Sangeeta Saxena et al., 2014).

The full potential of alkaline proteases in industrial processes is yet to be fully exploited (Cowan, 1996; Kumar and Takagi, 1999; Shampa Sen et al., 2009; Nirmal et al., 2011; Nilgun Tekin1 et al., 2012; Lakshmi et al., 2013; Jayasree et al., 2013).

Disadvantages of alkaline proteases:
Although enzymatic technology is very promising, it has limitations (Abdul Razzaq et al., 2019). Microbes can reproduce and increase their population in order to consume a large amount of substrate, but extracellular enzymes like alkaline protease cannot (Beti Vidmar and Maša Vodovnik (2018)). Enzymes cannot reproduce themselves, meaning that any increase in enzyme population must come from outside of the system namely, humans adding more enzymes to the system (Ayla Sant'Ana da Silva et al., 2020). It has also been shown that these alkaline proteases lose some reactivity after they interact with pollutants and could eventually become completely inactive (Xiao-lin Ao et al., 2018).

Advantages of alkaline proteases:
Among the different types of microbial proteases the most commercially important are the alkaline proteases, especially those from the fungal sources and were obtained from Numerous moulds especially belonging to the genera Aspergillus species such as Aspergillus sydowi (Danno and Yoshimura 1967), A.mellieus (Ito and Sugira 1968; Luisetti et al., 1991) and Aspergillus oryzae (Ito and Sugira 1968), A.fumigatus (Monod et al., 1991; Larcher et al., 1992; Roja Rani et al., 2012), A.flavus (Malathi and Chakraborthy 199), some strains of A. niger (Heneri et al., 1988) and A.sojae (Hayashi et al., 1967; Abdul Razzaq et al., 2019). Fungi elaborate a wider variety of enzymes than do bacteria.

For example, Aspergillus oryzae produces acid, neutral and alkaline proteases (Padmavathi, 2013). Fungal proteases can conveniently be produced in solid-state fermentation process (Chander, Mukesh. (2019)).

Proteases as Detergent additives:
The use of proteases in laundry detergents accounts for approximately 25% of the total worldwide sales of enzymes (Mecikoglu et al., 2006; Temam Abrar Hamza (2017)). Alkaline proteases useful for detergent applications were mostly active in the pH range 8 - 12 and at temperatures between 50°C-70°C (AlShehri, 2004). Many investigators (Kim et al., 2001; Charu Lata et al., 2014) studied optimal culture condition for protease productivity. Proteases produced by Bacillus spp., find a wide variety of application in detergents, leather industries, food and pharmaceutical industries (Ammar, et al., 1991).

Alkaline proteases are of special interest as they have excellent washing performance at pH (9-11), which removes protein based stains in both laundry and automatic dish washing detergents (Saeki et al., 2007; Dias et al., 2008; Mukesh Kumar et al., 2012).Bacteria (Hamid Mukhtar; Ikram-ul-Haq, 2008) mainly produce the proteases used in detergent industries. 1991; Jany and Mayer 1985; Kwon et al., 1994).A combination of lipase, amylase and cellulase is expected to enhance the performance of protease in laundry detergents (Padmavathi, 2013).

Proteases in Leather Industry:
The proteases widely used in leather industries (Kalisz, 1988; Inhs et al., 1999; Kumar and Tagaki, 1999; Ramachandranet al., 2004; Hamid Mukhtar and Ikram-ul-Haq, 2008).

The conventional methods in the leather processing industry is one of the worst offenders of the environment, due to pre-tanning operations involves quite toxic chemicals such as hydrogen sulphide creating environmental pollution, effluent disposal (Rani et al., 2012) and safety hazards (Okumura, et al., 1977; Malathi and Chakraborthy, 1991; Shivasharana et al., 2012; Padmavathi, 2013). Proteases are used for selective hydrolysis of non-collagenases constituents of the skin and for removal of nonfibrillarproteins such as albumins and globulins (Padmavathi, 2013).Of particular importance are the proteases with activity at alkaline pH and high temperatures (Kumar and Takagi, 1999; Thangam and Rajkumar, 2002; Agrawal et al., 2012).

Thus, for environmental reasons, the biotreament of leather using an enzymatic approach is preferable as it offers several advantages, e.g. easy control, speed and waste reduction, thus being ecofriendly (Shampa Sen et al., 2009).

Alkaline proteases with elastolytic and keratinolytic activity can be used in leather-processing industries.

Alkaline proteases alone account for 20% of the world enzyme market with their predominant use in leather processing and detergent industries (Oberoi et al., 2001; Saeki et al., 2007; Dias et al., 2008; Mukesh Kumar et al., 2012 Agrawal et al., 2012).

Proteases in Food and Pharmaceuticals:
Proteases are widely used in brewing, baking, tenderization of meat, dairy industry, synthesis of aspartame, developing effective therapeutic agents, treatment of wounds (Kalisz, 1988; Cowan, 1996; Kumar and Takagi, 1999; Inhs et al., June 1999; Kumar and Tagaki, 1999; Ramachandran et al., 2004; Alya Sellami-Kamoun et al., 2006; Hamid Mukhtar and Ikram-ul-Haq, 2008; Mayuri Sharma et al., 2019). Proteases are invariably used in tonics, especially for indigestion. Fungal alkaline proteases are also used in food protein modification (Rao et al., 1998; Southan, 2001; Padmavathi, 2013; Charu Lata et al., 2014).

Proteases to be used in the food industry are mainly produced by fungi, whereas the proteases used in leather or bacteria (Hamid Mukhtar and Ikram-ul-Haq, 2008; Noora Barzkar (2020)) mainly produce detergent industries. Alkaline proteases are of special interest as they could be used in manufacture food, such as

cheese making baking, preparation of soya hydrolysates, and meat ten-derization pharmaceuticals (Ferrero et al., 1996; Saeki et al., 2007; Dias, et al., 2008 and Mukesh Kumar et al., 2012).

Dairy Industry:
The major application of proteases in the dairy industry is in the manufacture of cheese (Petra Philipps-Wiemann (2018)).

The proteases produced by GRAS (genetically regarded as safe)-cleared microbes such as Mucor michei, Bacillus subtilis, and Endothia parasitica are gradually replacing chymosin in cheesemaking (Rao et al., 1998). In 1988, chymosin produced through recombinant DNA technology was first introduced to cheesemakers for evaluation. Genencor International increased the production of chymosin in Aspergillus niger var. awamori to commercial levels (Xiangyang Liu and Chandrakant Kokare (2017)).

Uses of proteases in Baking Industry:
Endo- and exoproteinases from Aspergillus oryzae are extensively used in production of foods such as syrups, alcohol, fruit juices, brewing, chocolate syrup, baking and meat tenderizing (Luis Carlos Gioia et al., 2017).

Synthesis of Aspartame:
The use of aspartame as a no calorific artificial sweetener has been approved by the Food and Drug Administration. An immobilized preparation of thermolysin from Bacillus thermoprotyolyticus is used for the enzymatic synthesis of aspartame (Charu Lata et al., 2014; Andreas Abelsson (2020)).

Brewing Industry:
The brewing industry is a major user of proteases. In the production of brewing wort Bacillus subtilis protease are used to solublize protein from barley adjuncts, thereby releasing peptides and amino acids which can fulfil the requirement of the nitrogen supply (Agrahari,2011; Rani et al., 2012, Kanupriya et al., 2017).

Medical Usage:
Kudrya and Simonenko (1994) exploited the elastolytic activity of B. Alkaline-fibrinolytic protease has been reported to preferentially degrade fibrin suggesting its future application in thrombolytic therapy and anticancer drugs (Mukherjee and Rai, 2011; Simkhada et al., 2010b; Charu Lata et al., 2014).

Therapeutics:
The use of immobilized alkaline protease from Bacillus subtilis possessing therapeutic properties has been studied for development of soft gel based medicinal formulas, ointment compositions, gauze, non-woven tissues and new bandage materials. Oral administration of proteases from Aspergillus oryzae has been used as a diagnostic aid to correct certain lytic enzyme deficiency syndromes (Rao et al., 1998). Alkaline-fibrinolytic protease has been reported to preferentially degrade fibrin suggesting its future application in thrombolytic therapy and anticancer drugs (Mukherjee and Rai, 2011; Simkhada et al., 2010b; Charu Lata et al., 2014).

Pharmaceutical Industry:
Proteases are useful in the field of medicine also where they have some diagnostic and therapeutic applications (Ward, 1985). Oral administration of proteases from Aspergillus oryzae (Luizym and Nortase) has been used as a digestive aid to correct certain lytic enzyme deficiency syndromes (Rani et al., 2012). An asparginase isolated from E. coli is used to eliminate asparginc from the bloodstream in the various forms of lymphocytic leukemia (Rao et al., 1998; Rani et al., 2012).Proteases are useful in the field of medicine also where they have some diagnostic and therapeutic applications (Ward, 1985).

Proteases in silk degumming in Textile Industry:
Serein, which is about 25%, is conventionally removed from the inner core of fibroin by conducting shrink proofing and twist-setting for the silk yarns, using starch (Kanehisa, 2000).

Photographic Industry-Silver recovery:
Alkaline protcases arc used in silver rccovery from used X-ray films by decomposing the gelatinous coating on them (Cowan, 1996; Kumar and Takagi, 1999; Anwar and Saleemuddin, 2000; Alya Sellami-Kamoun et al., 2006).

Used X-ray film contains approximately 1.5% to 2.0% (by weight) silver in its gelatin layers. Conventionally, this silver is recovered by burning the films, which causes undesirable environmental pollution (Ray, 2012; Charu Lata et al., 2014), hence the enzymatic hydrolysis of the gelatin layers on the X-ray film enables the recycling of both silver and poly-ester film base (Debette, 1991; Shampa Sen et al., 2009). Alkaline protease from B. subtilis decomposed the gelatin layer within 30 min at 50-60°C and released the silver.

Alkaline Proteases, a tool to clean environment/Waste Management:
Extracellular enzymes have ability to enhance bioremediation because of break down bonds within organic compounds and/or catalyse and transform into less toxic and more biodegradable forms (Vipul Kumar et al., 2019). Alkaline proteases are being able to reduce pathogen counts, solids content, and increase deflocculation in sludge (Passanai, (2020)).

Management of Industrial and House-hold wastes:
Protein-based residues usually represent the most significant potential foul ants within food bioprocess sectors, such as milk- and meat-processing operations. Pretreatment with NaOH, mechanical disintegration, and enzymatic hydrolysis resulted in total solubilization of the feathers. The ended product was a heavy, grayish powder with a very high protein content, which could be used as a feed additive (Mayuri Sharma et al., 2019). Similarly, many other keratinolytic alkaline proteases were used in feed technology for the production of amino acids.

Peptide Synthesis:
Enzymatic peptide synthesis offers several advantages over chemical methods, e.g. reactions can be performed stereo specifically and reactants do not require side-chain protection, increased solubility of non-polar substrates, or shifting thermodynamic equilibria to favor synthesis over hydrolysis (Wuts, Peter and Greene, Theodora (2006) In addition to demonstrating high organic tolerance, alkaline proteases from B.pumilus strain CBS and Streptomyces sp. strain AB1 are potential strong canditates for use in peptide synthesis in low water systems (Jaouadi et al., 2011; Mikawlrawng (2016).

CONCLUSION AND FURTHER APPLICATIONS:

There is a renewed interest in proteases as targets for developing therapeutic agents against relentlessly spreading fatal diseases such as cancer, malaria, and AIDS.

Expansion of biotechnology tools offers a constructive position in the development of proteases facilitate their applications to provide a sustainable environment achieved to attain the healthy human life. Acknowledgments: I would like to thank Dr. Suresh Vundavalli for his continuous help and encouragement to complete this article. For the provision of required amenities and facilities of the J.M.J.College, Tenali, Guntur, Andhra Pradesh and India to carry out the research and bring this article to print form.

REFERENCES:

I. Abdalla Mohamed Abdullah Maitig., Mohamed A.M. Alhoot and Kartikya Tiwari (2018). Isolation and Screening of Extracellular Protease Enzyme from Fungal Isolates of Soil, J Pure Appl Microbiol. Vol (12)4:2059-2067.

II. Abdul Razzaq., Sadia Shamsi., Arfan Ali., Qurban Ali., Muhammad Sajjad., Arif Malik and Muhammad Ashraf (2019). Microbial Proteases Applications, Front. Bioeng. Biotechnol., Vol (12).

III. Abdul Razzaq., Sadia Shamsi., Arfan Ali., Qurban Ali., Muhammad Sajjad., Arif Malik and Muhammad Ashraf (2019). Microbial Proteases Applications, Front Bioeng Biotechnol, Vol (7):110.

IV. Abdullah, R., Ashraf, H. and Haq, I (2003). Optimization and kinetic analysis of carbon sources on the production of alpha amylase by Saccharomyces cerevisiae. J. Food Technol. 1(4), 187-190.

V. Adinarayana, K. and Ellaiah, P (2002). Response surface optimization of the critical medium components for the production of alkaline protease by a newly isolated Bacillus sp.J. Pharm. Pharmaceut. Sci. 5(3), 272-278.

VI. Adinarayana-Kunamneni., Ellaiah Poluri. and Davuluri, Siva Prasad., (2003). Purification and partial characterization of thermostable serine alkaline protease from a newly isolated Bacillus subtilis PE-11. AAPS. Pharm. Sci. Tech, 4 (4), 56.

VII. Adriano A. Mendes, Heizir F. de Castro Raquel de L. C. Giordano, Screening of organic supports and activation protocols for immobilization and stabilization of lipase from Thermomyces lanuginosus (2013). Quím. Nova 36:2.

VIII. Agarwal, D., Patidar, P., Banerjee, T.and Patil, S., (2004). Production of alkaline protease by Penicillium sp. Under SSF conditions and its application to soy protein hydrolysis. Process Biochem, 39, 977-981.

IX. Agrawal R, R. Singh, A. Verma, P. Panwar and A.K. Verma, (2012). Partial Purification and Characterization of Alkaline Protease from Bacillus sp. Isolated from Soil World Journal of Agricultural Sciences 8 (1): 129-133, 2012.

X. Akimkina, T. V., E. A. Nosovskaya, and S. V. Kostrov. (1992). Cloning and expression of the neutral proteinase gene of Bacillus cereus in Bacillus subtilis cells. Mol. Biol. 26:418–423. 101.

XI. Aline Machado de Castro., Anderson Fragoso dos Santos., Vasiliki Kachrimanidou., Apostolis A.Koutinas., Denise M.G.Freire (2018).Chapter 10 - Solid-State Fermentation for the Production of Proteases and Amylases and Their Application in Nutrient Medium Production, Current Developments in Biotechnology and Bioengineering, Current Advances in Solid-State Fermentation:185-210.

XII. Allen, C., V. K. Stromer, F. J. Smith, G. H. Lacy, and M. S. Mount. (1986).

XIII. Ammerer, G., C. P. Hunter, J. H. Rothman, G. C. Saari, L. A. Valls, and T. M. Stevens. (1986). PEP4 gene of Saccharomyces cerevisiae encodes proteinase A, a vacuolar enzyme required for processing of vacuolar precursors.Mol. Cell. Biol. 6:2490–2497.

XIV. Antonov. (1988). Cloning, expression and structure of the truncated functionally active lon gene of Escherichia coli. Bioorg. Khim. 14:408–411.

XV. Argos, P. (1987). A sensitive procedure to compare amino acid sequences. J. Argos, P., G. Kamer, M. J. H. Nicklin, and E. Wimmer. (1984). Similarity in gene organization and homology between proteins of animal picornaviruses and a plant comovirus suggest common ancestry of these virus families. Nucleic Acids Res. 12:7251–7267.

XVI. Ashitha A.; Jyothis Mathew. and Radhakrishnan E. K., (2016) Alkaline protease production by Bacillus sp. hap-1, a Halophilic bacterium isolated from

a solar evaporation pond. World Journal of Pharmacy and Pharmaceutical Sciences, Vol 5(2): 808-815.

XVII. Atsumi, Y., S. Yamamoto, K. Morihara, J. Fukushima, H. Takeuchi, N. Mizuki, S. Kawamoto, and K. Okuda. (1989). Cloning and expression of the 102 alkaline proteinase gene from Pseudomonas aeruginosa IFO 3455. J. Bacteriol.171:5173–5175.

XVIII. Austew, B. M., and E. L. Smith. (1976). Action of staphylococcal proteinase on peptides of varying chain length and composition. Biochem. Biophys.Res. Commun. 72:411–417. Avakov, A. S., A. P. Bolotin, and S. V. Sorokin. (1990). The structure of Bacillus brevis metalloprotease gene. Mol. Biol. 24:1363–1372.

XIX. Ayla Sant'Ana da Silva, Roberta Pereira Espinheira, Ricardo Sposina Sobral Teixeira, Marcella Fernandes de Souza, Viridiana Ferreira-Leitão and Elba P. S. Bon (2020) Constraints and advances in high-solids enzymatic hydrolysis of lignocellulosic biomass: a critical review, Biotechnology for Biofuels, Vol Vol(13)58.

XX. Balakumaran M.D. and P.T. Kalaichelvan, (2012). Production and Purification of Alkaline Protease fromBacillus sp. MPTK 712 Isolated from Dairy Sludge Global Veterinaria 8: 433-439.

XXI. Ballinger, M. D., J. Tom, and J. A. Wells. (1996). Furilisin: a variant of subtilisin BPN9 engineered for cleaving tribasic substrates. Biochemistry 35:13579–13585.

XXII. Battaglino R. A. , M. Huergo, A. M. R. Pilosof, G. B. Bartholomai (1991). Culture requirements for the production of protease by Aspergillus oryzae in solid state fermentation, Applied Microbiology and Biotechnology., 35: 292-296.

XXIII. Beg, K.B., Sahai, V., and Gupta, R., (2003). Statistical media optimization and alkaline protease production from Bacillus mojavensis in a bioreactor. Process Biochem, 30: 203-209.

XXIV. Berger A, Schechter I. (1970). Mapping the active site of papain with the aid of peptide substrates and inhibitors. Philos Trans R Soc B 12:249–64. 103.

XXV. Beti Vidmar and Maša Vodovnik (2018). Microbial Keratinases: Enzymes with Promising Biotechnological Applications, Food Technol Biotechnol, Vol (56)3: 312–328.

XXVI. Carlile, M. J., Watkinson, S. C. And Goody, G. W., (2001). The fungi. Ed. 2nd , Academic Press, London. 475-476. • Chakrabarti, S.K., Matsumura, N. And Ranu, R.S., (2000). Purification and characterization of an extracellular alkaline serine protease from Aspergillus terreus. Curr. Microbiol., 40: 239-243.

XXVII. Chaloupka, J (1985). Temperature as a factor regulating the synthesis of microbial enzymes. Microbiol Sci 2:86-90.

XXVIII. Charles,P., Devnathan, V., Periasamy, A.,Kalaichelvan, P.T., and Hur, B.K. (2008).Purification, characterization and crystallization of an extracellular alkaline protease from Aspergillus nidulans HA-10, Journal of basic microbiology.48:347-352.

XXIX. Chen, M. Federico and M. Cettolo. (2007). Better Nbest Translations through Generative n-gram Language Models. In Proceeding of MT Summit XI. Copenhagen, Denmark.

XXX. Christensen, M. (1981). A synoptic key and evaluation of species in the Aspergillus flavus group. Mycologia 73: 1056–1084.

XXXI. Complementation of an Erwinia carotovora subsp. carotovora protease mutant with a protease encoding cosmid. Mol. Gen. Genet. 202:276–279.

XXXII. Conn, E. E., Stumpf, P. K., Bruening, G. And Doi, R. H., (1987). Outlines of biochemistry. 5 : 115-164, John Wiley & Sons, Inc. Singapore.

XXXIII. Cowan D. (1996). Industrial enzyme technology. Trends Biotechnology. 14: 177-178. 104 Dalel Daassi, Hela Zouari-Mechichi Lassaad Belbahri Jorge Barriuso Maria Jesus Martinez Moncef Nasri Tahar Mechichi (2016). Phylogenetic and metabolic diversity of Tunisian forest wood-degrading fungi: a wealth of novelties and opportunities for biotechnology,3 Biotech (2016) 6:46.

XXXIV. Danno, G&Yoshimura S. (1967). Studies on an alkaline proteinase of Aspergillus sydowi part 1: Purification and some properties of the proteinase. Agri Bio chem. 31:1151-1158.

XXXV. Dayanandan, A., J. Kanagaraj, L. Soundarray, R. Govindaraju and G. S. Rajkumar (2003). Application of alkaline protease in leather processing: an eco-friendly approach, J. Cleaner Production, 11: 533-536.

XXXVI. De Coninck J, Bouquelet S, Dumortier V, Duyme F, Verdier-Denantes I (2000). Industrial media and fermentation processes for improved growth and protease production by Tetrahymena thermophila. J Ind Microbiol Biotech 24:285–290.

XXXVII. Dias, D.R., D.M. Vilela, M.P.C. Silvestre and R.F. Schwan, (2008). Alkaline protease from sequencing of Bacillus sp. isolated from coffee bean grown on cheese whey. World J. Microbiol. Biotechnol. 24: 2027-2034.

XXXVIII. Domsch, K.H., Gams, W. and Anderson, TW. (1993). Compendium of Soil Fungi. IHWVerlag Press.

XXXIX. Dubey, K. V., Monu, P., Bishal, K. S. and Medichan, V.J., (2007). Papain-like proteases: Applications of their inhibitor. African Journal of Biotechnology, 6(9), 1077-1086.

XL. Elaine K. Luo, M.D. (2018). Enzymes: How they work and what they do, Tim Newman.

XLI. Ellaiah, P., B. Srinivasulu and K. Adinarayana, (2002). A review on microbial alkaline proteases. J. Sci. Ind. Res., 61: 690-704.

XLII. El-Zalaki, E.M., Mohasse, Z.S. And Mohamed, M.S., 1974. Partial purification of proteased by Aspergillus oryzae. Alexanderian J. agric. Res., 22: 63-71.

XLIII. Fogarty, W.M.; Dooyle, E.M.; Kelly, C.T. (1999). Comparison of the action pattern 2 high maltose forming alpha amylase on linear malto oligosaccharides. Enzyme Microbiol. Technol. 25:330-335.

XLIV. Frankena J, Koningstein GM, van Verseveld HW et al (1986). Effect of different limitations in chemostat cultures on growth and production of exocellular protease by Bacillus licheniformis. Appl Microbiol Biotechnol106- 112.

XLV. Gado, H.M., A.Z.M. Salem, P.H. Robinson and M.Hassan, (2009). Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Anim. Feed Sci. Technol., 154: 36-46.

XLVI. Gallezot, Pierre. (2008). Catalytic Conversion of Biomass: Challenges and Issues. ChemSusChem, Vol (1): 734-7.

XLVII. Ganapati D. Yadav and Deepali B. Magadum (2017). Kinetic Modelling of Enzyme Catalyzed Biotransformation Involving Activations and Inhibitions, Enzyme Inhibitors and Activators.

XLVIII. Gaston Ezequiel Ortiz, Diego Gabriel Noseda, Maria Clara Ponce Mora, Matías Nicolas Recupero, Martin Blasco, and Edgardo Alberto, (2016). A Comparative Study of New Aspergillus Strains for Proteolytic Enzymes Production by Solid State Fermentation, Enzyme Research, 2016 (2016): 11

XLIX. Gaurab Karki (2020). Microbial Proteases: Industrial Application and production process.

L. Gavrilescu, M & Chisti, Y 2005, 'Biotechnology - a sustainable alternative for chemical industry', Biotechnology Advances, 23: 471–499.

LI. Genckal H, C. Tari (2006). Alkaline protease production from alkalophilic Bacillus sp. isolated from natural habitatsEnzyme and Microbial Technology, 39: 703–710.

LII. Gessesse, A., (1997). The use of nug meal as a low-cost substrate for the production of alkaline protease by the alkaliphilic Bacillus sp. AR- 009 and some properties of the enzyme. Bioresour. Technol. 62: 59–61.

LIII. Global Enzymes Market Expected to Reach $10,519 Million by 2024, Allied Market Research (PRNewsfoto/Allied Market Research) News Provided, Allied Market Research(2018).

LIV. Gomma, M.A., Mostafa, M.M., Abouzied, M.M. and Habashy, M.E.L., (1989). Production of semi-alkaline proteases by Aspergillus sp. Egypt. J. Fd. Sci., 16: 9-26.

LV. Govardhan CP, Margolin AL. (1995). Extremozymes for industry— from nature and by design. Chem Ind 2:689–693. 107 • Grassin, C. and P. Fauquembergue. (1996). Wine; and Fruit juices. In: Industrial Enzymology (2ndEd.) (Eds): T. Godfrey and S. West. Macmillan Press Ltd.

LVI. Guleria, S., Walia, A., Chauhan, A. and Shirkot, C. K. (2016), Purification and characterization of detergent stable alkaline protease from Bacillus amyloliquefaciens SP1 isolated from apple rhizosphere. J. Basic Microbiol., 56: 138–152.

LVII. Gupta R, Beg QK, Khan S, Chauhan B (2002b). An overview on fermentation, downstream processing and properties of microbial alkaline proteases, Appl Microbiol Biotechnol 60:381–395.

LVIII. Gupta R, Beg QK, Lorenz P (2002a). Bacterial alkaline proteases: molecular approaches and industrial applications. Appl Microbiol Biotechnol 59:15–32.

LIX. Gupta S, Jain U and Chauhan N (2017) Laboratory Diagnosis of HbA1c: A Review. J Nanomed Res 5(4): 00120.

LX. H.S. Joo, C.G. Kumar, G.C. Park, K.T. Kim, S.R. Paik, C.S. Chang (2002).Optimization of the production of an extra cellular alkaline protease from Bacillus horikoshii Process Biochemistry, 38:155–159.

LXI. Hamid Mukhtar and Ikram-ul-Haq, (2012). Concomitant production of two proteases and alpha-amylase by a novel strain of Bacillus subtilis in a microprocessor controlled bioreactor. Braz J Microbiol. 43: 1072–1079.

LXII. Haq, I, Abdullah, R, Ashraf, H, Shah, A.H. (2002). Isolation and screening of fungi for the biosynthesis of alpha amylase. J. Biotechnol. 1: 61-66.

LXIII. Haq, I. And Mukhtar, H., (2007). Biosynthesis of acid protease by Penicillium griseoroseum IH-02 in solid-state fermentation. Pak. J. Bot., 39: 25-28.

LXIV. Haq, I., Mukhtar, H., Daudi, S., Ali, S. And Qadeer, M.A., (2003). Production of proteases by a locally isolated mould culture under lab conditions. Biotechnology, 2:30-36.

LXV. Haq, I.; Mukhtar, H. (2004). Biosynthesis of protease by Rhizopus oligosporous IHS13 by solid-state fermentation in a low cost medium. J. Basic Microbiol. , 44: 280-287.

LXVI. Hayashi.K.Fukushima.D.Shogi.K. (1967). Isolation of alkaline proteinase from Aspergillus sajae Physio-chemical properties amino acid composition and molecular confirmation. Agric Biol Chem., 31: 1237-41. 109.

LXVII. Hemachander, C & Puvanakrishnan, R 2000, 'Lipase from Ralstonia pickettii as an additive in laundry detergent formulations', Process Biochemistry; 35:809-814.

LXVIII. Hemlata Bhosale, Uzma Shaheen and Tukaram Kadam, (2016). Characterization of a Hyperthermostable Alkaline Lipase from Bacillus sonorensis 4R Hindawi Publishing Corporation Enzyme Research, (2016): 11.

LXIX. Hernandez-Martin, E. and C. Otero, (2008). Bioresour. Technol. 99, 277.

LXX. Hoa H.T, I. Baccigalupi, A. Huxham, A. Smertenko, S. Van, E. Ammendola (2000). Characterization of Bacillus species used for oral bacteriotherapy

and bacterioprophylaxis of gastrointestinal disorders Applied and Environmental Microbiology, 66: 5241–5247.

LXXI. Houbraken J, Frisvad JC, Samson RA. (2011).Taxonomy of Penicillium section Citrina. Stud Mycol; 70:53-138.

LXXII. Ibrahim, A.S.S.; Al-Salamah, A.A.; El-Toni, A.M.; Almaary, K.S.; ElTayeb, M.A.; Elbadawi, Y.B.; Antranikian, G. (2016) Enhancement of 110 Alkaline Protease Activity and Stability via Covalent Immobilization onto Hollow Core-Mesoporous Shell Silica Nanospheres.Int. J. Mol. Sci., 17, 184.

LXXIII. Industrial Uses Of Enzymes, Infinita Biotech, (2019).

LXXIV. Inhs, D.A.; Schmidt, W.; Richter, F.R. (1999). Proteolytic enzyme cleaner. US Pat. 5961366.

LXXV. International Union of Biochemistry and Molecular Biology (1992). Enzyme Nomenclature. New York: Academic Press.

LXXVI. Ito.,M and Sugira M.(1968).Purification and characterization of alkaline protease from Aspergillus oryzae. Yakaguku Zasshi., 88:1576-1582.

LXXVII. Iva Colter (2020). Detailed Introduction to the Functions and Roles of Enzymes, global pharma News and Resources, Pharmiweb.com.

LXXVIII. Jayant Parpalliwar, P. Patil, I.D. and Aaisha Parin Sayyad, (2016). Fermentative Production of Bacillus Protease using Vegetable Waste as Substrate, International Conference on Global Trends in Engineering, Technology and Management (ICGTETM-2016): 386.

LXXIX. Jayasree, D., Sandhya Kumari, T.D., Kavi Kishor, P.B., Vijaya Lakshmi, M., Lakshmi Narasu, M. (2009).Optimization of production protocol of alkaline protease by Streptomyces pulvereceus. International Journal of Research and Industry – Science and Technology 1: 79-82. 111.

LXXX. Jellouli K, Bougatef A, Manni L, Agrebi R, Siala R, Younes I, (2009). Molecular and biochemical characterization of an extracellular serine-protease from Vibrio metschnikovii J. J Ind Microbiol Biotechnol, 36:939–48.

LXXXI. Jimenez, L, Navarro, E, Ferrer, JLF & Ariza, LJ (1999). 'Biobleaching of cellulose pulp from wheat straw with enzymes and hydrogen peroxide', Process Biochemistry, 35:149-157.

LXXXII. Kalisz, H.M. (1988). Microbial proteinases, in: A Fietcher (Ed.), Advances in Biochemical Engineering/Biotechnology (enzyme studies), 36:3–61.

LXXXIII. Kalisz, H.M. (1988). Microbial Proteinases. Adv. Biochem. Eng. Biotechnology. 36, 17-55.

LXXXIV. Kandasamy, N, Velmurugan, P, Sundarvel, A, Raghava, RJ, Bangaru,C & Palanisamy, T (2012). 'Eco-benign enzymatic dehairing of goatskins utilizing a protease from a Pseudomonas fluorescens species isolated from fish visceral waste', Journal of Cleaner Production;25:27-33.

LXXXV. Kanupriya Miglani Sharma., Rajesh Kumara., Surbhi Panwar., Ashwani Kumar (2017).Microbial alkaline proteases: Optimization of production parameters and their properties, Journal of Genetic Engineering and Biotechnology, Vol(15)1:115-126.

LXXXVI. Karuna, J. And Ayyanna, C., (1993). Production of semi-alkaline protease enzyme from Aspergillus spp. Proceedings of the Ninth National Convention of Chemical Engineers and International symposium on H. Mukhtar And I. Haq 260 Importance of Biotechnology in coming decades. Viskhapatnam India: 8-11.

LXXXVII. Keay, L. P.W. Moser and B.S. Wildi.(1970). Proteases of the genus Bacillus I alkaline proteases, Biotechnology. Bieng: 12. 213.

LXXXVIII. Kembhavi, A.A., Kulkarni, A., Pant, A., (1993). Salt-tolerant and thermostable alkaline protease from Bacillus subtilis NCIM No. 64. Appl. Biochem. Biotechnol. 38, 83–92.

LXXXIX. Kirk-Othmer (2005), Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley & Sons, New York. • Klich MA, Pitt JI. (1988). A laboratory guide to common Aspergillus species and their teleomorphs.North Ryde, Australia: CSIRO Division of Food Processing.

XC. Klich MA. (2002). Identification of common Aspergillus species. Utrecht, Netherlands: CBS, Fungal Biodiversity Centre.

XCI. Kubackova, M., Karacsonyi, S. And Varadi, J.. (1975). Studies on xylanase from Basidiomycetes. Selection of strains for the production of xylanase. Folia Microbiol. , 20: 29-37. 113.

XCII. Kumari HBV, Nagarathna S, ChandramukiA. 2007. Antimicrobial resistance pattern among aerobic gram-negative bacilli of lower respiratory tract illness in the community. Thorax, 56:109-114.

XCIII. Larcher G Bouchara J PAnnaixV Symeoens F, Chabasse D Tronchin G. (1992). purification and characterization of a fibrinogenolytic serine proteinase from Aspergillus fumigatus culture filtrate. FEBS Lett. , 308:65-69.

XCIV. Larcher G, Cimon B, Symoens F, Tronchin G, Chabasse D, Bouchara JP. (1996). A 33 kDa serine proteinase from Scedosporium apiospermum. Biochem J, 315:119–26.

XCV. Layla Filiciotto and Gadi Rothenberg, (2020). Biodegradable Plastics: Standards, Policies, and Impacts, ChemSusChem.

XCVI. Li, F.; Yang. L. ; Lv, X.; Liu, D.; Xia. H.; and Chen S (2016) Purification and characterization of a novel extracellular alkaline protease from Cellulomonas bogoriensis. Protein Expr Purif. Vol 2(121):125-132.

XCVII. Lin JS, Lee SK, Chen Y, Lin WD, Kao CH. (2011). Purification and characterization of a novel extracellular tripeptidyl peptidase from Rhizopus oligosporus. J Agric Food Chem 59:11330–7.

XCVIII. Lods, LM, Dres, C, Johnson, C, Scholz, DB & Brooks, GJ (2001). 'The future of enzymes in cosmetics', International Journal of Cosmetic Science; 22: 85-94.

XCIX. López-Otín C, Bond JS. (2008). Proteases: Multifunctional enzymes in life and disease. J Biol Chem. 283:30433–7.

C. Luis M. Real Hernandez and Elvira Gonzalez de Mejia (2019). Enzymatic Production, Bioactivity, and Bitterness of Chickpea (Cicer arietinum) Peptides, Comprehensive Reviews in Food Science and Food Safety.

CI. Mayuri Sharma, Yogesh Gat, Shalini Arya, Vikas Kumar, Anil Panghal, and Ashwani Kumar (2019). A Review on Microbial Alkaline Protease: An Essential Tool for Various Industrial Approaches, Industrial Biotechnology, Vol.(15)2.

CII. Mayuri Sharma, Yogesh Gat, Shalini Arya, Vikas Kumar, Anil Panghal, and Ashwani Kumar (2019). A Review on Microbial Alkaline Protease: An Essential Tool for Various Industrial Approaches, Industrial Biotechnology, Vol (15)2.

CIII. Mehwish Akram., Sarah Shafaat., Dilara Abbas Bukhari and Abdul Rehm (2014).Characterization of a Thermostable Alkaline Protease from Staphylococcus aureus S-2 Isolated from Chicken Waste. Zoology and wildlife conservation Pakistan Journal of Zoology.

CIV. Mikawlrawng, K. (2016). Chapter 18 - Aspergillus in Biomedical Research, New and Future Developments in Microbial Biotechnology and Bioengineering, Aspergillus System Properties and Applications: 229-242.

CV. Mohamed, A.; Abdel, N.; Ismail, A.M.S.; Ahmed, S.A.; Ahmed F.; Fattah, A.; (1998). Production and Immobilization of alkaline protease from Bacillus Mycoides. Bioresource Technol. 64:205-210.

CVI. Mohan F N, Dileep D, Deepthi D (2005). Potential application of protease isolated from Pseudomonas auriginosa PD 100. Biotechnol.Ind,.8:19.

CVII. Monod M Togni G Rahalison L frenk E. (1991). Isolation and characterization of an extracellular alkaline protease of Aspergillus fumigates. J Medical Microbiol.,35:23

CVIII. Mothe Vishnuvardhan Reddy and Sultanpuram, (2016). Production, purification and characterization of a thermotolerant alkaline serine protease from a novel species Bacillus caseinilyticus Thirumala, 3 Biotech, (2016) 6:53

CIX. Mothe. T and Sultanpuram VR (2016) Production, purification and characterization of a thermotolerant alkaline serine protease from a novel species Bacilluscaseinilyticus. 3 Biotech. 2016;6(1):53.

CX. Muhammad zia-ul-Haq, Sanja Avar, Mughalqayum, Inamullah Khan, Shakeelahmad (2013). Chemical composition and antioxidant potential of Acacia leucophloea Roxb. Acta Bot. Croat. 72(1):133–144.

CXI. Mukesh Kumar D.J., Premavathi V enkatachalam, N.Govindarajan, M.D. Balakumaran and P.T. Kalaichelvan (2012). Production and Purification of Alkaline Protease from Bacillus sp. MPTK 712 Isolated from Dairy Sludge Global Veterinaria 8 (5): 433-439. Mukhtar, H. And Haq, I., (2008). Production of alkaline protease by Bacillus subtilis and its application as a depilating agent in leather processing. Pak. J. Bot., 40: 1673-1679.

CXII. Murakami K, Ishida Y, Masaki A, Tatsumi H. (1991). Isolation and characterization of the alkaline protease gene of Aspergillus oryzae. Agric Biol Chem; 55:2807–11.

CXIII. Murthy MVR and Lonsane BK. (1993). Effect of the composition of conidial inoculum development agar media on promoting production of proteinase by Aspergillus oryzae CFTRI 1480 in solid state fermentation system.Chem Mikrobiol Technol Lebensm, 15:179–84.

CXIV. Nai-Wan Hsiao, Yeh Chen, Yi-Chia Kuan, Yen-Chung Lee, Shuo-Kang Lee, Hsin-Hua Chan, Chao-Hung Kao (2014). Purification and characterization of an aspartic protease from the Rhizopus oryzae protease extract, Peptidase R V, 17: 89–94.

CXV. Nakadai T, Nasuno S, Iguchi N. (1973). Purification and properties of alkaline proteinase from Aspergillus oryzae.Agric Biol Chem; 37:2685–94.

CXVI. Nakao Y, Suzuki M, Kuno M, Maejima K. (1973). Production of alkaline protease from n-paraffins by a kabacidinresistant mutant strain of Fusarium sp. Agric Biol Chem, 37:1223–24.

CXVII. Nascimento, W.C.A. and Martins, M.L.L. (2004). Production and properties of an extracellular protease from thermophilic Bacillus sp. Braz. J. Microbiol., 35(1-2), 91-96. Nasuno S and Ohara T. (1971). Hyperproduction of proteinase and some hydrolytic enzymes by mutants of Aspergillus sojae. Agric Biol Chem, 35:829–35.

CXVIII. Neetu, Jabalia., Mishra, and Chaudhary, Nidhee. P.C. (2014). Applications, Challenges and Future Prospects of Proteases: An Overview.. Journal of Agroecology and Natural Resource Management Vol 1(3):179-183.

CXIX. Nguyen, D, Zhang, X, Jiang, Z, Audet, A, Paice, MG, Renaud, S and Tsang, A (2008). Bleaching of kraft pulp by a commercial hpoxer accessory enzymes degrade hexenuronic acids', Enzyme & Microbial Technology, Vol (43):130-136.

CXX. Nilgun Tekin, Arzu Cabuk Cihan, Zekiye Sampl Takac, Canan Yağci Tuzun,Kenan Tunc and Coskun Cokmus (2012). Alkaline protease production of Bacillus cohnii APT5 Turk J Biol 36:430-440.

CXXI. Nisha N.S. and Divakaran J. (2014). Optimization Parameters for Alkaline protease Production using Bacterial isolates from different coastal regions of Tamil Nadu, India, Int.J.Curr.Microbiol.App.Sci Vol 3 (8):500-505.

CXXII. Oberoi, R., Q.K. Beg, S. Puri, R.K. Saxena and R.Gupta, (2001). Characterization and wash performance of an SDS-resistant alkaline protease from a Bacillussp. World J Microbiol. Biotechnol. , 17: 493-97.

CXXIII. Oikonomopoulou K, Hansen KK, Saifeddine M, Vergnolle N, Tea I and Diamandis EP, (2006). Proteinase-mediated cell signalling: Targeting

proteinase-activated receptors (PARs) by kallikreins and more. Biol Chem; 387:677–85.

CXXIV. Okamura-Matsui Tokumitsu, Tomomi Tomoda, Shoko Fukuda and Masahiro Ohsugi (2003). Discovery of alcohol dehydrogenase from 117 mushrooms and application to alcoholic beverages Journal of Molecular Catalysis B: Enzymatic 23: 133–144.

CXXV. Panke, S, Held, M & Wubbolts, M 2004', Trends and innovations in industrial biocatalysis for the production of fine chemicals', Current Opinion in Biotechnology; 15:272-279.

CXXVI. Passanai (2020). Technologies for Value Addition in Food Products and Processes.

CXXVII. Patila, M.; Kouloumpis, A.; Gournis, D.; Rudolf, P.; Stamatis, H.(2016) Laccase-Functionalized Graphene Oxide Assemblies as Efficient Nanobiocatalysts for Oxidation Reactions. Sensors, 16, 287.

CXXVIII. Peter K. Robinson (2015). Enzymes: principles and biotechnological applications, Essays Biochem, Vol (59): 1–41.

CXXIX. Poldermans, B. (1990). Proteolytic enzymes. In: Proteolytic enzymes in industry: production and applications. Ed. Gerhartz W., VCH Publishers, Weinheim, Germany. pp. 108-123.

CXXX. Polgár L. (2005). The catalytic triad of serine peptidases. Cell Mol Life Sci; 62:2161–72.

CXXXI. Potumarthi R and Ch. Subhakar, A. (2007). Jetty Alkaline protease production by submerged fermentation in stirred tank reactor using Bacillus Licheniformes NCIM-2042: effect of aeration and agitation regimes Biochemical Engineering Journal, 34: 185–192.

CXXXII. Potumarthi R., Ch. Subhakar, A. Jetty (2007). Alkaline protease production by submerged fermentation in stirred tank reactor using Bacillus Licheniformes NCIM-2042: effect of aeration and agitation regimes Biochemical Engineering Journal, 34:185–192.

CXXXIII. Pourrat, H., Regerat, F., Lamaison, J.L. And Pourrat, A., (1979). Proteases production by A. flavus. Ann. Pharm. France., 37: 441.

CXXXIV. Pourrat, H., Regerat, F., Lamaison, J.L. and Pourrat, A., (1988). Production of semi-alkaline protease by Aspergillus niger. J. Ferment. Technol., 66: 383-388.

CXXXV. Prakasham, R.S., C.S. Rao, R. SreenivasRao, S. Rajesham and P.N. Sarma, (2005). Optimization of. Optimization of alkaline protease production by Bacillus sp. using bacteria. Taguchi methodology. Appl. Biochem. Biotechnol., 120: 133-144.

CXXXVI. Pushpam PL, Rajesh T and Gunasekaran P (2011). Identification and characterization of alkaline serine protease from goat skin surface metagenome. AMB Express, 1:1–3.

CXXXVII. Quratulain Shafique.; Muhammad Nadeem. and Mamoona Naz, (2016) Production of alkaline protease from Aspergillus oryzae via static liquid surface culture technique and its potential application as a detergent additive, IJBPAS, January, 2016, 5(1): 227-245.

CXXXVIII. Rai S.K. and A.K. Mukherjee, (2009). Ecological significance and some biotechnological application of an organic –solvent stable alkaline serine protease from Bacillus subtilis strain DM-04, Bioresour. Technol. 100, 2642–2645.

CXXXIX. Ramachandran, S.; Patel, A.K. Nampoothiri, K.M.; Chandran, S.; Szakacs, G.; Secol, C.R. and Pandey, A. (2004). Alpha amylase from a fungal culture grown on oil cakes and its properties. Braz. Arch. Biol. Technol. 47: 309-317.

CXL. Ramnani P., S.S. Kumar and R. Gupta, (2005). Concomitant production and downstream processing of alkaline protease and biosurfactant from B. licheniformis RG1: bioformulation as detergent additive Process Biochemistry, 4:3352–3359.

CXLI. Ramos, OS and Malcata, FX (2011). Food-grade enzymes. In: MooYoung, M, Butler, M, Webb, BC, Moreira, A, Grodzinski, B, Cui, ZF, Agathos, S (Eds.), Comprehensive Biotechnology, Academic Press, Burlington.

CXLII. Raper, K.B. and Fennell, D.I. (1965). The Genus Aspergillus. Baltimore, MD: Williams and Wilkins.

CXLIII. Rawlings ND and Barrett AJ. (1993). Evolutionary families of peptidases. Biochem J 290:205–18.

CXLIV. Razzaq, Abdul., Shamsi, Sadia., Ali, Arfan., Ali, Qurban., Sajjad, Muhammad., Malik, Arif and Ashraf, Muhammad. (2019). Microbial Proteases Applications. Frontiers in Bioengineering and Biotechnology, Vol(7)10:3389.

CXLV. Riddhi Sawant and Saraswathy Nagendran (2014). Protease: an enzyme with multiple industrial applications, world journal of pharmacy and pharmaceutical sciences, Vol 3(6): 568-579.

CXLVI. Robert J. R. Elliott., Ingmar Schumacher and Cees Withagen (2020). Suggestions for a Covid-19 Post-Pandemic Research Agenda in Environmental Economics, Environmental and Resource Economics, Vol (76):1187–1213.

CXLVII. Robertson DE, Mathur EJ, Swanson RV, Marrs BL and Short JM. (1996). The discovery of new biocatalysts from microbial diversity. SIM News, 46:3-4.

CXLVIII. Roja Rani M and N.N.Prasad, (2012). Strain Improvement of Aspergillus flavus for enhanced production of alkaline protease enzyme,International Journal of Science Innovations and Discoveries,2:113- 120.

CXLIX. Sabotič J and Kos J. (2012). Microbial and fungal protease inhibitors— current and potential applications. Appl Microbiol Biotechnol, 93:1351–75.

CL. Saeki, K, Ozaki, K, Kobayashi, T and Ito, S (2007). 'Detergent alkaline proteases: enzymatic properties, genes, and crystal structures', Journal of Bioscience and Bioengineering, 103:501-508.

CLI. Samantha Phan and Christine K.Luscombe (2019).Recent Advances in the Green, Sustainable Synthesis of Semiconducting Polymers, Trends in Chemistry, Vol (1)7:670-681.

CLII. Samson RA and Varga J. (2007). Aspergillus systematics in the genomic era. Utrecht, Netherlands: CBS Fungal Biodiversity Centre.

CLIII. Sangareswari M. (2013). Isolation and Identification of Agar wood Fungi, Souvenir of 3rd International Science Congress ISC-2013 Coimbatore, India, 8th-9th Dec.

CLIV. Saravanabhavan, S, Aravindhan, R, Thanikaivelan, P, Raghava RaoUU, J and Unni Nair, B (2004). 'A source reduction approach: integrated biobased tanning methods and the role of enzymes in dehairing and fibre opening', Clean Technologies and Environmental Policy, 7:3-14.

CLV. Shivakumar Srividya and Majumdar mala, (2011). Influence of process parameters on the production of detergent compatible alkaline protease by a newly isolated Bacillus sp. Turk J Biol 35:177-182.

CLVI. Shivasharana C.T and Naik. G. (2012). Ecofriendly applications of thermostable alkaline protease produced from a Bacillus sp.JB-99 under Solid State Fermentation G.R International Journal of Environmental Sciences; 3:964.

CLVII. Shrinivas, D. and Naik, G.R., (2011). Characterization of alkaline thermostable keratinolytic protease from thermoalkaliphilic Bacillus halodurans JB 99 exhibiting dehairing activity. Int. Biodeter.Biodegrad. 65:29–35.

CLVIII. Shruti Gamya Dash (2017). Microbial Characterization and Optimization of Protease Production by Bacillus SP. Isolated from Soil. International Journal of Innovations in Engineering and Technology (IJIET).

CLIX. Sindhu, R., Suprabha, G.N. and Shashidhar, S. (2009). Optimization of process parameters for the production of alkaline protease from Penicillium godlewskii SBSS 25 and its application in detergent industry. African J. Microbiol Res. 3(9): 515-522.

CLX. Su, Y.C. and Chen, W.P. (1970). Studies on milk-clotting enzymes from microorganisms. Part I. Screening tests and the production of the enzymes, J. Chin. Agric. Chem. Soc. 8:73–83.

CLXI. Sumanth, G.T., Waghmare, B.M. and Shinde, S.R., (2010). Incidence of mycoflora from the seeds of Indian main spices. AJAR, Vol 5(22):3122-3125.

CLXII. Sumita Thakur (2012). Lipases, its sources, Properties and Applications: A Review, International Journal of Scientific & Engineering Research, 3(7):1-29.

CLXIII. Swapna.V and Sreenivas Rao.P (2012). Optimization of Alkaline protease production from locally isolated Bacillus Sp. Bacillus firmus from soil microorganisms in batch culture using statistical design. International Journal of Engineering Research and Applications 2:917-924.

CLXIV. Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods.Mol Biol Evol. Vol 28:2731–2739.

CLXV. Taylor, M.M., D.G.Bailey, and S.H.Feairheller. (1987). A review of the uses of enzymes in the tannary. J.Am.Leather Chem.Assoc., 81:85-102.

CLXVI. Temiz, H., Okumus, E., Aykut, U., Dervisoglu, M. And Yazici, F., (2008). Partial purification of pepsin from turkey proventriculus. Wld. J. Microbiol. Biotech. , 24: 1851–1855.

CLXVII. Thakur, M.S. Karanth, N.G and Krishna, N. (1990). Production of fungal rennet by Mucor michei using solid state fermentation, Appl. Microbiol. Biotechnol. Vol 32:409–413. Thangam, E.B. and G.S. Rajkumar, (2002). Purification and characterization of alkaline protease from Alcaligenes faecalis. Biotechnol. Appl. Biochem., 35: 149-154.

CLXVIII. Tsai, Y. C. Juang, R. Y. Lin, S. F. Chen, S. W. Yamasaki, M. and Tamura, G. (1988). "Production and further characterization of an alkaline elastase produced by alkalophilic Bacillus strain Ya-B, Applied and Environmental Microbiology, vol. 54:3156–3161.

CLXIX. Tubesha, Z.A. and Al-Delaimy, K.S. (2003). Renin-like milk coagulant enzyme produced by a local isolate of Mucor. Int. J. Dairy Technol. 56: 237–341.

CLXX. Tunga R, Banerjee R and Bhattacharyya BC. (2001). Optimization of some additives to improve protease production under SSF. Indian J Exp Biol. 39: 1144-8.

CLXXI. Vaishali Choudhary and Jain P.C. (2012). Isolation and identification of alkaline protease producing fungi from soils of different habitats of Sagar and Jabalpur District (M.P), J. Acad. Indus. Res. 1(3): 109-112.

CLXXII. Valeika, V, Beleska, K, Valeikiene, V and Kolodzieskis, V (2009). 'An approach to cleaner production: from hair-burning to hair saving using a limefree unhairing system', Journal of Cleaner Production; 17:214-221.

CLXXIII. Van Den Hombergh JPTW, Van de Vondervoort PJI, Fraissinet-Tachet L, and Visser J. (1997). Aspergillus as host for heterologous protein production: the problem of proteases. Trends Biotechnol; 15:256–63.

CLXXIV. Vankar, P. S. (2007). Handbook on natural dyes for industrial applications, National Institute of Industrial Research, ISBN 81-89579-01-0 ,New Delhi, India.

CLXXV. Vida Maghsoodi,Akhtar Kazemi, Parvin Nahid, Soheila Yaghmaei, Mohammad Amin Sabzevari,(2013). Alkaline protease production by immo-

bilized cells using B. licheniformis Scientia Iranica C; 20: 607–6.

CLXXVI. Vijayaraghavan Ponnuswamy, Sophia Lazarus, and Samuel Gnana Prakash Vincenta (2014). De-hairing protease production by an isolated Bacillus cereus strain AT under solid-state fermentation using cow dung: Biosynthesis and properties Saudi J Biol Sci. 21(1): 27–34.

CLXXVII. Vipul Kumar., Yadav Veer Singh and Vishal Mishra (2019).Alkaline Protease: A Tool to Manage Solid Waste and Its Utility in Detergent Industry, Microbial Genomics in Sustainable Agroecosystems, 231-254.

CLXXVIII. Wang B, Liu X, Wu W, Liu X, Li S. (2009). Purification, characterization, and gene cloning of an alkaline serine protease from a highly virulent strain of the nematodeendoparasitic fungus Hirsutella rhossiliensis. Microbiol Res; 164:665–73.

CLXXIX. Woodley, JM (2008). 'New opportunities for biocatalysis: making pharmaceutical processes greener', Trends in Biotechnology; 26:321-327.

CLXXX. Wuts, Peter and Greene, Theodora. (2006). Protective Groups In Organic Synthesis, Vol(10):1002.

CLXXXI. Xiao-lin Ao, Xi Yu, Ding-tao Wu, Chao Li, Tong Zhang, Shu-liang Liu, Shujuan Chen, Li He, Kang Zhou and Li-kou Zou (2018). Purification and characterization of neutral protease from Aspergillus oryzae Y1 isolated from naturally fermented broad beans, AMB Express, Vol(8)96.

CLXXXII. Yegin S, Fernandez-Lahore M, Gama-Salgado AJ, Guvenc U, Goksungur Y and Tari C. (2011). Aspartic proteinases from Mucor sps. in cheese manufacturing. Appl Microbiol Biotechnol; 89:949–60.

CLXXXIII. Zhao G, Zhou A, Lu G, Meng M, Sun M and Bai Y. (2013). Identification and characterization of Toxoplasma gondii aspartic protease 1 as a novel vaccine candidate against toxoplasmosis.Parasites Vectors; 6:175.

CLXXXIV. Zhu, D, Wu, Q and Wang, N (2011). 'Industrial enzymes', Comprehensive Biotechnology, 3:3-13.

CLXXXV. Kanupriya., Miglani., Sharma., Rajesh Kumar., Surbhi, Panwar., Ashwani Kumar (2017). Microbial alkaline proteases: Optimization of production parameters and their properties. Journal of Genetic Engineering and Biotechnology, Vol (15)1:115-126.

CLXXXVI. Chander, Mukesh. (2019). Recent Advances in Microbial Production of Proteases. International Journal of Environmental Analytical Chemistry. 6. 65Z.

CLXXXVII. Abdalla Ali., Salima Sanja., Grbavči., Nataša Šekulji., Andre Stefanović., Sonja Jakovetić., Tanas ković., Nevena Luković., Zorica and Knežević-Jugović (2017).Production of enzymes by a newly isolated Bacillus sp. TMF-1 in solid state fermentation on agricultural by-products: The evaluation of substrate pretreatment methods, Bioresource Technology, Vol(228): 193-200.

CLXXXVIII. What Is Protease Enzyme? It's Uses In Dteregent Industry, Detergent Enzymes, Infinita Biotech (2019).

CLXXXIX. Temam Abrar Hamza (2017).Isolation and Screening of Protease Producing Bacteria from Local Environment for Detergent, American Journal of Life Sciences, Vol (5)5:116-124.

CXC. Vipul Kumar., Yadav Veer Singh and Vishal Mishra (2019). Alkaline Protease: A Tool to Manage Solid Waste and Its Utility in Detergent. Microbial Genomics in Sustainable Agroecosystems: 231-254.

CXCI. Andreas Abelsson (2020). Artificial and Non-sugar Sweeteners: the Good, the Bad, and the Science.

CXCII. Kanupriya., Miglani Sharma., Rajesh Kumar., Surbhi Panwar and Ashwani Kumar (2017).Microbial alkaline proteases: Optimization of production parameters and their properties, Journal of Genetic Engineering and Biotechnology, Vol (15)1:115-126.

CXCIII. Noora Barzkar (2020). Marine microbial alkaline protease: An efficient and essential tool for various industrial applications, International Journal of Biological Macromolecules, Vol (161)15:1216-1229.

CXCIV. Petra Philipps-Wiemann (2018). Chapter 13 - Proteases—human food, Enzymes in Human and Animal Nutrition Principles and Perspectives:267-277.

CXCV. Xiangyang Liu and Chandrakant Kokare (2017).Chapter 11 - Microbial Enzymes of Use in Industry, Biotechnology of Microbial Enzymes, Production, Biocatalysis and Industrial Applications (2017):267-298.

CXCVI. Mayuri Sharma, Yogesh Gat, Shalini Arya, Vikas Kumar, Anil Panghal, and Ashwani Kumar (2019). A Review on Microbial Alkaline Protease: An Essential Tool for Various Industrial Approaches, Industrial Biotechnology,Vol. (15)2.

CXCVII. Luis Carlos Gioia, Jose Ricardo Ganancio and Caroline Joy Steel (2017). Food Additives and Processing Aids used in Bread making.

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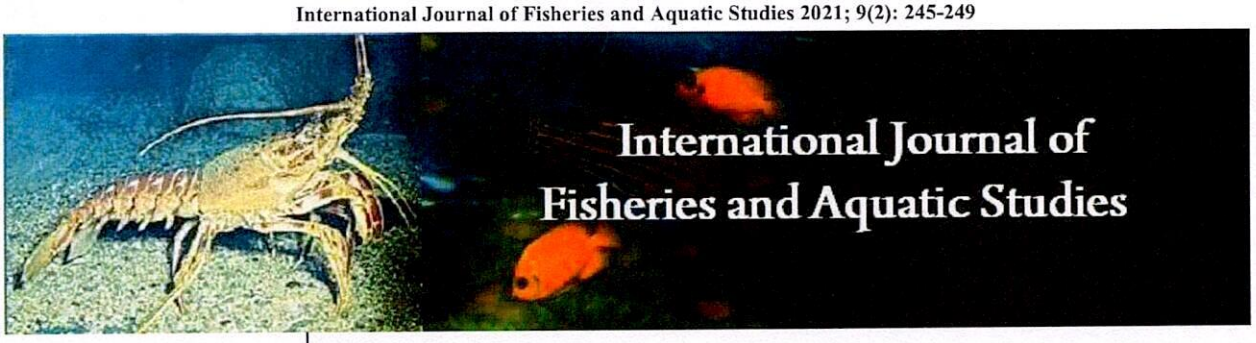
International Journal of Fisheries and Aquatic Studies 2021; 9(2): 245-249



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A case study on infection of trematodes pathogenicity in wild *Channa striata* collected from various freshwater ponds in Guntur district rural, semirural and urban areas

Aruna Mallavali

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Abstract
The Murrels (Channa striatus) is an indigenous, predatory freshwater fish. It is also known as lay man fish food with pharmacological benefits in treating wound and pain and in boosting energy of the sick. Amino acids include glycine, lysine and arginine and fatty acids are namely arachidonic acid, palmitic acid and docosahexaenoic acid are used for the preparation of several types of bioactive molecules commercially. Antinociceptive activity or toxic inhibitor, anti-depressant and neuroregenerative agent and it has wide-ranging medical uses. Henceforth the present study aimed to conduct the general survey by collecting various fresh water wild Channa striatus in Guntur district to investigate to identification of pathogenicity of trematodes and its affect on Channa striatus morphological, physiological and anatomical changes leads to the loss of nutritional, medicinal properties. Furthermore creating awareness to the target area where collected the samples about the pathogenicity impact on human health and their influences. Our results demonstrate that intestinal flukes are common in farmed fish in this area, suggesting that reservoir hosts such as dogs, cats, and pigs are more important in sustaining the life cycles of these fluke in fish farms than human hosts. This has implications for the effectiveness of control programs focused mainly on treatment of humans.

Keywords: human health, economic food, traditional nutritive food, amino acids, bioactive compounds, medicinal properties, hosts, fresh water ponds, pathogenicity, trematodes, awareness camp, pharmacological benefits, Channa striatus

Introduction
India is endowed with many freshwater resources, rich fish genetic biodiversity (2,200 fish species) and ranks 9th in terms of freshwater mega diversity (Rubina Mondal and Anuradha Bhat, (2020) [8]. Nevertheless, a significant portion of the freshwater fish production in India is still based on the harvest from wild population (Vijay Anand P.E. (2019) [9]. In tropical regions, parasites are major concern to freshwater and marine fishes (Morales-Serna *et al.*, 2019) [10]. They constitute a major limiting factor to the growth of farmed fish (Prangnell, David *et al.*, 2016) [11]. They play a vital role in devaluation of nutrients alteration of biology and behaviour inducing blindness and in decreasing immunity reduction of growth and fecundity, increasing mortality and morbidity and they also causes mechanical injuries based on number and site of infection (Bichi *et al.*, (2020) and Nmor *et al.*, (2020).
Moreover, parasites may also control host population dynamics and manipulate community structure (Marcogliese David, (2004) [14]. Parasitology is an ever going discipline in research. The host parasite associations are unique in the sense that of the two organisms it is only the parasites that is benefited while the host suffers (Stothard, *et al.,* 2018) [16]. The valuable information pertaining to the ecological aspects of freshwater fishes was contributed by several parasitologists of national and international status (Gozlan *et al.,* 2010 and Kiruba-Sankar *et al.,* 2018) [17, 18]. At present, very few records of parasitic helminths in the study were documented (Garcia *et al.,* 2018) [15]. The present research focused on to bring out the community characteristics of the metazoan parasite fauna identification in wild Channa species of freshwater fish collected from various ponds located in Guntur district of Andhra Pradesh.

Methodology

The present investigation dealing with parasitic diseases particularly trematode parasites take hosting of Channa *striata* collected from different ponds located in Guntur district, Andhra Pradesh been selected for a period of one year from July 2017- June 2018. Fish sampling was done by cast netting; usually in the morning hours between 6 to 9am and a minimum of 50 fish were netted each time. Examined the collected Fish for any disease symptoms were investigated. Samples were transferred to the zoology laboratory, J.M.J. College for women (A) in live and also moribund condition to conduct autopsy for further studies. Each fish was weighed and its total length was measured. It was then dissected the open viscera and observed for the trematode parasites. Each intestine was opened with a mid-ventral incision along the ventral bold vessel and intestine mucosa was exposed so as to examine surfaces of each chambers. All the parasites were collected and kept in the petridish with saline solution separately. For identification the trematodes were fixed, stained and whole mount were prepared according to the conventional technique.

To know the monthly changes of trematodes infection rates, prevalence, one an intensity, density of infection have been calculated for the each month in the study period July, 2017 to June, 2018. Data on monthly prevalence of infection, seasonal distribution and severity and type of infection were recorded. A standard data sheet was prepared to take details like total number of fish collected, number of infected and uninfected fish.

Results and Discussion

The present work was under taken the incidence of and seasonal prevalence of trematode parasites of murrel Channa striata from various fresh water ponds located in the Guntur district Andhra Pradesh. Data of prevalence of trematode infection with different genus of trematodes was recorded in a period of one year. The assessment of the prevalence was based on the parasites obtained from internal organs of the fish.

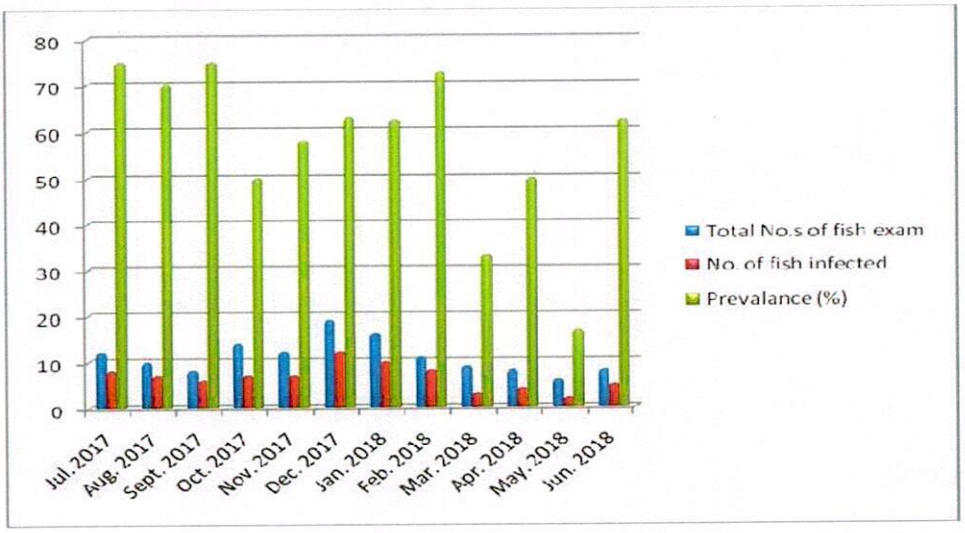

Fig 1: Monthly variations in overall prevalence of trematode in fection during 2017-118

Studies of prevalence: A total of 133 fishes were collected from various ponds of Guntur district. Out of 133, 79 fishes are infected. Months wise prevalence and mean, intensity was given (Table 1).

Table 1: Prevalance and mean intensity of trematodes parasite in *Channa striata* from of Guntur district during different months.

Month	Total No.s of fish exam	No. of fish infected	Total No. of parasites	Prevalance (%)	Mean intensity
July, 2017	12	8	14	75	1.75
August, 2017	10	7	16	70	2.28
September, 2017	8	6	18	75	3.0
October, 2017	14	7	20	50	2.85
November, 2017	12	7	18	58	3.6
December, 2017	19	12	21	63	2.6
January, 2018	16	10	29	62.5	2.9
February, 2018	11	8	13	72.7	2.1
March, 2018	9	3	8	33.3	2.6
April, 2018	8	4	12	50	3.0
May, 2018	6	2	4	16.6	2.0
June, 2018	8	5	12	62.5	2.4
Total:	133	79	185	688.6	31.08

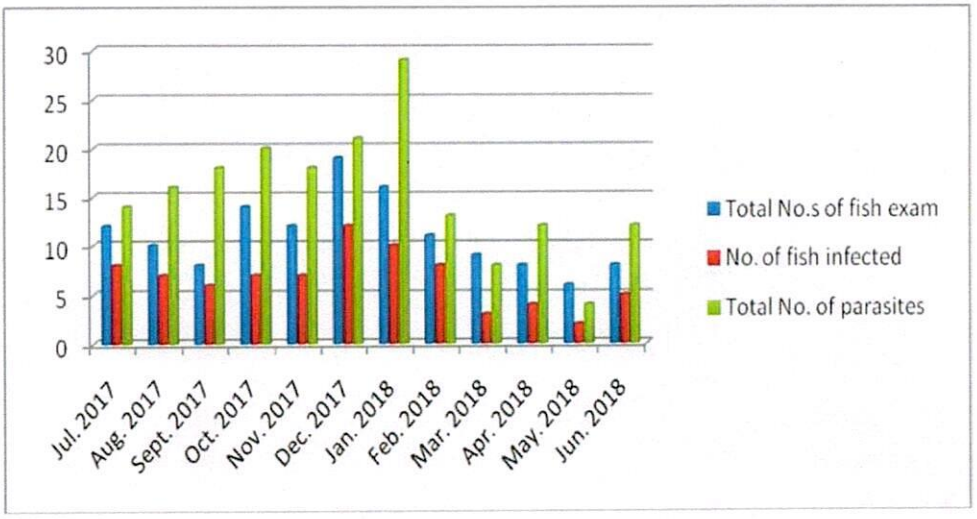

Fig 2: monthly variation total number of parasites infection during 2017-18

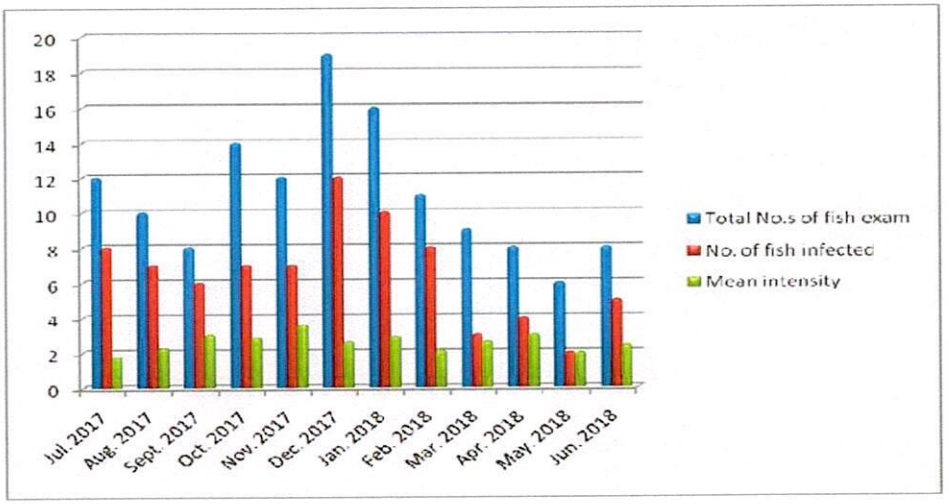

Fig 3: Monthly-variations in mean intensity of trematode infection during 2017-18

The trematode infection of *Channa stiata* from ponds/creeks and canals of Guntur district during different seasons was given Table 2.

Table 2: Percentage trematodes parasite infection of channa striata of Guntur district during different seasons

Season	Total No. of fish exam	No. of fish infected	Percentage of Infection
Rainy	**44**	**28**	**63.6**
Winter	**58**	**37**	**63.7**
Summer	**31**	**14**	**45.1**
Total:	**133**	**79**	**172.4**

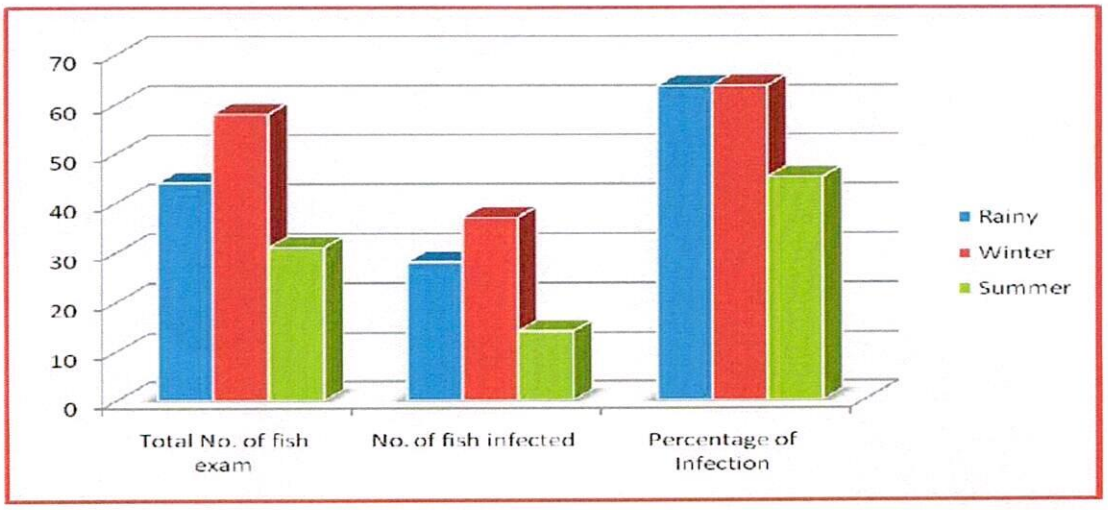

Fig 4: Seasonal variation in overall prevalence of trematode infection during 2017-18

The percentage of in the rainy season goes to 63.6%, winter goes to 63.7% and summer was goes to 45.1%. Monthly variations of infection of parasites were given (Fig 3.1).

Discussion

In the present examination the prevalence of infection was highest recorded in the both months of July and September, 2017. In the rainy seasons infection was higher percentage than winter. The lowest percentage was found in summer season. Feeding activates of the host may also be one of the reason (Auda Fares (2013) [1]. Furtherly found infection rate percentage was high during the periods of low temperature and low percentage infection during the high temperature (Kumari Gautam *et al.*, 2018) [2]. The factors responsible for influencing the seasonal cycle of parasitic infection were namely temperature, host feeding habits, availability of infective intermediate hosts, and parasite maturation (Joanne Cable *et al.*, 2017) [3]. Such parameters can easily be studied in a terrestrial or freshwater host (Binh cao and Pascale S. Guiton (2018) [4] and Krishna and Sreeramulu, (1996) studied the prevalence and intensity of infection with Didymozoid parasite in *Priacanthus ha*mrur, from waltair coast.

According to Rohde, (1993), temperature affects parasite faunas in two ways, 1) species numbers are greatly increased in warm seas and 2) species are different in cold and warm seas. Thus temperature is the most important factor responsible for differences in species numbers as well as variability in species.

The association of seasonal maturation with seasonal mortality occurs commonly amongst parasites of fish. Life cycle of these parasites may be short and not more than one single season (Nico *et al.,* 2019) [5]. Temperature brings a change in host behavior which indirectly effects the parasite recruitment and flow of parasites (Kennedy, 1975). The period of highest incidence of infection of parasites may be assumed as the lowest recruitment season as well as due to the elimination of parasites due to mortality. Rainy and winter seasons seem to be favourable for the parasite for infection. According to Bell and Burt, (1991) and Ellis *et al.*, (2020) [6]; Vincenzo *et al.,* (2020) [7] parasite community diversity is positively correlated with hosts local prevalence. Manter, (1966) stated that if hosts are sparse, they may lack parasites, although other conditions may be favourable. He stated that a certain concentration of hosts and parasites is needed to assure the completion of life cycles.

Conclusion and Future Scope: Snakehead has vital functions with medicinal and pharmaceutical fresh water fish. In generally it is been used to treat wounds, alleviate pain, boosts energy and endowed with remarkable anti-inflammatory, anti-nociceptive, platelet aggregation, as well as mild antimicrobial and antifungal properties. And also used as a therapeutics and nutritional supplements. Treating neurological diseases and in inducing regenerative potential of organs and cells. With reference of these applications we creating awareness to the remote area people and rural people who regularly consume murrels, due to indigenous helminthes pathogenicity may harm and causes different parasiting infections leads to the human abnormalities. Apart from have many advantages and many applications under research.

Acknowledgement

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References

1. Auda Fares. Factors Influencing the Seasonal Patterns of Infectious Diseases Int J Prev Med 2013;4(2):128-132.
2. Kumari Gautam N, Kumar Misra P, Murari Saxena A. Seasonal Variation in Helminth Parasites of Snakeheads Channa Punctatus and Channa Striatus (Perciformes: Channidae) in Uttar Pradesh, India, Helminthologia, 2018;55(3):230-239.
3. Joanne Cable, Iain Barber, Brian Boag, Amy R Ellison, Eric R Morgan, Kris Murray, Emily L Pascoe *et al.* Global change, parasite transmission and disease control: lessons from ecology, Philos Trans R Soc Lond B Biol Sci 2017;372(1719).
4. Binh Cao, Pascale S Guiton. Important Human Parasites of the Tropics, frontiers for young minds 2018.
5. Nico J. Smit, Niel L. Bruce, and Kerry A. Hadfield Life Cycle and Life History Strategies of Parasitic Crustacea, Parasitic Crustacea 2019;3:179-266.
6. Ellis Vincenzo, Huang Xi, Westerdahl, Helena, Jönsson Jane, Hasselquist Dennis *et al.* Explaining prevalence, diversity, and host specificity in a community of avian

haemosporidian parasites. Oikos 2020, 129. 10.1111/oik.07280.
7. Vincenzo A, Ellis Xi Huang Helena, Westerdahl Jane Jönsson Dennis, Hasselquist Júlio M, Neto Jan-Åke Nilsson Johan, *et al*. Explaining prevalence, diversity and host specificity in a community of avian haemosporidian parasites, Oikos 2020.
8. Rubina Mondal, Anuradha Bhat. Temporal and environmental drivers of fish-community structure in tropical streams from two contrasting regions in India, Plos one 2020.
9. Vijay Anand PE. Intelligence The fish farming industry of India, Second-largest freshwater fish producer has potential for further development, Global aqua culture alliance 2019.
10. Morales-Serna FN, Rodríguez-Santiago MA, Gelabert R. *et al*. Parasites of fish *Poecilia velifera* and their potential as bioindicators of wetland restoration progress. Helgol Mar Res 2019;73(1) https://doi.org/10.1186/s10152-019-0522-1.
11. Prangnell, David, Castro, Leandro, Ali, Abdulmehdi *et al*. Some Limiting Factors in Superintensive Production of Juvenile Pacific White Shrimp, Litopenaeus vannamei, in No-water-exchange, Biofloc-dominated Systems. Journal of the World Aquaculture Society 2016;47:10.1111/jwas.12275.
12. Bichi A, Yelwa Sadiq. Incidence of piscine parasites on the gills and gastrointestinal tract of *Clarias gariepinus* (teugels) at bagauda fish farm, Kkano. Bayero Journal of Pure and Applied Sciences 2010, 3. 10.4314/bajopas.v3i1.58732.
13. Nmor JC, Egwunyenga AO, AKE Jeg. Observations on the intestinal helminth parasites of cichlids in the upper reaches of River Orogodo, a freshwater body in Delta State, Southern Nigeria. Tropical Freshwater Biology. 2003, 12. 10.4314/tfb.v12i1.20883.
14. Marcogliese, David. Parasites: Small Players with Crucial Roles in the Ecological Theater. Eco Health. 2004;1:151-164. 10.1007/s10393-004-0028-3.
15. Garcia LS, Arrowood M, Kokoskin E, Paltridge GP, Pillai DR, Procop GW *et al*. Laboratory diagnosis of parasites from the gastrointestinal tract. Clin Microbiol Rev 2018;31:e00025-17. https:// doi.org/10.1128/CMR.00025-17.
16. Stothard J, Littlewood DTJ, Gasser R, Webster, Bonnie. Advancing the multi-disciplinarity of parasitology within the British Society for Parasitology: Studies of host-parasite evolution in an ever-changing world. Parasitology. 2018:145:1-6. 10.1017/S0031182018001476.
17. Gozlan, Rodolphe, Britton, Rob, Cowx, Ian *et al*. Current knowledge on non-native freshwater introductions. Journal of Fish Biology. 2010:76:751-786. 10.1111/j.1095-8649.2010.02566.x.
18. Kiruba-Sankar R, Raj J, Saravanan K, Kumar, Lohith, Angel *et al*. Invasive Species in Freshwater Ecosystems–Threats to Ecosystem Services 2018. 10.1016/B978-0-12-813064-3.00009-0.

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Max 30100/30102 Sensor Implementation to Viral Infection Detection Based On Spo2 and Heartbeat Pattern

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ABSTRACT

Introduction- Oxygen level present in the human body is a measure that shows the amount of Oxygen Saturation (SpO2) present in Red Blood Cells (RBC). It is as essential to check blood oxygen level as checking the body temperature level for wellbeing concerns. Particularly during the current circumstance when the viral infection and its outbreak is way normal. The recent episode of destructive infections like COVID-19, Middle East Respiratory Syndrome (MERS), Ebola, Lassa Fever, Zika Virus, Yellow Fever, and latest Influenza A [H3N2 and H1N2]warns us to stay ready for forthcoming virus attacks.[1] It is apparent from ongoing researches that the immune system of our body is likewise demonstrated by the SpO2 levels. All in all, we can say that the blood oxygen level is the non-intrusive method of recognizing the resistance capacity of an individual against the virus attack [2]. Oxygen level degradation is one of the major lethal explanations behind the passings of deaths in the recent COVID-19 cases. It was taught to ceaselessly screen the blood SpO2 level, as blood oxygen loss is generally not distinguished. Till the patient arrives at the emergency clinic, they normally breakdown due to lack of oxygen and respiration problems. Our paper proposes the Heart Beat and SpO2 sensor [Max 30100/30102] interfacing to check the immunity level of individuals. The framework is associated with an ESP8266 based IoT module to screen the body parameters like SpO2 and Heartbeat, live on the web. It very well may be profoundly valuable for the specialists to screen their patients from various wards of clinics as well as from any edge of the world. In our country, whereas of now, there is a shortage of doctors, this framework can assist with arriving at their patients remotely. This gadget can be a deliverer for individuals to get unaffected from a late flare-up of infections as the majority of individuals even get infected by different patients in hospitals too.

Keywords
Oxygen Saturation (SpO2), Red Blood Cells (RBC), Middle East Respiratory Syndrome (MERS)

INTRODUCTION

The year 2020 will be known as the COVID pandemic era due to the severity, communicable nature, and deadly impact of COVID-19 over humanity. As of today, the total cases of coronavirus is 108,812,516 of which 2,395,970 deaths and 80,841,562 recoveries are there. Death and recovery mainly depend on 2 parameters. Immunity level of the body [*durability of the body against virus*] and timely identification of viral infection. Most of the people died in coronavirus cases due to a lack of ventilation system provided to them in China. This numbers to only 20% of people who died of novel coronavirus infections in China got ventilation. 80% of patients who died of COVID-19 could not receive ventilation at the time of need. This is the report of April 2020 when COVID was not spread worldwide. The lack of ventilation support occurred as one of the biggest reasons for COVID-19 deaths worldwide.[3]

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In our paper, we deal with the SpO2 sensor which can live track the heartbeat and blood oxygen level. We can broadcast the sensor parameters in a real-time numerical and graphical format to the web servers. Early detection of degrading oxygen level and lower immunity due to lower oxygen level are the two major identification of critical situations in any viral disease pandemic. After COVID there are many viral diseases in the line to shake humanity like the SFTS virus.

Fig.1: Lack of Ventilators and Coordination: Main reason for Deaths during the COVID era [4]

We can be ready to combat these viruses we the help of technology. Adequate SpO2 level is between 95-100. A healthy person with good immunity always has the optimum SpO2 level [95-100] but when the value goes below 90 there is an urgent need to provide ventilation. Any further delay can be fatal and causes severe breathlessness which can further result to causes death. In the case of COVID patients it happened the same, till their blood oxygen saturation came below 90 they felt minor breathing difficulty, negligence of which further reached to minimum SpO2 level and ultimately deaths.

LITERATURE REVIEW

[1]. EsratJahan et al. explained in detail the pulse oximeter system. They explained that they have used the heartbeat and blood oxygen saturation as the parameters to analyze the health of a human being. The information about how the heartbeat and blood oxygen percentage is explained as the light absorption rate of the sensor. They provided the different heart rates for several categories and explained that for a healthy person the heartbeat should be in a prescribed range. The effectiveness of the healthy respiratory system is determined by the oxygen percentage present in the red blood cell of the blood. The device developed by EsratJahan et al. is a primary work in the field where the peripheral interface controller (PIC) microcontroller-based system detects the blood oxygen level and heartbeat. They use the transmittance method of body parameter measurement to detect the above-said parameters. It is found from their practical observation that the results obtained are very close to the preset value. The preset value of SpO2 is around 95-100 for the healthy person at the same time it is around 159- more than 100 for different age groups. One thing is observable that the heartbeat decreases with increasing age. This paper helps us to have different values of heart rate and SpO2 to match with our body parameters. [5]

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[2]. Avneendra K. Kanva et al. observed detailed information about SpO2 and heart rate using a smartphone. Their main work involved using a phone camera as the SpO2 detection tool. The variation in the color pattern of the finger placed on the camera lens can also calculate the accurate heartbeat. They also proposed the comparative accuracy test of their proposed phone-based SpO2 and heartbeat detection system with the noninvasive sensors available in the market. The optical video monitoring and detection of heartbeat and SpO2 are observed minutely. This enables the system to observe accurate and reliable outcomes. At first, they recorded the small video of the finger against a heavy light source from behind to accurately capture the amount of light passing through the finger. In the second step, around 600 frames are extracted from our video recorder for further analysis. In the next step, each image is processed for its red and blue particles of formation. The mean of red and blue color components are calculated and calculated to identify the standard deviation of the same red and blue components. The calculated value of all 600 frames is formed in the form of a graph. The SpO2 level calculated is linked with the heart rate with ± 15 of the ideal heart rate. This system provides another perspective to our study to create a much accurate calculative study over the reduced hardware for body parameter calculations. [6]

[3]. M.T Tamam et al. demonstrated in their paper the noninvasive method of heart rate and oxygen saturation and body temperature detection with the help of sensors. They mentioned that heart disease is the number 1 cause of death all over the world. Their main parameter deals with identifying heartbeat, body temperature, and blood oxygen saturation level. They designed a pulse oximeter of clip type. They calculated the result of body temperature, heartbeat, and SpO2 level on many participants. They used a heart rate sensor, oxygen saturation sensor, and temperature sensor. The Arduino board is used as the logic device in the system. The software system used was MULTISIM and BASCOM simulation software. The calculation error is comprised of SpO2-0.89% BPM-3.095% and temperature-0.78% this shows that the system is highly accurate in comparison to other methods. The real-time matching of body parameters and adding temperature sensors is what gives the almost similar consent but with additional display and IoT broadcast our result. [7]

OPERATION AND WORKING PRINCIPLE

3.1 Operation

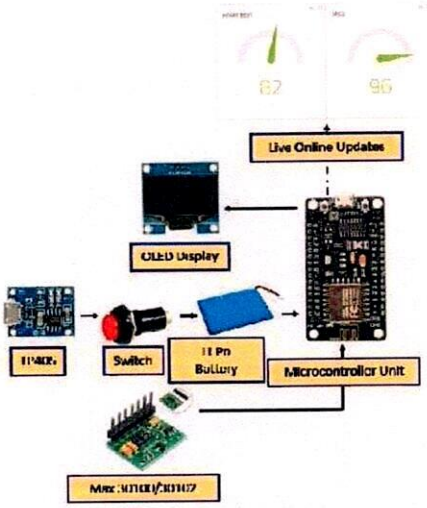

Fig.2: Operation Diagram

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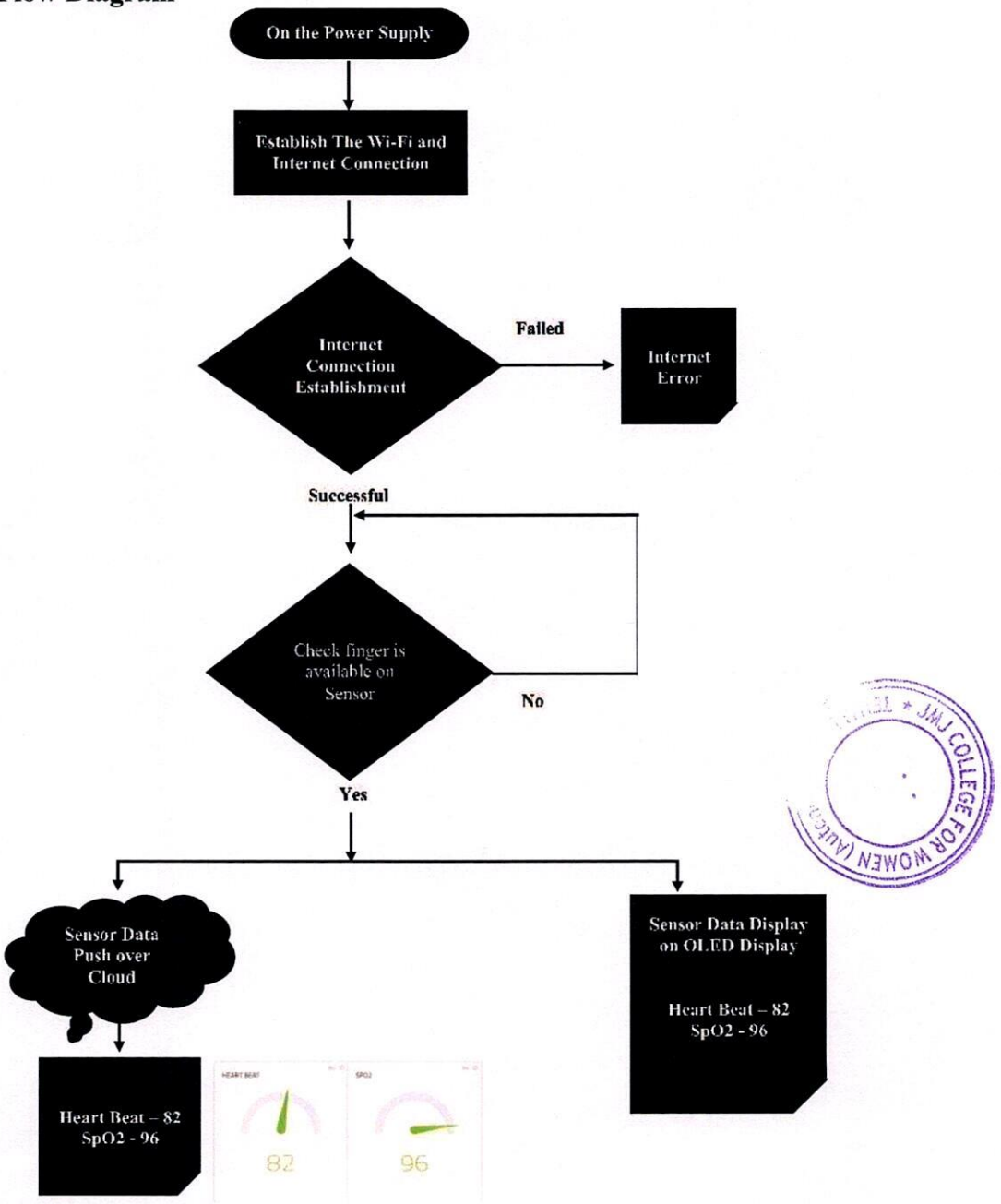
Our Max30100/30102 and IoT system deals with the detection of heartbeat and SpO2 levels. The real-time update is processed on the webserver using the Node MCU microcontroller system. The overall system is designed in the form of a portable device that is powered by a 2700 mAh Lithium Polymer (LiPo) battery. The rechargeable battery is charged and managed by the recharging controller TP4056. The charging controller can be powered by any phone charger or even through USB power from any PC, laptop, or even power banks. The internet connectivity can be provided with a WiFi connection from our internet modems or even by Hotspot of our phones.

3.2 System Flow Diagram



3.3 Microcontroller Program

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 2053 - 2061
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```
#include "FS.h"
#include <Wire.h>
#include "MAX30100_PulseOximeter.h"
#include "Adafruit_GFX.h"
#include "OakOLED.h"
#define CAYENNE_PRINT Serial
#include <CayenneMQTTESP8266.h>
#define REPORTING_PERIOD_MS 10000        // set to 10secs. Set as appropriate.
OakOLEDoled;
PulseOximeter pox;
float BPM, SpO2;
uint32_ttsLastReport = 0;
String hrData = "";
unsigned long timems =0;
 // WiFi network credentials.
charssid[] = "iotdata";
charwifiPassword[] = "12345678";
char username[] = "885805e0xxxx-f259-11e6-8577-0128e408a1ba";
char password[] = "f2400465a9c0c177xxxx28a6b0f5d6bad99594a0ef51";
charclientID[] = "3406xxx9f70-5dd9d-11eb-a2we4-b32ea624e442";
//char username[] = "885805e0-f259-11e6-8577-0128e408a1ba";
//char password[] = "f2400465a9c0c17728a6b0f5d6bad99594a0ef51";
//char clientID[] = "b86f9790-4cfd-11eb-a2e4-b32ea624e442";
const unsigned char bitmap [] PROGMEM=
{
0x00, 0x00, 0x00, 0x00, 0x01, 0x80, 0x18, 0x00, 0x0f, 0xe0, 0x7f, 0x00, 0x3f, 0xf9, 0xff, 0xc0,
0x7f, 0xf9, 0xff, 0xc0, 0x7f, 0xff, 0xff, 0xe0, 0x7f, 0xff, 0xff, 0xe0, 0xff, 0xff, 0xff, 0xf0,
0xff, 0xf7, 0xff, 0xf0, 0xff, 0xe7, 0xff, 0xf0, 0xff, 0xe7, 0xff, 0xf0, 0x7f, 0xdb, 0xff, 0xe0,
0x7f, 0x9b, 0xff, 0xe0, 0x00, 0x3b, 0xc0, 0x00, 0x3f, 0xf9, 0x9f, 0xc0, 0x3f, 0xfd, 0xbf, 0xc0,
0x1f, 0xfd, 0xbf, 0x80, 0x0f, 0xfd, 0x7f, 0x00, 0x07, 0xfe, 0x7e, 0x00, 0x03, 0xfe, 0xfc, 0x00,
0x01, 0xff, 0xf8, 0x00, 0x00, 0xff, 0xf0, 0x00, 0x00, 0x7f, 0xe0, 0x00, 0x00, 0x3f, 0xc0, 0x00,
0x00, 0x0f, 0x00, 0x00, 0x00, 0x06, 0x00, 0x00, 0x00, 0x00, 0x00, 0x00, 0x00, 0x00, 0x00, 0x00,
0x00
};
// Callback (registered below) fired when a pulse is detected
voidonBeatDetected()
{
Serial.println("Beat Detected!");
oled.drawBitmap( 60, 20, bitmap, 28, 28, 1);
oled.display();
}
void setup() {
Serial.begin(115200);
oled.begin();
oled.clearDisplay();
oled.setTextSize(2);
oled.setTextColor(1);
```

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```
oled.setCursor(0, 0);
oled.println("PLEASE WAIT!!!!!!!!!");
oled.display();
pinMode(2, OUTPUT);
Cayenne.begin(username, password, clientID, ssid, wifiPassword);
Serial.print("Initializing Pulse Oximeter..");
pinMode(16, OUTPUT);
if (!pox.begin())
   {
Serial.println("CONNECTION FAILED");
oled.clearDisplay();
oled.setTextSize(1);
oled.setTextColor(1);
oled.setCursor(0, 0);
oled.println("CONNECTION FAILED ");
oled.display();
for(;;);
   }
else
   {
oled.clearDisplay();
oled.setTextSize(2);
oled.setTextColor(1);
oled.setCursor(0, 0);
oled.println("  Heart beat and SpO2 monitoring");
oled.display();
Serial.println("WAITING FOR INPUT");
digitalWrite(2, HIGH);   //Turn off in-built LED
    }
pox.setOnBeatDetectedCallback(onBeatDetected);
pox.setIRLedCurrent(MAX30100_LED_CURR_24MA);
if(!SPIFFS.begin()){
Serial.println("An Error has occurred while mounting SPIFFS");
return;
  }
}
void loop()
{
pox.update();
  BPM = pox.getHeartRate();
  SpO2 = pox.getSpO2();
if (BPM < 40 || SpO2 == 0)
  {                              // Neglects low readings and starts loop again.
Serial.println(F("No Finger on Sensor!!!!!"));
return;
  }
if (millis() - tsLastReport> REPORTING_PERIOD_MS)
```

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```
  {
digitalWrite(2, LOW);                                  // Turn ON LED everytime reading is saved
  // Cayenne.loop();
Serial.print("Heart rate: ");
Serial.print(BPM);
timems = millis();
hrData = String(timems) + String(",") + String(BPM) + String(",") + String(SpO2);
//convert variable from integer to string
Serial.print(" bpm , SpO2:");
Serial.print(SpO2);
Serial.println(" %");
  File file = SPIFFS.open("/HR_test.txt", "a");
Cayenne.virtualWrite(0, BPM, "counter","p" );
Cayenne.virtualWrite(1, SpO2, "O2", "p")
oled.clearDisplay();
oled.setTextSize(1);
oled.setTextColor(1);
oled.setCursor(0,11);
oled.println(pox.getHeartRate());
oled.setTextSize(1);
oled.setTextColor(1);
oled.setCursor(0, 0);
oled.println("Heart BPM");
oled.setTextSize(1);
oled.setTextColor(1);
oled.setCursor(0, 30);
oled.println("Spo2");
oled.setTextSize(1);
oled.setTextColor(1);
oled.setCursor(0, 0);
oled.println("          HB+SPO2");
oled.setTextSize(1);
oled.setTextColor(1);
oled.setCursor(0, 11);
oled.println("          DETECTION");
oled.setTextSize(1);
oled.setTextColor(1);
oled.setCursor(0, 21);
oled.println("            UNIT");
oled.setTextSize(1);
oled.setTextColor(1);
oled.setCursor(0,45);
oled.println(pox.getSpO2());
oled.display();
if(!file)
  {
Serial.println("Failed to open file for writing");
```

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Received 20 January 2021; Accepted 08 February 2021.

```
return;
  }
file.println (hrData);
file.close();
digitalWrite(2, HIGH);
tsLastReport = millis();
  }
}
```

IMPLEMENTATION AND OUTPUT

4.1 Hardware Unit

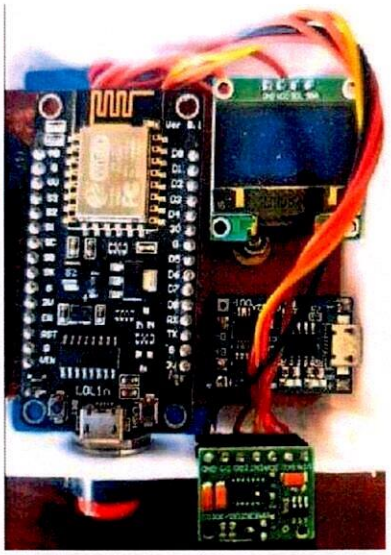

Fig.3: Working Module

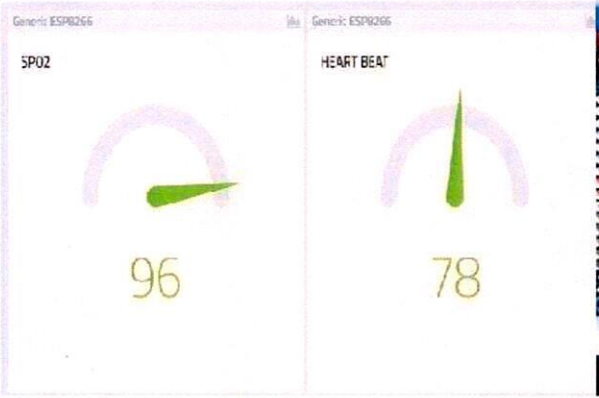

Fig.4: Online Output

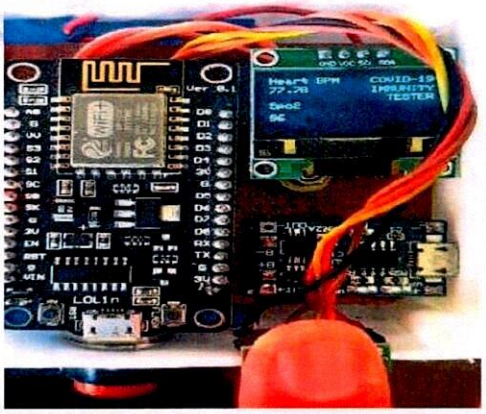

Fig.5: Offline Output

V. DISCUSSION AND CONCLUSION

Thus we developed a system of Live heartbeat and SpO2 tracking which is crucial for the current situation as well as for the future. Already in the market SpO2 and Heart Beat detection systems are available but they have limited features and their accuracy is mainly not tested. Our developed product is highly accurate and it has the additional feature of IoT connectivity which

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provides it worldwide coverage of the data transmission. The system is possible to implement in realtime and it can easily solve the problems like scarcity of doctors, the sudden need for ventilators, the worry of elder people living alone with health concerns, updates about patients getting treated, etc. We hope the positive implementation of technology will bring change for the health sector and enable us to fight against the forthcoming viral diseases.

REFERENCES

[1] Disease outbreaks by year. *World Health Organisation,* www.who.int, 2021.

[2] US National Library of Medicine. *Pulse-oximetry accurately predicts lung pathology and the immune response during influenza infection*. National Institutes of Health, June 2009.

[3] The Economic Times. *Many who died of COVID-19 may have had delayed ventilator support: Study,April 2020.*

[4] The Times of India. *Covid-19 hospitals struggling to make effective use of ventilators in Andhra Pradesh September 9, 2020.*

[5] EsratJahan et al. *An overview on heart rate monitoring and pulse oximeter system,* International Journal of Latest Research in Science and Technology ISSN (Online): 2278-5299,Volume 3, Issue 5: Page No 148-152. September-October 2014.

[6] Avneendra K Kanva et al. *Determination of SpO2 and Heart-rate using Smart-phone Camera, 2014* international Conference on Control, Instrumentation, Energy & Communication.

[7] M T Tamam et al. *Design a system of measurement of heart rate, oxygen saturation in blood and body temperature with non-invasive method,* IOP Conference Series: Materials Science and Engineering, 2018.

[8] I. D. Soubache et al. Diabetic Foot Ulcer Treatment Device Using Peltier and Embedded Electronics, Journal of Physics: Conference Series, 2020 J. Phys.: Conf. Ser. 1712 012041.

[9] Ms. Sarah EzhilKelna Edwin. Galvanic Skin Response And Heart Beat Sensor Coordination To Track The Health Parameter, International Journal of Grid and Distributed Computing, Vol. 13, No. 2, (2020), pp. 2557-2562.

[10] Saravanan J., Raj A.C., Singh R.K., Taye A. (2019) *An Efficient Security Implementation with Power Cane for Visually Challenged*. In: Mekuria F., Nigussie E., Tegegne T. (eds) Information and Communication Technology for Development for Africa. ICT4DA 2019. Communications in Computer and Information Science, vol 1026. Springer, Cham. https://doi.org/10.1007/978-3-030-26630-1_23

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Water Pollution: Psychological effect on human health & live reporting using IoT Technology

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Abstract: India is a country which is named after the river Indus which represents a rich culture well connected with water bodies [1]. It is the nation with 18% of the world's population and it also accounts for 4% of water resources available worldwide. The 4% water for 18% population provides only 1720 cubic meters of fresh water per person every year [2]. The biggest threat to water resources in India is water pollution. The most immediate and extensive reason for water pollution in India is sewage and its improper treatment. The industrial, agriculture, medical, radioactive, and solid wastes are directly dumped into rivers which causes the maximum damage to the quality of water present in different water bodies. The biggest sewage producing cities in India produce 38, 354 Million Litres per Day (MLD) sewage but out of that around 30.78% only gets treated under Urban Sewage Treatment (UST) and the remaining meets the freshwater to make it highly polluted and poisonous for human and aquatic creatures [3]. Our proposed system is an initiative to identify the quality of water using pH level of water using high precision pH analyzers and report this content using worldwide coverage connectivity medium, Internet of Things (IoT). Internet of things. The system proposed is a portable device that is linked with a Wi-Fi-based IoT system to report and update the quality of water present in any water body where it is planted. The live reporting can be broadcasted world-wise to aware about their water body is safe to use or not. This can also alarm the public against any dangerous chemical mix-up with water and save their lives.

Keywords: Million Litres per Day (MLD), Urban Sewage Treatment (UST), aquatic creatures, pH, Internet of Things (IoT)

1. Introduction

When the water of river Ganga begins at *Devprayag* where it is in its purest form no one can guess it will become one of the most polluted rivers all over the world [4]. With the increasing length of the Ganga from the Himalayas to Bangladesh, the distance of 2525 Kms. The holy river keeps contaminating.

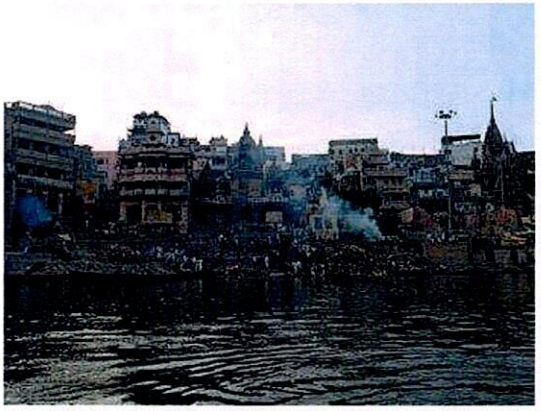

Figure 1. Pollution of Rivers in India [10]

There are some points where the pollution attains the level of 31 million bacteria in just 100 millimeters of water. The longest river of India which recharges the groundwater and provides water for drinking, agriculture, and household works is polluted to its maximum capacity means in many ways we intake this pollution into our bodies as well.

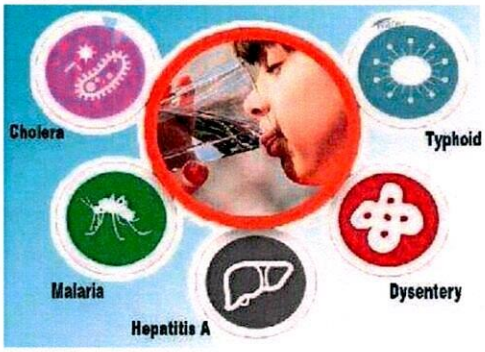

Figure 2. Pollution of Rivers in India [11]

Most of the disease like diarrhea, dysentery, typhoid, cholera, and poliomyelitis kills more than 500 thousand worldwide and they are the water-born disease. The major water pollutants are comprised of pesticides, viruses, parasites, chemicals, plastics, polythenes, phosphates, nitrates, and a lot more unknown substances [5]. Improper sewage treatment, dirt dumping, clothes washing in river steers, and waste disposal are the most immediate cause of water pollution in India. In recent times government started many initiatives to kick start the stagnant process of river cleaning. ''Namami Ganga'' is also such an initiative to free the longest river of India from dirt and pollution. The initial process to clear Ganga was initially started in 198 itself with the name of Ganga action plan 1, a hefty sum of 4000 crores has already been invested till 2014 but Ganga became dirtier and dirtier despite several plans and initiatives [6]. Cleaning a 2525 km long river is not possible by any government plan alone but, is a matter of public responsibility. Our proposed paper deals with research on the identification of the most impure water bodies their location and level of pollution. The reporting of the pollution level is demonstrated during the research. We also deal with several impurities and concerned diseases produced by water pollution. In our next session, we deal with the literature survey about the paper with all detailed analyses of previous works carried out in the field so far.

2. Literature Survey

[1] *Kofi Sarpong Adu-Manu et al.* explained in their paper about smart river monitoring systems with the help of wireless sensor networks. The study was conducted in Weija Dam, Ghana. To increase the power capacity of sensor nodes solar panels were used. The water quality was measured on different parameters like pH, conductivity level, calcium level, Temperature, Fluoride level, and Oxygen content. The result was cross-referred for the condition of aquatic life and water plants available in the water body during the test. Multiple sensors were deployed in the water body to capture several parameters in the real-time scenario. The wireless transmitted data is then received on the local monitoring station (BS) using local communication. Then through an internet connection, the complete data is remotely transmitted to the remote monitoring station and cloud. The complete data is retrieved on the user's system for further processing of information. The overall information is processed for five months straight to test its usability and effectiveness. It was observed that due to the quality reduction in water, the contamination increased in some parts of the water body. This was clearly stated in the report. This resulted in a high number of deaths of fishes and aquatic plants. [7]

[2] *Derara Chalchisa et al.* wrote in his paper about the test of water quality in the storage tanks to ensure the safety of urban water supplies. They mainly took the case study of Ethiopia where water is a big issue to solve. Most of the health issues are based on drinking contaminated water. In Urban areas mainly there are pipelined water connections but the water contamination in the distribution process mainly this issue is to be analyzed in detail. They reached the fact that all the samples of drinking water were contaminated by microbes. The samples collected from the exit of water tanks had more microbes compared to the microbes present at the entry of the water tanks. This result demonstrates the leakage in water tank and pipelines where microbes enter the water supply chain. Water contamination causes severe health issues and deaths as well. The water quality analysis obtained from the sample was matched with the WHO standard of drinkable water but none of the samples matched the quality. All the water samples were found to be substandard and unsafe for drinking. The major reason for the damaged water quality was due to leaks in the valve seals, uncapped connections, uncovered storages, exposed pipes, and damaged support/tanks. It was also proposed that if these issues are properly analyzed then this can solve the water contamination to its maximum extent. [8]

[3] *K. A. Mamun et al.* explained in detail the smart system of water quality monitoring. They took the case study of Fiji surface water. In their analysis, they explained that in recent years, water around Fiji has been reasonably contaminated and needs proper observation to predict the damage already done. Fiji is located in the Pacific Ocean and it requires different parameters to analyze the water quality in different locations. The water quality needs to be matched against the standards provided by Fiji national drinking water quality standards (NDWQS). The main parameters to be analyzed for water quality identification are the potential of Hydrogen (pH0, Temperature, Oxidation Reduction Potential (ORP), and conductivity. the proposed research on based on the five locations of Fiji to analyze the water quality using the above 4 parameters. the sensors are powered by solar panels and batteries to provide uninterrupted reporting of the water quality report. the water surface data is analyzed from the sensors and provided to the cloud storage system. then the cloud-stored data is further segregated into the form of graph representations and provided to the users through android applications and web pages. all the sensor nodes replicate the same process and report the concerned data from their geography for reliable results and analysis. [9]

3. Operation and Working Principle

In this system, we use different sensors for measuring the position of aquaculture. The pH sensor is the key sensor to consider the water quality by dipping it in the water. If the water is polluted the pH value drops and water becomes much acidic. If the water acidity increases or the pH value drops further from pH value 5 then the automatic motor starts to replace water. It also has an IoT feature to upload data online and maintain a record of pH value and automatic motor starting. It has an automatic water level controller to control the water level to its optimum level. This study gives the preferred data at any moment from any part of the world and screening their concern instantly at any part of the location.

3.1. Circuit Diagram

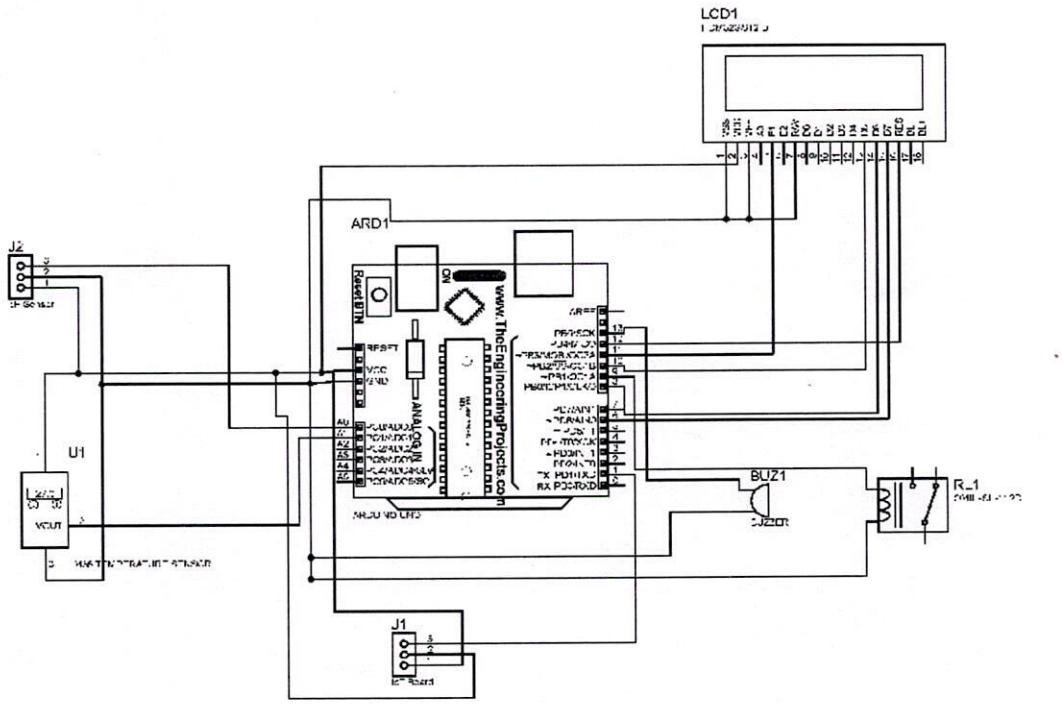

Figure 3. Overall System Circuit Diagram

The circuit diagram represents the overall system connection and pin configurations. The components and their assembling with a line diagram are drawn in the figure. It represents the Atmega microcontroller as the central intelligence core and thinker of logic controls. The relay connection, IoT page, display, and sensing unit is well labeled and demonstrated in the circuit diagram.

3.2. Block Diagram

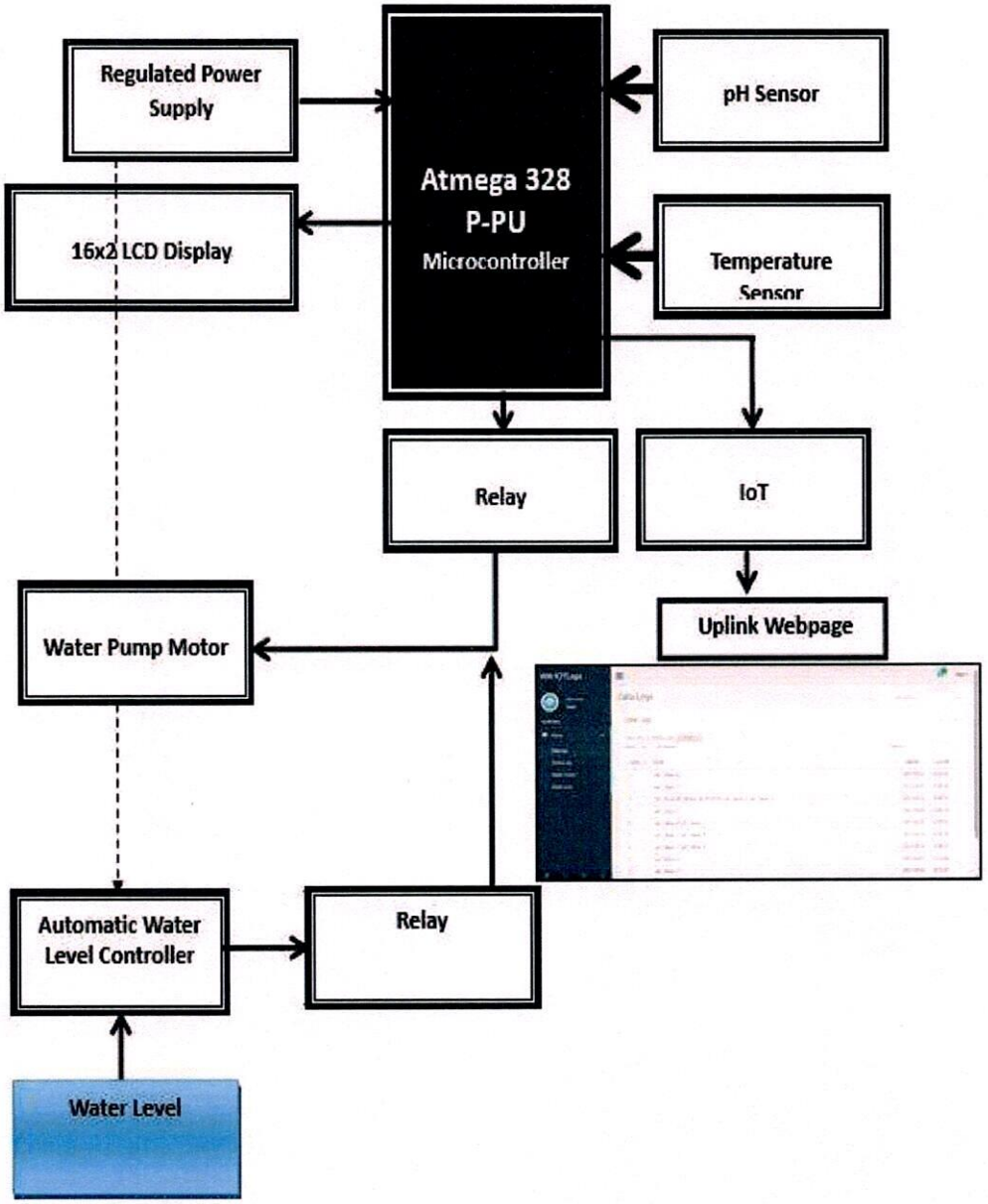

Figure 4. Overall System Block Diagram

Block diagram represents the overall system functionality and working. The components and their schematics are drawn in the diagram. It represents the Atmega microcontroller as the brain of the overall functionality. The IoT updates on the page demonstrate the working of the reporting system as well.

3.3. Microcontroller Program

```
#include <LiquidCrystal.h>
LiquidCrystal lcd(12, 11, 5, 4, 3, 2);
const int analogInPin1 = A0;
int sensorValue1 = 0;
long ll;

void setup()
{
Serial.begin(9600);
pinMode(8, OUTPUT);
pinMode(9, OUTPUT);
digitalWrite(8, LOW);
digitalWrite(9, LOW);
lcd.begin(16, 2);
lcd.setCursor(0, 0);
lcd.print("WATER QUALITY MONITORING")
```

```
lcd.setCursor(0, 1);
lcd.print("MONITORING UNIT");
delay(2000);
lcd.setCursor(0, 1);
lcd.print("RING AND CONTROL ");
delay(1000);
lcd.setCursor(0, 1);
lcd.print("LING SYSTEM FOR ");
delay(1000);
lcd.setCursor(0, 1);
lcd.print("AQUACULTURE");
delay(1000);
lcd.setCursor(0, 1);
lcd.print(" USING IOT------");
delay(1000);
lcd.clear();
lcd.setCursor(0, 0);
lcd.print("PH SENSOR STATUS");
Serial.print("*");
Serial.print(" PH SENSOR MODE ACTIVATES");
delay(2000);
Serial.print("#");
}

int hb,hbt,hbtt;
void loop()
{
Serial.print("*");
lcd.setCursor(0, 1);
lcd.print(" PH Value:-          ");
Serial.println("pH Value:-");
sensorValue1 = analogRead(analogInPin1);
lcd.setCursor(11, 1);
delay(20);
lcd.print(sensorValue1/12.5);
Serial.println(sensorValue1/12.5);
delay(5000);
Serial.print("#");
}
```

3.4. Major Advantages

- pH Sensor is the most reliable water quality sensor as it can be dipped in water
- IoT used is the most advanced communication technology
- Possible to maintain a database of all data records
- Possible to control the motor remotely using IoT

4. Discussion and Conclusion

Thus our proposed system of water quality testing and live reporting using IoT is designed. The paper in detail analyses the quality of water quality analysis using IoT systems and reports for public safety. The proposed system is also usable for any geographical location and can be of great use for the high water pollution containing water bodies. As a future enhancement, we can have the attachment of a turbidity sensor to also analyze the physical, visibility-based clarity of water identification. This would make our proposed system more accurate and reliable.

References

1. Wikipedia, "Names for India", https://en.wikipedia.org/wiki/Names_for_India
2. Wikipedia "Water resources in India", https://en.wikipedia.org/wiki/Water_resources_in_India
3. Wikipedia "Water pollution in India", https://en.wikipedia.org/wiki/Water_pollution_in_India
4. Wikipedia "Ganges", https://en.wikipedia.org/wiki/Ganges
5. IBERDROLA "Water pollution: how to protect our source of life", https://www.iberdrola.com/sustainability/water-pollution
6. Banjot Kaur "Namami Gange: 5 reasons why Ganga will not be clean by 2020", 15 October 2018 https://www.downtoearth.org.in/coverage/pollution/namami-gange-5-reasons-why-ganga-will-not-be-clean-by-2020-61891
7. Kofi Sarpong Adu-Manu et al., "*Smart River Monitoring Using Wireless Sensor Networks*" Hindawi, Wireless Communications and Mobile Computing, Volume 2020, Article ID 8897126, 19 pages, https://doi.org/10.1155/2020/8897126
8. Derara Chalchisa et al., "*Assessment of the quality of drinking water in storage tanks and its implication on the safety of urban water supply in developing countries*" Chalchisa et al. Environ Syst Res (2017) 6:12 DOI 10.1186/s40068-017-0089-2
9. K. A. Mamun et al., "*Smart Water Quality Monitoring System Design and KPIs Analysis: Case Sites of Fiji Surface Water*" Sustainability 2019, 11, 7110; doi:10.3390/su11247110.
10. Wikipedia "Pollution of the Ganges", https://en.wikipedia.org/wiki/Pollution_of_the_Ganges
11. Facebook "*Aqua Solution India*", https://www.facebook.com/aquasolutionsindia
12. ID Soubache, T Thirumurugan, B Annapurna, RK Singh, *"Diabetic Foot Ulcer Treatment Device Using Peltier and Embedded Electronics",* Journal of Physics: Conference Series 1712 (1), 012041, 2020
13. Dr. B. Annapurna, Asha Priyadarshini Manda, A. Clement Raj, Dr. R. Indira, Dr. Pratima Kumari Srivastava, Dr. V. Nagalakshmi, "*Max 30100/30102 Sensor Implementation to Viral Infection Detection Based On Spo2 and Heartbeat Pattern*", Annals of RSCB, vol. 25, no. 2, pp. 2053–2061, Mar. 2021.
14. B. Annapurna, T Rama Reddy, Ch. V. Raghavendran, Raushan Kumar Singh and Vedurai Veera Prasad, *"Coordinate Access System for Live Video Acquisition"*, Journal of Physics: Conference Series, doi:10.1088/1742-6596/1712/1/012034, 2020.
15. B. Annapurna, Raushan Kumar Singh, J. Saravanan, Durai Pandurangan, *"A tribrid Paradigm against Inundation wild animal attack and fire disasters",* International Journal of Innovative Technology and Exploring Engineering (IJITEE), ISSN: 2278-3075, Volume-9 Issue-2S4, December 2019.

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Bioaccumulation of Lead and Cadmium and its Impact on fresh water catfish, *Heteropneustes fossilis*

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Abstract: Heavy metals are key components to cause environmental pollution and heightened level of concentration is toxic to the living organisms. In generally food is the main source of accumulation of toxic metals day by day from various source, they include through soil, water and atmospheric dust. Many disorders like cancer, gene mutation, physiological malformation or physical deformations in pregnant women or very young children. May be one of the reason of consumption of indigenous food. Presently people are fascinate to have fish is the one of the most important ingredients in the daily diet due to this rapid growth of consumption and different practices to produce the aqua products based on the demand. Hence forth the present study focused on intoxication studies on the consumption of freshwater catfish namely *Heteropneustes fossilis*, collected from different aqua farming cultures. Aim of the work is to predict the different adverse effects of LC_{50} of lead and cadmium and mortality, behavioural and haematological studies.

Key words: Food Chain, Toxicity, Catfish, LC_{50}, Haematological, Lead, Cadmium, Mortality, Behavioural.

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Introduction: Pollution of aquatic environments with heavy metals has seriously increased worldwide and under certain environmental conditions fish may concentrate large amounts of some metals from the water in their tissues (Vhahangwele Masindi and Khathutshelo L. Muedi (2018)). The heavy metal contamination of aquatic ecosystem has attracted the attention of researchers all over the world owing to their toxicity at very low levels, persistence in the environment and ability to get incorporated in the tissues of organisms (Rai, Prabhat. (2008)). All these factors make the toxicants deleterious to the aquatic environment and consequently to humans who depend on aquatic products as source of food (Paul *et al.*, 2014). Heavy metals can accumulate in the tissues of aquatic animals and as such tissue concentrations of heavy metals are of public health concern to both animals and humans (Tembo Rostern (2017)).

Bioaccumulation of non-essential metals in tissues leads to intoxication, decreased fertility, tissue damage and dysfunction of a variety of organs (Nitasha Khatri and Sanjiv Tyagi (2015)). Heavy metals cause adverse biological effects (Golovanova, Irina. (2008)). It was reported that metals are taken up through different organs of the fish and induce morphological, histological and biochemical alterations in the tissues which may critically influence fish quality (Mehjbeen Javed1 and Nazura Usmani (2019)). Common sub-lethal effects are behavioral (e.g. swimming, feeding, attraction-avoidance, and prey-predator interactions), physiological (e.g. growth, reproduction and development), biochemical (e.g. blood, enzyme levels), and histological changes (Sanaa Abdulaziz Mustafa (2020)).

Once heavy metals are accumulated by aquatic organisms, they can be transferred to upper class of food chain. Heavy metals generally do not degrade and tend to biomagnify in man through food chain

(Hadeel M Huseen and Ahmed J Mohammed (2019)). Thus human health eventually is threatened by the consumption of such food. There are two ways of penetration of heavy metals into the organism either by direct water absorption or by consuming fish as food (Michael A Clark *et al.*, 2020). Reported larger metal loads in the tissues of predatory fish species. The ecological specificity of metal pollutant is that there are practically no self-cleaning mechanism known for them when present in water, they pass through the trophic chain of aquatic communities (Elisabet Lindgren *et al.*, 2018).

Heavy metals affect specific vital organs such as liver, gill and kidney. Liver contains highest metal concentration because it is an organ of storage and detoxification of metal. Heavy metals have the ability to bioaccumulate in the liver and kidney, the target organs of heavy metal pollution and also body's detoxification organs. Changes in histological structure of specific vital organs due to exposure of sub-lethal concentration of metal in various fishes have been reported by many workers (Gandhewar and Zade (2019)). The body constantly tries to eliminate heavy metals *via* the available exit routes: the liver, kidney and skin. Detoxification mechanism includes acetylation, sulfonation, oxidation, etc. Liver is a detoxification organ and essential for both the metabolism and excretion of toxic substances. Liver has the ability to degrade the toxic compounds but its regulating mechanism can be overwhelmed by elevated concentration of these compounds and could subsequently result in structural damage. Here most of the products are expelled through the bile into the small intestine and should leave the body *via* the digestive tract. Histological analysis is crucial in determining cellular changes that may occur in target organs, such as gill, liver and kidney (Ting Liu *et al.*, 2020).

Heavy metals can cause genetic mutation. They disrupt the metabolic process. Heavy metals alter prooxidant-antioxidant balance and bind to free sulphydryl groups resulting in inhalation of glutathione, metabolism, numerous enzyme and hormone functions. Chemically reactive pollutants such as electrophiles react with different nucleophilic biological molecules. Depending on its electrophilicity, an electrophilic pollutant reacts with soft nucleophile, such as thiol groups in protein and peptides or harder nucleophiles, such as nucleotides in DNA. They also pointed out that reaction with peptides and proteins interfere with the cellular reducing capacity through conjugation with glutathione or interfere with the enzyme activity, while DNA damage leads to mutation (Richard et al., 2019).

Lead is the nonessential and most toxic metal which is widely distributed in the aquatic environment and earth's crust. Heavy metals such as lead, mercury, and cadmium are considered to cause public health hazards. Nonessential components of lead may cause nephrotoxicity, neurotoxicity, decrease growth rate, survival, metabolisms and development, and several adverse health effects. Consumption of such metal-contaminated fishes by a human can cause serious health issue. Metals deteriorate the ecological balance of the aquatic environment because fish are at the end of the aquatic tropic level and they have a higher tendency to accumulate metals in their body (Richard *et al.*, 2014). The aim of the present study was to investigate heavy metal bioaccumulation and alteration in hematological indices and red blood cell and nucleus morphology and in different organs like gills and muscles of grass carp exposed with different concentrations of heavy metals.

MATERIALS AND METHODS

Animal: (8.5±5.5cm; 9.5±6.5g) were transported in oxygenated bags (50 fish per bag) from carp hatchery of Mardan and Peshawar to the lab. The fish were treated with 0.2% $KMnO_4$ solution for two minutes to remove any external infection.

Acclimatization: Fishes were acclimatized to the laboratory conditions in large fiber glass tanks with unchlorinated ground water for 3 to 4 weeks at a room temperature of 28 ± 2°C. As these catfishes are benthic in nature, overcrowding was avoided by keeping small numbers of fishes in each tank. Water was changed on alternate days. Tanks were covered with fish netting to prevent the escape of fishes.

Selection of sub-lethal concentrations: In the present study $1/10^{th}$ of the 96h LC_{50} value was taken as sub-lethal concentration (A). The two other doses, B & C, used were a reduction in concentration of the sub-lethal concentration (A) in a graded manner. The half concentration of the sub-lethal concentration a (50% reduction) was used as the second dose (B) while the third dose (C) was 50% reduction in concentration of the second dose B (Kayode *et al.*, 2016).

Haematological studies: After determining 96 h LC50 value, 3 sub-lethal concentrations (A, B, C) of Cadmium chloride were taken and 10 fishes were introduced in each concentration. For each sub-lethal exposure, five replicates were maintained. The water was changed every day in the control and renewed in the treatment group, so that the concentration of cadmium chloride remained the same during the experimental period. *Heteropneustes fossilis* was exposed to sub-lethal concentration of Cadmium for 21 days. At the end of 7th, 14thand 21st day sampling was done. At the end of the exposure period, blood was

taken by the following method. The fish were caught very gently using a small dip net, one at a time with least disturbance. Each fish was held and wrapped with a clean, dry towel and the posterior half of its body was blotted with a clean coarse filter paper. Blood from the Control and Cadmium chloride treated fishes were obtained by severance of caudal peduncle and collected in Eppendorf tubes containing 1% of Ethylene diamine tetra acetic acid (EDTA) as anticoagulant (Mgbenka *et al.,* 2003). Haematological parameters were estimated by standard methods as described by Hesser (1960) and Blaxhall and Daisley (1973).

Tissue Digestion for Accumulation: Estimation of heavy metals was carried out by following the tissue digestion. Tissue samples were thawed, rinsed in distilled water, and blotted with blotting paper. After blotting, the samples were transferred to 100 ml volumetric flasks. The entire flask was washed properly and rinsed with distilled water, before transferring the tissue samples. Then, the known weights of each tissue were transferred to these volumetric flasks. Samples digestion was carried out according to the methods presented. A slight modification was made in the procedure; instead of putting 10 ml nitric acid (60%) and 5 ml per chloric acid (70%) at the time of digestion, 5 ml nitric acid (60%) and 1 ml per chloric acid (70%) were added to each flask and the flasks were then kept overnight. The next day, a second dose of 5 ml nitric acid (60%) and 4 ml per chloric acid (70%) was added to each flask. The flasks were kept on a hot plate, covered with Pyrex glass cover, and allowed to digest at 200 to 250°C until a clear transparent solution was observed. Initially, dark brown fumes appeared followed by white fumes. The dense white fumes from the flask, after brown fumes, were an intimation of completion of the digestion process. By this method, digestion was accomplished in about 30 minutes instead of 3 to 4 hours as described in. After digestion, the samples were cooled, filtered through Whatman 42 filter paper and diluted to 100 ml with distilled water by proper rinsing of the digestion beakers.

Histological Studies: After the fish dissection, portions of tissues (gills and muscles) were preserved in 10% formalin for histological studies. The preserved tissues were processed in various grades of ethanol, cleared in xylene, and impregnated with wax (mp; 58°C). Five-micron-thick sections were cut using a rotary microtome (Leica RM 2165) at 100x. Tissue sections were stained with hematoxylin and eosin (H&E). Stained slides were observed and photographed under a high-resolution microscope (Leeia, Japan) fitted with a digital camera.

Statistical Analysis*:* the statistical analysis was performed using IBM SPSS (version 20).

RESULT AND DISCUSSION

Mortality and probit mortality of Lead and Cadmium (Toxicological Studies): Toxicological studies were conducted to evaluate the rate of mortality and probed mortality of *H. fossilis* under various known concentrations of cadmium in the aquaria. In the Aquarium or ecological pond (static and continuous flow) gradual increment of cadmium, Lead concentrations with time intervals proportional increment had been observed rate of mortality and probit mortality and plotted to determine the LC_{50} value and vulnerability rate of the fish (Table 4.4 and 4.5). Acute toxicity was found at 96 h LC_{50} of cadmium, lead in static was 20.68619 ppm; 65.00476 ppm and continuous flow was 16.66384 ppm 58.42679 ppm and lethal concentration (LC_{50}). Rate of mortality at 95% confidential levels includes both the cases of lower and upper at 96hours of exposure was observed good mortality rate at lower concentration for longer periods of time was more toxic and caused complete death resulted in both static and continuous flow system (static lower, upper 18.55514; 24.44486; continuous lower and upper 14.55514; 20.44486 ppm. Similar reports were observed in the case of salmonids, *Oncorhynchus mykiss, Salvelinus confluentus* and *Oncorhynchus tshawytscha* (Finalayson and Verrue, 1982; Hansen *et al*., 2002), guppy, *Poecilia reticulate (*Yilmaz *et al*., 2004), Cyprinus *carpio (*Muley *et al*., 2000; Dardenne *et al.,* 2007), Nile tilapia, *Oreochromis niloticus* (Mahnaz Sadat Sadeghi and Sadegh Peery(2018),Garcia *et al*., 2006) and Rohu, *Labeo rohita* (Dutta and Kaviraj, 2001). It also concurs the Canadian Environmental Protect Act, 1994 report in which it has been suggested that toxicity of cadmium in fish varies from species to species. Cadmium has shown toxic effects on the *H. fossilis* (Nilalohit *et al.,* 1981; Henary and Atchison, 1990; Brown *et al.*, 1994; Maruthayanagam *et al.,* 2002; Sobha *et al.*, 2007; Kasherwani *et al.*, 2009).The mortality due to the absorption and bio-accumulation of cadmium. The variations observed in the 24, 48, 72 and 96 h LC_{50} values between *H. fossilis* and other fishes may be attributed to the fact that metal induced changes in physiology and survival of aquatic organisms under metallic stress differ from metal to metal, species to species and from one experimental condition to other. The exact causes of death due to heavy metal poisoning are multiple and depend mainly on time-concentration combination. The 96 h LC_{50} values for cadmium were recorded as

5.36 mg L^{-1}. Sobha *et al.* (2007) reported 96 h LC_{50} of *Catla Catla* for Cd, Lead as 4.53 mg L^{-1}. El-Moselhy (2001) reported decrease in the *Heteropneustes fossilis.*

Table 4.1.2 : The LC_{50} values of cadmium exposed to *H. fossilis* for 24, 48, 72 and 96 h in Static system

S.No	Exposure period	Conc. (ppm)	LogConc.	No. of fish exposed	No of fish alive	No of fish dead	Percent Mortality	Probit Mortality	LC50
1	24 h	27.5	1.439333	10	9	1	10	3.7184	32.68056
2		29.0	1.462398	10	8	2	20	4.1584	
3		30.5	1.484300	10	7	3	30	4.4756	
4		32.0	1.505150	10	6	4	40	4.7467	
5		33.5	1.525045	10	4	6	60	5.2533	
6	48 h	24.5	1.389166	10	9	1	10	3.7184	28.14915
7		26.0	1.414973	10	8	2	20	4.1584	
8		27.5	1.439333	10	6	4	40	4.7467	
9		29.0	1.462398	10	4	6	60	5.2533	
10		30.5	1.484300	10	3	7	80	5.8416	
11	72 h	21.5	1.332438	10	8	2	20	4.1584	23.69800
12		23.0	1.361728	10	6	4	40	4.7467	
13		24.5	1.389166	10	4	6	60	5.2533	
14		26.0	1.414973	10	3	7	80	5.8416	
15		27.5	1.439333	10	2	8	90	6.2816	
16	96 h	18.5	1.267172	10	8	2	20	4.1584	20.68619
17		20.0	1.301030	10	6	4	40	4.7467	
18		21.5	1.332438	10	4	6	60	5.2533	
19		23.0	1.361728	10	2	8	80	5.8416	
20		24.5	1.389166	10	1	9	90	6.2816	

Table 4.1.3: The LC_{50} values of cadmium exposed to *H. fossilis* for 24, 48, 72 and 96 h in Continuous flow through system

S.No	Exposure period	Conc. (ppm)	Log Conc.	No. of fish exposed	No. of fish alive	No of fish dead	Percent Mortality	Probit Mortality	LC_{50}
1	24 h	23.5	1.371068	10	9	1	10	3.7184	28.68277
2		25.0	1.397940	10	8	2	20	4.1584	
3		26.5	1.423246	10	7	3	30	4.4756	
4		28.0	1.447158	10	6	4	40	4.7467	
5		29.5	1.469822	10	4	6	60	5.2533	
6	48 h	20.5	1.311754	10	9	1	10	3.7184	24.13725
7		22.0	1.342423	10	8	2	20	4.1584	
8		23.5	1.371068	10	6	4	40	4.7467	
9		25.0	1.397940	10	4	6	60	5.2533	
10		26.5	1.423246	10	3	7	80	5.8416	
11	72 h	17.5	1.243038	10	8	2	20	4.1584	19.68146
12		19.0	1.278754	10	6	4	40	4.7467	
13		20.5	1.311754	10	4	6	60	5.2533	
14		22.0	1.342423	10	3	7	80	5.8416	
15		23.5	1.371068	10	2	8	90	6.2816	
16	96 h	14.5	1.161368	10	8	2	20	4.1584	16.66384
17		16.0	1.204120	10	6	4	40	4.7467	
18		17.5	1.243038	10	4	6	60	5.2533	
19		19.0	1.278754	10	2	8	80	5.8416	
20		20.5	1.311754	10	1	9	90	6.2816	

Table 4.1.12: 95% Confidence levels of Cadmium exposed to H. *fossilis* for 24, 48, 72 and 96 h in static and continuous flow through methods

S.No	Exposure period	95% Confidence levels			
		Static Method		Continuous flow through Method	
		Lower	Upper	Lower	Upper
1	24 h	27.55514	33.44486	23.55514	29.44486
2	48 h	24.55514	30.44486	20.55514	26.44486
3	72 h	21.55514	27.44486	17.55514	23.44486
4	96 h	18.55514	24.44486	14.55514	20.44486

Table 4.1.13. The LC_{50} values of Lead exposed to *H. fossilis* for 24, 48, 72 and 96 h in Static system

S.No	Exposure period	Conc. in ppm	Log Conc.	No. of fish exposed	No. of fish alive	No. of fish dead	Percent Mortality	Probit Mortality	LC_{50}
1		72.0	1.857332	10	9	1	10	3.7184	
2	24 h	75.0	1.875061	10	8	2	20	4.1584	
3		78.0	1.892095	10	7	3	30	4.4756	82.35547
4		81.0	1.908485	10	6	4	40	4.7467	
5		84.0	1.924279	10	4	6	60	5.2533	
6		68.0	1.832509	10	9	1	10	3.7184	
7	48 h	71.0	1.851258	10	8	2	20	4.1584	
8		74.0	1.869232	10	6	4	40	4.7467	75.33431
9		77.0	1.886491	10	4	6	60	5.2533	
10		80.0	1.903090	10	3	7	80	5.8416	
11		64.0	1.806180	10	8	2	20	4.1584	
12	72 h	67.0	1.826075	10	6	4	40	4.7467	
13		70.0	1.845098	10	4	6	60	5.2533	68.44619
14		73.0	1.863323	10	3	7	80	5.8416	
15		76.0	1.880814	10	2	8	90	6.2816	
16		61.0	1.785330	10	8	2	20	4.1584	
17	96 h	64.0	1.806180	10	6	4	40	4.7467	
18		66.0	1.819544	10	4	6	60	5.2533	65.00476
19		69.0	1.838849	10	2	8	80	5.8416	
20		72.0	1.857332	10	1	9	90	6.2816	

Table 4.1.14. The LC_{50} values of Lead exposed to *H. fossilis* for 24, 48, 72 and 96 h in Continuous flow through system

S.No	Exposure period	Conc. in ppm	Log Conc.	No .of fish exposed	No. of fish alive	No. of fish dead	Percent Mortality	Probit Mortality	LC_{50}
1		66.0	1.819544	10	9	1	10	3.7184	
2	24 h	69.0	1.838849	10	8	2	20	4.1584	
3		72.0	1.857332	10	7	3	30	4.4756	76.35705
4		75.0	1.875061	10	6	4	40	4.7467	
5		78.0	1.892095	10	4	6	60	5.2533	
6		62.0	1.792392	10	9	1	10	3.7184	
7	48 h	65.0	1.812913	10	8	2	20	4.1584	
8		68.0	1.832509	10	6	4	40	4.7467	69.32508
9		71.0	1.851258	10	4	6	60	5.2533	
10		74.0	1.869232	10	3	7	80	5.8416	
11		58.0	1.763428	10	8	2	20	4.1584	
12	72 h	61.0	1.78533	10	6	4	40	4.7467	
13		64.0	1.80618	10	4	6	60	5.2533	62.43528
14		67.0	1.826075	10	3	7	80	5.8416	
15		70.0	1.845098	10	2	8	90	6.2816	
16		54.0	1.732394	10	8	2	20	4.1584	
17	96 h	57.0	1.755875	10	6	4	40	4.7467	
18		60.0	1.778151	10	4	6	60	5.2533	58.42679
19		63.0	1.799341	10	2	8	80	5.8416	
20		66.0	1.819544	10	1	9	90	6.2816	

Table 4.1.23. 95% confidence Levels of Cadmium exposed to H. *fossilis for* 24, 48, 72 and 96 h in static and continuous flow through methods

S.No	Exposure period	95% Confidence levels			
		Static Method		Continuous flow-through Method	
		Lower	Upper	Lower	Upper
1	24 h	72.11027	83.88973	66.11027	77.88973
2	48 h	68.11027	79.88973	62.11027	73.88973
3	72 h	64.11027	75.88973	58.11027	69.88973
4	96 h	61.08835	71.71165	54.11027	65.88973

Behavioural Studies (Gill and Tissue): Various sub-lcthal concentrations of cadmium and lead shown adverse effects on behaviour of fish. In the both cases of stagnant and continuous flow culture. Observable dysfunctions were found which includes sluggish movement due to this condition fish slowly moved to the bottom of the aquarium. Swim independently and trying to jump out of the water in the 7, 14 and 21 day. This kind of behaviour is taking as index to measure the physiological and biochemical alterations of an organism. Inability to take feed or reduced quantity consumption by the animal, position and defend nature was altered ((Prashanth *et al.*, 2011)). In the second day onwards animal secretes mucous all over the body, more in gill region. Physiological responses were measured based on the moderate and mild gulping of air and moderate opercular movement was observed. The animal, *H. fossilis* showed various behavioral changes at different cadmium and lead concentrations. The type, rate and duration of the behavioral changes increased with increase in concentrations. Animal is Hyperactive and attempted to escape from the tank during the first hours of all treatments. The behavioural disorders included loss of balance, respiratory difficulty, slowness of motion; frequent surfacing activity and increased mucus secretion were observed after 48 h of exposure. These toxic effects increased as the dose increased. After 72 h of exposure in higher concentration, the secretion of mucus increased and the fish turns upside down in the water, became motionless, sideways swimming and loss of balance were observed concurrent with the reports of Puvaneswari and Karuppasamy (2007), Asim Ullah et al., (2016). The anal fin, the anus and the area around the eyes were bloody. The fish behaviour in laboratory can be a sensate marker of toxicant-induced stress (Smita Srivastava *et al.*, 2007). The target organ most frequently involved in systemic toxicity is the CNS (brain and spinal cord) (Klaassen, 2008), resulting in loss of coordination and locomotion, instability followed by hyper excitability, tremors and convulsions (Wouters and Vanden Brecken, 1978).

Table 4.2.1.Behavioural responses of *H. fossilis* exposed to various sub-lethal concentrations of Cadmium at different periods of exposure

Exposure Periods	Sub-lethal conc.	Behavioural Responses					
		Surface visit	Jumping	Fast swimming	Mucous secretion	Air gulping	Opercular movement
7th Day	Control	++	++	++	-	++	++
	A	++	++	++	-	++	++
	B	++	++	++	-	++	++
	C	++	++	++	-	++	++
14th Day	Control	++	++	++	-	++	++
	A	+	+	-	+	+	++
	B	++	+	+	-	++	++
	C	++	++	++	-	++	++
21st Day	Control	++	++	++	-	++	++
	A	+	-	-	+	+	++
	B	+	-	-	+	+	++
	C	++	+	+	-	++	++

A = Sub-lethal conc. (2.068 ppm); B = 50% SL of A (1.034 ppm); C = 50% SL of B (0.517 ppm) Notes: - none, + mild and ++ moderate.

Table 4.2.2. Behavioural responses of *H. fossilis* exposed to various sub-lethal concentrations of Lead at different periods of exposure

Exposure Periods	Sub-lethal conc.	Behavioural Responses					
		Surface visit	Jumping	Fast swimming	Mucous secretion	Air gulping	Opercular movement
7th Day	Control	++	++	++	-	++	++
	A	++	++	++	-	++	++
	B	++	++	++	-	++	++
	C	++	++	++	-	++	++
14th Day	Control	++	++	++	-	++	++
	A	+	+	-	+	+	++
	B	++	+	+	-	++	++
	C	++	++	++	-	++	++
21st Day	Control	++	++	++	-	++	++
	A	+	-	-	+	+	++
	B	+	+	+	+	++	++
	C	++	+	+	-	++	++

A = Sub-lethal conc. (6.50 ppm); B = 50% SL of A (3.25 ppm); C = 50% SL of B (1.625 ppm)Notes: - none, + mild and ++ moderate.

Haematological Studies: Haematogram was carried out to determine the erythrocytes (RBC), leucocytes (WBC) and thrombocytes. Total 82 animals belong to the H. *Fossilis* were used for this study. The animal weight was approximately 54±4g. RBC was immature at low temperature count of the RBC found to be 3.26±0.63 millions/mm^3 for males and 3.24±0.76 millions/mm^3 for females and leucocytes (WBC) were found to be 3.74±2.25 millions/mm^3 for males and 3.76±2.16 millions /mm^3 for females. Hemoglobin (Hb) 10.52±1.62 g/dl in males and 10.66 ±1.72 g/dl in females were predicted, during the breeding season and the minimum when the gonads were immature. All blood cells were found highest value where gonads were maturing at highest temperature in summer months. Variations found in the haematological studies

depend on the body weight, physiological condition of the animal, temperature and season. Haematological parameters more quickly reflect the health status of fish than any other commonly measured parameters (Arun Thomas *et al.*, 2017; Atkinson and Judd, 1978).

Table 4.3.1. Haematological profile of *H. fossilis* (Male and Female)

	RBC (millions)	Hb (g/dl)	PCV (%)	WBC (millions)	MCV (μm^3)	MCH (pg)	MCHC (%)
Male (N=42)							
Mean±SD	3.26±0.63	10.52±1.62	39.16±3.28	3.74±2.25	120.12±2.57	32.26±2.25	26.86±4.93
Range	2.52 – 4.36	7.12 – 14.98	32.12 – 46.56	3.56 -3.86	106.78- 127.46	28.25 - 34.35	22.1 – 32.17
Female (N=40)							
Mean±SD	3.24±0.76	10.66 ±1.72	39.34±2.16	3.76±2.16	121.41±2.26	32.90±2.66	27.08±3.98
Range	2.38 – 4.22	7.16 – 15.26	32.14 – 48.12	3.58 – 3.85	114.02 – 135.04	30.08 – 36.16	22.27 – 31.71

Haematologically significant changes were seen when subjected with various concentrations of Cadmium and Lead exposure of *H. fossilis* for 7, 14 and 21 days has shown significant decrease in haematocrit and haemoglobin concentration, Red blood cell counts with increased count of white blood cell (Table 4.3.2). Where decline stat of MCHC found when compared to control. But opposite levels of increment in the case of both MCV and MCH values respectively. . Increase in erythrocyte number during spawning season has been reported by Ezzat *et al.* (1973); Yazan et al., (2014) for *Tilapia zilli* and Fourie and Hattingh (1976) for carp. More number of erythrocytes is needed for the high energy demands associated with gonadial maturation. Cameron (1970) has shown that changes in RBC counts in pin fish are of some importance in meeting seasonal increase in respiratory demands (Srivastava and Sanjeev Choudhary (2011)).

Table 4.3.2. Cadmium induced changes in Hematological parameters of *H. Fossilis*

Treatments	Exposure period	Experiment						
		RBC (millions)	Hb (g/dl)	PCV (%)	WBC (millions)	MCV (μm^3)	MCH (pg)	MCHC (%)
Control	7th Day	3.10±0.112	10.52±0.16	38.94±1.16	3.72±0.160	119.80±3.26	33.93±1.22	27.01±0.34
	14th Day	3.11±0.342	10.64±0.68	38.98±1.18	3.78±0.226	121.15±4.12	34.21±1.02	27.29±0.64
	21th Day	3.11±0.645	10.72±0.12	38.98±0.96	3.78±0.468	121.92±2.62	34.46±1.46	27.50±0.46
A	7th Day	2.08±0.342	8.28±0.34	37.18±0.84	3.98±0.234	199.42±2.88	39.80±1.20	22.27±0.48
	14th Day	1.66±0.112	7.02±0.48	35.06±0.82	4.09±0.146	260.60±3.12	42.28±1.16	20.02±0.74
	21th Day	1.22±0.126	5.72±0.34	32.88±0.68	4.24±0.248	367.86±3.78	46.88±1.28	17.39±0.66
B	7th Day	2.42±0.246	9.64±0.28	38.18±0.66	3.90±0.106	164.38±4.12	39.83±0.86	25.27±0.78
	14th Day	1.98±0.242	8.16±0.46	36.68±0.96	3.98±0.426	210.50±3.46	41.21±0.96	22.24±0.84
	21th Day	1.78±0.422	7.20±0.68	35.26±0.88	4.08±0.346	240.78±2.86	42.69±1.08	20.41±0.56
C	7th Day	2.76±0.116	9.82±0.24	38.85±0.86	3.82±0.420	140.76±2.68	35.57±1.28	25.27±0.86
	14th Day	2.48±0.112	9.10±0.26	37.86±0.68	3.88±0.268	160.72±2.78	36.69±1.02	24.03±0.66
	21th Day	2.04±0.246	8.46±0.42	36.24±0.84	3.96±0.262	202.15±3.26	41.47±0.98	23.34±0.82

*Each value is represented as mean ± SD (n=5); Values are significant at $p<0.05$ (based on t-test)
A = Sub-lethal conc. (2.068 ppm); **B** = 50% SL of A (1.034 ppm); **C** = 50% SL of B (0.517 ppm)

Table 4.3.10. Lead induced changes in Haematological parameters of *H. fossilis*

Treatments	Exposure period	Experiment						
		RBC (millions)	Hb (g/dl)	PCV (%)	WBC (millions)	MCV (μm^3)	MCH (g)	MCHC (%)
Control	7th Day	3.10±0.112	10.52±0.16	38.94±1.16	3.72±0.160	119.81±1.88	33.94±0.46	27.01±0.34
	14th Day	3.11±0.342	10.64±0.68	38.98±1.18	3.78±0.226	121.16±2.12	34.21±0.86	27.29±0.64
	21th Day	3.11±0.645	10.72±0.12	38.98±0.96	3.78±0.468	121.93±2.22	34.47±0.78	27.50±0.46
A	7th Day	2.16±0.348	8.78±0.14	37.88±0.84	3.94±0.342	185.06±2.08	40.50±0.82	23.17±0.46
	14th Day	1.78±0.124	7.32±0.82	35.86±0.82	4.05±0.416	235.28±2.26	41.12±0.68	20.41±0.36
	21th Day	1.29±0.142	5.98±0.42	33.68±0.68	4.18±0.242	340.16±2.32	46.36±0.62	17.75±0.28
B	7th Day	2.56±0.222	9.84±0.18	38.68±0.66	3.86±0.222	152.27±1.68	38.44±0.78	25.24±0.52
	14th Day	2.03±0.242	8.66±0.62	36.98±0.96	3.94±0.260	201.38±1.98	42.66±0.88	23.95±0.46
	21th Day	1.84±0.226	7.52±0.68	35.86±0.88	4.04±0.412	228.04±2.12	43.40±0.58	20.97±0.54
C	7th Day	2.82±0.142	10.08±0.40	38.80±0.86	3.80±0.280	137.06±1.86	35.74±0.82	25.98±0.46
	14th Day	2.56±0.136	9.58±0.16	37.92±0.68	3.86±0.408	154.14±1.68	37.42±0.38	25.26±0.44
	21th Day	2.12±0.264	8.86±0.24	36.48 ±0.84	3.94±0.320	192.17±1.22	41.79±0.88	24.28±0.64

*Each value is represented as mean ± SD (n=5); Values are significant at $p<0.05$ (based on t-test)
A = Sub-lethal conc. (6.50 ppm); **B** = 50% SL of A (3.25 ppm); **C** = 50% SL of B (1.625 ppm).

Conclusion: The present study implies that the cadmium and Lead are at increased dosage subjected, toxicities, initiation of inflammation and redness. Prolonged exposure causes acute physiological and anatomical changes could result. Henceforth the present investigation depicts that with accordance of pollution rate and toxicity, enhanced in the fish. So that degree of harmfulness increased day bay day leads to cause many alternations by consuming such kind of food regularly, to avoid the toxicity of the above said metal free or required quantity aquarium or ecological cultivation must be maintained and recommended to practise to give or supply the vital with good protienacious food.

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References:

1. Elisabet Lindgren, Francesca Harris, Alan D. Dangour, Alexandros Gasparatos, Michikazu Hiramatsu, Firouzeh Javadi, Brent Loken, Takahiro Murakami, Pauline Scheelbeek & Andy Haines (2018). Sustainable food systems—a health perspective, Sustainability Science Vol (13):1505–1517.
2. Gandhewar S. S. and Zade S. B. (2019).Bioaccumulation Of Some Heavy Metals In The Fish,Clarias Batrachus (Linn.) RJLBPCS.
3. Golovanova, Irina. (2008). Effects of heavy metals on the physiological and biochemical status of fishes and aquatic invertebrates. Inland Water Biology. Vol(1): 93-101.
4. Hadeel M Huseen and Ahmed J Mohammed (2019). Heavy Metals Causing Toxicity J. Phys.: Conf. Ser. 1294.
5. Mehjbeen Javed1 and Nazura Usmani (2019). An Overview of the Adverse Effects of Heavy Metal Contamination on Fish Health, Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. Vol 89(2):389–403.
6. Michael A Clark, Marco Springmann, Jason Hill, and David Tilman, (2019). Multiple health and environmental impacts of foods, Vol 116 (46) 23357-23362.
7. Nitasha Khatri and Sanjiv Tyagi (2015). Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas, Publication Cover, Frontiers in Life Science, Vol 8(1):23-39.
8. Paul B Tchounwou, Clement G Yedjou, Anita K Patlolla, and Dwayne J Sutton (2014). Heavy Metals Toxicity and the Environment, EXS; Vol 101: 133–164

9. Rai, Prabhat. (2008). Heavy Metal Pollution in Aquatic Ecosystems and Its Phytoremediation Using Wetland Plants: An Ecosustainable Approach. International journal of phytoremediation. Vol 10: 131-58.
10. Richard M. LoPachin, Brian C. Geohagen, B.S., and Lars U Nordstroem (2019). Mechanisms of Soft and Hard Electrophile Toxicities, Toxicology.Vol 418: 62–69.
11. Sanaa Abdulaziz Mustafa (2020). Histopathology and heavy metal bioaccumulation in some tissues of *Luciobarbus xanthopterus* collected from Tigris River of Baghdad, Iraq, Egyptian Journal of Aquatic Research journal homepage, Vol 46, 123-129.
12. Tembo Rostern N (2017) The Effects of Some Metals in Acidified Waters on Aquatic Organisms, Fish & Ocean Opj 4(4).
13. Ting Liu, Xue Liang, Chao Lei, Qinhong Huang, Weiqi Song, Rong Fang, Chen Li, Xiaomei Li, Hui Mo, Ning Sun, Haoran Lv and Zhihua Liu (2020).High-Fat Diet Affects Heavy Metal Accumulation and Toxicity to Mice Liver and Kidney Probably via Gut Microbiota, Front. Microbiol.
14. Vhahangwele Masindi and Khathutshelo L. Muedi (2018). Environmental Contamination by Heavy Metals, intechopen.
15. Arun Thomas, E.T. Bhagya, S. and Abdul Majeed, (2017). Clinical Utility of Blood Cell Histogram Interpretation, J Clin Diagn Res; Vol 11(9): OE01–OE04.
16. Asim Ullah, Hameed Ur Rehman, Waqar Saeed, Muhammad Faheem Quraish, Zakir Ullah, Haroon, Riaz Ur Rehman, Sajid Awais, Raqeebullah, Waqar Ahmad and Kausar Saeed (2016). Determination of 96-hr LC50 value of cadmium for a fish, Labeo rohita, Journal of Entomology and Zoology Studies; Vol 4(5): 380-382.
17. Srivastava S. and Sanjeev K. Choudhary (2011). Haematological and Biochemical Reference Intervals for *Heteropneustes fossilis*, Asian Fisheries Science 24 (2011):314-320.
18. Mahnaz Sadat Sadeghi and Sadegh Peery(2018). Evaluation of toxicity and lethal concentration (LC50) of silver and selenium nanoparticle in different life stages of the fish Tenualosa ilish (Hamilton 1822); Oceanography Fish Open Access J, Vol 7(5).
19. Yazan M. Alomari, Siti Norul Huda Sheikh Abdullah, Raja Zaharatul Azma, and Khairuddin Omar (2014). Automatic Detection and Quantification of WBCs and RBCs Using Iterative Structured Circle Detection Algorithm, Vol 2014:1-17.

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PERCENTAGE CONCENTRATION OF NUCLEOTIDES IN GENOME DATA OF SARS - CORONA VIRUSES

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ABSTRACT

As the era of big data is coming, many genomic consortia are generating an enormous amount of data to characterize the functional roles of genetic variants and these data are widely available to the public. To reveal novel genomic insights from this data within a reasonable timeframe, traditional data analysis methods may not be sufficient or scalable, forcing the need for big data analytics to be developed for genomics. Genome data of fifty SARS -Corona Virus are analyzed for finding out common feature among them. A novel feature called "Percentage Concentration of Nucleotides" denoted as pA, pT, pG and pC are evaluated for each genome data and cross verified with other data whether all of them possess the same genetic features or not. Adjoints of a genome data are four independent binary sequences corresponding to the nucleotides of adenine, thymine, guanine and cytosine. For example, the adjoint of adenine of a genome sequence is a binary sequence consisting of 1's in the place of adenine in the genome sequence and 0's in all other places. The adjoint of thymine of a genome sequence is a binary sequence consisting of 1's in the place of thymine in the genome sequence and 0's in all other places. The adjoint of guanine of a genome sequence is a binary sequence consisting of 1's in the place of guanine in the genome sequence and 0's in all other places. The adjoint of cytosine of a genome sequence is a binary sequence consisting of 1's in the place of cytosine in the genome sequence and 0's in all other places. Adjoint arrays of all nine genome data are pair wise correlated, segregation of similar genomes into different classes is done and then result is reported in this paper.

Key words: SARS- Corona Viruses, Genome, Machine Learning Algorithms

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1. INTRODUCTION

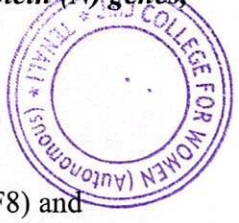
Irrespective of the size of the enterprise, either big or small, accessing the data continuous to be precious and irreplaceable asset. Data is exists internally to the enterprise and also exists outside the four walls and firewalls of the enterprise. Data is present in homogeneous sources as well as heterogeneous sources. The need of the hour is to understand, manage, process and take the data for analysis to draw valuable insights [1]. Big data is defined as the large data sets that are too large or complicated to be processed by traditional data applications. Big Data applies to information that can't be processed or analyzed using traditional processes or tools. Increasingly, organizations today are facing more and more big data challenges. The big data era is in full force today because the world is changing. They have access to wealth of information, but, they don't know how to get values out of it because it is sitting in its most raw form or in a semi structured or unstructured format; and as a result, they don't know even whether it is worth keeping or not [2]. Genomic medicines attempts to build individualized strategies for diagnostic or therapeutic decision making by utilizing patients genomic information. Big data analytics uncovers hidden patterns, unknown correlations and other insights through examining large scale various datasets [3]. A genome is an organisms complete set of DNA including all of its genes. Each genome contains all the information needed to build and maintain that organisms. In human, a copy of the entire genome more than 3 billion DNA base pairs is contained in all cells that have a nucleus [4]. Each cell in a body for example skin cell, liver cell contains the instructions in our genome are made up of DNA. Within DNA is unique chemical code that guides our growth, development and health. This code is determined by the order of four nucleotide bases that make up DNA. Adenine, Thymine, Guanine and Cytosine are the four nucleobases in the nucleic acid of DNA that are represented by A-T-G-C. DNA has twisted structure of a double helix. It has the functions in protein synthesis and chemical component of DNA and RNA. RNAs are termed as viruses. They are constructive as well as destructive. Constructive Viruses act in the Forward Process and destructive Viruses act in the reverse process. [5]

2. DATA USED FOR ANALYSIS

Fifty complete genome of viruses as given in the NCBI website: https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/ are considered here for data analysis. These genome data are assumed to be genuine and analysis carried out on them. [6]

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2 / human / IND / Assam-JMC4/2020 ORF8 protein (ORF8) and nucleocapsid phosphoprotein (N) genes, partial cds

GenBank: MT429168.1



LOCUS : MT429168 323 bp RNA linear VRL 05-MAY-2020
DEFINITION : Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IND/Assam-JMC4/2020 ORF8 protein (ORF8) and nucleocapsid phosphoprotein (N) genes, partial cds.
ACCESSION : MT429168
VERSION : MT429168.1

SOURCE : Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2)
ORGANISM :Severe acute respiratory syndrome coronavirus 2
Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
Orthocoronavirinae; Betacoronavirus; Sarbecovirus.
REFERENCE : 1 (bases 1 to 323)
AUTHORS : Borkakoty,B., Bali,N.K., Barua,P., Hazarika,R., Sharma,M.D. and Phukon,P.
TITLE : TSP PCR for identification of L and S type of SARS-CoV-2
JOURNAL Unpublished
REFERENCE : 2(bases 1 to 323)
AUTHORS : Borkakoty,B.,Bali,N.K.,Barua,P.,Hazarika,R.,Sharma,M.D.Phukon,P.
TITLE : Direct Submission
JOURNAL :Submitted (05-MAY-2020) Microbiology, Regional Medical Research Centre (ICMR), Dr. B Borkakoty, Scientist-E, Regional Medical Research Centre For NE Region (ICMR), Bokel, Dibrugarh, Assam 786001, India
COMMENT : ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES :Location/Qualifiers

ORIGIN

ggtaattata cagtttcctg tttacctttt acaattaatt gccaggaacc taaattgggt
agtcttgtag tgcgttgttc gttctatgaa gacttttag agtatcatga cgttcgtgtt
gttttagatt tcatctaaac gaacaaacta aaatgtctga taatggaccc caaaatcagc
gaaatgcact ccgcattacg tttggtggac cctcagattc aactggcagt aaccagaatg
gagaacgcag tggggcgcga tcaaaacaac gtcggcccca aggtttaccc aataatactg
cgtcttggtt caccgctctc act

Genome data of MT429168.1 Numerical Sequence

0001100101 0100000000 0001000000 1011001100 0001001100 0111000000
1000000010 0000000000 0000010011 0100000010 1001001001 0000000000
0000010100 0010001110 0110111001 1110000001 0110001000 0111100100
0111000100 0000100100 0000000010 0000101000 1100000100 1100101100
0101100010 0000000001 0011110110 0000000001 1000001000 1101101000
0000000000 0100000000 100

Similarly other fifty genomes that are chosen for analysis can also be coded as shown above.

3. PERCENTAGE CONCENTRATION OF NUCLEOTIDES IN GENOME SEQUENCE

Similar calculation of pH value of a solution, the values of pA, pT, pG and pC of a genome sequence and composition of these values like the proportion pA : pT : pG : pC is a method to classify and categorize genome sets. The Percentage Concentration of Nucleotides calculation

is as follows: "Given a genome sequence, the number of particular nucleotide, say A, present in that genome sequence is counted and the sum is divided by the total number of nucleotides in that genome sequence. The fraction when multiplied by 100 yields the percentage concentration of Adenine pA". Similarly one can evaluate pT, pG, and pC. Now, pA, pT, pG and pG were calculated for all fifty virus genome data [7]. To find out novel genomic insights traditional methods of analyzing data will not be sufficient, forcing the need for big data analytics to be developed for genomics. So, certain Machine Learning Algorithms are used to analyze this genome data and inferences are drawn for fifty genomes [8].

3.1. Basic details of all the Fifty Virus Genome are given below:

Table 1

SNO	GENE BANK ID	Length of Nucleotides	No. of Adenine (A)	No. of Thymine (T)	No. of Guanine (G)	No. of Cytosine(C)
1	LC522350.1	182	60	52	35	35
2	LC523807.1	357	110	73	86	88
3	LC523808.1	357	79	73	86	88
4	LC523809.1	357	110	73	86	88
5	LC528233.1	29902	8933	9601	5869	5499
6	LC529905.1	29903	8954	9596	5864	5489
7	LC534418.1	29878	8931	9595	5861	5491
8	LC534419.1	29874	8932	9590	5860	5492
9	LR757995.1	29872	8932	9589	5861	5490
10	LR757996.1	29868	8933	9586	5861	5488
11	LR757998.1	29866	8937	9584	5859	5486
12	MN908947.3	29903	8954	9594	5863	5492
13	MN938384.1	29838	8909	9586	5859	5484
14	MN938385.1	287	98	82	53	54
15	MN938386.1	287	98	82	53	54
16	MN938387.1	107	38	33	22	14
17	MN938388.1	107	38	33	22	14
18	MN938389.1	107	38	33	22	14
19	MN938390.1	107	38	33	22	14
20	MN970003.1	290	79	99	57	55
21	MN970004.1	290	79	99	57	55
22	MN975262.1	29891	8942	9595	5863	5491
23	MN975263.1	287	98	82	53	54
24	MN975264.1	287	98	82	53	54
25	MN975265.1	287	98	82	53	54
26	MN975266.1	107	38	33	22	14
27	MN975267.1	107	38	33	22	14
28	MN975268.1	107	38	33	22	14
29	MN985325.1	29882	8933	9595	5863	5491
30	MN988668.1	29881	8932	9594	5863	5492
31	MN988669.1	29881	8932	9594	5863	5492
32	MN988713.1	29882	8933	9591	5862	5488
33	MN994467.1	29882	8933	9595	5861	5493
34	MT371574.1	29756	8889	9561	5843	5463
35	MT372481.1	29898	8961	9594	5857	5485
36	MT374105.1	29900	8952	9595	5861	5492
37	MT374110.1	29900	8952	9597	5863	5488
38	MT374112.1	29901	8952	9601	5861	5487

39	MT374116.1	29901	8954	9597	5861	5489
40	MT375457.1	29788	8929	9559	5844	5456
41	MT375460.1	29848	8912	9593	5859	5484
42	MT375481.1	29899	8952	9598	5862	5487
43	MT375482.1	29880	8949	9592	5858	5481
44	MT375483.1	29871	8924	9599	5861	5487
45	MT396247.1	29853	8865	9538	5773	5421
46	MT429168.1	323	90	96	67	70
47	MT435084.1	29800	8895	9573	5854	5474
48	MT435085.1	29800	8896	9582	5852	5470
49	MT435086.1	29800	8887	9565	5838	5469
50	MT439597.1	29303	8747	9408	5760	5385

4. FORMULA TO CALCULATE PA, PT, PG, PC

The formula to calculate pA, pT, pG, pC as shown below:

pA= (Number of Adenine / Length of Nucleotides) * 100
Example:
pA for genome ID LC522350.1 is = 60/182*100 = **32.97**

pT= (Number of Thymine / Length of Nucleotides) * 100
Example:
pT for genome ID LC522350.1 is = 52/182 = **28.57**

pG = (Number of Guanine / Length of Nucleotides) * 100
Example:
pG for genome ID LC522350.1 is = 35/182 = **19.23**

pC = (Number of Cytosine / Length of Nucleotides) * 100
Example:
pC for genome ID LC522350.1 is = 35/182 = **19.23**

Similarly pA, pT, pG, pC is calculated for all fifty genomes.

Table 2

SNO	GENE BANK ID	No. of Adenine (A)	No. of Thymine (T)	No. of Guanine (G)	No. of Cytosine (C)	Length of Nucleotides	pA	pT	pG	pC
1	LC522350.1	60	52	35	35	182	32.97	28.57	19.23	19.23
2	LC523807.1	110	73	86	88	357	30.81	20.45	24.09	24.65
3	LC523808.1	79	73	86	88	357	22.13	20.45	24.09	24.65
4	LC523809.1	110	73	86	88	357	30.81	20.45	24.09	24.65
5	LC528233.1	8933	9601	5869	5499	29902	29.87	32.11	19.63	18.39
6	LC529905.1	8954	9596	5864	5489	29903	29.94	32.09	19.61	18.36
7	LC534418.1	8931	9595	5861	5491	29878	29.89	32.11	19.62	18.38
8	LC534419.1	8932	9590	5860	5492	29874	29.90	32.10	19.62	18.38
9	LR757995.1	8932	9589	5861	5490	29872	29.90	32.10	19.62	18.38
10	LR757996.1	8933	9586	5861	5488	29868	29.91	32.09	19.62	18.37
11	LR757998.1	8937	9584	5859	5486	29866	29.92	32.09	19.62	18.37
12	MN908947.3	8954	9594	5863	5492	29903	29.94	32.08	19.61	18.37

13	MN938384.1	8909	9586	5859	5484	29838	29.86	32.13	19.64	18.38
14	MN938385.1	98	82	53	54	287	34.15	28.57	18.47	18.82
15	MN938386.1	98	82	53	54	287	34.15	28.57	18.47	18.82
16	MN938387.1	38	33	22	14	107	35.51	30.84	20.56	13.08
17	MN938388.1	38	33	22	14	107	35.51	30.84	20.56	13.08
18	MN938389.1	38	33	22	14	107	35.51	30.84	20.56	13.08
19	MN938390.1	38	33	22	14	107	35.51	30.84	20.56	13.08
20	MN970003.1	79	99	57	55	290	27.24	34.14	19.66	18.97
21	MN970004.1	79	99	57	55	290	27.24	34.14	19.66	18.97
22	MN975262.1	8942	9595	5863	5491	29891	29.92	32.10	19.61	18.37
23	MN975263.1	98	82	53	54	287	34.15	28.57	18.47	18.82
24	MN975264.1	98	82	53	54	287	34.15	28.57	18.47	18.82
25	MN975265.1	98	82	53	54	287	34.15	28.57	18.47	18.82
26	MN975266.1	38	33	22	14	107	35.51	30.84	20.56	13.08
27	MN975267.1	38	33	22	14	107	35.51	30.84	20.56	13.08
28	MN975268.1	38	33	22	14	107	35.51	30.84	20.56	13.08
29	MN985325.1	8933	9595	5863	5491	29882	29.89	32.11	19.62	18.38
30	MN988668.1	8932	9594	5863	5492	29881	29.89	32.11	19.62	18.38
31	MN988669.1	8932	9594	5863	5492	29881	29.89	32.11	19.62	18.38
32	MN988713.1	8933	9591	5862	5488	29882	29.89	32.10	19.62	18.37
33	MN994467.1	8933	9595	5861	5493	29882	29.89	32.11	19.61	18.38
34	MT371574.1	8889	9561	5843	5463	29756	29.87	32.13	19.64	18.36
35	MT372481.1	8961	9594	5857	5485	29898	29.97	32.09	19.59	18.35
36	MT374105.1	8952	9595	5861	5492	29900	29.94	32.09	19.60	18.37
37	MT374110.1	8952	9597	5863	5488	29900	29.94	32.10	19.61	18.35
38	MT374112.1	8952	9601	5861	5487	29901	29.94	32.11	19.60	18.35
39	MT374116.1	8954	9597	5861	5489	29901	29.95	32.10	19.60	18.36
40	MT375457.1	8929	9559	5844	5456	29788	29.98	32.09	19.62	18.32
41	MT375460.1	8912	9593	5859	5484	29848	29.86	32.14	19.63	18.37
42	MT375481.1	8952	9598	5862	5487	29899	29.94	32.10	19.61	18.35
43	MT375482.1	8949	9592	5858	5481	29880	29.95	32.10	19.61	18.34
44	MT375483.1	8924	9599	5861	5487	29871	29.88	32.13	19.62	18.37
45	MT396247.1	8865	9538	5773	5421	29853	29.70	31.95	19.34	18.16
46	MT429168.1	90	96	67	70	323	27.86	29.72	20.74	21.67
47	MT435084.1	8895	9573	5854	5474	29800	29.85	32.12	19.64	18.37
48	MT435085.1	8896	9582	5852	5470	29800	29.85	32.15	19.64	18.36
49	MT435086.1	8887	9565	5838	5469	29800	29.82	32.10	19.59	18.35
50	MT439597.1	8747	9408	5760	5385	29303	29.85	32.11	19.66	18.38

5. INFERENCES DRAWN FROM PERCENTAGE NUCLEOTIDE CONCENTRATION OF ABOVE 50 GENOMES

The data of the percentage nucleotide concentration gives potential inferences when we talk about the segregation of different viruses into different classes. The data when color coded can be seen below.

Table 3

SNO	GENE BANK ID	No. of Adenine	No. of Thymine	No. of Guanine	No. of Cytosine	Length of Nucleotides	pA	pT	pG	pC
1	LC522350.1	60	52	35	35	182	32.97	28.57	19.23	19.23
2	LC523807.1	110	73	86	88	357	30.81	20.45	24.09	24.65
3	LC523808.1	79	73	86	88	357	22.13	20.45	24.09	24.65
4	LC523809.1	110	73	86	88	357	30.81	20.45	24.09	24.65
5	LC528233.1	8933	9601	5869	5499	29902	29.87	32.10	19.62	18.39
6	LC529905.1	8954	9596	5864	5489	29903	29.94	32.09	19.61	18.35
7	LC534418.1	8931	9595	5861	5491	29878	29.89	32.11	19.61	18.37
8	LC534419.1	8932	9590	5860	5492	29874	29.89	32.10	19.61	18.38
9	LR757995.1	8932	9589	5861	5490	29872	29.90	32.10	19.62	18.37
10	LR757996.1	8933	9586	5861	5488	29868	29.90	32.09	19.62	18.37
11	LR757998.1	8937	9584	5859	5486	29866	29.92	32.09	19.61	18.36
12	MN908947.3	8954	9594	5863	5492	29903	29.94	32.08	19.60	18.36
13	MN938384.1	8909	9586	5859	5484	29838	29.85	32.12	19.63	18.37
14	MN938385.1	98	82	53	54	287	34.15	28.57	18.47	18.82
15	MN938386.1	98	82	53	54	287	34.15	28.57	18.47	18.82
16	MN938387.1	38	33	22	14	107	35.51	30.84	20.56	13.08
17	MN938388.1	38	33	22	14	107	35.51	30.84	20.56	13.08
18	MN938389.1	38	33	22	14	107	35.51	30.84	20.56	13.08
19	MN938390.1	38	33	22	14	107	35.51	30.84	20.56	13.08
20	MN970003.1	79	99	57	55	290	27.24	34.14	19.66	18.97
21	MN970004.1	79	99	57	55	290	27.24	34.14	19.66	18.97
22	MN975262.1	8942	9595	5863	5491	29891	29.92	32.10	19.61	18.37
23	MN975263.1	98	82	53	54	287	34.15	28.57	18.47	18.82
24	MN975264.1	98	82	53	54	287	34.15	28.57	18.47	18.82
25	MN975265.1	98	82	53	54	287	34.15	28.57	18.47	18.82
26	MN975266.1	38	33	22	14	107	35.51	30.84	20.56	13.08
27	MN975267.1	38	33	22	14	107	35.51	30.84	20.56	13.08
28	MN975268.1	38	33	22	14	107	35.51	30.84	20.56	13.08
29	MN985325.1	8933	9595	5863	5491	29882	29.89	32.11	19.62	18.38
30	MN988668.1	8932	9594	5863	5492	29881	29.89	32.11	19.62	18.38
31	MN988669.1	8932	9594	5863	5492	29881	29.89	32.11	19.62	18.38
32	MN988713.1	8933	9591	5862	5488	29882	29.89	32.10	19.62	18.37
33	MN994467.1	8933	9595	5861	5493	29882	29.89	32.11	19.61	18.38
34	MT371574.1	8889	9561	5843	5463	29756	29.87	32.13	19.64	18.36
35	MT372481.1	8961	9594	5857	5485	29898	29.97	32.09	19.59	18.35
36	MT374105.1	8952	9595	5861	5492	29900	29.94	32.09	19.60	18.37
37	MT374110.1	8952	9597	5863	5488	29900	29.94	32.10	19.61	18.35
38	MT374112.1	8952	9601	5861	5487	29901	29.94	32.11	19.60	18.35
39	MT374116.1	8954	9597	5861	5489	29901	29.95	32.10	19.60	18.36
40	MT375457.1	8929	9559	5844	5456	29788	29.98	32.09	19.62	18.32
41	MT375460.1	8912	9593	5859	5484	29848	29.86	32.14	19.63	18.37
42	MT375481.1	8952	9598	5862	5487	29899	29.94	32.10	19.61	18.35
43	MT375482.1	8949	9592	5858	5481	29880	29.95	32.10	19.61	18.34
44	MT375483.1	8924	9599	5861	5487	29871	29.88	32.13	19.62	18.37
45	MT396247.1	8865	9538	5773	5421	29853	29.70	31.95	19.34	18.16
46	MT429168.1	90	96	67	70	323	27.86	29.72	20.74	21.67
47	MT435084.1	8895	9573	5854	5474	29800	29.85	32.12	19.64	18.37
48	MT435085.1	8896	9582	5852	5470	29800	29.85	32.15	19.64	18.36
49	MT435086.1	8887	9565	5838	5469	29800	29.82	32.10	19.59	18.35
50	MT439597.1	8747	9408	5760	5385	29303	29.85	32.11	19.66	18.38

The data has been color coded on the percentage nucleotide concentrations of all nucleotides. A similarity in color code for different genomes for percentage nucleotide concentrations reveals that the concentrations for different genomes can form a base to segregate the genomes into different classes.

The genomes have been segregated by coloring the SNO Column cells with the same color. The BRIGHT RED colored SNO cells do not come under any class according to their non-similarity in percentage nucleotide concentrations with other genomes or with each other.

The classes can be defined as below and have been given a color corresponding to the class defined by a colored band after the class number.

Class 1:

S.NO	GENE BANK ID	No. of Adenine	No. of Thymine	No. of Guanine	No. of Cytosine	Length of Nucleotides	pA	pT	pG	pC
2	LC523807.1	110	73	86	88	357	30.81	20.45	24.09	24.65
4	LC523809.1	110	73	86	88	357	30.81	20.45	24.09	24.65

5.1. The Graphical Representation of Class – 1 is shown in figure-1

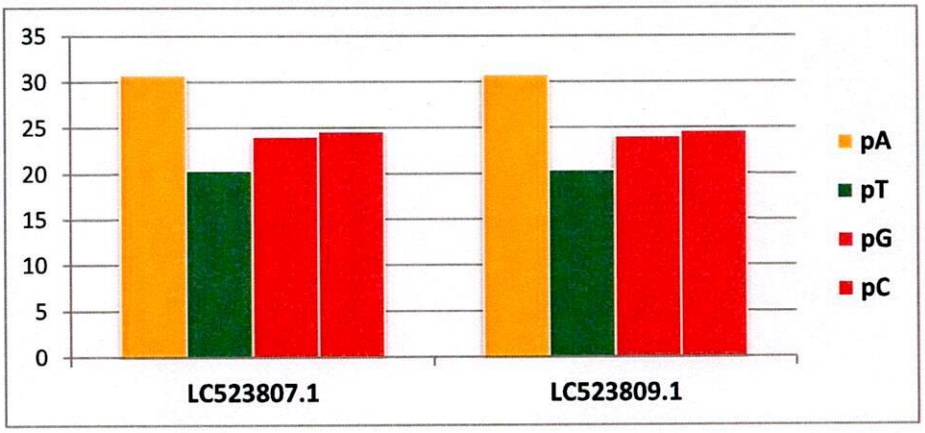


Figure 1 Representation of class -1

Class 2:

SNO	GENE BANK ID	No. of Adenine	No. of Thymine	No. of Guanine	No. of Cytosine	Length of Nucleotides	pA	pT	pG	pC
5	LC528233.1	8933	9601	5869	5499	29902	29.87	32.10	19.62	18.39
6	LC529905.1	8954	9596	5864	5489	29903	29.94	32.09	19.61	18.35
7	LC534418.1	8931	9595	5861	5491	29878	29.89	32.11	19.61	18.37
8	LC534419.1	8932	9590	5860	5492	29874	29.89	32.10	19.61	18.38
9	LR757995.1	8932	9589	5861	5490	29872	29.90	32.10	19.62	18.37
10	LR757996.1	8933	9586	5861	5488	29868	29.90	32.09	19.62	18.37
11	LR757998.1	8937	9584	5859	5486	29866	29.92	32.09	19.61	18.36
12	MN908947.3	8954	9594	5863	5492	29903	29.94	32.08	19.60	18.36
13	MN938384.1	8909	9586	5859	5484	29838	29.85	32.12	19.63	18.37
22	MN975262.1	8942	9595	5863	5491	29891	29.92	32.10	19.61	18.37
29	MN985325.1	8933	9595	5863	5491	29882	29.89	32.11	19.62	18.38
30	MN988668.1	8932	9594	5863	5492	29881	29.89	32.11	19.62	18.38
31	MN988669.1	8932	9594	5863	5492	29881	29.89	32.11	19.62	18.38
32	MN988713.1	8933	9591	5862	5488	29882	29.89	32.10	19.62	18.37
33	MN994467.1	8933	9595	5861	5493	29882	29.89	32.11	19.61	18.38
34	MT371574.1	8889	9561	5843	5463	29756	29.87	32.13	19.64	18.36
35	MT372481.1	8961	9594	5857	5485	29898	29.97	32.09	19.59	18.35
36	MT374105.1	8952	9595	5861	5492	29900	29.94	32.09	19.60	18.37
37	MT374110.1	8952	9597	5863	5488	29900	29.94	32.10	19.61	18.35
38	MT374112.1	8952	9601	5861	5487	29901	29.94	32.11	19.60	18.35
39	MT374116.1	8954	9597	5861	5489	29901	29.95	32.10	19.60	18.36
40	MT375457.1	8929	9559	5844	5456	29788	29.98	32.09	19.62	18.32
41	MT375460.1	8912	9593	5859	5484	29848	29.86	32.14	19.63	18.37
42	MT375481.1	8952	9598	5862	5487	29899	29.94	32.10	19.61	18.35
43	MT375482.1	8949	9592	5858	5481	29880	29.95	32.10	19.61	18.34
44	MT375483.1	8924	9599	5861	5487	29871	29.88	32.13	19.62	18.37
47	MT435084.1	8895	9573	5854	5474	29800	29.85	32.12	19.64	18.37
48	MT435085.1	8896	9582	5852	5470	29800	29.85	32.15	19.64	18.36
49	MT435086.1	8887	9565	5838	5469	29800	29.82	32.10	19.59	18.35
50	MT439597.1	8747	9408	5760	5385	29303	29.85	32.11	19.66	18.38

5.2. The Graphical Representation of Class – 2 is shown in figure-2

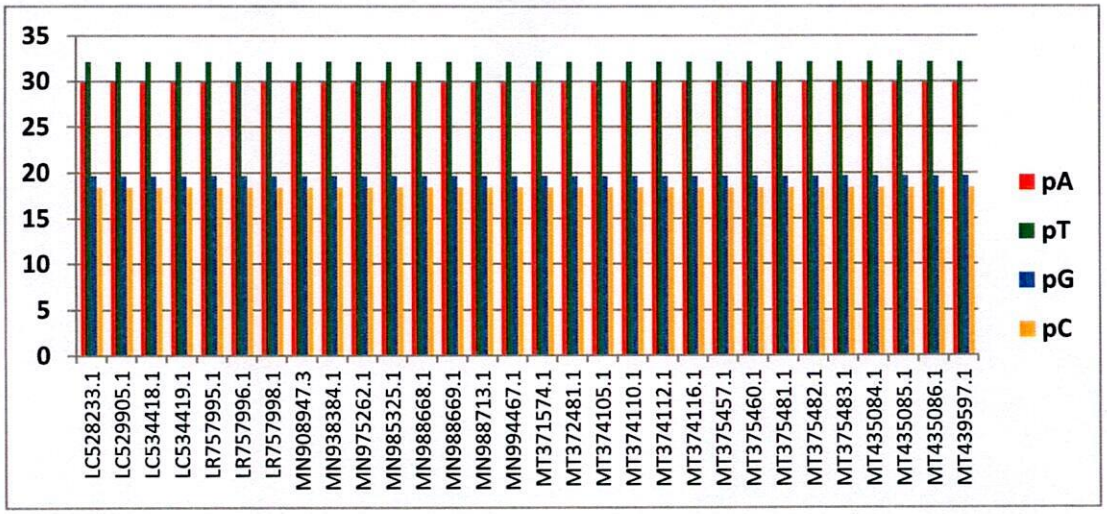


Figure 2 Representation of class -2

Class 3:

SNO	GENE BANK ID	No. of Adenines	No. of Thymine	No. of Guanine	No. of Cytosine	Length of Nucleotides	pA	pT	pG	pC
14	MN938385.1	98	82	53	54	287	34.15	28.57	18.47	18.82
15	MN938386.1	98	82	53	54	287	34.15	28.57	18.47	18.82
23	MN975263.1	98	82	53	54	287	34.15	28.57	18.47	18.82
24	MN975264.1	98	82	53	54	287	34.15	28.57	18.47	18.82
25	MN975265.1	98	82	53	54	287	34.15	28.57	18.47	18.82

5.3. The Graphical Representation of Class – 3 is shown in figure - 3

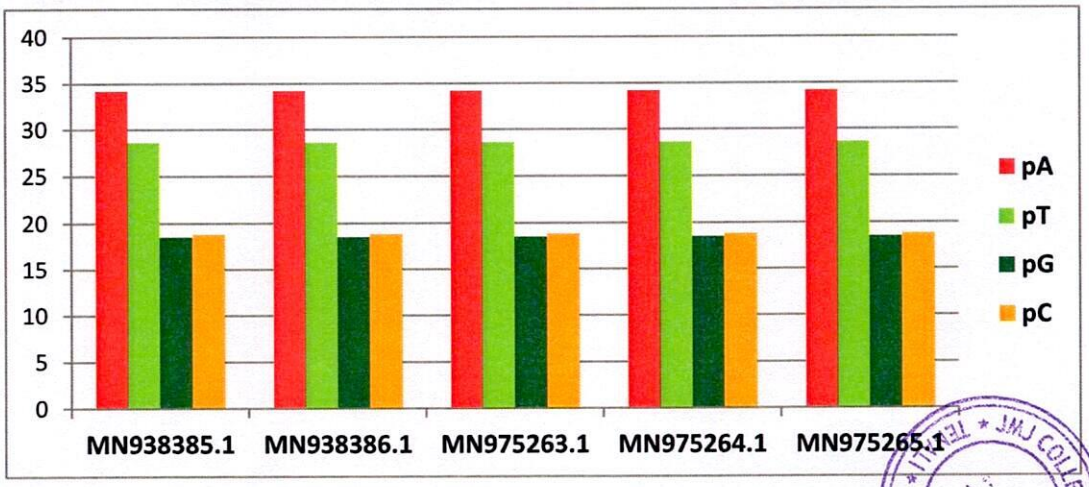


Figure 3 Representation of class -3

Class 4:

SNO	GENE BANK ID	No. of Adenine	No. of Thymine	No. of Guanine	No. of Cytosine	Length of Nucleotides	pA	pT	pG	pC
16	MN938387.1	38	33	22	14	107	35.51	30.84	20.56	13.08
17	MN938388.1	38	33	22	14	107	35.51	30.84	20.56	13.08
18	MN938389.1	38	33	22	14	107	35.51	30.84	20.56	13.08
19	MN938390.1	38	33	22	14	107	35.51	30.84	20.56	13.08
26	MN975266.1	38	33	22	14	107	35.51	30.84	20.56	13.08
27	MN975267.1	38	33	22	14	107	35.51	30.84	20.56	13.08
28	MN975268.1	38	33	22	14	107	35.51	30.84	20.56	13.08

5.4. The Graphical Representation of Class – 4 is shown is figure -4

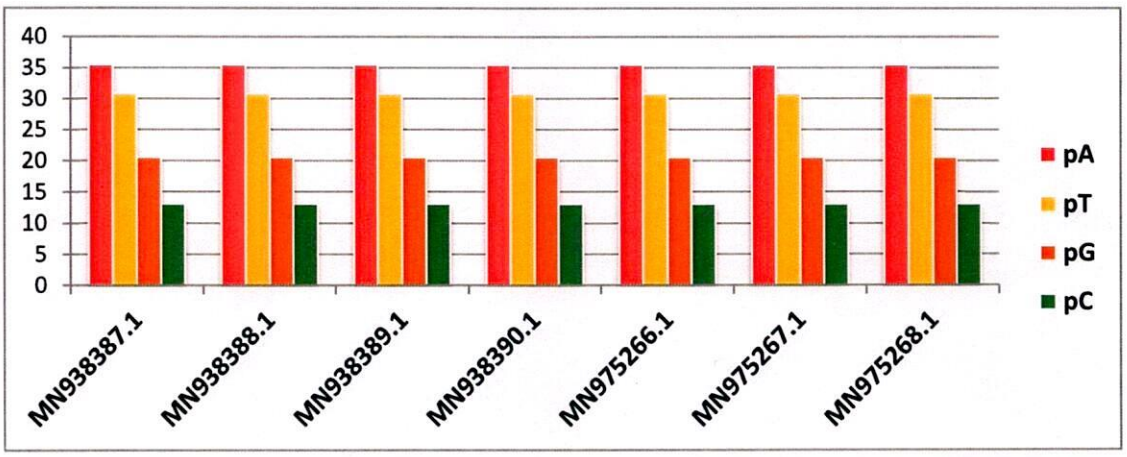


Figure 4 Representation of class -4

Class 5:

SNO	GENE BANK ID	No. of Adenine	No. of Thymine	No. of Guanine	No. of Cytosine	Length of Nucleotides	pA	pT	pG	pC
20	MN970003.1	79	99	57	55	290	27.24	34.14	19.66	18.97
21	MN970004.1	79	99	57	55	290	27.24	34.14	19.66	18.97

5.5. The Graphical Representation of Class – 5 is shown is figure -5

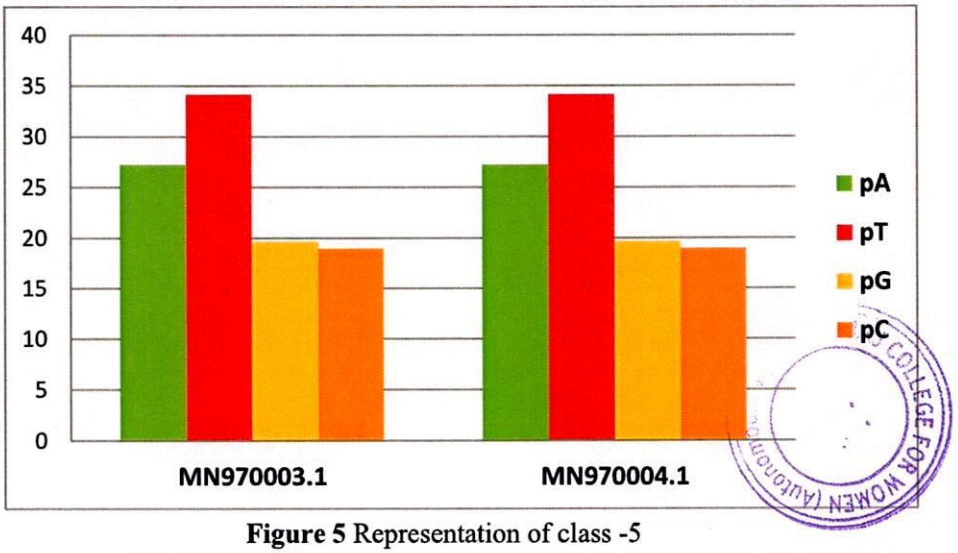


Figure 5 Representation of class -5

NO SIMILARITIES:

The following genomes show no similarities in percentage nucleotide concentrations with above classes or with each other. Therefore, they have been placed in a separate class.

SNO	GENE BANK ID	No. of Adenine	No. of Thymine	No. of Guanine	No. of Cytosine	Length of Nucleotides	pA	pT	pG	pC
1	LC522350.1	60	52	35	35	182	32.97	28.57	19.23	19.23
3	LC523808.1	79	73	86	88	357	22.13	20.45	24.09	24.65
45	MT396247.1	8865	9538	5773	5421	29853	29.70	31.95	19.34	18.16
46	MT429168.1	90	96	67	70	323	27.86	29.72	20.74	21.67

5.6. The Graphical Representation of No Similarities is shown is figure -6.

There is no similarity in percentage nucleotide concentrations for this class. The graph also shows the dissimilar percentages of pA, pT, pG, pC for the specified four genomes.

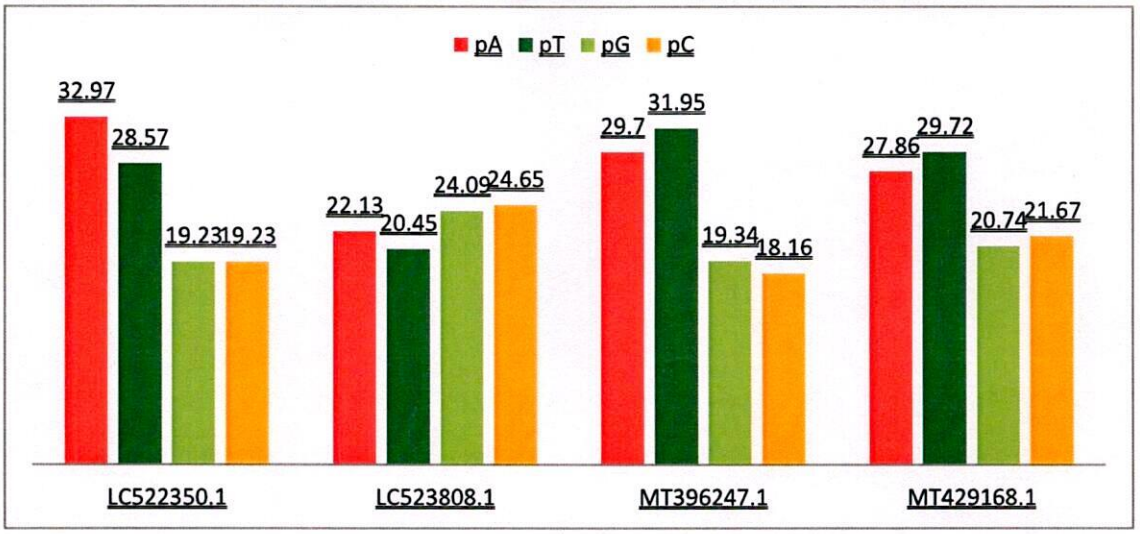


Figure 6 Representation of No Similarities in percentage nucleotide concentrations for 4 genomes

6. CONCLUSION

As a result of a systematic study of certain valid SARS-Corona virus data, we arrive at some findings. The % nucleotide concentrations of the virus data under study are observed to be varying within tolerable limits. This means that the viruses have similar kind of morphological structures. The question that arises here is whether the functional behavior of these viruses remains same or not. One cannot come to conclusions just by comparing similarity in morphological structures alone. A detailed intra cellular study is required now to understand the behavioral patterns of these viruses.

REFERENCES

[1] Seema Acharya, Subhashini Chellappan, "Big Data and Analytics", Wiley India Pvt.Ltd, 2nd Edition, 2018

[2] Chris Eaton, Dirk Deroos, Tom Deutsch, George Lapis, Paul C. Zikopoulos, "Understanding Big Data Analytics for Enterprise Class Hadoop and Streaming Data", McGraw Hill Education (India) Edition, 2015.

[3] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5343946/

[4] https://ghr.nlm.nih.gov/primer/hgp/genome

[5] https://www.yourgenome.org/facts/what-is-a-genome

[6] https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/

[7] Rajan E G., et al, "Pairwise Spatial Correlation of SARS-Corona Viruses", London Journals Press, 2020

[8] https://www.researchgate.net/publication/319096778_Big_data_analytics_in_genomics

ADVANCED ICT TOOLS FOR IMPLEMENTATION OF THE COMPLEX CALCULATIVE STUDY

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Abstract: India being a nation of Mathematicians like Srinivasa Ramanujam, Aryabhata, Shakuntala Devi, and so forth is constantly celebrated for worldwide numerical commitments throughout time. In the current circumstance, math is a subject that most understudies attempt to dodge because of its extensive counts and complex definitions. Numerous nations around the globe have done several turns of events and developments for mathematical calculations in the field of Chemistry, Physics, Electronics, Computer Science, and so forth with the assistance of cutting-edge Information Communication Technology (ICT) apparatuses. Indian mathematical subsystems also need to evolve and upgrade to meet the global professional competition from the grassroots level. There is a genuine need to join ICT instruments for mathematics in school and college education to have a superior agreement. The joining of ICT instruments can change the course of calculative study to calculative upgrade which further prompts higher concept understanding and perspective development. This paper discusses the new improvement in the field of advanced calculation and how it is limiting the weight on understudies. As per Prytherch (2000), "ICTs are networks that provide new opportunities for teaching, learning, and training through the delivery of digital content. "Integration technology in education means giving experiential learning to the students. Software tools for calculation are utilized in the study hall for improving intellectual capacity and abilities in students especially to diminish computation stress among them.

1. Introduction

In the era of globalization, the education society is going through many opportunities and challenges. ICT integration in education was a role changer for the teaching-learning process but implementing it was a challenge as well. The trend of the classroom has been shifted from teacher-cantered to student-cantered and the teacher became a guide, facilitator, blackboards turned into white-board, SMARTBOARD, Artificial intelligence & Virtual reality tools being used for imparting knowledge.

Integrating ICT in curriculum encouraging the use of applications for Teaching – Learning. (NCTM, 2000) (Is, 1998) National Council of Teachers of Mathematics suggested that using technology is part of teaching strategies. NCTM states that" It is important for teachers and students should have access to technologies that support and advance mathematical sense-making, reasoning, problem-solving, and communication. Effective teachers optimize the potential of technology to develop students' understanding, stimulate their interest, and increase their proficiency in mathematics. When teachers use technology strategically, they can provide greater access to mathematics for all students" (NCTM, 2000).[1]

2. Literature Survey

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(Kilicman et al., 2010) Done a study on, "Teaching and Learning Mathematics with tool". He had done the review on the recent Italian tools used in Mathematics'teaching. He reviewed the studies related to the software like: 'The DGE: Dynamic Geometry Software, The CAS: Computer Algebra Systems, Spreadsheet for mathematic knowledge. He concluded his study by saying that only tools feature is not the parameter to decide the integration of technology in the classroom also features like classroom activity, interaction among teachers and students & between the students are also important.

(Barry, 2017) Have entitled his study "Alleviating Math Anxiety through the Integration of Technology in Elementary School" This study discusses the strategies, outcome, and challenge in integrating technology in Mathematics teaching on primary school students. This study aims to answer questions related to factors influencing technology-integrated teaching strategies and student outcome and engagement. As a sample two elementary school was taken and data was collected through a semi-structured questionnaire. The research concluded his study by saying teacher knowledge and belief toward technology.

(Das, 2019) named his study " Role of ICT for Better Mathematics Teaching" Study aim to explore the aspect of technology integration in mathematics teaching. The researcher has done a document-based analytical study for drawing the conclusion. He believed the role of mathematics is not limited to the academic domain only. He discusses the integration of ICT in Mathematics at the teacher training level. This study mentioned the drawbacks as poor infrastructure, teacher focus on PowerPoint Presentation creativity rather than content knowledge, Lack of technical skills in teachers.

(Sevari, 2018) Entitled his study "The effectiveness of mathematics software aided learning tool with performance assessment on student independence and student learning outcomes" done to verify the effect of mathematical software on performance of students. This was an experimental study done on the Mathematics Education Department of FMIPA Undiksha, 49 integral calculus students were a sample for this study. Result of this research shows those student performances were higher who were using mathematics software-assisted device in comparison to a conventional learning tool.

3. ICT Tools for Teaching and Learning Mathematics

Mathematics tools help students in concept building to perform calculation-related problems, which makes mathematics easier & understandable.

Maple

Figure.1 Logo of Maple

The tool is used for symbolic and numeric calculation data analysis, visualization in Mathematics. This serves only in 2d processing. This tool open source and used for mathematics and engineering has a mathematics library as well. It is particularly useful for illustrating interval algorithms in the field of validating computing. Maple software is generally used for college students. It contains almost 45 tutorial which is used for teaching-leaning process for undergraduate students. This tool contains mathematics concepts related to, calculus, algebra, complex variable, vector, this tool helps in understanding the steps of calculation while solving the problem.Maple2020 has updated the software by including some new features and also upgraded for solving the problems, document creation, interactive problem-solving.This

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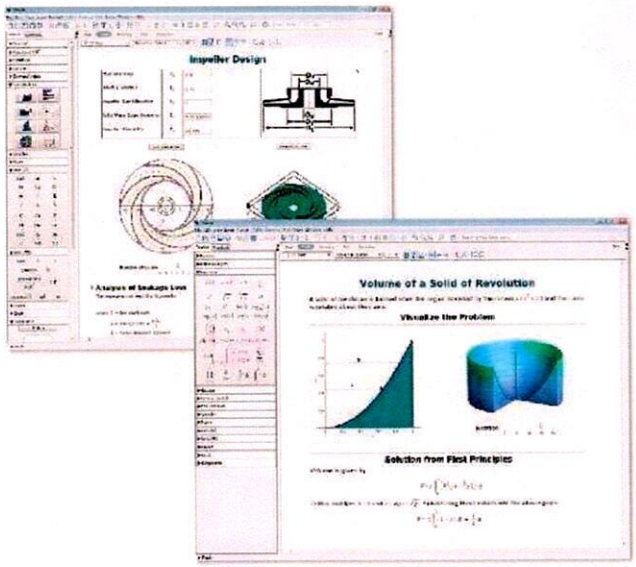
software needless programming in comparison to other software.

Output



Mathematica

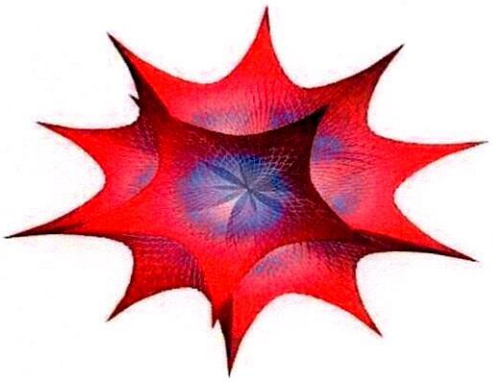
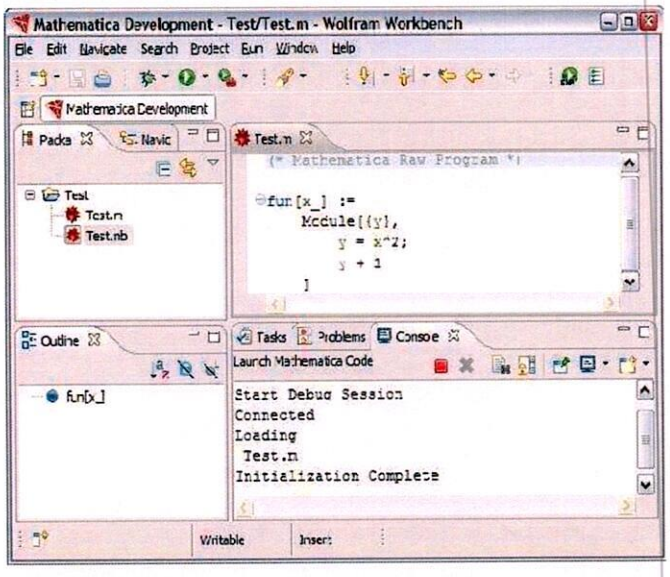


Figure.2 Logo of Mathematica

This software is constructed to solve numerical and symbolic problems in mathematics. This tool is generally used in the field of engineering, Science, computing fields. It provides excellent visualization in 2D and 3D. Mathematica is developed by Wolfram Research of Champaign, and he grants free access and accounts on the latest version of the software. This tool can be used for self-learning with the help of tutorials.

Output



MatLab

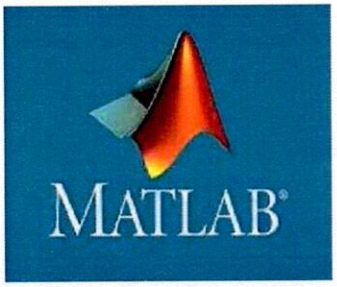


Figure.3 Logo of MatLab

It is an interactive system which helps you in solving complex computing problem, particularly with matrix and vector difficulties. Toolboxes of MatLab help you to learn and employ technology. This software can be used to accomplish multiple tasks related to Math and computation, Algorithm development, Modeling, simulation, and prototyping Data analysis, exploration, and visualization Scientific and engineering graphics Application development, including Graphical User Interface building. This software is not accessible for free.

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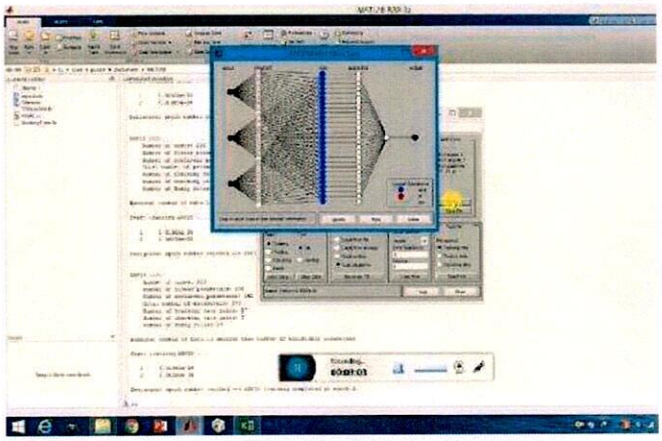
Output

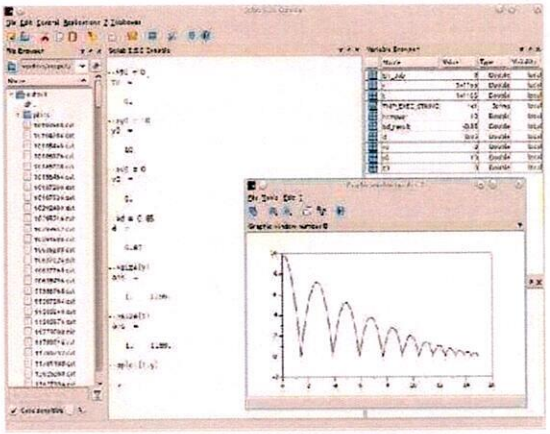


SciLab

Figure.4 Logo of Scilab

This is free and open software used for complex calculations. It uses scientific computing techniques. Scilab has a separate module for image processing. This software is most easy to use and provides various techniques for numerical calculation. A user can develop their module and execute their specific problem. Previously Scilab focused only on linear algebra but at present, it is covering many topics related to scientific computing. It provides a set of plotting functions and graphic features. The Xcos setting provides a hybrid dynamic systems modeler and simulator.

Output



GeoGebra

Figure.5 Logo of GeoGebra

GeoGebra is a tool that can be used for middle school to college levels students for Mathematics education. This tool contains all sections of mathematic. One can perform all the calculations related to geometry, algebra, spreadsheets, graphing, statistics, and calculus in this tool. It is free software where a student can explore experiments with objects and parameters for concept building in the mathematical curriculum. GeoGebra provides bi-directional representations of geometry and algebra.

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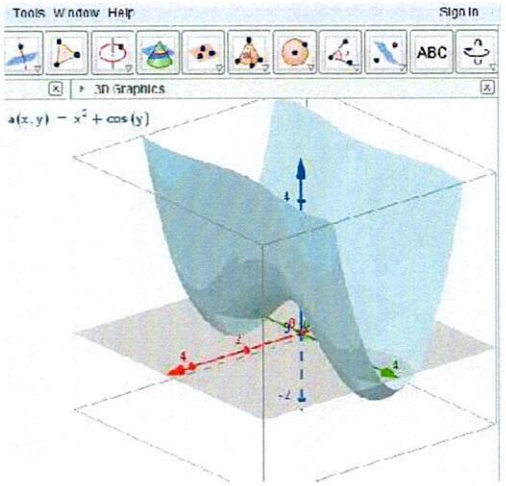


Winplot

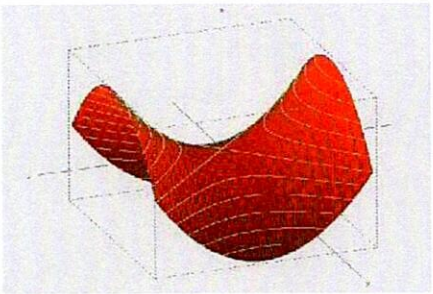
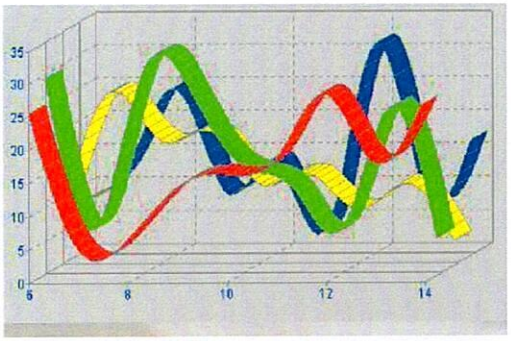


Figure.6 Logo of Winplot

Winplot is a plotting utility for Microsoft Windows, It can draw and animate mathematical curves and surfaces presented in a variety of formats. learners can drag and move their objects without redoing the drawing. So that learners have more time to think about the concept building rather than spending time restructure the drawing. Winplot can perform many procedures on functions like generating graphs of cross-sectional solid, detect trajectory on a slope, calculating line and surface integral.

Output



4. Real-time Programming and Examples

Example 1 - Manual calculation vs. CAS calculation

Find the determinant of a matrix $A = \begin{bmatrix} 7 & 6 & 9 & 5 & 6 \\ 3 & 8 & 7 & 5 & 7 \\ 5 & 4 & 6 & 8 & 7 \\ 1 & 2 & 3 & 8 & 7 \\ 7 & 5 & 3 & 1 & 5 \end{bmatrix}$.

This will take huge time manually and if we calculated the answer still we need to verify it.

Software Used – Scilab

Just 2 lines of code can bring the answer in few seconds.

```
--> A = [7 6 9 5 6; 3 8 7 5 7; 5 4 6 8 7; 1 2 3 8 7; 7 5 3 1 5]
--> det(A)
```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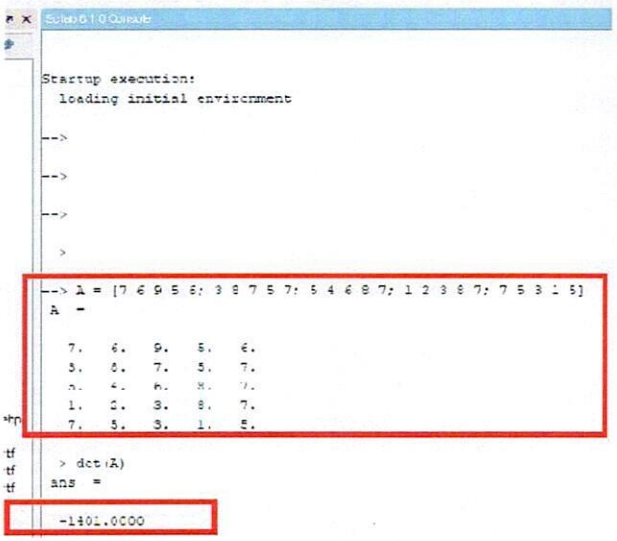


Figure.7 Scilab

Example 2 – SIN 3D Plot

Plot 3D Plot of SIN function. It's practically impossible to plot SIN 3D plot from all direction and rotate it on paper manually. CAS software can do it effortlessly.

Software Used – MATLAB

Just 8 lines of code can bring the answer in few seconds.

Program

```
a=0:0.01:1;
b=sin(2*pi*a);
c=b'*b;
surf(c)
xlabel('X AXIS')
ylabel('Y AXIS')
zlabel('Z AXIS')
title('SIN 3D PLOT')
```

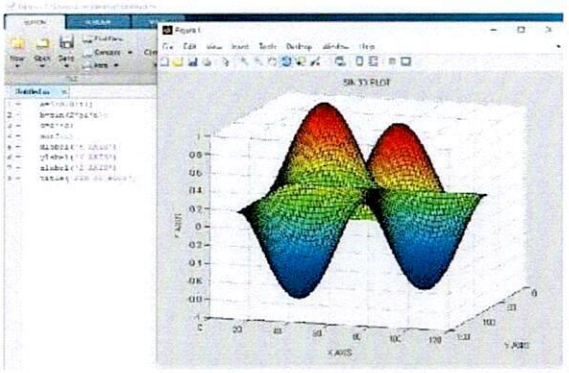


Figure.8 MatLab

Example 3 – Find the solution to the following set of linear equations

2x-3y+4z = 5

y+4z+x = 10

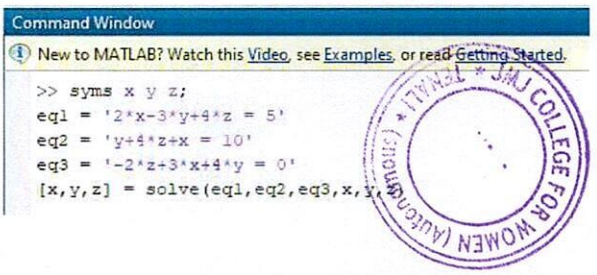
-2z+3x+4y = 0

Software Used – MATLAB

Just 5 lines of code can bring the answer in few seconds.

Program

```
syms x y z;
eq1 = '2*x-3*y+4*z = 5'
eq2 = 'y+4*z+x = 10'
eq3 = '-2*z+3*x+4*y = 0'
[x,y,z] = solve(eq1,eq2,eq3,x,y,z)
```



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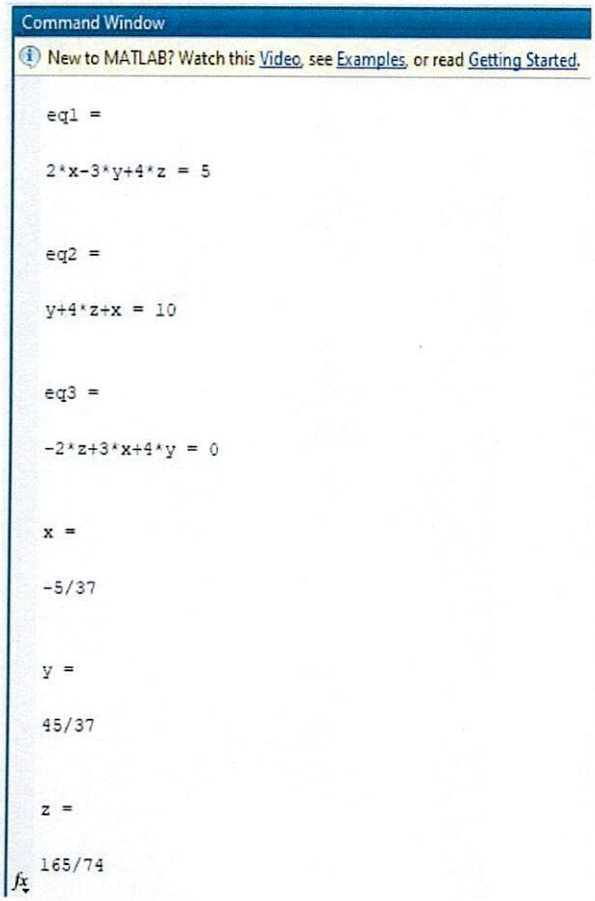

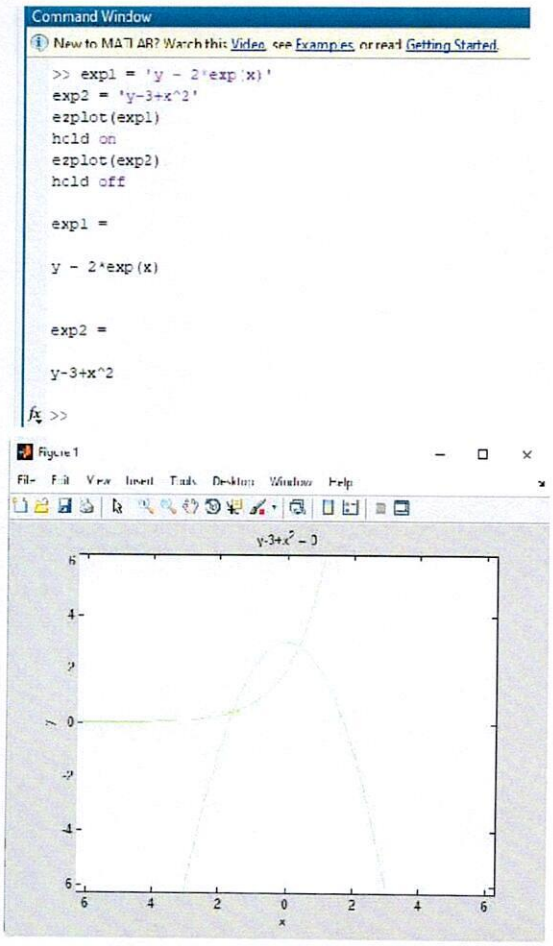

Example 4 – Find the solution by plotting these two equations and looking at their intersections.

exp1 = 'y - 2*exp(x)'

exp2 = 'y-3+x^2'

Software Used – MATLAB

Just 7 lines of code can bring the answer in few seconds.

Program

```
exp1 = 'y - 2*exp(x)'
exp2 = 'y-3+x^2'
ezplot(exp1)
hold on
ezplot(exp2)
hold off
```

5. Significance

- Solve & explain math problems easily and perfectly
- Solve any complex problems related to Mathematics from any kind of complex
- Create an interactive & stress-free environment for learning.
- Make learning experiential.
- Enhance student cognitive skills of students.
- Reduce fear and anxiety of students from the mathematics curriculum.

5. Conclusion

In traditional teaching-learning, mathematics calculations were taking longer time taking process. After all the time when the calculations give you the wrong output, it

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demotivates the students. The concept of mathematics (formulas/theorem) was not easy to recall. All these reasons were creating fear in student's minds for mathematics. Integrating Technology-enhanced learning in school has created a hassle-free environment for students. The implementation of these Computer Aided Software can ease up the mathematical calculations for the staffs and students from departments like Physics, Chemistry, Mathematics, Computer Science, Zoology, electronics and engineering domains as well.

REFERENCES

1. Strategic Use of Technology in Teaching and Learning Mathematics - National Council of Teachers of Mathematics. (n.d.). Retrieved February 26, 2021, from https://www.nctm.org/Standards-and-Positions/Position-Statements/Strategic-Use-of-Technology-in-Teaching-and-Learning-Mathematics/(International,n.d.) International, S. (n.d.). Effects of Technology on Classrooms and Students.
2. Barry, A. (2017). Alleviating Math Anxiety through the Integration of Teacher Beliefs in Senior School. Journal Basic of Education, 3(April), 335–342.
3. Das, K. (2019). Role of ICT for better Mathematics Teaching. Shanlax International Journal of Education, 7(4), 19–28. https://doi.org/10.34293/education.v7i4.641
4. Is, W. (1998). Pssm. Journal of Equine Veterinary Science, 18(11), 719. https://doi.org/10.1016/s0737-0806(98)80482-6
5. Kilicman, A., Hassan, M. A., & Husain, S. K. S. (2010). Teaching and learning using mathematics software "the new challenge." Procedia - Social and Behavioral Sciences, 8, 613–619. https://doi.org/10.1016/j.sbspro.2010.12.085
6. Sevari, K. (2018). The Effectiveness of Math Educational Software on Creativity and Academic Achievement. Psychology and Behavioral Science International Journal, 8(4). https://doi.org/10.19080/pbsij.2018.08.555741
7. (Campbell et al., 2010)Campbell, S. L., Chancelier, J.-P., Nikoukhah, R., Campbell, S. L., Chancelier, J.-P., & Nikoukhah, R. (2010). Introduction to Scilab. Modeling and Simulation in Scilab/Scicos with ScicosLab 4.4, 9–71. https://doi.org/10.1007/978-1-4419-5527-2_2

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USING Of TECHNOLOGY TO IMPROVE COMMUNICATION AND SOFT SKILLS OF ESL LEARNERS

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Abstract

Technology makes the classroom active, interactive and student centred. Students learn faster and easier than before because of the use of technology in the class room. Technology is increasingly becoming popular and it is being exploited for teaching and learning all over the world mostly because of its flexibility in terms of time, place and pace. While technology can play an important role in supporting and enhancing language learning, the effectiveness of any technological tool depends on the knowledge and expertise of the qualified language teacher who manages and facilitates the language learning environment in the classroom. This paper discusses the impact of using technology to improve the communication and soft skills of the ESL learners. Computers and language teaching have walked hand in hand for a long time and technology has contributed as a teaching tool in the language classroom. In language teaching and learning, the teacher can choose from technology: Radio, TV, CD player, Computers, The internet, Electronic Dictionary and so on. Hence, this paper also intends to make English teachers aware of the strategies to use technology in the classroom in an effective manner.

Keywords:ESL learners, classroom, technology, communication, soft skills.

Introduction

Technology plays an important role in today's human society development. Based on this fact, it is indispensable to take advantage of the modern technological facilities in aiding the task of English language education. Students trying to learn English as a second language need further language support. They need to practice in hearing language, reading language, speaking language, and writing language in order to develop their experience and skills (Ybarra & Green, 2003). For doing such tasks, they are in need of using various tools which can help them learn the language easily and effectively. Each technological tool has its specific benefits and application with one of the four language parts (speaking, listening, reading, and writing). However, in order to use these techniques successfully, the students should be familiar with using computers and internet, and capable of interacting with these techniques. The effect of technology has become huge in teaching and learning the language in addition to the instructor's role. In other words, the role of the instructor together with the role of the technology can lead to advanced learning results (Sharma, 2009)

Review of literature on using Technology in Teaching, Learning Process

Technology is very much part of language learning throughout the world at all different levels, The ICT Test Bed evaluation (Underwood 2006) provides evidence that many teachers use ICT to support innovative pedagogy. It states: "New technologies that provide a good fit with existing practices, such as interactive whiteboards are first to be embedded, but others like video conferencing, digital video and virtual learning environments are now being incorporated, providing evidence of on-going learning by the workforce. ICT allow for a higher quality lessons through collaboration with teachers in planning and preparing resources (Ofsted, 2002). Students learn new skills: analytical, including improvements in reading comprehension (Lewin et al, 2000). ICT also develop some writing skills: spelling, grammar, punctuation, editing and re-drafting (Lewin et al, 2000). Still new technologies encourage independent and active learning, and students' responsibility for their own learning (Passey, 1999) ICT proves that students who used educational technology felt more successful in school they are more motivated to learn more and have increased self- confidence and self-esteem. It is also confirmed that many students found learning in a technology-enhanced setting more stimulating and much better than in a traditional classroom environment (Pedretti and Mayer-Smith 1998).

Furthermore, it has been proved that technologies have many other benefits as given below;The biggest reason for incorporating technology into education is the overall changes in global communication. Technology opens doors to many more opportunities by linking the world together. The olden days of limited options for education are long gone and all thanks to technological advances. Students will not only have flexibility with online schooling but also have access to more resources.

Virtual Community

A pivotal aspect of learning English is to how to communicate with others in a social setting. Students presently have a virtual community of learners to discuss topics with, seek advice, or gain leadership skills by helping others. Interactive whiteboards for instance, are a simple but invaluable way for English learners to access helpful resources or lessons. Instructors may include previous topics that are extremely important to progress to the next level. In addition, Interactive whiteboards help a great deal to support an online education and are a perfect example of how to incorporate technology and learning English.

Inspire Great Interest

Another benefit of mixing technology and learning English is the possibility of heightening interest. A traditional classroom setting is often not conducive to learning because the rote strategies do not challenge or interest students. But technology has completely changed the game and makes it easier for English learners to focus because the content can be presented in a number of ways. Lessons that include computer-based instruction, visual aids, and technologically advanced materials help students achieve more in less time.

Accessibility

Technology is certainly not beneficial only for education because just about everything has become more accessible nowadays. But especially for learning English, having technical assistance and more flexibility is the key to success. Traditional learning in a classroom is extremely limited and can only be on-going as long as students are present. However, technology and learning English has allowed students to use mobile phones or laptops for example, to access require information anytime they need. This not only helps students to absorb the material but also offers valuable practice on the proper ways to use tools of technology.

Build Confidence for Success

Students often feel discouraged in a classroom setting because falling behind can be embarrassing and discouraging. But with a course that incorporates technology and learning English, students can learn with privacy and learn the vital skills of success. Technology cannot be ignored even by English learners because all communication in social or business networks use the internet in some form or another.

Importance of Soft Skills

Students prefer to blend technology and learning English because of the valuable skills acquired throughout the lessons. Computer software and online tools help learners to absorb the material much more easily and also hone language and soft skills that are useful their life achievement. Soft skills like critical thinking, analyzing, and problem-solving – are crucial for learning and attaining a job. Soft skills like self-awareness motivation, curiosity, teamwork, grit, resilience and adaptability are very essential for the students to acquire to live in job marketing world. An important feature of soft skills is that the students will have the potential to affect learning ability and job environment. Soft skills foster the development of cognitive abilities that further boost learning (Cunha & Heckman, 2007).

Use of Technology in Reading and Writing

The most basic form of technology for reading and writing is that of word processing. A study by Al-Harbi (2008) notes that "using technology has a positive impact on using the Internet for ESL student reading and writing skills with a word processor, students build upon natural connections between reading, writing and thinking" (2008, p. 29). This is the traditional use of technology for the reading and writing strands, but there are other more developed and modern uses for technology when teaching these two elements of English language learning. A more recent study by Kasapoglu-Akyol (2010) attempted to discover the ways that educational technology tools could help to improve language and communication skills for ESL learners at Michigan University, basing the study within the international student community. The overall results "of the study suggest that students are using technological tools in their daily lives for many purposes, especially for their education.

It also is seen that using educational technology tools that will help the students and the teachers to be more successful, efficient and practical people in their lives" (2010, p. 225). His

work also outlined the importance of using technology to develop reading and writing skills, particularly if students of English were behind their class or required level. The study also supports the use of word processing tools as a way of enabling students to develop their reading and writing skills. He notes "word processors, including some that are bilingual, are an excellent way to further writing development and motivate students to write" (2010, p.229). This belief is strengthened by the research conducted by Peregoy and Boyle (2012). Their study found that students were able to more appropriately learn English at a quicker and more efficient rate using technology to aid their reading and writing skills. Their study found that the use of the Internet (which uses English as its primary language, particularly for ESL sites), helped to immerse students in the language far more than traditional classroom learning. Through these beliefs, it is clear that the use of technology, through both word processing programs, online bilingual dictionaries and the use of the Internet more generally, can aid students in their learning.

Use of Technology in Speaking and Listening

The development and diffusion of software for producing, uploading, downloading and playing digital audio files (i.e., podcasts) make the flexible use of a wide range of audio material easier than ever for language learners. Hegelheimer and O'Bryan (2009) conducted a review of podcast resources and technologies for second language education, highlighting one resource, ESLpod.com, which includes more than 500 free downloadable audio files, organized by topic and developed especially for English language learners. Other premade podcasts are available to promote academic listening skills, facilitate preparation for listening tests, provide grammar tips or cover business English topics. As O'Bryan and Hegelheimer (2007) point out, beyond providing listening material for in-class use, podcasts can be a repository of classroom discussions or lectures for use outside of class to extend and amplify autonomous learning. The argument exists that technology was being used in the ESL classroom prior to the advent of the Internet, with rudimentary technology such as cassette tapes and CDs that enabled students to hear native speakers of English, thereby improving their overall speaking and listening skills in the ESL classroom (Zhao, 2005).

It is apparent that these technologies are still used to a certain extent and that the CD in particular is still a fundamental and central feature of ESL learning. However, the literature also acknowledges that more engaging and personal technological tools have been developed to help students engage with their learning of English as a second language. A study by Nomass (2013) outlines that there are a variety of technological tools that can be used to help improve speaking and listening including "online English language learning Web sites, computer assisted language learning programs, presentation software, electronic dictionaries, chatting and email messaging programs, listening CD-players, and learning video-clips" (p.111). His study outlines a number of possible technological tools that can be used to help develop English language ability, particularly within the speaking and listening elements. There are other tools like video chat programs such as Skype that can link up ESL speakers

with English native speakers, either in the same country or at an international level. These tools underline that there has been a great development in the technology that can be used in the ESL classroom.

Other tips to improve Communication Skills

Effective communication is one of the most important life skills we can learn—yet one we don't usually put a lot of effort into. Communication is one of the most important skills we can ever learn. It leads everything that we do—whether we're communicating at work to meet deadlines and achieve results, or communicating with friends, family and partners to build strong relationships.

Some of essential tips to communicate effectively are given below;

- **Be a Good Listener**
  Listening is an essential part of communication: not only does it help you to build rapport with other people, it's also a way of demonstrating respect for others. When people feel respected, it's very easy to build long, happy relationships.

- **Never talk over people**
  This demonstrates a real lack of respect. By talking over someone what we are basically saying is **"I don't care what you're saying—what I have to say is more important".**

- **Don't finish other people's sentences**
  Research has shown by doing this we are dis-empowering the other person, so we need to bite our tongue.

- **Maintain eye contact.**
  By looking the other person in the eye, we are proving that you're interested in what they're saying. This also looks at us and less distracted.

- **Slow Down the Speaking Speed**
  Learners are often told not to worry about the mistakes they're making, however, it is easy to understand why we would like to make a good impression on our audience. To overcome this difficulty, we may try slowing down our speaking speed.

Technology in ESL Classes

In the 21st century, technology is everywhere. Especially the new generation is growing up with technology and gets familiar with it. Computer technologies have dramatically changed the way people reach information, do research and communicate with people all around the world. Because of this reason, schools and teachers need to be aware of improving their technological tools and skills to be able to catch the students' attentions and interests. Using technology in classrooms also makes the lesson more efficient. To be able to improve their language skills, like writing, reading, listening and speaking, English language learners use computers, software programs to check their work and correct themselves, improve their

language skills; use Internet, e-mails to search information, join in threats, publish their work, read technology texts, communicate each other even worldwide.

Conclusion

Rapid technological advancement increases the learning potentialities of the students. And the use of technology would improve the learning and communication and soft skills of the students to fit into a job marketing world. In order to meet the changing demands of the industrial world, the learners have to acquire computer skills, communication skills and soft skills. As an educationist, we need to create a learning environment for the students with latest advancement in technology and life skills and this learning has to be continuous to win the job marketing world.

References

Al-Harbi, A. (2008). Comparative study between two ESL writing approaches: Computer processing Microsoft Word vs hand writing of two freshman college Saudi student groups. New York: ProQuest.

Cunha, F., & Heckman, J. (2007). The technology of skill formation. *American Economic Review*, 97(2), 31–47.

Hsu, C., Sun, J., Boticki, I. & Wong, L. (2013). 'How flexible grouping affects the collaborative patterns in a mobile-assisted Chinese character learning game?' Educational Technology and Society 16(2), pp.174-187.

Kasapoglu-Akyol, P. (2010). Using educational technology tools to improve language and communication skills of ESL students. Research on Youth and Language 4(2), p.225-241.

Kukulska-Hulme, A. & Shield, L (2008). 'An overview of mobile assisted language learning: From content delivery to supported collaboration and interaction'. ReCALL, 20(3), pp. 271–289.

Kukulska-Hulme, A. & Traxler, J. (2005). Mobile learning: a handbook for educators and trainers. Abingdon: Routledge.

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SOLVING KNAPSACK PROBLEM BY USING SUPER-INCREASING SEQUENCE METHOD

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Abstract :In this Paper, we will study the Knapsack Problem whose solutions are obtained by using super-Increasing Sequence with some basic number theory properties like fundamental theorem of arithmetic, modular arithmetic, modular multiplication, congruence's, Pair of relatively primes, Inverse modulo etc., with counter example, for a given sequence the knapsack problem solution will be presented some types they are inspection method, the ciphers describe based on transformed super-increasing sequences, the enciphering and deciphering procedures of the knapsack cipher based on modular arithmetic and a multiplicative knapsack problem will be solved by analytically.

Keywords – Super increasing sequence, fundamental theorem of arithmetic, modular arithmetic; modular multiplication, pair of relatively primes, Inverse modulo, encipher and decipher

I. INTRODUCTION

The **knapsack problem** is a problem in combinatorial optimization: Given a set of items, each with a weight and a value, determine the number of each item to include in a collection so that the total weight is less than or equal to a given limit and the total value is as large as possible. It derives its name from the problem faced by someone who is constrained by a fixed-size knapsack and must fill it with the most valuable items. The problem often arises in resource allocation where the decision makers have to choose from a set of non-divisible projects or tasks under a fixed budget or time constraint, respectively.

The knapsack problem has been studied for more than a century, with early works dating as far back as 1897[1].The name "knapsack problem" dates back to the early works of the mathematician Tobias Dantzig (1884–1956) [2], and refers to the common place problem of packing the most valuable or useful items without overloading the luggage.Publickey cryptography was invented by Whitfield diffie, Martin Hellman and Ralph Merkle in 1970 [3, 4]. The cipher system was invented by Merkle and Hellman [4], Shamir [4,6] has shown that knapsack ciphers are not satisfactory for public-key cryptography, A method for obtaining digital signatures and public-key cryptosystems invented by R. L. Rivest, A. Shamir, and L. M. Adleman [5],Shamir investigated how to share secret[6],

II. GENERAL METHOD/INSPECTION METHOD

Given a set of positive integers $a_1, a_2, a_3, ...a_n$ and a Sum **S** of a subset of these integers, the knapsack problem asks which of these integers add together to give S. Another way to phrase the knapsack problem is to ask for the values of $x_1, x_2, x_3, ...x_n$ each either 0 or 1, such that

$$S = a_1x_1 + a_2x_2 + a_3x_3 + ... + a_nx_n \quad (1)$$

Example: Let all subsets of the integers $(a_1, a_2, a_3, a_4, a_5, a_6, a_7) = (2,3,4,7,11,13,16)$ that have 18 as their sum. we see that there are four subsets of these six integers that add together to give 18 namely 2+16 = 18, 7+11 = 18, 3+4+11 = 18 and 2+3+13 = 18.
Equivalently, there are exactly four solutions to the equation
$2x_1 + 3x_2 + 4x_3 + 7x_4 + 11x_5 + 13x_6 + 16x_7 = 18$ With $x_i = 0$ or 1 for i=1,2,3,4,5,6,7.

namely

$x_1 = x_7 = 1, x_2 = x_3 = x_4 = x_5 = x_6 = 0$

$x_4 = x_5 = 1, x_1 = x_2 = x_3 = x_6 = x_7 = 0$

$x_2 = x_3 = x_5 = 1, x_1 = x_4 = x_6 = x_7 = 0$

$x_1 = x_2 = x_6 = 1, x_1 = 1 = 0$

To verify equation (1) hold, whether each x_i is either 0 or 1, requires that we perform at most n additions. On the other hand, to search by trial and error solutions of equation (1), may require that we check all 2^n possibilities for $(x_1, x_2, x_3, ...x_n)$.

The best method known for finding a solution of the knapsack problem requires $O(2^{\frac{n}{2}})$ bit operations by using recurrence relation solving, which makes a computer solution of general knapsack problem extremely infeasible even n=100.Certain values of the integers $a_1, a_2, a_3, ...a_n$ make the solution of the knapsack problem much easier than the solution in the general case or inspection method.

For the instance if $a_j = 2^{j-1}$ to find the solution of $S = a_1x_1 + a_2x_2 + a_3x_3 + ... + a_nx_n$ where $x_i = 0$ or 1 for i = 1, 2, 3, 4.....,n, simply requires that we find the binary expression of S. We can also produce easy knapsack problems by choosing the integers $a_1, a_2, a_3, ...a_n$ so that the sum of the first (j-1) of these integers is always less than the j^{th} integer i.e., so that

$$\sum_{i=1}^{j-1} a_i < a_j \qquad j = 1, 2, 3, ...n$$

If a sequence of integers $a_1, a_2, a_3, ...a_n$ satisfies this inequality we call the sequence *Super Increasing*

III.SUPER INCREASING SEQUENCE METHOD

1. Definition of Super increasing sequence:

The sequence $a_1, a_2, a_3, ...a_n$ is super increasing if it satisfies the following inequality

$a_2 > a_1, a_3 > a_2 + a_1, a_4 > a_3 + a_2 + a_1 .....a_n > a_{n-1} + a_{n-2} + ... + a_3 + a_2 + a_1$. then corresponding x value is 0 otherwise 1.

By Super increasing sequence method:

Example: Let us find the integers $(a_1, a_2, a_3, a_4, a_5, a_6, a_7) = (2, 3, 4, 7, 11, 13, 16)$ that have 18 as their sum.

First, we note that since 2+3+4+7+11>18, a sum of integers from this set can only be less than 18 if the sum contains the integers 18.

$2x_1 + 3x_2 + 4x_3 + 7x_4 + 11x_5 + 13x_6 + 16x_7 = 18$ with each $x_i = 0$ or 1, we must have $x_7 = 1$ and

$2x_1 + 3x_2 + 4x_3 + 7x_4 + 11x_5 + 13x_6 = 2$ since 13>2, $x_6 = 0$.

Since $2x_1 + 3x_2 + 4x_3 + 7x_4 + 11x_5 = 2$ since 11>2, $x_5 = 0$

Since $2x_1 + 3x_2 + 4x_3 + 7x_4 = 2$ since 7>2, $x_4 = 0$

Since $2x_1 + 3x_2 + 4x_3 = 2$ since 4>2, $x_3 = 0$

Since $2x_1 + 3x_2 = 2$ since 3>2, $x_2 = 0$

Since $2x_1 = 2$ obviously we have $x_1 = 1$.

Therefore the solution is 18 = 2 + 16.

In general to solve the knapsack problems for super increasing sequence $a_1, a_2, a_3, ...a_n$ i.e., to find the values of $x_1, x_2, x_3, ...x_n$ with $S = a_1x_1 + a_2x_2 + a_3x_3 + ... + a_nx_n$ and $x_i = 0$ or 1 for i=1,2,3,…….n. when S is given, we use the following algorithm

.

2. Algorithm:

Step: 1

First find x_n by noting that

$$x_n = \begin{Bmatrix} 1 : if\ "S \geq a_n" \\ 0 : if\ "S \leq a_n" \end{Bmatrix}$$

Step: 2

Then find $x_{n-1}, x_{n-2}, ...x_1$ in succession using the equations

$$x_j = \begin{Bmatrix} 1 : if\ "S - \sum_{i=j+1}^{n} x_i a_i \geq a_j" \\ \sum_{j=1}^{n} x_j a_j = S \end{Bmatrix} \text{ for j= n-1, n-2, ... 1.}$$

3. Working Procedure of the Algorithm:

First note that if $x_n = 0$ when $S \geq a_n$, then $\sum_{i=1}^{n} x_i a_i \leq \sum_{i=1}^{n} a_i < a_n \leq S$ contradicting the condition $\sum_{j=1}^{n} x_j a_j = S$.

Similarly if $x_j = 0$ when $S - \sum_{i=j+1}^{n} x_i a_i \geq a_j$ then

$$\sum_{i=1}^{j-1} x_i a_i \leq \sum_{i=1}^{j-1} x_j + \sum_{i=j+1}^{n} x_i a_i < a_j + \sum_{i=j+1}^{n} x_i a_i \leq S$$

Which is again contradiction using this algorithm knapsack problems based on super-increasing sequence can be solved extremely quickly.

IV.THE CIPHERS THAT WE DESCRIBE HERE ARE BASED ON TRANSFORMED SUPER-INCREASING SEQUENCES:

This cipher system was introduced by Merkle and Hellman and was considered a good choice Key cypher system.

1. Procedure:

Step1: Let $a_1, a_2, a_3, a_4, a_5 a_6, ...a_n$ be super-Increasing Sequence

Step2: Let m be a positive integer with $\mathbf{m > 2a_n}$

Step3: Let w be an integer relatively prime to m with $\overline{w}$ modulo m

Step4: Form the sequence $b_1, b_2, b_3, ...b_n$ where $b_j \equiv wa_j \pmod{m}$ and $0 < b_j < m$.

Step5: We cannot use a special technique to solve a knapsack problem of the type

$S = \sum_{i=1}^{m} b_i x_i$ Where S is a positive integer, since the sequence $b_1, b_2, b_3, ...b_n$ is not super Increasing.

However when $\overline{w}$ is known we can find $\overline{w}s = \sum_{i=1}^{m} \overline{w}b_i x_i = \sum_{i=1}^{m} a_i x_i \pmod m$

Since $\overline{w}b_j \cong a_j \pmod m$ form above equation we see that $S_0 = \sum_{i=1}^{n} a_i x_i$ Where S_0 is the least positive residue of $\overline{w}s$ modulo m

Step6: We can easily solve the equation $S = \sum_{i=1}^{n} b_i x_i$,Since $b_j \cong wa_j \pmod m$ and $0 \le b_j \le m$.

2. Example problem1:

The super Increasing sequence $(a_1, a_2, a_3, a_4, a_5) = (3, 5, 9, 20, 44)$

Now to find the m value based on the condition $m > 2a_n$ then m > 88 , let us take m=89.

Let us the value of w which is relatively prime to m that is w = 67,since (67,89) = 1

Now to find the sequence $(b_1, b_2, b_3, b_4, b_5)$ based on the $b_j \cong wa_j \pmod m, 0 \le b_j \le m$

$b_j \cong 67a_j \pmod{89}$

$j = 1, b_1 \cong 67(3) \pmod{89} \cong 201 \pmod{89} \Rightarrow b_1 = 23$

$j = 2, b_2 \cong 67(5) \pmod{89} \cong 335 \pmod{89} \Rightarrow b_2 = 68$

$j = 3, b_3 \cong 67(9) \pmod{89} \cong 603 \pmod{89} \Rightarrow b_3 = 69$

$j = 4, b_4 \cong 67(20) \pmod{89} \cong 1340 \pmod{89} \Rightarrow b_4 = 5$

$j = 5, b_5 \cong 67(44) \pmod{89} \cong 2948 \pmod{89} \Rightarrow b_5 = 11$

The sequence of $(b_1, b_2, b_3, b_4, b_5) = (23, 68, 69, 5, 11)$

Now to find $\overline{w}$ based on the formula $\overline{w}w \cong 1 \pmod m$

$w\overline{w} \cong 1 \pmod m \Rightarrow 67(4) \cong 1 \pmod{89} \Rightarrow \therefore \overline{w} = 4$

To solve the knapsack problem $23x_1 + 68x_2 + 69x_3 + 5x_4 + 11x_5 = 84$

We can multiply both sides of this equation by 4 an inverse 67 modulo 89.

$23\square 4x_1 + 68\square 4x_2 + 69\square 4x_3 + 5\square 4x_4 + 11\square 4x_5 = 84\square 4 \text{ mod } ulo 89$

$92x_1 + 272x_2 + 276x_3 + 20x_4 + 44x_5 = 336 \text{ mod } ulo 89$

$3x_1 + 5x_2 + 9x_3 + 20x_4 + 44x_5 = 69$

The solution of this easy knapsack problem is that $x_2 = x_4 = x_5 = 1, x_1 = x_3 = 0$.

Hence the original problem has its solution is that **68+5+11=84**.

3. Example problem2:

Find the sequence obtained from the super-increasing sequence (1,3, 5,10,20,41,80) when modular multiplication is applied with multiplier w : 17 and modulus m : 162.

Solution: The given super Increasing sequence

$(a_1, a_2, a_3, a_4, a_5, a_6, a_7) = (1, 3, 5, 10, 20, 41, 80)$ and w=17 , modulo m=162. Now to find the sequence $(b_1, b_2, b_3, b_4, b_5, b_6, b_7)$ based on the formula $b_j \cong wa_j \pmod{m}, 0 \le b_j \le m$

$j = 1, \Rightarrow b_1 \cong 17 \times 1 \pmod{162} \cong 17 \pmod{162} \Rightarrow b_1 = 17$

$j = 2, \Rightarrow b_2 \cong 17 \times 3 \pmod{162} \cong 51 \pmod{162} \Rightarrow b_2 = 51$

$j = 3, \Rightarrow b_3 \cong 17 \times 5 \pmod{162} \cong 85 \pmod{162} \Rightarrow b_3 = 85$

$j = 4, \Rightarrow b_4 \cong 17 \times 10 \pmod{162} \cong 170 \pmod{162} \Rightarrow b_4 = 8$

$j = 5, \Rightarrow b_5 \cong 17 \times 20 \pmod{162} \cong 340 \pmod{162} \Rightarrow b_5 = 16$

$j = 6, \Rightarrow b_6 \cong 17 \times 41 \pmod{162} \cong 697 \pmod{162} \Rightarrow b_6 = 49$

$j = 7, \Rightarrow b_7 \cong 17 \times 80 \pmod{162} \cong 1360 \pmod{162} \Rightarrow b_1 = 64$

The sequence of $(b_1, b_2, b_3, b_4, b_5, b_6, b_7) = (17, 51, 85, 8, 16, 49, 64)$

Now to find $\overline{w}$ based on the formula $\overline{w}w \cong 1 \pmod{m}$

$w\overline{w} \cong 1 \pmod{m} \Rightarrow 17(143) \cong 1 \pmod{89} \Rightarrow \therefore \overline{w} = 143$

To solve the knapsack problem, we can multiply both sides of this equation by 143 an inverse 17 modulo 162

$17 \times 143x_1 + 51 \times 143x_2 + 85 \times 143x_3 + 8 \times 143x_4 + 16 \times 143x_5 + 49 \times 143x_6 + 64 \times 143x_7 = 153 \times 143$

$2431x_1 + 7293x_2 + 12155x_3 + 1144x_4 + 2288x_5 + 7007x_6 + 9152x_7 = 21879 \bmod 162$

$x_1 + 3x_2 + 5x_3 + 10x_4 + 20x_5 + 41x_6 + 80x_7 = 9$

The solution of this easy knapsack problem is that $x_1 = x_2 = x_3 = 1, x_4 = x_5 = x_6 = x_7 = 0$

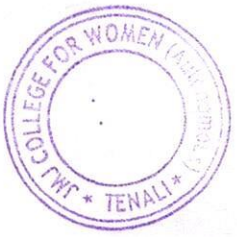
Hence the original solution is **17+51+85=153**

V.THE ENCIPHERING AND DECIPHERING PROCEDURES OF THE KNAPSACK CIPHER BASED ON MODULAR ARITHMETIC:

Step1: Each Individual choose a super increasing sequence of positive integers of specific length N. $a_1, a_2, a_3, a_4, ...a_N$ as well as modulus m with condition $m > 2a_N$ and multiplier w with (m,w) = 1.

Step2: The transformed sequence $b_1, b_2, b_3, b_4, b_5, ...b_N$ when $b_j \cong wa_j \pmod{m}, 0 \le b_j \le m$, for j=1, 2, 3, 4,.....N is made public.

Step3: When someone wishes to send a message P to this individual the message is first translated into a string of 0's and 1's using binary equivalence letters as shown below table.



letter	binary equivalent	letter	binary equivalent
A	00000	N	01101
B	00001	O	01110
C	00010	P	01111
D	00011	Q	10000
E	00100	R	10001
F	00101	S	10010
G	00110	T	10011
H	00111	U	10100
I	01000	V	10101
J	01001	W	10110
K	01010	X	10111
L	01011	Y	1 1000
M	01100	Z	11001

Step4:This string of zero's and one's is next split into segments of length N,if not, we can simply fill out the last block with all 1's.For each block ,a sum is computed using the sequence $b_1, b_2, b_3, b_4, b_5, ...b_N$; for instance of the block $x_1, x_2, x_3, ...x_n$;given $S = b_1x_1 + b_2x_2 + ... + b_Nx_N$ .Finally the sum generated by each block form the cipher message.

Step5:We note that the decipher text generated by knapsack cipher without knowledge of m and w require that a group of hard knapsack problems of the form $S = b_1x_1 + b_2x_2 + ... + b_Nx_N$ be solved on the other hand, when m and w are known the knapsack problem(S) can be transformed into an Easy knapsack problem.

$$\overline{w}S = \overline{w}b_1x_1 + \overline{w}b_2x_2 + ... + \overline{w}b_Nx_N$$

Since

$$\overline{w}S \cong a_1x_1 + a_2x_2 + ... + a_Nx_N \pmod m$$

where $\overline{w}b_j \cong a_j \pmod m$, $\overline{w}$ is an inverse of w modulo m,so that $S_0 = a_1x_1 + a_2x_2 + a_3x_3 + ... + a_Nx_N$

Where S_0 is the least positive integer residue of $\overline{w}S \pmod m$,we have equality (S_0),Since both sides of the equation are positive integers less than m which are congruent modulo m

.

1. Example the enciphering and deciphering procedure of knapsack cipher with an super-Increasing sequence:

We start with the super Increasing sequence

$(a_1, a_2, a_3, a_4, a_5, a_6, a_7, a_8, a_9, a_{10}) = (2, 11, 14, 29, 58, 119, 241, 480, 959, 1917)$

Take the value of m based on the condition $m > 2a_N = 2 \times 1917 = 3834$

Let us take m = 3837 as the enciphering modulus, so that $m > 2a_N$ and w = 1001 as the multiplier, so that (m,w) = 1 to transform the super increasing sequence into the sequence

$(2 \times 1001, 11 \times 1001, 14 \times 1001, 29 \times 1001, 58 \times 1001, 119 \times 1001, 241 \times 1001, 480 \times 1001, 959 \times 1001, 1917 \times 1001)$

$(2002, 11011, 14014, 29029, 58058, 119119, 241241, 480480, 959959, 1918917)$

After performing modulo 3837, we get the below sequence, i.e., the public key is

$(2002, 3337, 2503, 2170, 503, 172, 3347, 855, 709, 417)$

and the private key is $(2, 11, 14, 29, 58, 119, 241, 480, 959, 1917)$

The sender sends the message as follows:

To encipher the message "REPLY IMMEDIATELY"
First translate the letters of the message into their five digits binary equivalence in **above table and** group these digits into blocks of **ten**, to obtain the following
1000100100 0111101011 1100001000 0110001100 0010000011 0100000000 1001100100 0101111000
For each block of ten binary digits, we form a sum by adding together the approximate terms of the sequence
(2002, 3337, 2503, 2170, 503, 172, 3347, 855, 709, 417)
In the slots corresponding to positions of the block containing a digit equal to '1' this gives us
3360, 12986, 8686, 10042, 3629, 3337, 5530, 9529

Now the receivers decode the message as follows:

To decipher, we find the least positive residue modulo 3837 of 23 times each sum23 is an inverse of 1001 modulo 3837 and then solve the corresponding easy knapsack problem with respect to the original super increasing sequence
(2, 11, 14, 29, 58, 119, 241, 480, 959, 1917)
For deciphering the above block as follows
$3360 \times 23 \cong 540 (\text{mod } 3837)$

$12986 \times 23 \cong 3229 (\text{mod } 3837)$

$8686 \times 23 \cong 254 (\text{mod } 3837)$

$10042 \times 23 \cong 746 (\text{mod } 3837)$

$3629 \times 23 \cong 2890 (\text{mod } 3837)$

$3337 \times 23 \cong 11 (\text{mod } 3837)$

$5530 \times 23 \cong 569 (\text{mod } 3837)$

$9529 \times 23 \cong 458 (\text{mod } 3837)$
The decoded message is (540, 3229, 254, 746, 2890, 11, 569, 458)
Its equivalent binary decoded message is:
540 = 2+58+480 = 1000100100 =RE
3229 = 11+14+29+58+241+959+1917 = PL
254 = 2+11+241 = 1100001000 = YI
746 = 11+14+241+480 = 0110001100 = MM
2890 = 14+959+1917 = 0010000011 =ED
11=11 = 0100000000 = IA
569 = 2+29+58+480 = 1001100100 = TE
458 = 11+29+58+119+241 = 0101111000 = LY
The decoded message is: **REPLY IMM EDIATELY**.

2. Example:
Encipher the message BUY NOW using the knapsack cipher based on the sequence obtained from the super-increasing sequence (17, 19, 37, 81,160), by performing modular multiplication with multiplier w = 29 and modulus: m=331.

Sol: Given super Increasing sequence $(a_1, a_2, a_3, a_4, a_5) = (17, 19, 37.81, 160)$

and w = 29 and m = 331, here (m,w) = 1,so transfer the super increasing sequence into the sequence
$(29 \times 17, 29 \times 19, 29 \times 37, 29 \times 81, 29 \times 160)$

$(493, 551, 1073, 2349, 4640)$ mod(331)

$(162, 220, 80, 32, 6)$
The public key is: (162.220, 80, 32, 6) and Private Key is: (17, 19, 37, 81,160)

The sender sends the message as follows:

To encipher the message "BUY NOW"
First translate the letters of the message into their five digits binary equivalence in **above table and** group these digits into blocks of **five**, to obtain the following
00001 10100 11000 01101 01110 10110
For each block of ten binary digits, we form a sum by adding together the approximate terms of the sequence. In the slots corresponding to positions of the block containing a digit equal to '1' this gives us
(6,242,382,306,332,274) mod (331)
(6, 242, 51, 306, 1,274)

Now the receivers decode the message as follows:

To decipher, we find the least positive integer residue modulo 331, of 137 times sum 137 is an inverse of 29 modulo 331

i.e., $ww = 1 \bmod(331) \Rightarrow 29w = 1 \bmod(331) \Rightarrow w = 137$
and then solve corresponding easy knapsack problem with respect to the original super increasing Sequence
For deciphering the above block as follows
$6 \times 137 \cong 160 \bmod(331) \Rightarrow 160$

$242 \times 137 \cong 54 \bmod(331) \Rightarrow 54$

$51 \times 137 \cong 36 \bmod(331) \Rightarrow 36$

$306 \times 137 \cong 216 \bmod(331) \Rightarrow 216$

$1 \times 137 \cong 137 \bmod(331) \Rightarrow 137$

$274 \times 137 \cong 135 \bmod(331) \Rightarrow 135$
The decoded message is : (160,54,36,216,137,135)
Its equivalent binary decoded message is:
160 = 160 = 00001 = B
54 = 17 + 37 = 10100 = U
36 = 17 +19 =11000 = Y
216 = 19 +37 +160 = 01101 = N
137 = 19+37+81 = 01110 = O
135 = 17+37+81 = 10110 = W
The decoded message is**: BUY NOW**

VI.A SEQUENCE OF PAIRS OF RELATIVELY PRIME INTEGERS BY USING MODULAR MULTIPLICATION

There are several possibilities for altering this cipher system to avoid the weakness found by Shamir. One such possibility is to choose a sequence of **pairs of relatively prime integers** $(w_1, m_1), (w_2, m_2), (w_3, m_3), \ldots\ldots\ldots (w_r, m_r)$ and then form the series of sequences

$$b_j^{(1)} = w_1 a_j \pmod{m_1}$$

$$b_j^{(2)} = w_2 b_j^{(1)} \pmod{m_2}$$

$$b_j^{(3)} = w_3 b_j^{(2)} \pmod{m_3}$$

.

.

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$$b_j^{(r)} = w_r b_j^{(r-1)} \pmod{m_r}$$

for j = l, 2, ..., n. We then use the final sequence $(b_1^{(r)}, b_2^{(r)} \ldots\ldots\ldots b_n^{(r)})$ as the enciphering sequence. Involving sequences obtained by **iterating modular multiplications** with different moduli (although there are several promising methods for the production of such algorithms)

Example: **Find the sequence obtained by applying successively the modular multiplications with multipliers and moduli (7,92), (11,95), and (6,101), respectively, on the super-increasing sequence (3,4,8,I7,33,67)**

$$b_1^{(1)} \cong w_1 a_1 \pmod{m_1} \cong 7 * 3 (\mathrm{mod}\ 92) \cong 21 (\mathrm{mod}\ 92)$$

$$b_1^{(2)} \cong w_2 b_1^{(1)} \pmod{m_2} \cong 11 * 21 (\mathrm{mod}\ 95) \cong 41 (\mathrm{mod}\ 95)$$

$$b_1^{(3)} \cong w_3 b_1^{(2)} \pmod{m_3} \cong 6 * 41 (\mathrm{mod}\ 101) \cong 44 (\mathrm{mod}\ 101)$$

$$\therefore b_1^{(3)} = 44$$

$$b_2^{(1)} \cong w_1 a_2 \pmod{m_1} \cong 7 * 4 (\mathrm{mod}\ 92) \cong 28 (\mathrm{mod}\ 92)$$

$$b_2^{(2)} \cong w_2 b_2^{(1)} \pmod{m_2} \cong 11 * 28 (\mathrm{mod}\ 95) \cong 23 (\mathrm{mod}\ 95)$$

$$b_2^{(3)} \cong w_3 b_2^{(2)} \pmod{m_3} \cong 6 * 23 (\mathrm{mod}\ 101) \cong 37 (\mathrm{mod}\ 101)$$

$$\therefore b_2^{(3)} = 37$$

$b_3^{(1)} \cong w_1 a_3 (\text{mod } m_1) \cong 7 * 8(\text{mod } 92) \cong 56(\text{mod } 92)$

$b_3^{(2)} \cong w_2 b_3^{(1)} (\text{mod } m_2) \cong 11 * 56(\text{mod } 95) \cong 46(\text{mod } 95)$

$b_3^{(3)} \cong w_3 b_3^{(2)} (\text{mod } m_3) \cong 6 * 46(\text{mod } 101) \cong 74(\text{mod } 101)$

$\therefore b_3^{(3)} = 74$

$b_4^{(1)} \cong w_1 a_4 (\text{mod } m_1) \cong 7 * 17(\text{mod } 92) \cong 27(\text{mod } 92)$

$b_4^{(2)} \cong w_2 b_4^{(1)} (\text{mod } m_2) \cong 11 * 27(\text{mod } 95) \cong 12(\text{mod } 95)$

$b_4^{(3)} \cong w_3 b_4^{(2)} (\text{mod } m_3) \cong 6 * 12(\text{mod } 101) \cong 72(\text{mod } 101)$

$\therefore b_4^{(3)} = 72$

$b_5^{(1)} \cong w_1 a_5 (\text{mod } m_1) \cong 7 * 33(\text{mod } 92) \cong 47(\text{mod } 92)$

$b_5^{(2)} \cong w_2 b_5^{(1)} (\text{mod } m_2) \cong 11 * 47(\text{mod } 95) \cong 42(\text{mod } 95)$

$b_5^{(3)} \cong w_3 b_5^{(2)} (\text{mod } m_3) \cong 6 * 42(\text{mod } 101) \cong 50(\text{mod } 101)$

$\therefore b_5^{(3)} = 50$

$b_6^{(1)} \cong w_1 a_6 (\text{mod } m_1) \cong 7 * 67(\text{mod } 92) \cong 9(\text{mod } 92)$

$b_6^{(2)} \cong w_2 b_6^{(1)} (\text{mod } m_2) \cong 11 * 9(\text{mod } 95) \cong 4(\text{mod } 95)$

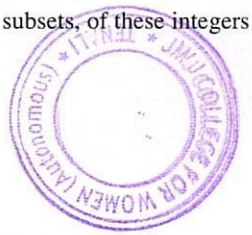
$b_6^{(3)} \cong w_3 b_6^{(2)} (\text{mod } m_3) \cong 6 * 4(\text{mod } 101) \cong 24(\text{mod } 101)$

$\therefore b_6^{(3)} = 24$

The final encipher sequence is : $(b_1^{(3)}, b_2^{(3)}, b_3^{(3)}, b_4^{(3)}, b_5^{(3)}, b_6^{(3)}) = (44, 37, 74, 72, 50, 24)$

VII.MULTIPLICATIVE KNAPSACK PROBLEM

A multiplicative knapsack problem is a problem of the following type: Given positive integers $a_1, a_2, a_3, a_4, a_5, ...a_n$ and a positive integer P, find the subset, or subsets, of these integers with product P, or equivalently, find all solutions of P , $P = a_1^{x_1} a_2^{x_2} a_3^{x_3} .....a_n^{x_n}$



Where $x_j = 0 or 1, for, j = 1, 2, 3, ...n$

Examples Problems

1. Find all products of subsets of the integers 2,3,5,6, and l0 equal to 60

Solution:
60 can be expressed as product of primes as follows

$60 = 2^{1}\square 3^{0}\square 5^{1}\square 6^{1}\square 10^{0} = 2\square 5\square 6 = 60$
$\therefore$ The possible subset is (2,5,6)

$60 = 2^{0}\square 3^{0}\square 5^{0}\square 6^{1}\square 10^{1} = 6\square 10 = 60$
$\therefore$ The possible subset is (6,10)

$60 = 2^{1}\square 3^{1}\square 5^{0}\square 6^{0}\square 10^{1} = 2\square 3\square 10 = 60$
$\therefore$ The possible subset is (2,3,10)
The required possible subsets are: (2,5,6), (6,10), (2,3,10)

2. Find all products of subsets of the integers 8, 13,17,21,95,121 equal to 15960
Solution:

$15960 = 8^{1}\square 13^{0}\square 17^{0}\square 21^{1}\square 95^{1}\square 121^{0} = 8\square 21\square 91 = 15960$
The required possible subset is: (8,21,91).

VIII. CONCLUSION

Knapsack encryption provides a good approach to creating public and private keys, where there private key is easy to use, while the public key is difficult to compute. The method was outlined by Ralph Merkle in his search for a trap door function [13], but the glory of the sustainable trap door went to RSA, and soon cracks began to show when Adi Shamir [4] published methods to crack it.
Knapsack problems appear in real-world decision-making processes in a wide variety of fields, such as finding the least wasteful way to cut raw materials, selection of investments and portfolios, selection of assets for asset-backed securitization, and generating keys for the Merkle–Hellman and other knapsack cryptosystems.

One early application of knapsack algorithms was in the construction and scoring of tests in which the test-takers have a choice as to which questions they answer. For small examples, it is a fairly simple process to provide the test-takers with such a choice. For example, if an exam contains 12 questions each worth 10 points, the test-taker need only answer 10 questions to achieve a maximum possible score of 100 points. However, on tests with a heterogeneous distribution of point values, it is more difficult to provide choices. Feuerman and Weiss proposed a system in which students are given a heterogeneous test with a total of 125 possible points. The students are asked to answer all of the questions to the best of their abilities. Of the possible subsets of problems whose total point values add up to 100, a knapsack algorithm would determine which subset gives each student the highest possible score.

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REFERENCES

[1] Mathews, G. B. (25 June 1897). "On the partition of numbers" (PDF). Proceedings of the London Mathematical Society. 28: 486–490. doi:10.1112/plms/s1-28.1.486.

[2] Dantzig, Tobias. Numbers: The Language of Science, 1930.

[3] M. E. Hellman, "The mathematic of public-key cryptography," Scientffic American, Volume 241-(1979), 146-157.

[4] Shamir, "A polynomial time algorithm for breaking the basic Merkle-Hellman cryptosystem," proceedings of the 23rd Annual symposium of the Foundations of computer science, 145-152.

[5] R. L. Rivest, A. Shamir, and L. M. Adleman, "A method for obtaining digital signatures and public-key cryptosystems," communications of the ACM, Volume 2t (1979), tZO-126.

[6] Shamir, "How to share a secret," communications of the ACM, Volume 22 0979), 612-6t3.

[7] Shamir, R. L. Rivest, and L. M. Adleman, "Mental poker" The Mathematical Gardner, ed. D. A. Klarner, wads worth International, Belmont, California, 1981, 37-43.

[8] Elementary Number Theory and lts Applications Kenneth H. Rosen AT&T Information Systems Laboratories (formerly part of Bell Laboratories.

[9] L M. Adleman, C. Pomerance and R. S. Rumely, "On distinguishing prime numbers from composite numbers," Annals of Mathematics, volume 117 (1983), 173-2A6

[10] H. C. Williams, "The influence of computers in the development of number theory Computers and Mathematics Applications, Volume 8 (1982),75-9

[11] D. M. Burton, Elementary Number Theory, Allyn and Bacon, Boston, t976

[12] T. A. Apostol, Introduction to Analytic Number Theory, Springer Verlag, New York, 1976

[13] Merkle, Ralph; Hellman, Martin (1978). "Hiding information and signatures in trapdoor knapsacks". Information Theory, IEEE Transactions on 24 (5): 525–530. doi:10.1109/TIT.1978.1055927.

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Ground Water Quality Analysis of Srikakulam District Andhra Pradesh, India for Domestication and Agricultural Practice
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Ground Water Quality Analysis of Srikakulam District Andhra Pradesh, India for Domestication and Agricultural Practice

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ABSTRACT

The present investigation focused on water samples collected from different places in Srikakulam District and analyzed for various physicochemical parameters. Such as water Temperature, Turbidity, Total Dissolved Solids, pH, Dissolved Oxygen, Free Carbon dioxide and Total Hardness, Chlorides, Alkalinity, Phosphate and Nitrates were analyzed for a period of two years from pre and post monsoon 2019 to 2020. All the Parameters were within the permissible limits. The results indicate that the samples were Non-polluted and can be used for Domestic, Irrigation and Fisheries.

Key Words: Physico-Chemical Parameters, Temperature, Turbidity, Total Dissolved Solids, Dissolved Oxygen, Free Carbon dioxide, Total Hardness, Chlorides, Alkalinity

INTRODUCTION:

Ground water has long been considered as one of the purest forms of water available in nature and meets the overall demand of rural and semi-urban people (Fienen and Arshad, (2016)). Large scale industrial growth has caused serious concern regarding the susceptibility of

ground water contamination due to waste materials (Peiyue Li, Karunanidhi, and Subramani, *et al.,* 2021). Waste materials at the factories when subjected to reaction with percolating rain water and reach the aquifer system and hence degrade the ground water quality (Navarro Ferronato and Vincenzo Torretta, (2019), Richard Espinoza. (2020)). Heavy metals constitute a very heterogenous group of elements widely varied in their chemical properties and biological functions (Vhahangwele Masindi and Khathutshelo Muedi, (2018)). They are persistant in nature, therefore get accumulated in soil and plants (Mahima Begum *et al.,* 2021). Dietary intake of many heavy metals through consumption of plants and drinking water has long term detrimental effect on human health (Obasi and Akudinobi (2020)). The transmission of water borne disease has been a matter of concern for many years (Kumar, Srivastava and Banerjee, (2022)). Hence bacteriological examination is also very important in the assessment.

Water is one of the abundantly available substances in nature. It is an essential constituent of all animal and vegetable matter and forms about 75% of the matter of earth's crust (Nicholas Le Pan; Bruno Venditti (2021)). It is also an essential ingredient of animal and plant life. Water is distributed in nature in different forms such as rainwater, river water, spring water and mineral water.

Man needs about 500 to 700 liters of water every day for domestic needs such as drinking, cooking, washing, bathing, flushing toilets, besides recreational purposes, commercial purposes, industrial needs and firefighting etc. At the same time water is a potential carrier of pathogenic microorganisms which can endanger human health and life. Water that is free of disease producing microorganisms and chemical substances deleterious to health is called potable water. In this universe it is the only inorganic fluid with a relative density of unity (EPA, United states Environmental protection Agency 2022). It is mainly because of this magical substance the earth's temperature is maintained reasonably uniform at an average of 16^0C, in the absence of which its temperature would have varied as on moon where it is 100^0C during daytime and -130^0C during nights. Man can survive for five weeks without food but cannot live more than 5 days without water (Tim Sharp and Doris Elin Urrutia (2022)). 75% of human body is water because of which only his/her specific gravity is about unity. 80% of milk, 87% of apples, 80% of fish, 77% of beef, 75% of potatoes and 66% of eggs are water (Sruthi M and Pallavi Suyog Uttekar (2021)). It is the only naturally occurring liquid compound on the surface of earth and is called universal solvent. Though its universally known chemical formula is H_2O, it has an unusual property of expanding on freezing and has

Table-1.

Normal range of water quality parameters according to the WHO: Table-1

Substances and characteristics	Acceptable concentration	Maximum Permissible unit
Colour	5 units	25 units
Odour	Unobjectionable	Unobjectionable
Taste	Unobjectionable	Unobjectionable
Turbidity	5 units	5 units
Dissolved solids	500 mg/L	1500 mg/L
Alkalinity	200	600 mg/L
pH range	7.0–8.5	----
Total Hardness	300 mg/L	600 mg/L
Chloride	250 mg/L	1000 mg/L
Sulphate	200 mg/L	400 mg/L
Nitrate	20 mg/L	45 mg/L
D O	5.0	-------
B O D	5.0	--------
C O D	10.0	------
Iron	0.1 mg/L	1.0 mg/L
Calcium	75.00	---
Magnesium	30.00	---
Fluoride	1.00	---
Electrical Conductivity(EC)	2000.00	---
Total dissolved Solids(TDS)	500.00	---
Total Hardness as $CaCO_3$	200 mg/L	---

Temperature and pH of water: Temperature of water samples taken at the time of collection ware in the range of 24 to 26°C. The maximum permitted standard of drinking water is 25°C. The pH value of water samples collected from well and bore well water were in the range of 7.34 to 8.61 reported for post monsoon 2019 samples.

Total Dissolved Solids (TDS): The maximum TDS range for water was found to be 842 mg/L and minimum was 112 mg/L and maximum TDS for 5th sample shown maximum levels of 842 mg/L and minimum was with 8th sample shown 112 mg/L (Table 2) for post monsoon 2019 samples.

Electrical Conductivity: The conductivity of water samples has shown in the results tables in both pre and post monsoon of 2019 to 2020. The results reveal that obtained value, the maximum EC range found for 20th sample 1862 mg/L and minimum was 512 mg/L 5th sample for post-monsoon 2019 samples.

Total Hardness: In the present investigation, maximum and minimum total hardness for collected water samples had shown in the pre and post monsoon 2019 501mg/L and gradually increased total hardness was reported and shown in post monsoon 2020 was 1200. The total hardness of collected water samples of 2019 both pre and post monsoon in comparison with post and pre monsoon 2020 were more. These high values may be due to the addition of calcium and magnesium salts.

COD and BOD: Chemical Oxygen Demands (COD) and Biochemical Oxygen Demand (BOD) is important parameters for oxygen required to degradation of organic matter. Acceptable levels were reported.

Alkalinity: The alkalinity range set by WHO is 500mg/L. Our results showed that alkalinity of given samples of both post and pre monsoon of 2019 and 2020 was not accordance with standard data.

Chlorides and DO: Chloride found high value for both the water samples. In the 2019 pre and post monsoon samples had shown 500 mg/L. It is reported that the higher value of chloride is associated with increased level of pollution and reported as 1362 in the post monsoon 2020. Dissolved oxygen is a most important aquatic parameter, whose existence is essential to aquatic fauna. It plays an important role in life process of animals. In this study DO values found 2.8 to 5.9 mg/L range found in the both the cases of pre and post monsoon of 2019 and 2020 collected samples were shown.

CONCLUSION: From physical and chemical properties of water samples collected from different places in Srikakulam District, Andhra Pradesh, India. Samples collected from in and around of 20 stations of Srikakulam district were analysed for water quality and utilization purpose both domestic and irrigation practice. The analysis reveals that the ground water of the area needs some degree of treatment before consumption.

REFERENCE:

1. Fienen, M.N., Arshad, M. (2016). The International Scale of the Groundwater Issue. In: Jakeman, A.J., Barreteau, O., Hunt, R.J., Rinaudo, JD., Ross, A. (eds) Integrated Groundwater Management. Springer, Cham. pp 21–48.
2. Peiyue Li, D. Karunanidhi, T. Subramani & K. Srinivasamoorthy (2021). Archives of Environmental Contamination and Toxicology
3. Navarro Ferronato and Vincenzo Torretta (2019). Waste Mismanagement in Developing Countries: A Review of Global Issues, volume 80, pages1–10.
4. Richard Espinoza. (2020). How Does Leachate Contaminate Our Water Supply.
5. Vhahangwele Masindi and Khathutshelo L. Muedi (2018). Environmental Contamination by Heavy Metals.
6. Mahima Begum, Bhaswati Sarmah, Gayatri Goswami Kandali, Sontara Kalita, Ipsita Ojha, Raktim Bhagawati and Lipika Talukdar (2021). Persistant Organic Pollutants in Soil and Its Phytoremediation, Biodegradation Technology of Organic and Inorganic Pollutants.
7. Obasi, P.N., Akudinobi, B.B. (2020). Potential health risk and levels of heavy metals in water resources of lead–zinc mining communities of Abakaliki, southeast Nigeria. Appl Water Sci 10, 184.
8. Kumar, P., Srivastava, S., Banerjee, A. (2022). Prevalence and predictors of water-borne diseases among elderly people in India: evidence from Longitudinal Ageing Study in India, 2017–18. BMC Public Health 22, 993.
9. Nicholas LePan; Bruno Venditti (2021). The one essential element needed to accelerate action on climate change, Visual Capitalist.
10. Types of Drinking Water Contaminants (2022). Contaminant Candidate List (CCL) and Regulatory Determination, EPA, United states Environmental protection Agency.
11. Tim Sharp and Doris Elin Urrutia (2022). The pits of craters at the lunar poles are home to the coldest temperatures on the moon. What is the temperature on the moon; space.com.
12. Sruthi M and Pallavi Suyog Uttekar (2021). How Long Does It Take to Die If You Don't Eat; Medicine Net.
13. Water Science School (2019). How Much Water is There on Earth; USGS,science for changing world.

Ground Water Quality Analysis of Srikakulam District Andhra Pradesh, India for Domestication and Agricultural Practice

Tables and Graphs

Post-monsoon-water analysis-2020

Table 1

Sl.No	pH	Conductivity	TDS	HCO_3	TH
1	7.58	2690	441.6	550	1260
2	8.82	400	2688	100	660
3	9.14	350	3864	240	300
4	8.77	1060	1290	280	400
5	8.29	1580	809	440	1220
6	6.82	4200	2609	780	1060
7	8.78	1510	324	90	580
8	8.59	1908	2160	590	1460
9	8.77	2280	859	170	880
10	8.5	1210	1340	40	260
11	8.46	1450	2980	90	780
12	8.47	1420	268	330	860
13	8.17	1580	371	120	1140
14	8.94	2900	1016	250	1000
15	8.69	1850	144	180	420
16	8.14	1440	201	190	760
17	8.49	1580	371	420	860
18	7.95	2600	664	320	480
19	7.9	2009	280	490	180
20	7.44	1210	198	150	120

Table 2

Sl.No	Cl	F	NO_3	SO_4	Na	K	Ca	Mg
1	150	0.44	25.38	128	42.64	5.16	72	29.45
2	710	0.53	33.21	192	657.6	12.2	168	131.3
3	260	0.66	20.18	156	109.9	52.6	180	48.62
4	290	0.99	16.84	156	108.1	260.9	188	43.76

5	240	0.89	19.85	194	98.65	11.9	104	63.21
6	710	0.81	42.84	122	657.6	12.2	168	131.3
7	160	0.26	41.45	171	44.98	11.3	140	29.45
8	470	0.94	22.22	147	218	20.8	112	68.07
9	290	0.76	28.33	125	125.8	16.3	156	58.34
10	130	0.52	34.18	99	24.6	10.53	116	24.86
11	170	0.72	40.21	121	19.1	16.2	132	24.31
12	130	0.36	31.53	157	27.24	10.68	148	19.72
13	190	0.88	11.29	176	85.84	11.34	124	19.45
14	400	0.79	21	163	189.3	172.4	188	43.76
15	100	0.51	2.37	120	90.62	39.88	132	34.03
16	140	0.82	13.35	158	29.86	10.86	140	34.59
17	160	0.48	41.38	146	54.4	23.98	132	39.45
18	420	0.62	12.33	212	402.6	178.8	164	53.48
19	400	0.6	47.56	197	208.6	140	138	260
20	140	0.57	30.18	110	28.16	10.94	124	39.72

Pre-Monsoon-water analysis-2020

Table 3

S.No.	pH	Conductivity	TDS	HCO_3	TH
1	8.92	1100	784	290	80
2	8.96	1700	648	126	4.7
3	8.87	1600	184	280	340
4	8.85	1010	170	60	80
5	8.9	1350	424	100	240
6	8.99	1800	640	140	240
7	8.73	4200	2248	220	920
8	8.55	1500	860	280	320
9	8.61	550	260	120	140
10	8.19	2400	886	60	260

11	8.51	800	156	180	280
12	8.06	700	412	40	220
13	7.98	3800	284	60	140
14	8.49	2400	996	110	240
15	7.98	300	284	60	100
16	8.77	700	584	70	140
17	8.65	930	167	140	251
18	8.52	2980	1228	220	120
19	8.64	2080	1980	130	280
20	8.93	520	505	160	220

Table 4

Sl.No	Cl	F	NO_3	SO_4	Na	K	Ca	Mg
1	192.19	0.88	14.76	154	8.51	2.2	56	36
2	156.73	1.17	45.64	120	20.4	0.76	74	13.6
3	156.73	0.41	2.836	110	25.83	0.9	52	32.4
4	114.18	1.03	13.06	110	5.05	4.12	58	27.2
5	128.36	0.58	1.673	124	0.85	1.4	52	14.4
6	192.19	0.86	10.65	134	6	17.5	52	24.4
7	496.44	0.38	37.53	185	3.42	14.4	54	90
8	212.76	0.58	25.24	109	8.51	11.9	66	43.2
9	135.46	1.4	10.95	110	0.78	10.8	68	28
10	177.3	1.02	25.13	165	4.62	30.9	62	28
11	128.37	0.45	20.25	17	8.44	1.94	82	32.4
12	142.55	0.31	13.71	17	12.93	1.84	70	21.6
13	170.92	0.13	11.53	18	15.58	0.66	68	25.2
14	177.3	0.41	8.873	159	9.72	3.5	52	32.4
15	199.29	1.02	14.18	126	17.91	9.22	56	20.8
16	135.46	0.49	30.22	116	10	0.86	46	27.2
17	190	0.21	13.1	163	63.06	1.14	64	24.31
18	354.6	0.25	10.43	117	9.11	114	66	46.8

19	425.52	0.46	18.65	124	98.8	1.25	68	43.2
20	121.27	0.34	11.82	186	11.02	4	42	25.2

Post-Monsoon-water analysis-2019

Table 5

S.No.	pH	EC(µs/m)	TDS(mg/l)	HCO_3mg/l)	TH(mg/l)
1	8.55	794	607	250	60
2	8.45	745	476	10	20
3	7.54	681	335	40	40
4	8.02	330	111	60	20
5	8.56	528	338	70	240
6	8.69	1021	597	100	304
7	8.19	830	931	170	200
8	7.58	1023	254	10.4	240
9	8.04	1320	544	10.028	200
10	8.28	683	337	65	40
11	8.04	696	345	40	80
12	8.08	722	262	0	120
13	8.88	683	337	65	40
14	8.36	1559	698	80	480
15	8.33	739	673	53	80
16	8.22	389	148	1.3	60
17	8.09	439	180	70	390
18	7.89	2025	896	30	220
19	7.88	1657	260	300	502
20	7.89	1330	451	135	320

Table 6

Sl.No	Cl	F	NO_3	SO_4	Na	K	Ca	Mg

	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)
1	155.3	0.89	0.42	133	138	0.8	29	39
2	110	0.14	1.82	62	73.96	1.2	28	176
3	147	0.79	1.68	129	31	3.2	49	200
4	130	0.28	4	130	14	0.78	62	104
5	160	0.15	6	45.82	6.9	0.39	59	224
6	100	0.38	38.4	104	73.28	1.56	89.6	258.4
7	154	0.89	11.6	62	87	35	45	160
8	43.4	0.38	1.8	139	135	1.2	45	200
9	122	0.67	18.2	249	188	2.7	39	168
10	76	1.3	12.8	76.8	48	1.56	49	100
11	57	0.48	4.2	153	60	1.6	48	132
12	82	0.39	8.4	86	96	2.3	74	96
13	76	1.36	12.8	76.8	48	1.56	64	100
14	130	0.49	17	182	110	35	112	368
15	102	0.66	3.4	144	116	25	58	72
16	70	0.42	5.6	57	21	0.39	64	136
17	136	0.81	3.64	57	21	10	48	252
18	500	0.25	6.02	110	215	160	44	280
19	170	0.16	18	154	131	3.1	100	400
20	165	0.82	25.7	152	129	42	66	304

Pre-Monsoon-Water Analysis-2019

Table 7

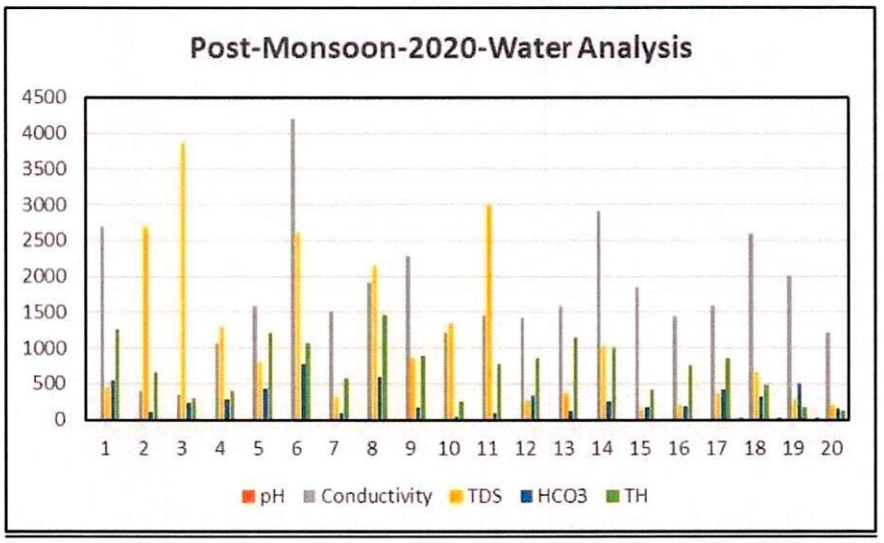
S.No.	pH	EC(µs/m)	TDS(mg/l)	HCO_3 (mg/l)	TH(mg/l)
1	7.95	1649	943	216	222
2	7.74	846	532	184	158
3	8.91	512	212	195	151
4	8.61	1548	824	362	155

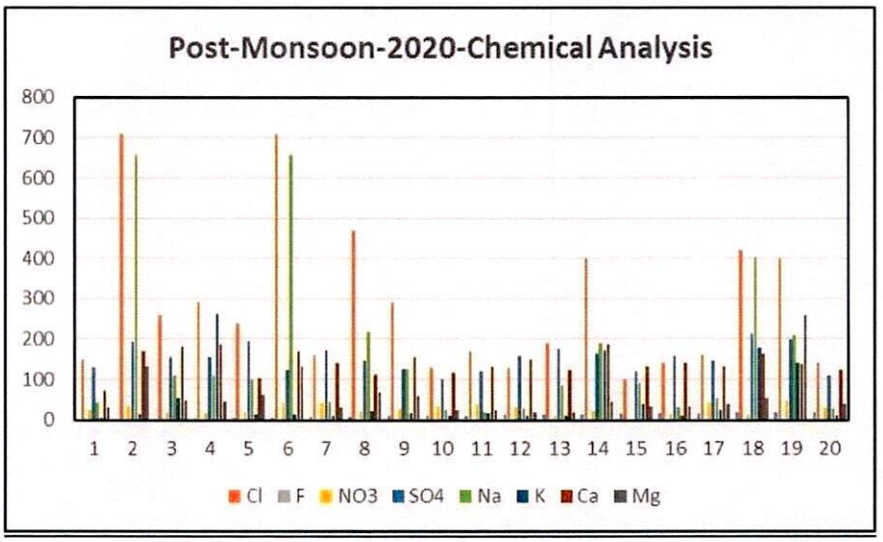
5	8.12	1284	642	164	560
6	8.49	948	431	221	158
7	8.72	946	384	184	164
8	8.14	1486	1000	198	228
9	8.27	948	528	221	294
10	7.99	647	284	184	154
11	8.29	1184	824	192	421
12	8.36	849	462	138	201
13	8.26	948	394	124	165
14	8.06	1346	955	286	201
15	8.22	624	384	168	191
16	8.38	856	198	194	164
17	8.14	948	328	251	201
18	8.01	1238	884	196	164
19	8.34	946	489	301	184
20	8.61	1862	1262	234	194

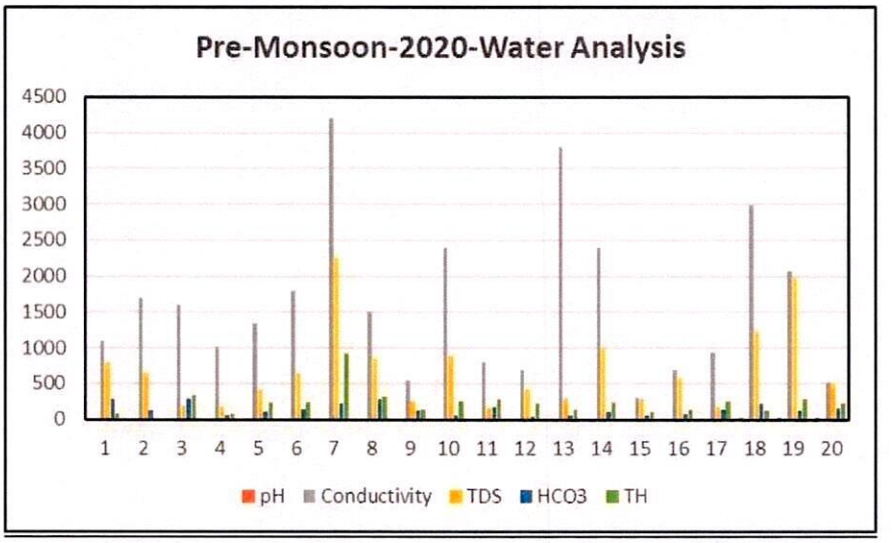
Table 8

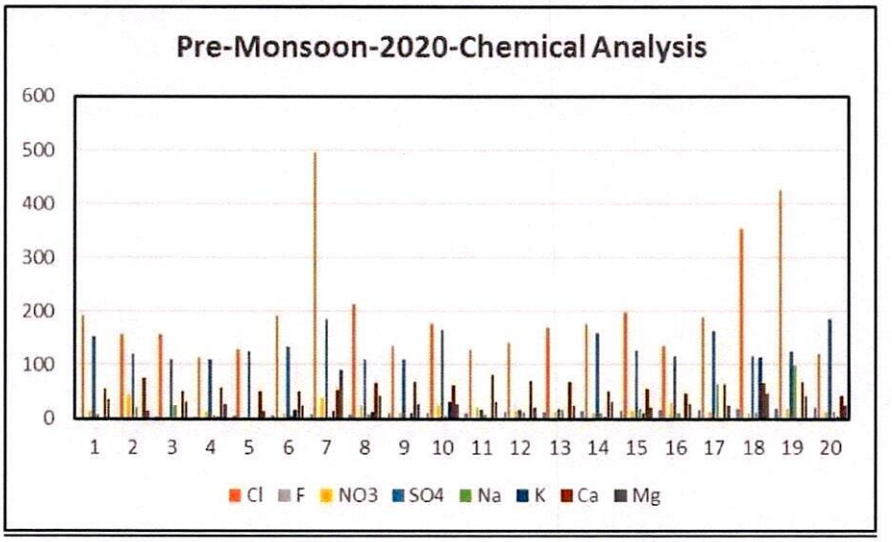
Sl.No	Cl (mg/l)	F (mg/l)	NO_3 (mg/l)	SO_4 (mg/l)	Na (mg/l)	K (mg/l)	Ca (mg/l)	Mg (mg/l)
1	165	0.14	32.16	138	206	38.16	28	101
2	195	0.42	7.52	131	51.02	2.64	42	123
3	112	0.48	8.12	141	801	8.46	21	21
4	141	0.31	14.67	184	92	38	201	501
5	168	0.52	31.65	101	301.24	6.94	191	301
6	152	0.32	23.49	112	18.64	3.84	132	1176
7	127	0.41	9.84	158	91	72	41	84
8	238	0.26	16.84	194	213	34	58	184
9	109	0.43	8.26	159	56	32.54	39	98
10	143	0.26	24.62	154	21.34	6.84	121	154
11	306	0.32	16.84	158	201	464	132	182
12	169	0.21	58.02	124	74.62	12.84	84	112
13	176	0.23	32.08	112	42.54	32.57	62	89
14	186	0.12	51.04	181	124.61	26	121	154

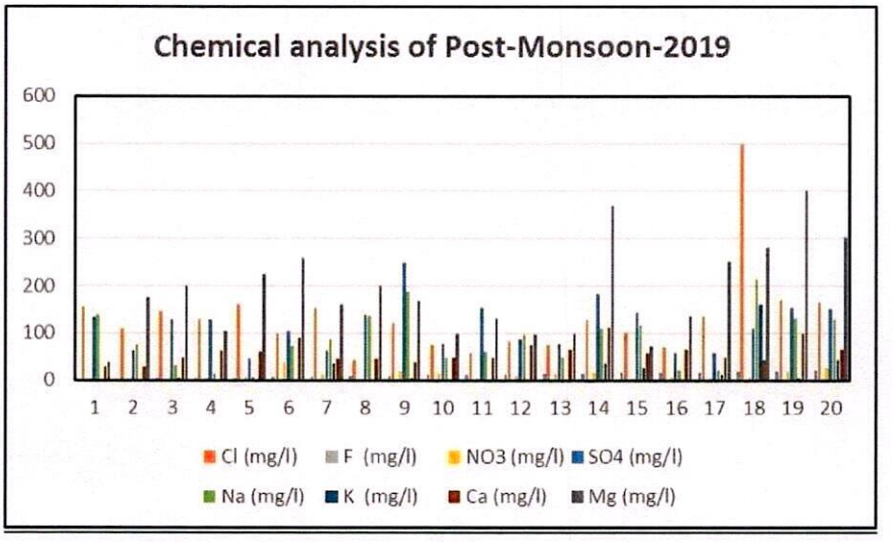
15	109.62	0.32	12.84	126	62.71	32	28	89
16	164	0.16	8.62	19	34.08	17.84	41	48
17	172	0.26	58.64	128	34.01	8.46	54	132
18	212	0.31	37.46	161	164.85	94.62	67	154
19	289	0.11	19.56	112	112	10.51	126	167
20	301	0.41	32.64	212	234	8.42	121	364

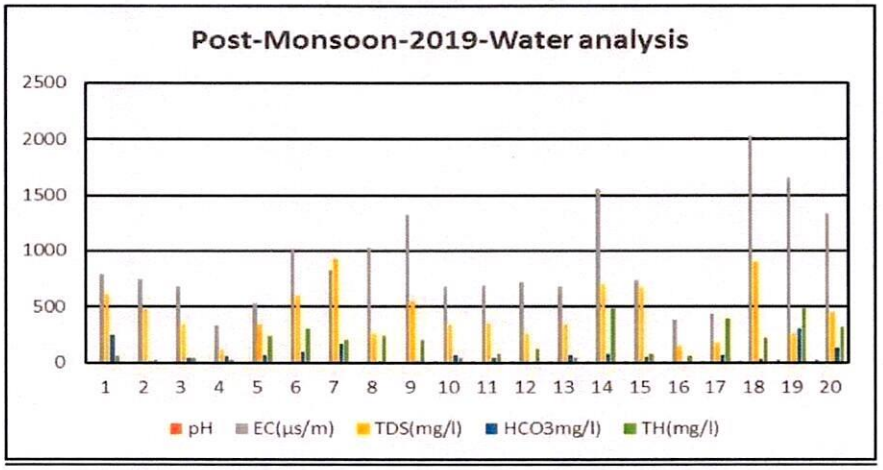


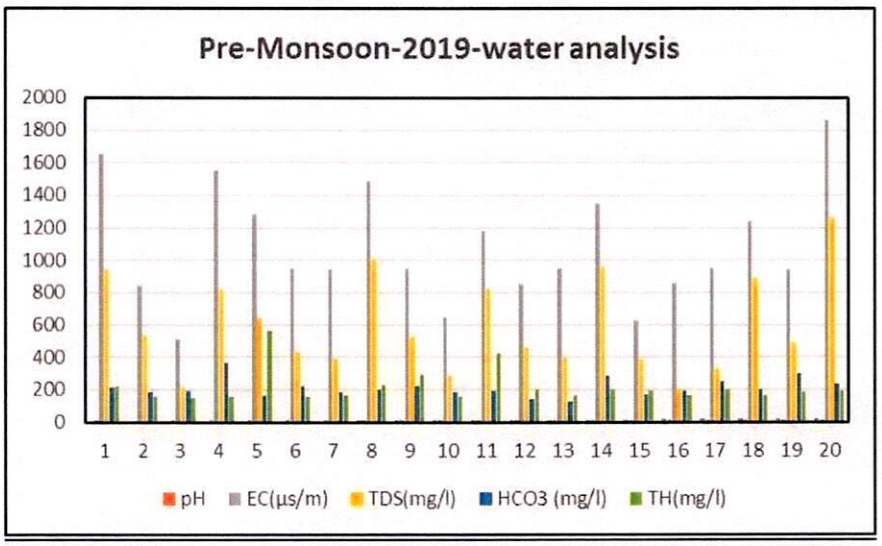


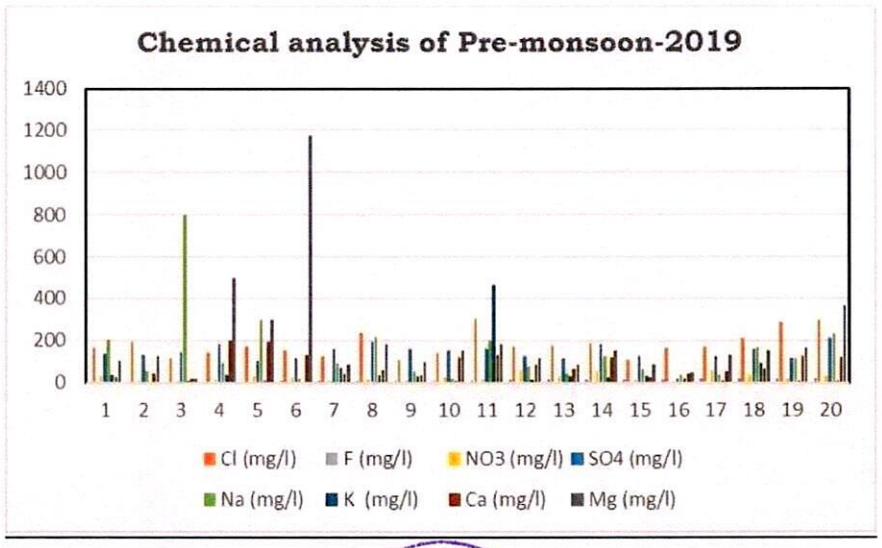












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Exploration of Alienated Self in Thrity Umrigar's If Today Be Sweet

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ABSTRACT: Family comprises of many relations like husband-wife, father-daughter, mother-son and brother- sister. In all these relationships the relation between husband and wife has always occupied a central place. Love, faith and understandings are the fundamental poles in the relationships. But the role of woman is always determined by the male society and treats secondary. Woman needs identity. Present day woman fight for her identity, freedom, discrimination and equality.

Women writers choose to focus their writings on the experiences of woman in the society. Mainly South Asian women writers write from almost anywhere in the world, from all parts of Asia, from USA, Canada, Australia, etc. Many of the diasporic writers focused on the immigrant life of woman in their new county. The themes of their fiction based on race, culture, home, family back ground, identity, loneliness, nationality and political status.

Thrity Umrigar is one such writer, who through her writings shows how India and Indianess figures in the diasporic writings from USA. She belongs to twentieth century Indian born American novelist, who contributed substantially to the diasporic women writings. Her works deal with major contemporary social issues like alienation, nostalgia, exploitation, racism, cultural clashes, discrimination, class deviation and poverty.

The present paper glances at one of her novels If Today be Sweet(2007) and explores the way of the female characters are represented. The prominence of the paper on the "Exploration of Alienated Self", the protagonist experiences from different world and struggles to cling in that culture in the alien land. Tehmina, the protagonist of the novel suffers from the pain of being far away from the home and the memories of her mother land.

Key words: Diaspora, immigrant, alienated self and culture.

INTRODUCTION

Thrity Umrigar is an Indian born American novelist, journalist and critic. She was born in Bombay, India, and moved to the USA at the age of twenty-one. After received her M.A. degree, she worked as a daily reporter for seventeen years. Present she is working at Case Western University. Her fiction powerfully explored the human relationships, family, power twists, poverty, identity, class difference, and life of immigrants in America. Most of her novels have diasporic elements. She has eight novels to her credit. Her debut novel is Bombay Time published in 2001, The Space between Us (2006), If Today be Sweet (2007), The Weight of Heaven (2009), The World we Found (2012), The Story Hour (2014), Everybody's Son (2017) The Secrets Between Us (2018) and a memoir First Darling of the Morning (2004).

Umrigar belongs to Parsi community, proud to be a Parsi, she portrays her community, customs and Zoroastrian life in the most of her novels. Inher novelsBombay Time, The Space between Us and If Today be Sweet, she depicts her love for Bombay and most of the characters are Parsis. Her fondness for Bombay and Parsi community is similar to that of Rohinton Mistry. In most of their novels they celebrated Bombay, the city of their birth.

In the past few years many prominent Indian women writers have made a mark not only in the field of Indian English Literature but also in the diasporic literature. The writers of diasporic literature focused on the lives of immigrants, problems faced in the alien land, rootlessness, homelessness, identity, isolation and cultural clash. Most of the writers expressed their personal experiences faced in the 'new country' through the characters of their works. Like Bharati Mukherjee, Chitra Banerji Divakaruni, Umrigar belongs to the genre of Indo-American Literature. Her fictional writings portray multi facets of Indian women, their plight, sufferings, and domestic space in the site of contact and conflicts between tradition and modernity.

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The present paper delineates how a widowed woman as an individual undergoes her psychological, and personal trauma which allows them to strengthen her emotional chords there by to stand up to the situation and audaciously accept the challenges of her life in the novel If Today be Sweet. It deals with these situations and analyzes them that protagonist experienced, and will bring to light the struggles, challenges, acceptance and resistance to the American life.

If Today be Sweet is a story about an Indian woman, who after her husband's death she moved to America where her only son settled with his family. The protagonist Tehmina is, in a dilemma whether to stay in America or in India. She experienced different situations and suffered lonely in the adopted land. It is hard to settle down in America and give up her own country. If she stays in America she has to change according to circumstances. The rough pictures that Umrigar draws of the sensitive moments which reflect the psychological pain that comes with the changes of culture and life that protagonist experienced. In her words;

"To give up the city of one's birth, old friends whom you grew up with, an apartment that you've decorated and cleaned and furnished, all this is very hard...."(156)

Tehmina Vakail, the woman protagonist of the novel, was born in Calcutta and the only child of a doctor Hoshang Vakail. Through a program at the school, she met Nilu, one of her friends from Bombay. To attend Nilu's birthday party, she begged her father to go Bombay. She is embarrassed with the feeling of unexpected pleasure of freedom, for the first being away from home and the thoughts of Bandra, where the movie stars, and Juhu Beach. She thinks;

"I'm in Bombay, she kept saying herself. These are all Bombayites. Everything she'd ever heard about Bombayites seemed true- these people were more mature, more sophisticated, more urbane than her crowd in Calcutta." (111)

She met Rustom Sethna in Bombay, one of common friends of Nilu, fell in love each other, and convinced their families to get marry. Eventually she moved to Bombay, the place she loves more than Calcutta. Their only son Sorab, a good, brave and courageous man, went to America for higher education. He is married to Susan, an American and settled there with her and their only son Cookie. Tehmina and Rustom visits America once a year in the Christmas vacation. The sudden death of Rustom is a terrible blow in her life and it changes her life a lot, her hope, and her identity. After the incident she has to move America unwillingly. Umrigar explores the life of an older Parsi woman, who comes to America from India and the analysis of widowed Tehmina how she tries to fit into the new world, and tries to decide what parts of her life she can retain.

This is a tender story of family, pain, love, loss, and widowhood of Tehmina, a sixty-six-years old Bombayite, with her loss and having to carve out a new life for herself in a new country. The dilemma that she faces whether to live in India with the memories of her husband or in the USA, where their only son settled. But she is unsure of what to do with rest of her life in America, and if she decides to live in Bombay, where everything is familiar and reminds her the memories of her husband, where they are around every corner of their apartment and the city, which she loved more than her birth place.

"In Bombay, I feel like a person- a person whose life has meaning, whose life follows a path." (Umrigar 32)

In this miserable condition as a widow and an immigrant she feels insecure, emotionally weak, stressed, and depressed. The difficult days she had followed her husband's funeral and reeling with grief and streaks of loneliness, has her reasonable anxieties within a world that has many turns, diversions challenges wager- like situations. She has to adjust herself and due to continuous emotional stress she loses her mental balance. Going back to India and continuing her life there, would feel at home with herself. But if she settled in America, it would give her a chance of living with her son and his family. Thuen asserts;

Widows reported lower levels of psychological wellbeing than married. After the death of spouse, her life becomes mismanaged and therefore, she has more difficulty to cope with her problems in such situation. In the social sphere, the condition of widow is much worse in India than what we see in many other countries. (Thuen 1997)

In this period of mourning over the loss of a husband, the old memories are reawakening and that leads to a re-evaluation of one's life. Moreover, the sphere of life may be shrinking and need to establish new relationships to overcome their loneliness and have to adjust to the unfamiliar surroundings.The changing circumstances make a person's life and choices ultimately shaped. Here, the protagonist is faces two types of problems, one is the loss of husband and another one is alienated from motherland. The process of decision making becomes a Himalayan task to her. The great distress she found herself a stranger in the new environment. She is totally confused to being an Indian or American. She bestrides between two

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cultures India and America, straddling between two worlds, familiar and alien, two ideologies, and two ways of life.

This dislocation costs her cultural isolation and social estrangement. This sense of alienation perceived a state of rootlessness and loneliness. These two variants of alienations are related because they come from the same basic condition of anomie. This situation doesn't arise merely in Tehmina's life, it takes place when a person stays in the alien land, as he feels that he is a stranger and has to struggles a lot to overcome his nostalgic feelings. Sometimes the migrants lose the complete sense of belongingness and encounters cultural differences and identity crisis. She thinks;

"And yet.... Bombay is my home. Here, I am afraid that I will always be a stranger that I will never get used to all these days..."(Umrigar 32)

The institution of marriage provides not only the companion in the life but it also provides psychological and emotional support. Rustom is a man of boundless confidence and make people happy around him. Due to his death Tehmina lose the source of getting emotional support. In this situation she becomes more isolated, feels alienated and insecure. Her life is easy, painless and unworried when she had her husband but in his absence she feels that her life is aimless, concave and sunken. Now she reminds Rustom; "My darling. Look how I am floundering without you. Look how I can't make the smallest decision without you." (Umrigar 91)

After Rustom's demise, she moved to America along with her son Sorab. Though it is not her first visit to the USA, but it is her first visit without her husband. Once, everything is familiar-Sorab, Susan and Cookie and the house, but now it is unfamiliar. She clearly felt the absence of her husband. Sometimes they conveyed their displeasure over her grief. She felt a pang of remorse about their irritation whenshe mentioned his name, it would be like breaking the social rule. She wondered that he has been banned from their live.

"Does everything in this country have an expiration date? She wondered even grief and mourning (Umrigar 130)

She getssolace only in the presence of her grandson Cookie, but she often feels as an intruder in her daughter-in-law's home and perturbed by the changes in her son Sorab, who is stressed from the corporate rat race. Sometimes he yells at her using foul language and his voice bitter and scary in its frustration. Her loneliness floods with the memories of her husband, community, her traditional ways – she found no small amount of purpose in America. In widows the fear and anxiety was produced by being alone, isolation would be an unbearable experience to them and sometimes loneliness may lead to depression and loss of hope. Besides the loss of Rustom's company, memories of her motherland and the loneliness in alien land humiliated her. They provide her everything food, shelter and clothing to fulfil their duty. But she wants their love, care and attention. Because she is in emotional need of love, care and respect of family members rather than just getting enough food, clothes and a place to live in. As a widow she feels that she loses her status in the family.Malkinson, Ruth, a reviewer of APA PsycNET asserts;

"Widows found a greater lack of emotional than instrumental support; people attitudes and comments rather than their actions were perceived as unhelpful and painful". (Malkinson, Ruth 1987)

Her life in India is entirely different from America, as a mother, wife, and daughter-in-law she fulfilled all her duties. She caressed her ill mother-in-law and helped elderly people of her neighborhood. She visits orphanages to serve poor children, and open handedly welcomes Percy when his mother died of cancer, whose father was alcoholic and could not adequately take care of him. Her life is busy with a blur of ringing doorbells and raised voices of fisher woman, newspaper boy, baker, and butcher all made her morning busy. The chain of unexpected visitors and relatives who dropped in without calling all made her breathless. But her life is completely contrasted, she is alone, unable to adjust in this new host country, gradually she becomes the victim of isolation, alienation, confusion and identity crisis.

She always wonders that how simple the way of Americans' life leading. Though they are individualistic and dynamic but they think that life is too much laughter and play, as the life in a Walt Disney movie, forward looking, optimistic and self-improvement but they are spending more money on therapists and grief counselors. She doesn't know why they have to pay the therapists to listen to them, because of the nuclear families, no grandparents andaunt or uncle in the family, her thoughts about Indian and American life;

"Life is a Bollywood melodrama-full of loss and sadness. And so everyone rejects Bollywood for Disney. Even my Sorab was seduced by Disney life-all this pursuit of happiness and pursuit of money, but this year I've learned a new lesson. May be the Indian way is better after all." (32)

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When Sorab decides to go to America for higher education, Tehmina had thought that going to America could broaden his horizons, would make him stand on the shoulders of his parents and see farther than they ever had. But instead the inconsistent had happened. In a strange way, he seemed to have shrunk and his world had narrowed. She remembering the college days of her son, the boy who had go upon the crowded, thunderous streets of Bombay, who had catch a train to college in the noisy crowds, who had eaten and drunk from roadside booth, and who had witnessed the whole human experience, the millionaires, the lepers and slum colonies. But he disappeared from the world what he had experienced in Bombay. Such a boy, now he could fence himself in a timid, clean, antiseptic world where there was free from germs, bacteria as well as passion and human misery. He is in a shell like a snail. She humiliates and thinks about Sorab; "how did he expect his sixty-six-year old mother to live in that world". (8). Susan, as an American woman, always struggles for a neat house and a clean bathroom, she fights with Sorab about his mother doesn't clean the house and bathroom. If Tehmina decided to settle with them, they going to ask servant to come and clean every week instead of two weeks, and also want to move in to a bigger house. She felt a chill in her heart that she heard the words from Percy about the need of bigger house, it meant that her presence was an inconvenience to them. She thought about her thoughtless habits (gargled loud in bathroom which Susan hates so much) and behaviors which affected Susan and Sorab and created abrasion between them. Although she tries to live subduedly in their house, and uses bathroom stealthily at night so not to wake them up. She feels a guest in the house, and how eager she wants to help Susan in any way she could, also disappear when necessary. Her heart sting with coldness at the image of her son's appeasing his frustrated wife Susanwife. Sometimes he also expresses his dissatisfaction;

"Maybe asking Mamma to live with us is not a good idea, maybe she need to go back home at the end of her six months". (63)

Tehmina loves to visit the Farmers' Market in the USA along with Eva, it gives pleasure and comfort to her. It is quite different from the antiseptic, air-conditioned, and clean supermarkets in America. The dirty floors, noisy, crowded streets, sweaty vendors and the rotting fruits, all remind her, the markets of Bombay. She feels;

"To bite an American apple or an orange was to taste disappointment. Nothing burst with flavor, nothing tasted as sweet or as tangy the way fruits did in Bombay. Even the roses of America had no perfume to them, a fact that Tehmina still couldn't quite accept." (34)

But being an American, Susan doesn't like Tehmina's visit to the Farmer's Market, often she expresses her disapproval; 'god knows why she came home loaded with fruits and vegetables' (59). Sometimes Sorab also irritates about this, 'Mamma could be passive-aggressive. All bloody Parsi women were'. (59) She thought that if she settled in America that would burdens Sorab and Susan's lives, who are busy withtheir professions and it is difficult to spend time to take care of her because of their busy schedule. She is alone all the day at home, no neighbors and no sidewalks. The only solace she got in the alien land is Eva Metzembaum, an American woman who comes to love and consider as her best friend. She feels comfortable and shares everything with her. Abandonment and neglect is one of the greatest miseries in age of Tehmina. Talott, Maria assert in their journal;

"The relationship of widows and their adult children and found that some widows seemed to feel that they annoy or irritate their children and that they are unwelcome in their children lives. Some widows felt emotionally dependent on their children." (Maria 1990)

The attitude towards death in America is entirely different, what Tehmina experienced her visit after departure of Rustom. Death did not constitute an important challenge to the individual Americans. Being an American Susan said to Tehmina about Rustom's death 'the sad stuff-if it just brings you down' (7). But according to Indian tradition death treats as sacred thing and the period of mourning immediately following the cremation which lasts thirteen days, it is vary according to the place, people, caste and circumstances. But the Americans talk to a therapist to overcome their grief. Even Sorab also suggest her to take pills in her mourning period. The cultural and traumatic shock she experiences in the alien land. She needs emotional support in this period otherwise she becomes more isolated. The people in this age and condition need a good company to console in the difficulties, worries and provide support in despair. They may face various psychological problems that affect their personality, behaviour and health. She wants to say to her daughter-in-law;

"You will know what it's like to miss someone so badly it's like your own organs betray

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you. Your heart, your skin, your brain, all turn into traitors." (8)

Umrigar's skilled and natural ways of storytelling captures the image of India through the stern plot twists the tragedies of her protagonist Tehmina fantastically wealthy. Her writings offer excellent insights into this important aspect of our changing times and mobile society which expresses itself in the alienation of her characters. She also attempts a graphic portrayal of cultural alienation owing to marital discords.

Umrigar provides her woman character the courage and strength to overcome her miseries and shows her the ways to fight in the difficult situations. A kind of woman Tehmina influences many people in America, makes new relationships and so it forms as a new family. During the course of six months stay in America, she begins to see the world in a new light. She emotionally bonds with the two neglected American boys from the next door, which caused to change her life. Tara is an arrogant mother of the two boys Jerome and Joshua, a sharp bird like faces who looks dirty than the poor boys in India, is the neighbor of Sorab. Tehmina often feeds them when they are hungry. But Susan strictly instructs her that she don't want the two boys to around her son Cookie. Susan instructs;

"I don't want any puking in my kitchen. You have a good heart mom, I really appreciate that but I want you to listen to me-I don't want to have those boys over again I hope you can respect my decision." (16,20)

But feeding the people gives Tehmina pleasure and satisfaction. She always remembers the words that Rustom used to say that two things are important to human being 'food and education, so never refuse to give them to people'. Her heart breaks at each time the boys opened their mouth with hungry. She wants to slit open her belly and hid the boys there and keeps them safe and warm forever. The mother of the two boys is so difficult, imperious and plays rap music at late night. She yells her children terrifically and calls them 'trouble'.(5) Tehmina always wondered at her treating of children, how could any mother in the world called her children 'trouble' and how could god gives her such precious gifts to.Her eyes sting with tears the way of Tara treating her two boys. She beats them and one of the boys is bleeding. She left the injured boy alone at home and drove away. Tehminalooked around and prayed for help, any neighbor would come and stop her madness, like the neighbors in Bombay who ran from all directions.

"No neighborhood woman come and held the sobbing boys to her breast; no neighborhood child looked at Tara with big, accusing, shaming eyes." (Umrigar 193)

Her heart melts with the image of the wounded boys. She decided to rescue the boys but one side the words of Susan whirling in her mind about not to contact with the next door family and another side the thought of the boys alone at home, finally she decided to save the boys, she was shivering with the cold and afraid when she eyed the six feet fence. The words of Susanthat not to contact them and the thinks of neighbors, if someone caught her crazy mission streaks in her mind, but she jumped off the fence.She forced herself to enter in to the house of Tara where the two boys sit in the corner of the room as quite as mice. She brings the boys to her home.

She wants to break the news to her son, but meantime her grandson Cookie called 911. Two officers came and after investigation, appreciate her crazy mission and takes the boys to their aunt's house. Everything is so different from her ways of life in India. Sorab's eyes narrows when he sees his mother's picture in the daily mirror and the news of jumping over the fence in head line "A Christmas Miracle". He thinks about his mother;

"Mamma, who refused to even go to the gym in the housing complex, mamma, who had to rest if they walked too fast in the park, yeah, he could just see his mother jumping over the fence. She might as well jump over the moon." (225)

When Sorab asks her about this incident she is afraid, like a heavy, clumsy bird with broken wings. But things have been changed for a short while. The Americans start praising her crazy mission, which is so alien to them and applauses her as an 'American hero'. The Mayor of the town, Sorab's boss and others start acclaim her bravery and courage. But once, America is the place, which is alien world to her, who felt lonely here. Now, America is the familiar world, and has a family here. She is a good natured woman that everyone could see a mother, grandmother, friend, and want to be around. She just demonstrates her goodness, virtue and integrity. She is a stranger to America but it praises her as;

"She is a visitor to America; she is stranger to this country. But to two frightened young Rosemont Heights boys, Bombay native Tehmina Sethna, 66, turned out to be 'A Christmas Angel'."(228)

Umrigar succeeds in its wonderful analysis of the woman's experience in a foreign land, who caught between two cultures, one is

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familiar and another is strange. Making decision-some decisions are small, some are large and some are life changing. Here in America she has her family, who needs her presence and love, Cookie needs her, as a grandmother who could give her love to him, Susan needs her to burnish her rough edges, remind her responsibilities, Sorab, Percy, Joe(Sorab's boss, who treats her as his mother), Eva need her, she has enough love for all of them.A sweet natured woman, Tehmina sprinkles everyone's life with a bit of sugar, so that everyone will live happily ever after.

Finally, she decides to stay in America on her own terms. The country teaches her how to live independently, dignifiedly and individually. She wants to live her rest of life alone in her own apartment. So there is no need to sell Sorab's house to buy a bigger one. Now she is brave, dare and courageous to lead her life alone. Rustom says;

"A woman who so adored me, who so relied on my strength and that she forgot to measure her own worth, who never new she carried the world, my world, in the palm of her hand." (3)

Umrigar handles the emotions of the central characters, the tragic beauty implied by her narrative art, and the feminine psyche of the novelist enlightened by experience of contemporary India. At the end of the novel, it is Tehmina, who stands apart for her extraordinary strength and flexibility, to with stand the terrible odds and pressures. Umrigar disposes keen insight in to the human psyche, and the striking talent that portraying the situations. Her art of narration is immense, in the novel the 'fence' resembles as decision making. Here the fence is a separator. To choose one between two things, two cultures, and two nations.

"When she had found the courage to jump, she had landed in more than Antonio's yard. She had landed in America. The fence had been the dividing line between the past and the future, between India and America." (293)

Umrigar draws the universal elements; love, loss, grief, acceptance belonging, family, isolation, uncertainty and community and brought them to the place where cultures conserve their identity and are traverse by their commonalities. She depicts the difficulties in compassionately revealing the wonderful humanity through the character of her protagonist. Tehmina's seeming alienation is strikingly described by the novelist. She is perhaps the most afflicted victim of the cultural alienation resulting from the East-West encounter depicted by Umrigar. The way she weaves the complexities of women especially as immigrants are vulnerable. Her narrative style is humane, sympathetic, compelling and evoking the pain and sufferings women faced in the society.

REFERENCES:

[1]. Umrigar,Thrity. If Today be Sweet, Harper Collins, Pub- 2008- ISBN – 978-0-06-124023-2.

[2]. Thuen, Frode, Reime, Marit, Hegg: Skrautoll, Kari (1997), The Effect of Widowhood on Psychological well-being and Social support in the Oldest Group of Elderly. Journal of Mental Health, UK,6, 265-274.

[3]. Malkinson, Ruth. (1987),Helping and being helped: The Support Paradox. Death Studies, 11, 205-219.

[4]. Talott, Maria M. (1990), The Negative side of the Relationship between Older Widows and their Adult Children: The Mothers Perspective. Gerontologist, 30, 595-603.

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Evaluation of Anti-inflammatory activites of Isorhamnetin 3-O-α-L- (6''-E-p-coumaroyl)-rhamnoside isolated from *Indigofera tinctoria*

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ABSTRACT:

The isolation isorhamnetin 3-O-α-L- (6''-E-p-coumaroyl)-rhamnoside was obtained from *Indigofera tinctoria*. *Indigofera tinctoria* flower has been found to rich in medicinal properties such as anti -inflammatory anticancer, anti diabetic, antimicrobial activity. The plant flower have been found to rich in nutrients and anti nutrients such as crude protein The present study has been under taken with an objective to determine the anti -inflammatory or immunosuppressive agent., antibacterial, antioxidant and cytotoxic activity of the flower methanol extract *Indigofera tinctoria*. The selected medicinal plant was collected from nearby region of cuddlaore. The compound present in isorhamnetin 3-O-α-L- (6''-E-p-coumaroyl)-rhamnoside the extract were identified by analysis of UV, IR, ^{1}H NMR, ^{13}C NMR, HPLC, TLC spectroscopy methods .

KEYWORDS: *Indigofera tinctoria*, Isorhamnetin, Methanol, Anti –inflammatory, Phytochemical Screening.

INTRODUCTION:

Indigofera tinctoria belongs to the family of Fabaceae which is commonly known as Avuri (OR) Neeli in Tamil nadu. It has light green pinnate leaves and sheafs of pink or violet flowers. The plant is alegume, so it is rotated in to fields to improve the soil (1). The juice of *Indigofera tinctoria* leaves, flowers and indigo powder mixed with honey is used for enlargement of liver and spleen. Juice is also used in asthma, whooping cough, lung diseases and kidney disorders as in dropsy (2). Experimental evidence suggests that *Indigofera tinctoria* possesses anti-inflammatory, hepatoprotective, anti-epileptic, anti-cancer and neuroprotective properties (3) The methanol extract was concentrated in a rotary evaporator. The concentrated methanol extract was used for anti-inflammatory activity (4). The present work has carried out to compare the efficacy of phytochemically extracted the methanol *Indigofera tinctoria* fraction of and also to study its pharmacological effect has been anti-inflammatory activity by carrageenan model. Methanol fraction isorhamnetin 3-O-α-L-(6''-E-p-coumaroyl)-rhamnoside were identified (Fig. 1) using HPLC, UV, and NMR by comparison with the published data.

Isorhamnetin-3-*O*-α-L-(6''-*E*-*p*-coumaroyl)-rhamnoside

Plant material:

Fresh flowers (2kg) of *Indigofera tinctoria* was collected during the August to December 2018 from the Cuddalore District in Tamilnadu, India. Dr. N. Ramakrishnan authenticated this species and the voucher specimen number is GUH7284, were deposited in Herbaium of the Botany Department, Government Arts College (Autonomous), Kumbakonam, Bharathidasan University, India.

Extraction and isolation Air dried flowers of *Indigofera tinctoria* (2.25kg) was extracted with 90% methanol (MeOH) in a Soxhlet apparatus. The hydro-alcoholic solution was concentrated under reduced pressure to dryness, and the residue was dissolved in hot water (1000mL) and kept in the cold overnight. After filtration, the clear solution was consecutively partitioned with petroleum ether, chloroform and ethyl acetate. *Indigofera tinctoria* flowers was reported on the identification isorhamnetin 3-O-α-L-(6''-E-p-coumaroyl)-rhamnoside in the methanolic crude extract was prepared by soaking a sample (50g) of powdered flowers material in 90% methanol (300mL) for 72 h. The extract was filtered using clean cloth and What man No. 1 filter paper. The filtrate was concentrated in vacuum at 30°C and stored in sterile sample containers at 4°C until further use. Double spot on TLC; Rf : 0.32,0.78

Spectroscopic methods:

Melting points were determined on a Fisher Scientific melting point apparatus and are uncorrected. The IR spectrum was measured on FT-IR spectrograph (Perkin Elmer Spectrophotometer, USA) with KBr tablets from 4000 to 400 $cm^{-1}$with resolution $2cm^{-1}$. ^{1}H and NMR experiments have performed on a Bruker AMX 400 instrument (Bruker Company, Faelladen, Switzerland) standard pulse sequences running at 400 MHz for ^{1}H NMR and ^{13}C NMR. Chemical shifts gave in δ (ppm) about TMS as internal standard material and the coupling constants (J) are in Hz. Column chromatography was performed on silica gel 60 as stationary phase (particle size 0.04 - 0.036mm, 230-400 mesh, ASTM E. Merck, Germany). The different solvent systems in volumetric ratios were employed (vol. ratios): $CHCl_3$/EtOAC/ MeOH (14:3:3). Flavonoids were visualized by UV light 363 nm, with NH3 vapors and by spraying with 1% $AlCl_3$ in MeOH. A sugar was detected by spraying with aniline phthalate solution in n-BuOH and heating at 105 °C. activated by heating at 110°C for one hour before to use. TLC was carried out on 0.25mm Brinkman percolated silica gel F254 plates (silica gel 60, 230-400 mesh, Merck, Germany). The different solvent systems were used for TLC analyses (Water: Chloroform: Methanol, 10:64:28v). spots were visualized by spraying with bromothymol blue in EtOH. A Shimadzu HPLC system (Columbia, MD), was used with UV

detection at 280 - 350nm. A chromatographic system comprising a Spectra Physics P-200 series gradient pump (Fremont, CA, USA), a rheodyne injector fitted with a 20- FL loop, the C18 column (250× 4.6mm) (phenomenex, Torrance, CA, USA) was used.

Animals:

Male albino mice (30-40g) and male albino rats (100- 150g) of Wistar strain were procured from the animal house (5) 2014, Department of Zoology, Government Arts College (Autonomous), Bharathidasan University, Kumbakonam, Tamilnadu, India. Animals were fasted overnight and were divided into control, standard and different test groups each consisting of five groups (six animals each). They housed in cages and maintained under standard conditions at 26±2°C and relative humidity 44-56% and 10 h light and 14 h dark cycles each day for one week before and during the experiments. All animals were fed with the standard rodent pellet diet, and water adlibitum. Before starting the experiment on animals, the experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee (IAEC). (Approval No. BDU/IAEC/2011/31/29.03.2011).

Anti-inflammatory activity Carrageenan induced paw:

The anti-inflammatory activity of the test compounds were evaluated in Wistar rats employing the method. The different test concentration of isolated isorhamnetin 3-O-α-L-(6"-E-p-coumaroyl)-rhamnoside. Group I served as control, Group II standard diclofenac sodium 100 mg. Group III methanol extract 200 and 300 mg, Group IV petroleum ether extract 200 and 300 mg, Group V Chloroform extract 200 and 300 mg, methanolic extracts of *Indigofera tinctoria* were administrated to the animals in the test groups at the dose of 200 mg/kg by oral route(6). Animals in the standard group received Diclofenac sodium at dose of 100mg/kg, by oral route. Control group animals were received 1% DMSO at the dose of 10ml/kg body weight. The acute inflammation was induced by the sub-plantar administration of 0.1ml of 1% carrageenan in the right paw. Paw volume was measured by using digital plethysmometer (Ugo Basile-Italy) before administration of carrageenan and after 1, 2, and 3 hrs intervals (7). The efficacy of different drug was tested on its ability to inhibit paw edema as compared to control group.

Volume of edema = Final Paw Volume - Initial Paw Volume

The Percentage inhibition of paw edema was calculated by the formula as below.

% Inhibition of Paw edema = [(VC – VT) / VC] x 100
Where, VC = Paw edema of control group and VT = Paw edema of treated group

STATISTICAL ANALYSIS:

The experimental results were expressed as multiple comparisons of Mean ± SEM were carried out by one way analysis of variance (ANOVA) followed by Dunnet Multiple Comparisons Test and statistical significance was defined as $P< 0.05$

RESULTS AND DISCUSSION:

Chemical identification

Isorhamnetin-3-O-α-L-(6"-E-p-coumaroyl)-rhamnoside - Yellow amorphous powder; m.p. 200 - 202 ^{o}C;RT 26.3min;; IR vmax(KBr): 3272, 2912, 2840, 1692, 1641, 1622, 1517, 1237,1064 and 592 cm^{-1} (8); ^{1}H NMR and ^{13}C NMR (400 MHz,DMSO-*d6*, δ ppm, J, Hz) The UV spectrum of the flavones glycoside from the methanol fraction showed two absorption peaks at 260nm and 340nm. Chemical constituents- Spectral data of compounds. This compound also gave positive color reactions for a hydroxyl flavone with several reagents (9). 4'-trihydroxy-3'- methoxyflavonol. The ^{1}H NMR spectrum showed five aromatic protons signals at (δ 6.23, d, J = 2.1 Hz, H-6; δ 6.23, d, J = 2.1 Hz, H-8; δ 6.56, d, J = 2.1 Hz, H-2'; δ 7.80, d, J = 2.1 Hz, H-5'; δ 6.90, dd, J = 8.5 are typical of an AX system in B ring and their corresponding carbon signals appear at δ 98.5, 93.2, 114.6, 115.4, 121.7, respectively. Also methoxy protons signal was present at δ 3.80 ppm (3H, s) which showed with the carbon resonance at δ 145.1 (C-3'). These data clearly confirmed the characteristic pattern of isorhamnetin as aglycone (13). In addition, an anomeric α- rhamnose proton was recognized in this spectrum as a doublet at δ 5.48 ppm with respect to the question of α-or β-linkage of the sugar moieties, it has been found the coupling constant J = 1.61 Hz. corresponded to the anomeric proton of α-linked rhamnose (14). The methyl protons of the sugar rhamnose appear at δ 1.15 ppm which is therefore assigned to a 6-deoxy sugar (rhamnose) and rest of the sugar protons appear in the range δ 3.48 (10).

The ^{1}H-NMR spectrum of 4 also showed signals ascribed to sugar moieties and a p-coumaroyl residue (Table 1). The arrangements of the sugar units were assigned after hydrolysis of 4 compared to those of reliable sugar samples. The lower field shifts of H_2-6''' (δ 7.36, J = 8.2 Hz) of one glycosyl unit suggested the substitution site of the pcoumaroyl unit. Also the signals at 6.36 and 7.61 ppm (both d, JAB = 15.8 Hz) assigned to Trans olefinic protons suggested the presence of p-coumaric acid as the acyl moiety. In the ^{13}C NMR spectrum, the signal at 167.6 ppm (s, C=O) supported this proposal. The ^{1}H NMR is suitable method to distinguish between both Z, E- isomerism of cinnamic acid. The E-cinnamoyl residue is detectable by a pair of doublets with shift values of 6.36 ppm for C_8-H and 7.61 ppm for C_7-H, the corresponding large coupling constant is about 16 Hz, which agrees well with findings of this study (11) The ^{13}C NMR spectrum contained 31 carbon signals, 15 of them has assigned to the flavonol aglycone and one was methoxy carbon signal verified the isorhamnetin, and remaining 15 signals has attributed to sugar rhamnose with addition of p-coumaroyl unit. The sugar moiety has proved to be acylated at C- to achieve 3 of the aglycone as deduced from the anomeric proton at δH 5.48 and δC 131.1 ppm, which were in close agreement compound 4 as isorhamnetin-3-O-α-L-(6"-E-p-coumaroyl)- rhamnoside.

Table 1. ^{1}H and ^{13}C NMR data of 3 and 4 (DMSO-d_6, 400 MHz)

Position	δ_C in ppm	δ_H [*J* (Hz)] in ppm
2	157.6	
3	134.2.	
4	177.5	
5	160.8	
6	98.5	6.29 (d, *J*=2.1 Hz)
7	164.4	
8	93.2	6.56 (d, *J*=2.1 Hz)
9	156.7	
10	103.8	
1′	121.9	
2′	114.6	7.80 (d, *J*=2.1 Hz)
3′	145.1	
4′	148.4	
5′	115.4	6.90 (d, *J*=8.5 Hz)
6′	121.7	7.62 (dd, *J*=2.1, 8.5 Hz)
1″	100.0	5.48 (brd, *J*=1.58 Hz)
2″	70.2	3.48 (dd, *J*=3.3, 1.58 Hz)
3″	70.7	3.56 (dd, *J*=9.5, 3.3 Hz)
4″	72.3	3.37 (d, *J*=9.5 Hz)
5″	68.8	3.48 (dq, *J*=9.5, 6.2 Hz)
6″	17.9	1.15 (s)
3-OH		9.38 (s)
5-OH		12.56 (s)
7-OH		10.78 (s)
4′-OH		9.72 (s)
3′-OMe	55.4	3.80 (s)
1‴	126.9	
2‴	131.1	7.38 (d, *J*=8.2 Hz)
3‴	116.7	6.80 (d, *J*=8.7 Hz)
4‴	161.3	
5‴	116.7	6.80 (d, *J*=8.7 Hz)
6‴	131.1	7.36 (d, *J*=8.2 Hz)
7‴	146.9	7.61 (d, *J*=15.8 Hz)
8‴	114.3	6.36 (d, *J*=15.8 Hz)
9‴	167.6	

Anti-inflammatory activity:

Inflammation is a response of living tissue to injuries that involve activation of various enzymes, mediator release, cell migration, tissue breakdown and repair (12). Carrageen an-induced hind paw edema is a suitable experimental animal model of acute inflammation (13). Carrageenan induced paw edema takes place in three phases, in the first phase (1 h after carrageenan induce) involves the release of serotonin and histamine from mast cells, in a second phase (2 h) was provided by kinins and the third phase (3 h) was mediated by prostaglandins, the cyclooxygenase and lipoxygenase products (14) .As shown in the results (Table 2), restraint of paw edema (after 3 h) for test compound (200mg) with 2.82±0.28mL paw volume, respectively. It shows the methonlic extracts compounds had a significant anti-inflammatory effect, and the results were compared with standard diclofenac sodium 100 mg/kg and showed the paw volume reduction of 2.62±0.13 without statistical significance ($p > 0.05$) between methonlic extracts compounds and diclofenac sodium. Results were also reported in % restraint of edema (protection against inflammation) after three-hour treatment in comparison with the control group (Table 2). It showed a maximum percentage reduction (58.23%) in paw edema at 3 hours. methonlic extracts compounds at the dose of 200mg/kg body weight showed the percentage of inhibition of paw edema at 3 h 49.45%, respectively. All the test and standard groups have reduced the thickness of edema of the hind paw in different percentages compared to the control group .The maximum inhibition (3.11±0.24; $p < 0.001$) elicited by the chloroform extract was recorded at 3 hours. In the carrageenan isorhamnetin 3-O-α-L- (6"-E-p-coumaroyl)-rhamnoside showed significant inhibitory effect on the oedema formation. This effect started from the first hour and was maintained in all the inflammatory phases, suggesting that the main mechanism of action of the tested compound may involve prostaglandin biosynthesis pathway and may influence other mediators of inflammation.

Table 2. Anti - inflammatory activity of isorhamnetin 3-O-α-L- (6"-E-p-coumaroyl)-rhamnoside From *Indigofera tinctoria*

Groups	Initial paw volume	Paw volume at different time interval (in ml)		
		1h	2h	3h
Control (1% DMSO)	2.28 ± 0.19	2.61 ± 0.04	2.90 ± 0.14	2.81 ± 0.15
Diclofenac Sodium (100 mg/kg)	2.26± 0.24	2.52 ± 0.07	2.70 ± 0.11	2.62 ± 0.13
Methanol extract (200 mg/kg)	2.27± 0.18	2.49± 0.32	2.92± 0.24	2.82± 0.28
(300 mg/kg)	2.29± 0.15	2.40 ± 0.12	2.59 ± 0.07	2.72 ± 0.8
Petroleum ether extract (200 mg/kg)	2.25± 0.27	2.87± 0.26	3.08± 0.13	2.99± 0.11
(300 mg/kg)	2.26± 0.14	2.72 ± 0.02	2.98 ± 0.14	2.91 ± 0.12
Chloroform extract (200 mg/kg)	2.27± 0.19	2.86± 0.28	3.31± 0.30	3.11± 0.24
(300 mg/kg)	2.28± 0.19	2.73 ± 0.03	3.13 ± 0.23	2.97± 0.05

All values are expressed in Mean ± SEM

Values are expressed in Mean ± Standard Deviation (n=6)

One-way ANOVA (Dunnetts method) Means for groups in homogeneous subsets are displayed. Subset for alpha = 0.05 level.

Phytochemical Screening:

The phytochemical screening of plant for the presence of glycosides, flavonoids, phenols, resin and tannins. This analysis revealed that the flower contained higher value of fat, protein, fiber and minerals (15).

Phytochemical Test	PE	CE	EE	ME	WE
Carbohydrates/glycosides	(-)	(-)	(-)	(+)	(+)
Alkaloid	(-)	(-)	(-)	(-)	(-)
Flavonoids	(-)	(-)	(-)	(+)	(+)
Saponins	(-)	(-)	(-)	(-)	(+)
Tannins	(-)	(-)	(-)	(+)	(-)
Phenolics compound	(-)	(-)	(-)	(+)	(+)
Protein and amino acid	(-)	(-)	(-)	(+)	(+)

PE= Pt. ether Extract; CE=Chloroform Extract ;
EE= Ethyl acetate Extract; ME= Methanolic Extract;
WE= Water Extract

CONCLUSIONS:

The present investigation, we confirm isorhamnetin 3-O-α-L- (6"-E-p-coumaroyl)-rhamnoside isolated from methanol crude extract of *Indigofera tinctoria* was better anti-inflammatory

Group:

Bioactive substances from this plant were employed to develop drugs for treating.

Inflammation:

This effect may be due to its flavonoids, quercetin, isorhamnetin and their Glycosides composition.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

REFERENCES:

1. Amrithpal Singh. Medicinal Plants of the World, Special indin edition, Oxford and IBH" Publishing Co.Pvt Ltd, , New delhi. 2006:168.
2. Nadkarni K M, Indian meteria medica, 3rd revised enlarged edition, Ramdas Bhatkat for Popular Prakashan Pvt. Ltd Mumbai. 2002; 680-681.
3. Asuntha G, Prasannaraju Y, Prasad KVSRG. Effect of ethanol extract of *Indigofera tinctoria* Linn. (Fabaceae) on lithium/pilocarpine-induced status epilepticus and oxidative stress in Wistar rats.Trop J Pharm Res, 2010; 9: 149–156.
4. Tyagi PK, Rai VK, Pahria AK, Kumar SS, Singh Y, Sharma M, Goval M. Preliminary phytochemical screening and evaluation of antiinflammatory activity of ethanolic extract of leaves of *Indigofera tinctoria* Linn. J Curr Pharm Res. 2010; 3: 47–50.
5. Sivagnanam Ilayaraja, Kalaivanan Prabakaran, Rajamanickam Manivannan Evaluation of Anti- Bacterial, Analgesic and Anti-Inflammatory activities of Oncocalyxone A isolated from Prenanthes sarmentosus Journal of Applied Pharmaceutical Science. 2014; 4 (10): 88-91.
6. Mossa, J.S., Rafatullah, S., Galal, A.M., Al-Yahya, M.A. Pharmacological Studies of Rhusretinoeehoea. International J Pharmacognosy. 1995; 33:242-246

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Data Article

Investigation on Co(II), Ni(II), Cu(II) and Zn(II) complexes derived from quadridentate salen-type Schiff base: Structural characterization, DNA interactions, antioxidant proficiency and biological evaluation

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ABSTRACT

Four mononuclear metal chelated complexes, **[Co(L)]** (**1**), **[Ni(L)]** (**2**), **[Cu(L)]** (**3**) and **[Zn(L)]** (**4**), were designed and synthesized from a novel quadridentate salen-type Schiff base ligand ($\mathbf{H_2L}$) derived from 2-hydroxy napthaldehyde and 4-fluoro-1,2-phenylenediamine. The chemical structures of synthesized compounds were fully characterized by various techniques. Spectral and analytical investigations clearly suggested that a square planar geometry around metal centre. The thermal analysis (TGA) of these complexes indicated greater thermal stability and various steps involved in thermal decomposition of metal complexes. The binding ability between the metal complexes and CT-DNA was investigated by UV-Vis, fluorescence spectroscopy and viscometric experiments which revealed an intercalation binding mode of binding. The cleavage property of metal complexes against pBR322 DNA has been explored. The antioxidant activity of M(II) complexes against active free radicals was determined by DPPH free radical scavenging experiment. Furthermore all compounds were screened against antimicrobial activity.

Specifications Table

Subject area	Bioinorganic chemistry
Compound	Quadridentate salen-type Schiff base
Data category	Synthesis, Spectral data, biological studies
Data acquisition format	NMR, FT-IR, UV–Visible, Mass spectra, Magnetic moment, Elemental analysis.
Data type	Analyzed.
Procedure	Transition metal complexes were synthesized and characterized by elemental and spectroscopic analysis. Compounds were subjected to their biological evaluation.
Data accessibility	Data is available with this article.

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1. Rationale

Schiff bases and their metal complexes are significant set of biologically active derivatives and have received huge attention of inorganic biochemists due to their bio-medicinal properties, including antibacterial, antifungal, antioxidant, antiinflamatory, anticancer and herbicidal activities [1–3]. The metal complexes with Schiff bases play an important role in coordination chemistry due to their variety of applications such as binding ability with DNA, capability of DNA cleavage, catalytic and biological properties, ease of synthesis, changeable structural confirmations [4–8].

The family of salen-type Schiff bases complexes were synthesized by condensation between aromatic or aliphatic diamines and phenolic aldehydes. Multidentate Schiff base ligands coordinate with transition metal ion to form complexes of special type of chelators and binds with metal ions through nitrogen atom of azomethine group (–C=N–) and oxygen of phenolic –OH group, which are intact to construct a variety of new frame works with interesting chemotherapeutic and bio-medical properties [9–11]. The metal complexes were thermally stable and actively participated in asymmetric catalysis, mainly involved in organic transformations as catalytic species and some of them are identified as oxygen carriers [12,13].

An extensive attention given towards the quadridentate Schiff base complexes due to their stupendous antioxidant and anticancer properties [14–16]. Free radicals ($^*OH^-$ and $^*O_2^-$) generated in human body induces pathophysiological abnormalities leads to a variety of cancers by breaking the several proteins and lipid contents of cells. Antioxidants were used to treat the human body from such abnormalities [9,17]. Therefore it is essential to develop the potential drugs with both strong antioxidant and anticancer properties for quick remedy from miscellaneous type of cancers.

Biologically active cobalt ion contributes an important role in many metabolic processes of living organisms. As a central metal ion in vitamin-B_{12}, regulates the normal functioning of brain and the nervous system. Earlier studies revealed that, most of the cobalt complexes significantly act as antioxidants, antimicrobial, antiproliferent and antiviral drugs [18,19]. The literature survey noticed that, the nickel complexes are vital in redox enzyme systems as active catalysts and also nickel supported complexes exhibit catalytic activity in hydrogenation reactions [20,21]. Many of copper complexes reported as pharmacological agents and considered as potent anticancer drugs. These complexes induce their anticancer property via blocking the aggressive expansion of cell division by rupturing the cell DNA through an oxidative mechanism and are the best alternative to Cis-Platin drug [22–24]. Zinc is the second most abundant element in human body and emerged as central metal ion in many biological active enzymes, carbonic anhydrase, carboxy peptidase and alcohol dehydrogenase. The literature suggests that several zinc complexes act as antidepresents, antiinflamatory and antialergic agents [25,26].

In the present investigation, we made a focus on synthesis and structural characterization of salen-type tetradentate Schiff base ligand and its Co(II), Ni(II), Cu(II) and Zn(II) complexes. All complexes were subjected to DNA binding (Complex – CT-DNA) and cleavage (Complex – pBR322 DNA) studies. Further, invitro DPPH free radical scavenging method employed to investigate antioxidant activity and finally antimicrobial activity was carried out against variety of bacterial and fungal strains, results were compared with standard drugs.

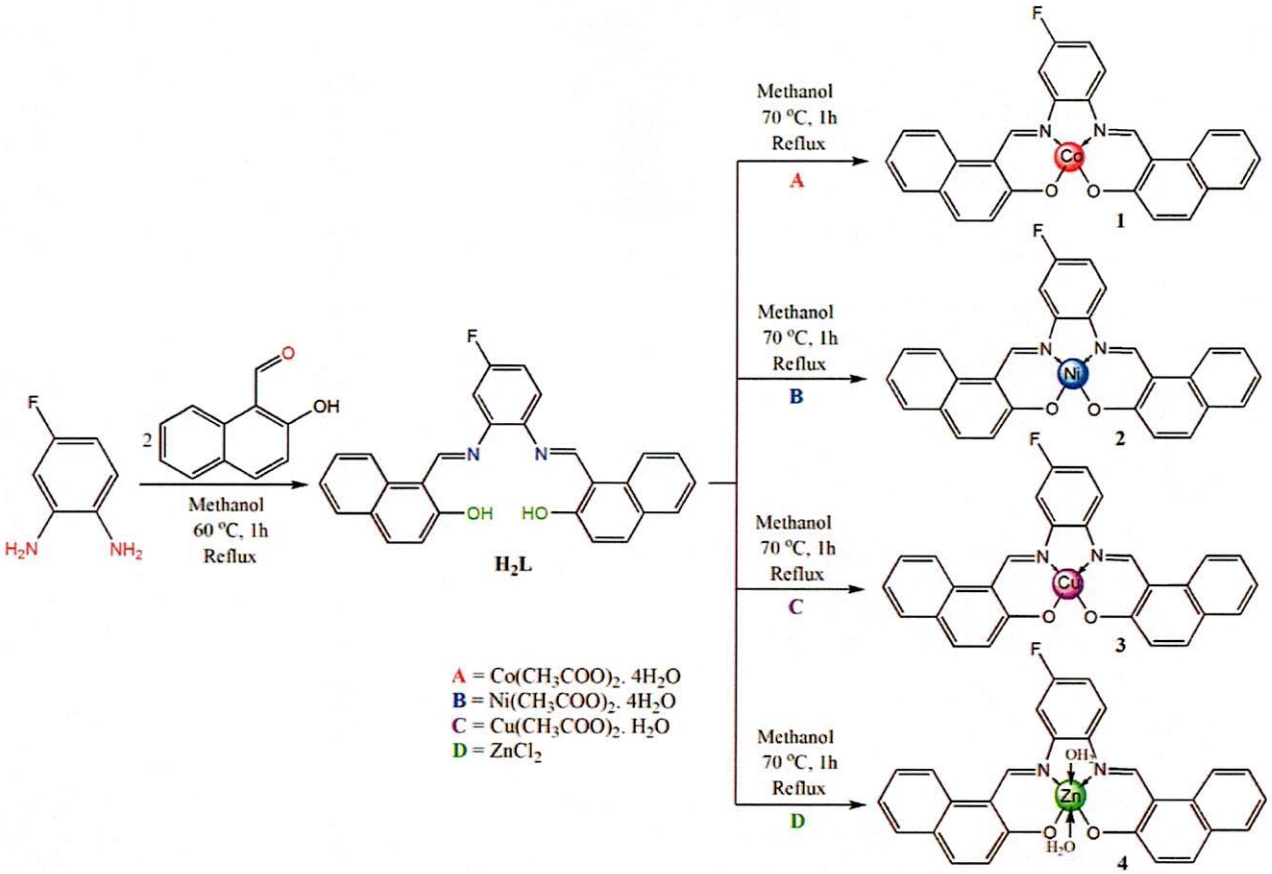
2. Procedure

2.1. Chemical materials

Chemicals, hydrated metal salts, $M(OAc)_2.xH_2O$ (M= Co, Ni and Cu) and Solvents utilized in this experimental work were of analytical grade purchased from Hi-Media Ltd., Merck, Finar and Sigma–Aldrich Chemicals. Standard methods were employed to distillation of solvents and used for synthesis of Schiff base and its metal complexes [27]. The Calf-thymus DNA (CT–DNA) and supercoiled pBR322 DNA (stored at 4°C) were procured from Genei, Bangalore, India. A Tris–HCl/NaCl buffer (Tris-buffer, pH=7.2) solution of CT–DNA give a ratio of 1.8–1.9 of UV absorbance at 260 and 280 nm, DNA used in this study was protein free [28].

2.2. Physical Instrumentation

Carbon, Hydrogen and Nitrogen elemental analysis of compounds were carried out by Perkin-Elmer 240C (USA) elemental analyzer. The metal percentage of the complexes was calculated by atomic absorption spectroscopy after treatment of complexes with HNO_3 on GBC Avanta 1.0 AAS. The ^{1}H-NMR, ^{13}C-NMR spectra of Schiff base ligand and its zinc complex were recorded on a Bruker 400 MHz NMR. FT-IR spectra of all compounds were recorded on Perkin–Elmer Infrared model 337 instruments in the range 4000–250cm^{-1} with KBr pellet method. Electronic spectra (200 to 800nm range) of all compounds were recorded on Shimadzu UV–2600 spectrophotometer. Gouy balance model 7550 was used to investigate the magnetic moment values of metal complexes and the calibrant, $Hg[Co(NCS)_4]$ was used in this investigation. ESR spectrum of Cu (II) complex was carried out in DMSO solvent using JEOL–Japan made JES–FA200 ESR Spectrometer at 77 K. Thermal stability of complexes was determined by themogravimetric analysis (TGA) and was carried out on Shimadzu TGA–50H thermogravimetric analyzer, in temperature range of 30–1200°C with 10°C min^{-1} heating rate. JSM–6360 LV (JEOL) scanning electron microscope instrument used to found the surface morphology of Schiff base ligand and its metal complexes at variable voltage and magnifications. Powder X-ray difraction analysis of compounds was explored by X'pert Pro X–Ray Diffractometer. VG AUTOSPEC mass spectrometer was used for detrmmination of mass spectral data of all compounds. The polmon instrument, model No. MP–96 was used to find out the melting points of all compounds. The solution state UV



Scheme 1. Synthesis pathway of ligand, **H_2L** and its **Co(II), Ni(II), Cu(II)** and **Zn(II)** complexes.

absorption studies and fluorescence quenching experiments were recorded on Shimadzu UV–2600 spectrophotometer and RF–5301PC (JASCO) spectrofluorometer respectively. Viscosity measurements were carried out on Vensil-Ostwald viscometer. DNA nuclease experiments were performed with GeNei gel electrophoresis tool and DNA bands containing gels were photographed by Bio-Rad gel imaging system.

2.3. Synthesis and procedures

*2.3.1. Preparation of Schiff base ligand (**H_2L**)*

synthesis of Schiff bases ligand, **H_2L** was carried out by mixing of hot methanolic solution of 2-hydroxy-napthaldehyde (2.0 mmol) to hot stirred methanolic solution of 4-fluoro-1,2-phenylenediamine (1.0 mmol) and the solution was refluxed for 1 hour at 60 °C on oil bath. Later the solution was allowed cool for 1-2 h leads to setting down of orange solid. The product was filtered and gently rinse with Pet-ether and recrystallized from methanol. TLC technique was helped to check the purity of product and dried in vacuum desiccator loaded with anhydrous $CaCl_2$ (Scheme 1).

*2.3.1(1). Ligand **H_2L***

Anal. Cal (%): C, 77.41; H, 4.41; N, 6.45. Found: C, 77.29; H, 4.32; N, 6.32. ^{1}H–NMR (400 MHz, $CDCl_3$): δ = 15.11 (s, 1 H), 14.97 (s, 1 H), 9.69 (m, 2 H), 8.55-8.59 (t, J = 7.90, 2 H), 7.95-8.00 (t, J = 9.41 Hz, 2 H), 7.88-7.91 (dd, J = 2.76 Hz, J = 2.76 Hz, 1 H), 7.81-7.86 (m, 3 H), 7.54-7.58 (t, J = 7.78 Hz, 2 H), 7.36-7.41 (m, 2 H), 7.26-7.31 (m, 1 H), 7.81-7.86 (m, 3 H), 7.11-7.13 (d, J = 9.03 Hz, 1 H), 7.03-7.05 (d, J = 9.03 Hz, 1 H) (**Fig. S1**). ^{13}C–NMR (400 MHz, $CDCl_3$): δ = 169.94, 167.19, 162.94, 160.52, 159.15, 157.92, 140.18, 137.76, 136.87, 136.37, 133.52, 133.34, 129.44, 128.67, 128.58, 127.50, 127.36, 124.25, 124.12, 122.11, 121.32, 114.23, 114.00, 109.90, 109.77, 107.12, 106.87, 79.65 (**Fig. S2**). FT–IR (KBr)(cm^{-1}): $\upsilon_{(OH)}$ 3449; $\upsilon_{(CH=N)}$ 1630; $\upsilon_{(C-O)}$ 1165. UV ($CHCl_3$) $\lambda_{max,}$ nm: 265, 328, MS (ESI): *m/z* = 434 $[M+H]^+$. Yield: 76%, M.P: 222-225 °C.

2.3.2. Synthesis of Co(II), Ni(II) Cu(II) and Zn(II) complexes [1–4]

To the hot stirred methanolic solution of Schiff base ligand (**H_2L**) (0.01 M), hot methanolic solution of metal acetates/chlorides [$Co(CH_3COO)_2$. $4H_2O$/ $Ni(CH_3COO)_2$. $4H_2O$/ $Cu(CH_3COO)_2$. H_2O/ $ZnCl_2$] (0.01 M) was added drop wise and refluxed for 1 hour at 70 °C leads to formation of coloured solid. The reaction mixture was left constant for 1-2 hrs for settling down the metal complex. The product was filtered and washed 2-3 times with methanol and petroleum ether. Filtered metal complexes were dried in vacuum desiccators loaded with anhydrous $CaCl_2$ (Scheme 1).

*2.3.2(1). [Co(L)](**1**)*

Anal. Cal (%): C, 68.44; H, 3.49; N, 5.70. Found: C, 68.30; H, 3.38; N, 5.57. FT–IR (KBr)(cm^{-1}): $\upsilon_{(C=N)}$ 1606, $\upsilon_{(C-O)}$ 1193, $\upsilon_{(M-O)}$ 491, $\upsilon_{(M-N)}$ 430. UV-Vis. (DMSO) λ_{max} (nm): 273, 335, 426, 468. μ_{eff} (BM): 2.15. MS-ESI (m/z): 493 $[M+2H]^+$. Yield = 71%. M.P: 310-313 °C.

*2.3.2(2). [Ni(L)](**2**)*

Anal. Cal (%): C, 68.47; H, 3.49; N, 5.70. Found: C, 68.36; H, 3.37; N, 5.61. FT–IR (KBr)(cm^{-1}): $\upsilon_{(C=N)}$ 1607, $\upsilon_{(C-O)}$ 1199, $\upsilon_{(M-O)}$ 491, $\upsilon_{(M-N)}$ 429. UV-Vis. (DMSO) λ_{max} (nm): 273, 338, 377, 481. μ_{eff} (BM): Dia Magnetic. MS-ESI (m/z): 493$[M+2H]^+$. Yield = 72%. M.P: 307-310 °C.

*2.3.2(3). [Cu(L)](**3**)*

Anal. Cal (%): C, 67.80; H, 3.45; N, 5.65. Found: C, 67.65; H, 3.32; N, 5.56. FT–IR (KBr)(cm^{-1}): $\upsilon_{(C=N)}$ 1614, $\upsilon_{(C-O)}$ 1194, $\upsilon_{(M-O)}$ 489, $\upsilon_{(M-N)}$ 427. UV-Vis. (DMSO) λ_{max} (nm): 265, 327, 387, 479. μ_{eff} (BM): 1.78. MS-ESI (m/z): 518 $[M+Na]^+$. Yield = 70%. M.P: 330-335 °C.

*2.3.2(4). [Zn(L)](**4**)*

Anal. Cal (%): C, 62.99; H, 3.96; N, 5.25. Found: C, 62.85; H, 3.85; N, 5.13. ^{1}H–NMR (400 MHz, $CDCl_3$): 9.75-9.77 (m, 2 H), 8.43-8.45 (m, 2 H), 8.18-8.29 (m, 2 H), 7.82-8.11 (m, 1 H), 7.71-7.88 (m, 3 H), 7.48-7.51 (m, 2 H), 7.21-7.65 (m, 3 H), 6.98-7.01 (m, 2 H), (**Fig. S1**). FT–IR (KBr)(cm^{-1}): $\upsilon_{(C=N)}$ 1613, $\upsilon_{(C-O)}$ 1167, $\upsilon_{(M-O)}$ 544, $\upsilon_{(M-N)}$ 443. UV-Vis. (DMSO) λ_{max} (nm): 271, 333, 417, 471. μ_{eff} (BM): Dia Magnetic. MS-ESI (m/z): 533$[M+H]^+$. Yield = 69%. M.P: 340-344 °C.

2. 4. DNA binding techniques

2.4.1. UV–Vis absorption titrations

Tris-HCl/NaCl buffer (pH=7.2) is used to determined the nature of interaction between metal complexes and DNA at room temperature. The titrations were carried out by maintaining the metal complex concentration (10 μM) as constant, whereas the Calf Thymus DNA (CT–DNA) concentration as variable (0–10 μM). At 260-280 nm wavelength range 1.8-1.9/1 ratio of UV-absorbance was shown by the buffer solution containing CT-DNA indicating that the CT-DNA was completely free of protein [29]. The metal complexes (**1-4**) are moderately soluble in buffer solution due to this reason, metal complexes were dissolved in dimethyl sulfoxide (DMSO) to prepare stock solution. The concentration of CT-DNA used in these experiments was determined by absorption spectroscopy with the known molar extinction coefficient of 6600 $M^{-1}Cm^{-1}$ at 260 nm [30] while determining the absorbance, to eliminate the absorbance of CT-DNA itself equivalent quantities of CT-DNA were added to complex solution as well as reference solution.

2.4.2. Fluorescence quenching assay

Fluorescence quenching assay was performed to further clear understanding of interaction between metal complex and DNA. Because of non-emissive nature of ethedium bromide (EB), the EB and CT-DNA were mixed evenly and kept constant for 15 mints at room temperature for fluorescence quenching study. The fluorescence emission intensities of EB (12.5 μM) bound CT-DNA were evaluated in the wavelength range of 500–750 nm with fixed excitation wavelength (λ=350 nm) by increasing complex concentration from 0–60 μM and CT-DNA concentration (125 μM) as constant. The Stern-Volmer constant, Ksv was calculated for each complex by using following equation [31].

$$I_0/I = 1+K_{sv}[Q]$$

Where, I_0 = fluorescence emission intensity in the absence of complex
I = fluorescence intensity in the presence of complex
K_{sv} = Stern–Volmer constant which is a measure of the efficiency of quenching
[Q] = [metal complex] (concentration of quencher).

2.4.3. Viscosity measurements

Ostwald capillary viscometer immersed in a thermostatic water bath is used to determine the changes in viscosity of solution containing metal complex and DNA. The thermostatic water bath maintains the solution temperature as constant in the range 30 ± 0.2 °C. Digital timer was used to measure the viscosity of metal complexes, while measuring, the concentration of CT-DNA is kept constant (100 μM) where as the variable concentrations of metal complex (0–100 μM) is used. Average flow time of each metal complex was calculated by measuring flow time of each metal complex about three times to get accurate flow value. The viscosity measurements were analysed as $(\eta/\eta_o)^{1/3}$ versus [complex]/[CT–DNA], where η and η_o are the CT–DNA viscosity in the presence and absence of metal complex. The functions "η" and "η_o" are calculated from $\eta = (t\text{-}t_o) / t_o$ and $\eta_o = (t^1\text{-}t_o) / t_o$ respectively, where t = flow time of CT-DNA solution in the presence of the complex, t^1 = flow time of CT-DNA solution in the absence of the complex, t_o = flow time of CT-DNA solution in buffer solution. Finally, viscosity measurements were calculated after correction of flow time of buffer alone.

2.5. DNA cleavage studies

Agarose gel electrophoresis technique was employed to explore the DNA cleavage ability of metal complexes. Photolytic cleavage (UV light irradiation) and oxidative cleavage (H_2O_2 involved) of super coiled pBR322 DNA (0.2μg/μL) by all metal complexes (20 μM) were investigated in Tris–HCl/NaCl buffer (pH = 7.2). The prepared samples were loaded in wells of 1% agarose gels and were electrophorsed at 37 °C by applying 70 Volts for 1 hour in TAE buffer solution mixed with 1.0 mg/ml ethedium bromide (EB). After completion of this electrophoresis process the gels were photographed in the presence of UV source to observe the DNA cleavage patterns.

2.6. Biological evaluation

Antimicrobial activity (antibacterial and antifungal) of synthesized ligand (**H_2L**) its complexes (**1–4**) were determined by *invitro* disc diffusion method [32]. DMSO dissolved 1.0 mg/ml concentrated sample stock solutions were utilized for this biological test. All compounds were screened against Gram-positive bacterial strains, *Bacillus amyloliquefaciens (B. amyloliquefaciens), Staphylococcus aureus* (S. aureus), Gram-negative bacterial strains, *Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli (E. coli)* and also against fungal strains, *Sclerotium rolfsii (S. rolfsii), Macrophomina phaseolina (M. Phaseolina).* The drugs, streptomycin and mancozeb were used as standard drugs to compare the antibacterial and antifungal activity respectively. Nutrient agar medium was prepared and inoculate with 0.1 mL of culture sample. This nutrient agar medium transfered into sterilized petridishes and incubate for proper development of culture medium. The paper discs having 6 mm diameter were soaked in test compounds and placed on the solidified medium by maintaining appropriate distance to avoid the merging of colonies with each other. The bacterial petridishes were autoclaved at 37 °C for 48 hours and fungal dishes were at 30 °C for 72 hours. The activities of tested compounds were evaluated interms of inhibition zones.

2.7. DPPH$^\bullet$ free radical scavenging activity

2, 2-diphenyl-2-picryl-hydrazyl (DPPH) solution was used to investigate the antioxidant property of all metal complexes (**1–4**) by Blois method [33] with some adjustments. On UV-Vis spectrophotometer, DPPH shows a strong absorption band at 517 nm due to odd electron. The metal complex samples were prepared in test tubes with variable concentrations (20-100 μM) along with standard (Ascorbic acid/ Vit-C). To each sample solution 1 ml of 0.3 μM concentrated DPPH solution was added and mixed thoroughly. The samples were incubated for 30-40 mints in dark. After incubation, the absorbances were recorded at 517 nm to investigate the antioxidant activity of metal complexes. The IC_{50} values (50% inhibition concentration) of complexes were calculated with regression lines.

3. Data, value and validation

The synthesized Schiff base ligand (**H_2L**) is orange in colour and its complexes (**1–4**) are coloured in nature. All complexes are stable and non-hygroscopic at room temperature. The prepared complexes are soluble in DMF and DMSO. From the analytical data, it is confirmed that the ratio of metal to ligand is 1:2.

3.1. Nuclear Magnetic Resonance spectroscopy

The ^{1}H–NMR spectroscopic data of the ligand (**H_2L**) and its Zn(II) complex (**4**) was determined at ambient temperature in $CDCl_3$ and DMSO-d_6 solvent respectively at 400 MHz. The ^{1}H–NMR spectrum of **H_2L (Fig. S1)** shows two characteristic singlet signals at δ ~ 15.11 ppm and δ ~ 14.97 ppm are attributed to two non-equivalent phenolic –OH protons. The other characteristic two azomethine –NH protons appear at δ ~ 9.69 ppm and all remaining aromatic protons observed in the region of δ ~ 7.03–8.55 ppm. In the ^{1}H–NMR spectra of Zn(II) complex, disappearance of two phenolic –OH signals indicated the displacement of phenolic–OH groups due to complexion with metal ion. Along with this, the azomethine protons signal is observed at δ ~ 9.66 ppm in Zn(II) complex which confirms the coordination of nitrogen of azomethine group to the metal ion. In Zn(II) complex, the aromatic proton signals are appeared at δ ~ 6.98–8.45 ppm region. The comparison of chemical shift values of the ligand, **H_2L** and its Zn(II) complex clearly shows disappearance of phenolic proton signals in Zn(II) complex and down field shift in azomethine nitrogen proton signal and aromatic proton signal. Therefore, the geometries proposed to these compounds are feasible and supported by the results of ^{1}H-NMR spectroscopy.

3.2. Electronic spectra and magnetic susceptibility

The electronic spectral transitions and magnetic moment values are helpful in determination of geometry of metal complexes. The magnetic moment values and electronic spectra of Schiff base ligand, **H_2L** and its metal complexes (**1–4**) were presented in **Table 1** and **Fig. S3,** respectively. In the present investigation, the ligand (**H_2L**) showed major characteristic bands at 265 nm (π-π^* transition) and 328–380 nm (n-π^* transitions), upon complexation these transitional bands are shifted to 270–275 nm (π-π^* transitions) and 327–426 nm (n-π^* transitions) respectively indicating that the binding sites of ligand is coordinated to metal ion [26]. Along with π-π^* and n-π^* bands, LMCT bands also found in metal complexes

Table 1
Electronic spectral and magnetic moment values of Schiff base ligand, $\mathbf{H_2L}$ and its complexes (**1**–**4**).

Compound	π-π^* (nm)	n-π^* (nm)	LMCT (nm)	d-d (nm)	μ_{eff} (BM)
$\mathbf{H_2L}$	265	328, 380	-	-	-
[Co(L)] (1)	275	335, 426	468	593	2.15
[Ni(L)] (2)	273	338, 377	478	648	-
[Cu(L)] (3)	270	327, 387	479	661	1.79
[Zn(L)] (4)	271	333, 417	471	-	-

Table 2
FT–IR data of ligands, $\mathbf{H_2L}$ and its metal complexes (**1**–**4**)

Compound	$\nu_{(OH)}$ (cm^{-1})	$\nu_{(C=N)}$ (cm^{-1})	$\nu_{(C-O)}$(cm^{-1})	$\nu_{(M-O)}$ (cm^{-1})	$\nu_{(M-N)}$ (cm^{-1})
$\mathbf{H_2L}$	3449	1630	1165	-	-
[Co(L)] (1)	-	1606	1193	491	430
[Ni(L)] (2)	-	1607	1199	491	429
[Cu(L)] (3)	-	1614	1194	489	427
[Zn(L)] (4)	3434	1613	1167	544	443

in the range 468-479 nm. In addition to all these bands, the Co(II) complex showed d-d band at 593 nm (transition : $^1A_{1g} \rightarrow {}^1B_{1g}$) [34], Ni(II) complex showed d-d band at 648 nm (transition : $^1A_{1g} \rightarrow {}^1A_{2g}$) [35], the Cu(II) complex showed d-d band at 661 nm (transition : $^2B_{1g} \rightarrow {}^2E_g$) [36] and no d-d transition was shown by the Zn(II) complex in electronic spectra due to its d^{10} electronic configuration [37]. The magnetic moment of complex was measured by following equation, $\mu_{eff} = 2.828\ [\chi_m.T]^{1/2}$ where χ_m = molar susceptibility and T= room temperature. The Co(II) and Cu(II) showed magnetic moment values as 2.15 BM and 1.79 BM respectively, indicating the paramagnetic nature where as Ni(II) and Zn(II) complexes are found to be diamagnetic in nature. Both the results (electronic and magnetic values) are in good aggrement with square planar geometry for Co(II), Ni(II) and Cu(II) complexes and octahedral geometry was assigned for Zn(II) complex.

3.3. FT–IR spectra

Functional groups present on the ligand and its metal complexes and binding sites of Schiff base ligand connected to metal ion were recognized by FT–IR spectroscopy using KBr pellets in the region 4000-400 cm^{-1}. FT–IR values are given in **Table 2** and **Fig. S4** represents for ligand ($\mathbf{H_2L}$) and its complexes (**1**–**4**). The broad band appeared at 3449 cm^{-1} region is correspond to phenolic –OH group of ligand, which is disappeared in its metal complexes, indicating the ligand coordinated to metal ion through phenolic oxygen atom. This is further confirmed by the shift in the υ(C–O) peak of $\mathbf{H_2L}$ from 1165 cm^{-1} to higher frequencies, 1193 cm^{-1}(**1**), 1199 cm^{-1}(**2**), 1194 cm^{-1}(**3**) and 1167 cm^{-1}(**4**) in its complexes. The FT–IR spectra of Zn(II) complex showed a broad diffuse band at 3434 cm^{-1} region, indicates the coordination sphere is associated with water molecules. The strong peak appeared at 1630 cm^{-1} region for ligand, assigned to azomethine group (–C=N), upon complexation this is shifted to lower wave number to the extent of 15–25 cm^{-1} indicating the nitrogen atom of azomrthine group (–C=N) is coordinated to the metal ion. Further, the non ligand bands appeared in the region of 489–544 cm^{-1} assigned to υ(M–O) bond and in the region 427–443 cm^{-1} assigned to υ(M–N) bond. These characteristic bands provide the additional support to coordination of ligand to metal ion via oxygen atom of phenolic –OH group and nitrogen atom of azomethine group respectively [38–40].

3.4. Mass spectra

In the present study, ESI-MS technique was employed to confirm the mass of the ligand, $\mathbf{H_2L}$ and its complexes (**1**–**4**) by observing the intense molecular ion peaks in the spectra and shown at m/z = 435 $[M+H]^+$ ($\mathbf{H_2L}$), 493 $[M+2H]^+$ (**1**), 493 $[M+2H]^+$ (**2**), 518 $[M+Na]^+$ (**3**), 533 $[M+H]^+$ (**4**). From the ESI-MS results, the molecular ions confirms, 1:1 stoichiometric ratio for metal to ligand as satisfactorily (**Fig. S5**).

3.5. Thermal study

Thermogravimetric analysis of all metal complexes (**1**–**4**) was carried out to determine the thermal stability over a temperature range from 30-1200 °C. The analysis was accomplished at an increased heating rate of 10 °C per minute under the nitrogen environment. Fig. 1 represents the thermogravimetric curves of Co(II), Ni(II), Cu(II) and Zn(II) complexes. From the results of thermogravimetric analysis, it is observed that, the Co(II), Cu(II) and Zn(II) complexes showed two step decomposition process where as the Ni(II) complex decomposes in three steps. All complexes showed high thermal stability up to the range of 200–500 °C except Zn(II) complex. The Zinc complex shows decomposition below the 100 °C indicating water molecules associated in the coordination sphere. In the first step decomposition process of cobalt and copper complexes, the

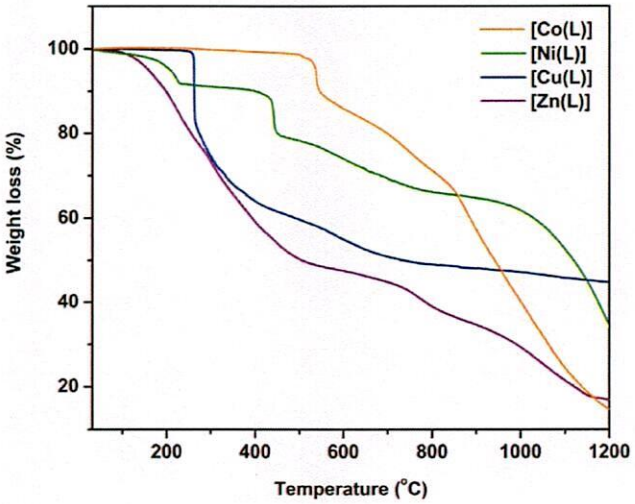


Fig. 1. Thermogravimetric curves of **Co(II), Ni(II), Cu(II)** and **Zn(II)** complexes.

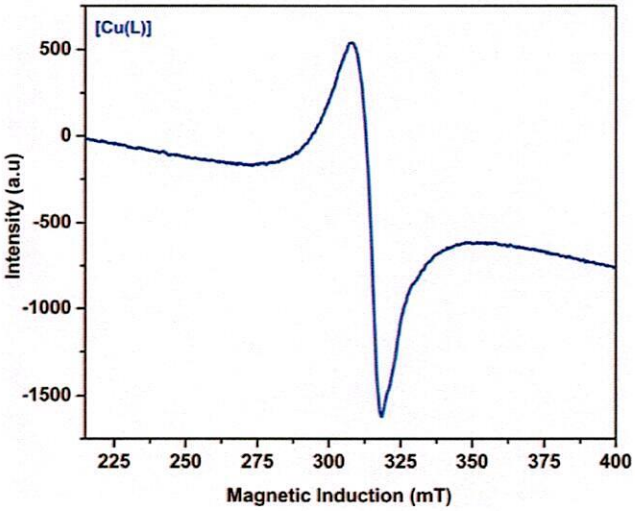


Fig. 2. ESR spectrum of **Cu(II)** complex.

weight loss observed in temperature range 250–490 °C and the Zinc complex shows its first step decomposition in the range of <100–490 °C indicating the partial loss of ligand moiety. In second step, the weight loss observed in temperature region 290–850 °C for cobalt and copper complexes and 490–805 °C for zinc complex indicating the complete decomposition of organic moiety. Nickel complex shows its weight loss in the temperature region of 185–230 °C (first step), 407–480 °C (second step) and 880–1096 °C (third step). The horizontal line above the 1000 °C may due to the complete decomposition of metal complex, resulting metal oxide (MO) moiety.

3.6. ESR spectra

Geometrical environment around the Cu(II) ion in Cu–complex was determined by the analysis of Electron Spin Resonance (ESR) spectral data obtained from ESR spectroscopy. The ESR spectroscopy was carried out at liquid nitrogen temperature (77 K) and in DMSO solvent. The ESR spectrum of Cu–complex (**1**) was shown in Fig. 2. "g" tensor values are determined by the analysis of ESR data and were used in identifying the ground state of the copper complex. The calculated $g_{\parallel}$, $g_{\perp}$ and G values are 2.143, 2.072 and 1.987 respectively. From the data, it is observed that the $g_{\parallel}$, $g_{\perp}$ and g_e (2.0023) values follow the order $g_{\parallel} > g_{\perp} > g_e$ (2.0023), which is an indicative of square planar geometry for copper complex in which the unpaired electron is in $d_{x^2-y^2}$ orbital with ^2B_1g ground state [41]. Further, the $g_{\parallel}$ value is less than 2.3 suggesting the metal–ligand bond is covalent in nature [42]. Kneubuhi's method is employed to found the "G" values [$G = (g_{\parallel}-2) - (g_{\perp}-2)$] to know the exchange coupling interactions between Cu(II)–Cu(II) ions. In the present study, the "G" value of Cu-complex found to be 1.987 and according to Hathway–Billings concept, it is found that the considerable exchange interactions exist between the Cu(II) ions [43].

3.7. Powder X–ray diffraction study

As single crystals of synthesized compounds were not obtained, powder X–ray diffraction (PXRD) technique was performed to find out the nature of all compounds by taking 2θ scale in the range of 10–80 ° degrees. Fig. 3 explores the powder XRD difractograms of synthesized ligand and its Co(II), Ni(II), Cu(II) and Zn(II) complexes. From the results, all the compounds showed sharp peaks indicates their crystalinity in nature. The average crystallite sizes of all compounds were determined from the d_{XRD} patterns. The Debye-Scherrer's formula, $D = 0.9\ \lambda\ /\ \beta\ \cos\theta$ was exploited to calculate the crystallite size [44]. In the formula, D = particle size, 0.9 is the shape factor constant, λ = wavelength of X-ray, β = full width at the half-maximum (FWHM) and θ = diffraction angle for hkl plane. The average grain sizes of ligand **H_2L** and its metal complexes **1**–**4** were found to be 36.6 nm, 22.3 nm, 25.4 nm, 24.7 nm and 28.1 nm respectively.

3.8. SEM and EDX study

Scanning electron microscopy (SEM) is a well known and useful technique to ascertain the surface morphology of synthesized compounds. The SEM micrographs of ligand and its metal complexes exhibits dissimilar morphological appearence from each other [45]. The SEM micrographs of **H_2L** and its complexes (**1**–**4**) shown in Fig. 9. The ligand **H_2L** showed a long dimensional rod like structures with rough surface where as its Co(II), Ni(II), Cu(II) and Zn(II) complexes depicts agglomerate of smaller globular like structres (**1**), non–uniformed small sponge like structures (**2**), irregular shapes of wooden like pieces with sharp ends (**3**) and smaller and larger rods with different size scattered in zigzag manner (**4**) respectively.

The energy dispersive X-ray diffraction (EDX) analysis is used to know the chemical composition of ligand, **H_2L** and its complexes (**1**–**4**). The elements present in the ligand and its complexes are shown in Fig. 4 as EDX graph along with SEM images. In the EDX graph of ligand, C, H, N, F element peaks are observed and in its complexes along with these peaks Co (**1**), Ni (**2**), Cu (**3**) and Zn (**4**) elemental peaks also observed.

3.9. DNA binding techniques

3.9.1. UV–Vis absorption titrations

The mode of binding and strength of binding of the metal complexes with DNA were determined by globally acceptable electron absorption method, in which considerable changes in absorbance and wavelength were observed [46]. In this investigation, the increased concentration of DNA leads to decrease in π-π^* transition intensity bands by 26.5–34.5 % (hypochromism) with 2–4 nm red shift. The DNA binding absorption spectra of Co(II), Ni(II), Cu(II) and Zn(II) complexes are presented in Fig. 5. The red shift/bathochromic shift is an indication for strong stacking interactions i.e., intercalative interactions between the active portion of metal complex and nitrogenous base pairs of DNA [19]. Mainly the non-covalent π-π^* interactions are involved in formation of interactive binding between metal complex and CT–DNA leading to hypochromism. This is evidenced by π-π^* transitions between π^* orbital of complex to π orbital of base pairs of DNA which results lowering of π-π^* transition energy and leads to bathochromic shift [24]. Further, the intrinsic binding constants, Kb values of metal complexes with CT–DNA were explored from following equation by using UV–vis absorption spectral data

$$[DNA]/(\varepsilon_a-\varepsilon_f)=[DNA]/(\varepsilon_b-\varepsilon_f)+1/K_b(\varepsilon_b-\varepsilon_f)$$

Where [DNA] = concentration of DNA in the base pairs, ε_a = apparent coefficient of the complex in presence of DNA, ε_b = molar extinction coefficient of DNA bound form of complex, ε_f = molar extinction coefficient of free complex, K_b = intrinsic binding constant, calculated from the plot drawn between [DNA]/ (ε_a–ε_f) Vs [DNA]. In the present study, the K_b values are found to be $4.62 \pm 0.02 \times 10^4\ M^{-1}$(**1**), $8.87 \pm 0.01 \times 10^4\ M^{-1}$(**2**), $9.44 \pm 0.01 \times 10^4\ M^{-1}$ (**3**), $3.25 \pm 0.02 \times 10^4\ M^{-1}$ (**4**) respectively. From the result, the copper complex strongly interact with CT-DNA than Co(II), Ni(II) and Zn(II) complexes .

3.9.2. Fluorescence quenching assay with Ethidium bromide

The binding nature of metal complexes (**1**–**4**) with CT-DNA base pairs was additionally evidenced by fluorescence quenching investigations. Ethedium Bromide (EB) is a strong fluorescence active probe and emits strong fluorescence intensity in presence of CT–DNA where as it is non-emissive and shows weak fluorescence intensity in absence of DNA, due to quenching of free EB fluorescence nature by solvent molecules. The strong fluorescence intensity is because of strong intercalative mode of binding [47,48]. Generally, the other DNA binding agents/ substances can quench the fluorescence intensity in their higher concentrations. The gradual quenching of fluorescence intensity of EB–DNA adducts/system was observed by successive increments of metal complex concentration. This is due to displacement of EB from CT–DNA and clearly showed the potential competence of metal complex with EB in binding with CT–DNA. The emission spectra of EB–DNA system in the presence and absence of quencher (metal complex) were presented in Fig. 6. The Stern-Volmer quenching constant, K_{sv} values were determined from the slop of plot I_0/I Vs [Q]. In the present study, the K_{sv} values found to be $1.38 \pm 0.01 \times 10^4\ M^{-1}$ (**1**), $1.60 \pm 0.02 \times 10^4\ M^{-1}$ (**2**), $4.61 \pm 0.01 \times 10^4\ M^{-1}$ (**3**), $4.34 \pm 0.02 \times 10^3\ M^{-1}$ (**4**) respectively. The results revealed that the Cu(II) complex showed higher fluorescence quenching activity than other complexes. The K_{sv} values are well reliable with the UV–DNA binding constant (K_b) values.

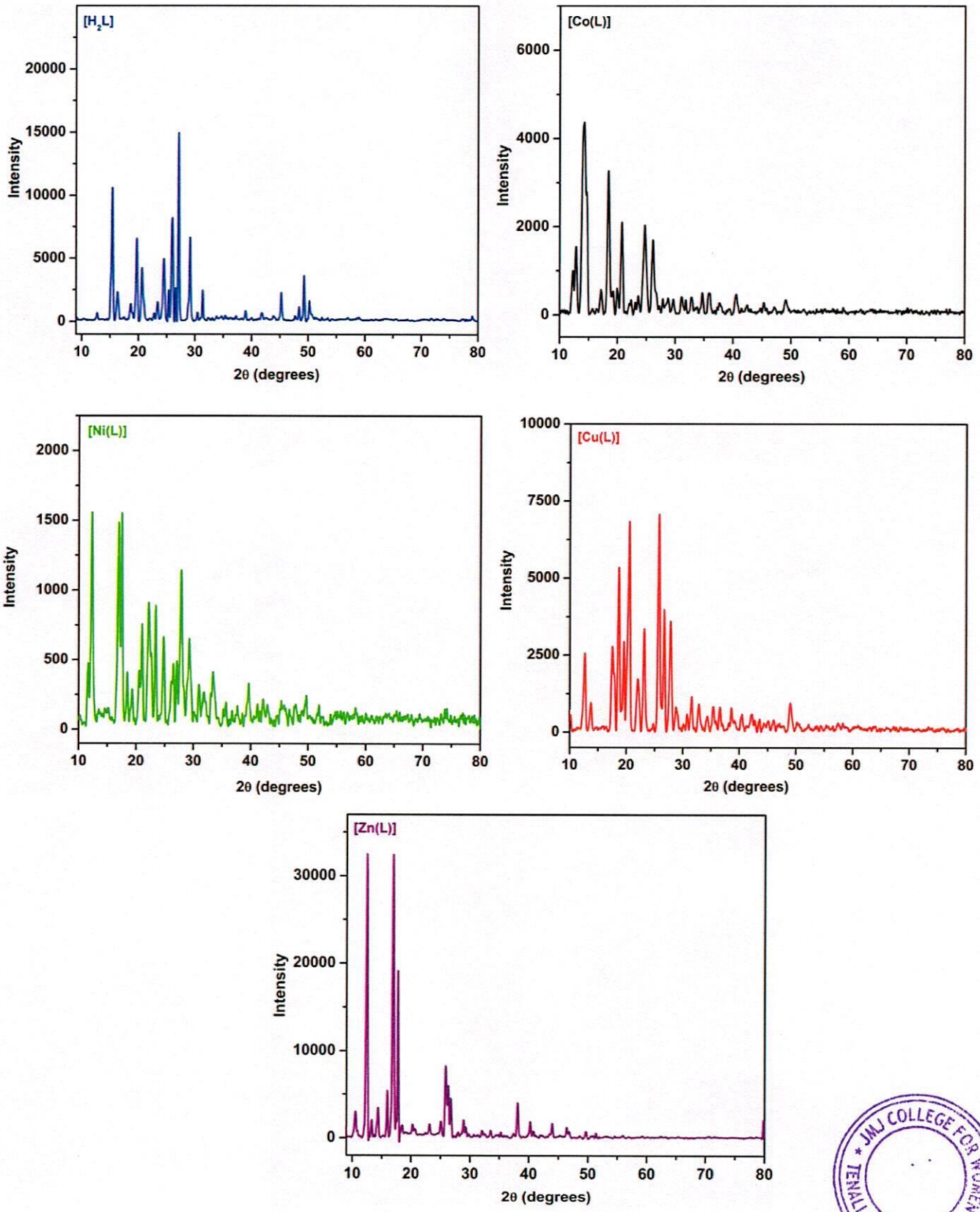
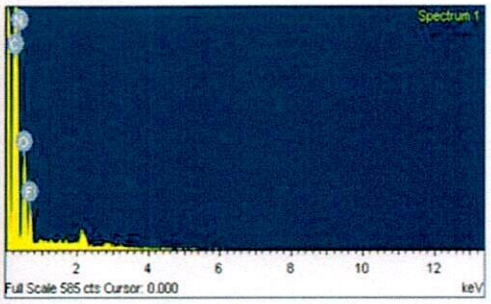
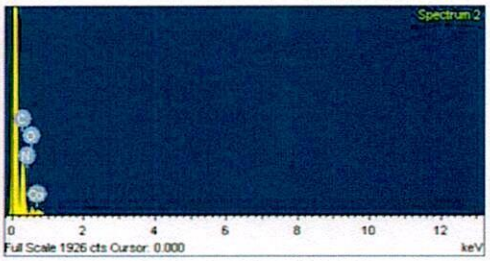


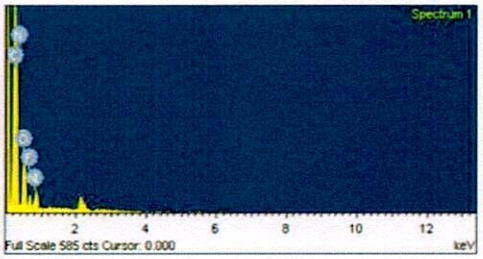
Fig. 3. Powder XRD patterns of ligand, H_2L and its complexes **1**–**4**.



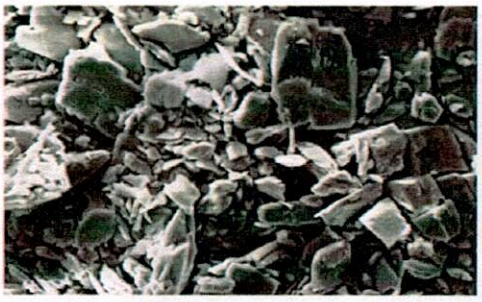
SEM micrograph of H_2L EDX graph of H_2L

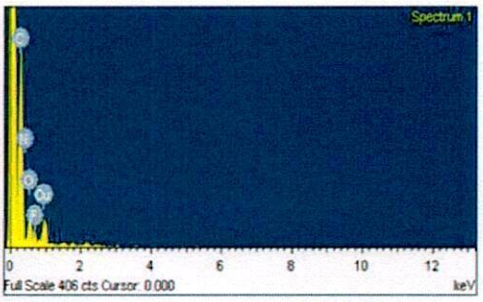


SEM micrograph of [Co(L)] (1) EDX graph of [Co(L)] (1)

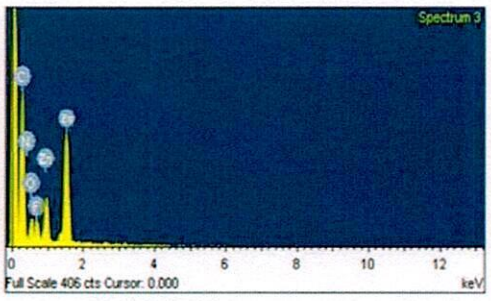


SEM micrograph of [Ni(L)] (2) EDX graph of [Ni(L)] (2)





SEM micrograph of [Cu(L)] (3) EDX graph of [Cu(L)] (3)



SEM micrograph of [Zn(L)] (4) EDX graph of [Zn(L)] (4)

Fig. 4. SEM and EDX graphs of ligand, H_2L and its complexes **1**–**4**.

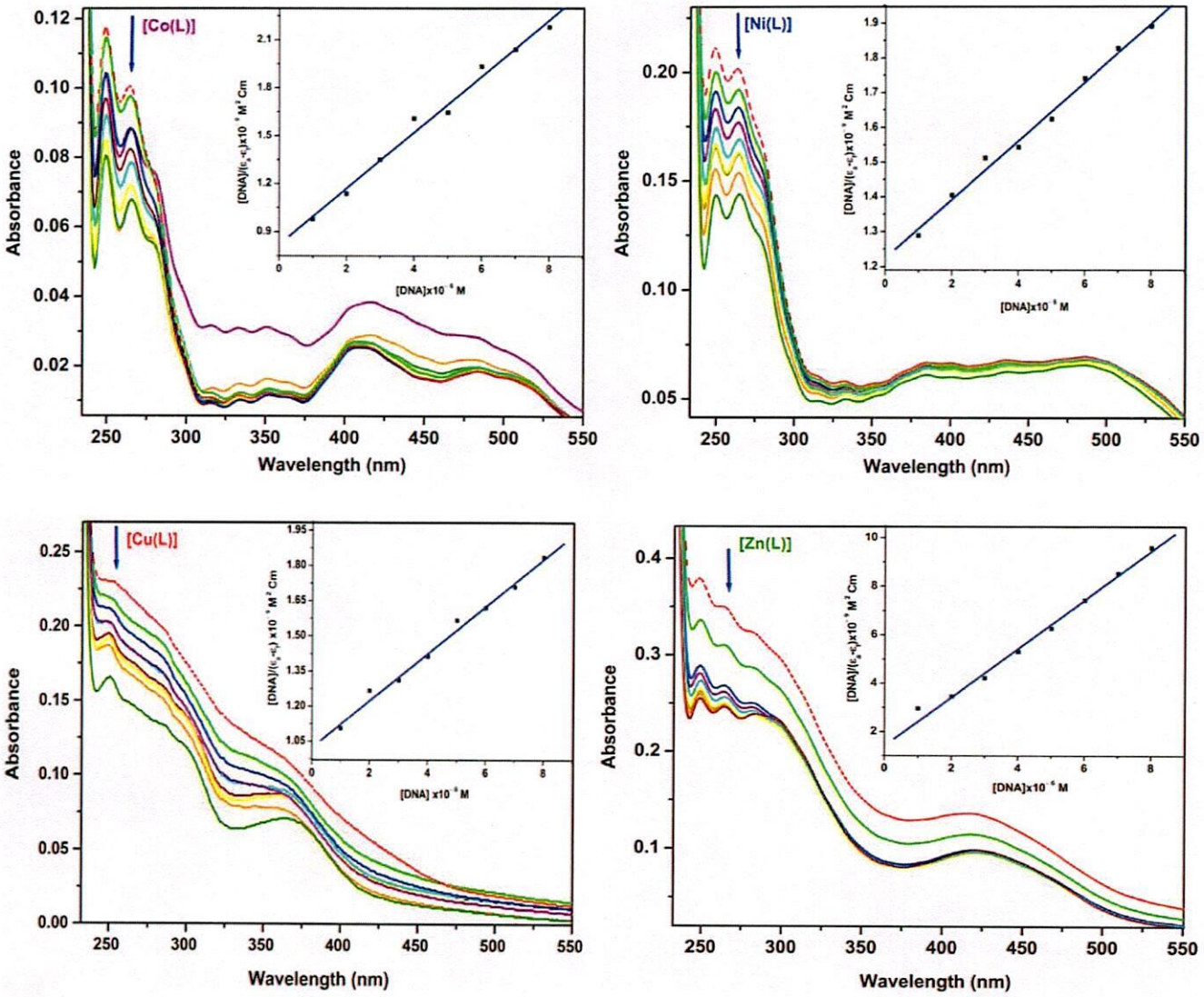
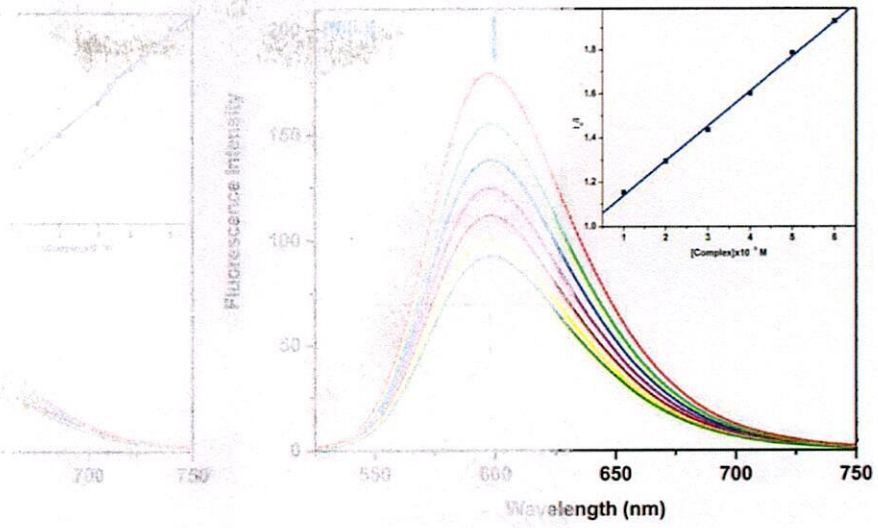
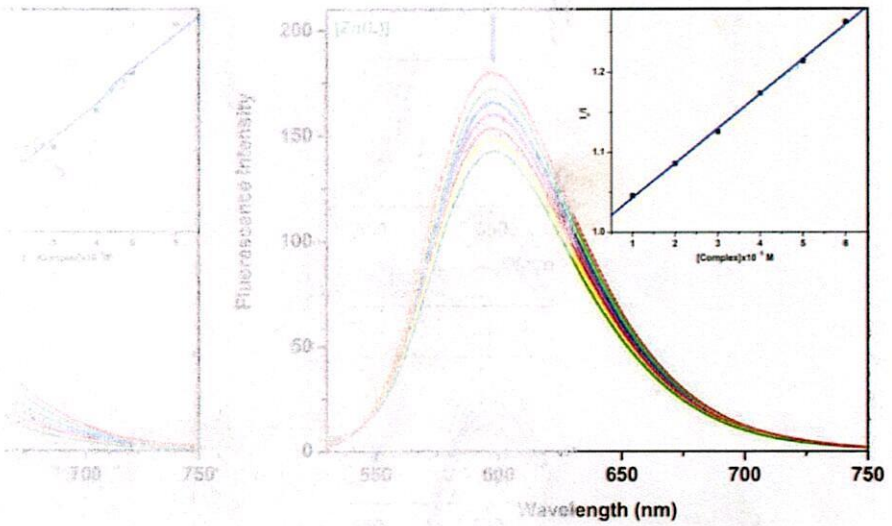


Fig. 5. Electronic absorption spectra of complexes, **1**–**4** in the absence (red curve) and presence (other curves) of CT–DNA, added in increment wise at room temperature in Tris–HCl/NaCl buffer (pH=7.2). Conditions: [complex] = 10 μM and [CT–DNA] = 0–10 μM. Arrow (↓) shows the absorbance change upon increasing CT–DNA concentration Inset: plot of $(\varepsilon_a-\varepsilon_f)/(\varepsilon_b-\varepsilon_f)$ vs. [DNA] for the absorption titration of CT–DNA with the complexes.

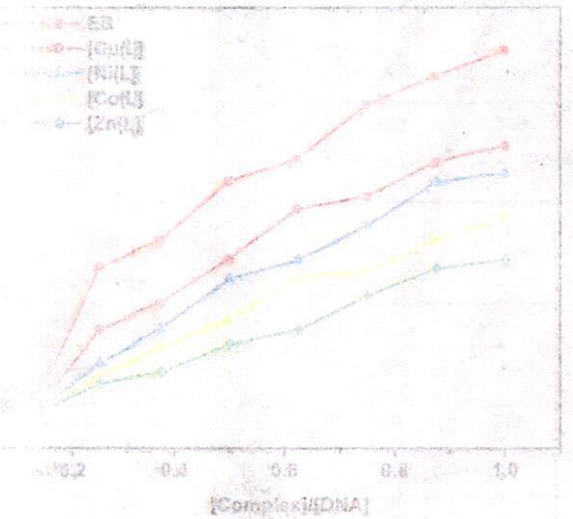
3.9.3. *Viscosity measurements*

UV-vis absorption studies and fluorescence quenching experiments were essential but not enough to establish the binding nature of metal complex to CT–DNA, further it was resolved by employing the viscosity measurement studies, as it was most important experiment to identify the nature of binding in solution state. In the viscometric investigations, decrease or increase of DNA viscosity is observed, it depends upon the nature of binding and type of interactions i.e., non-classical or classical intercalators. The non-classical intercalators may involve in bending/ kinking of DNA double helix via intercalation (non-classical) in grooves of DNA which leads to decrease in viscosity of DNA [49,50]. In a classical intercalation nature of binding it is expected increase in viscosity of DNA helix due to incorporation of classical intercalator in DNA base pairs, which leads to increase in the space between DNA base pairs (due to separation) and lengthening of DNA helix was monitored. In this investigation, the binding nature of metal complexes (**1**–**4**) with CT–DNA was determined by measuring the relative specific viscosity of CT–DNA. In this experiment, the variable concentration of metal complex was employed in successive increments where as constant concentration of CT–DNA was maintained. The Fig. 7 showed the relative specific viscosities of metal complexes. From the results, it is observed that the extension of CT–DNA helix was found while increasing the complex concentration leading to increase in CT–DNA viscosity indicates intercalation binding mode exists between metal complex and CT–DNA [51]. The viscosity measurements reveal that the increase is more in the case of Cu(II) complex suggesting Cu(II) complex more effectively intercalates with CT-DNA than remaining complexes and these results are well consistent with UV–DNA absorption and fluorescence results.





...3 -4 in the absence (red curve) and presence (other curves) of EB bound to CT–DNA. Conditions: ...50 μM.The arrow (↓) shows the intensity changes with increasing concentrations of the complex.



...ractants of EB, complexes 1 –4 on the relative specific viscosity of CT-DNA.

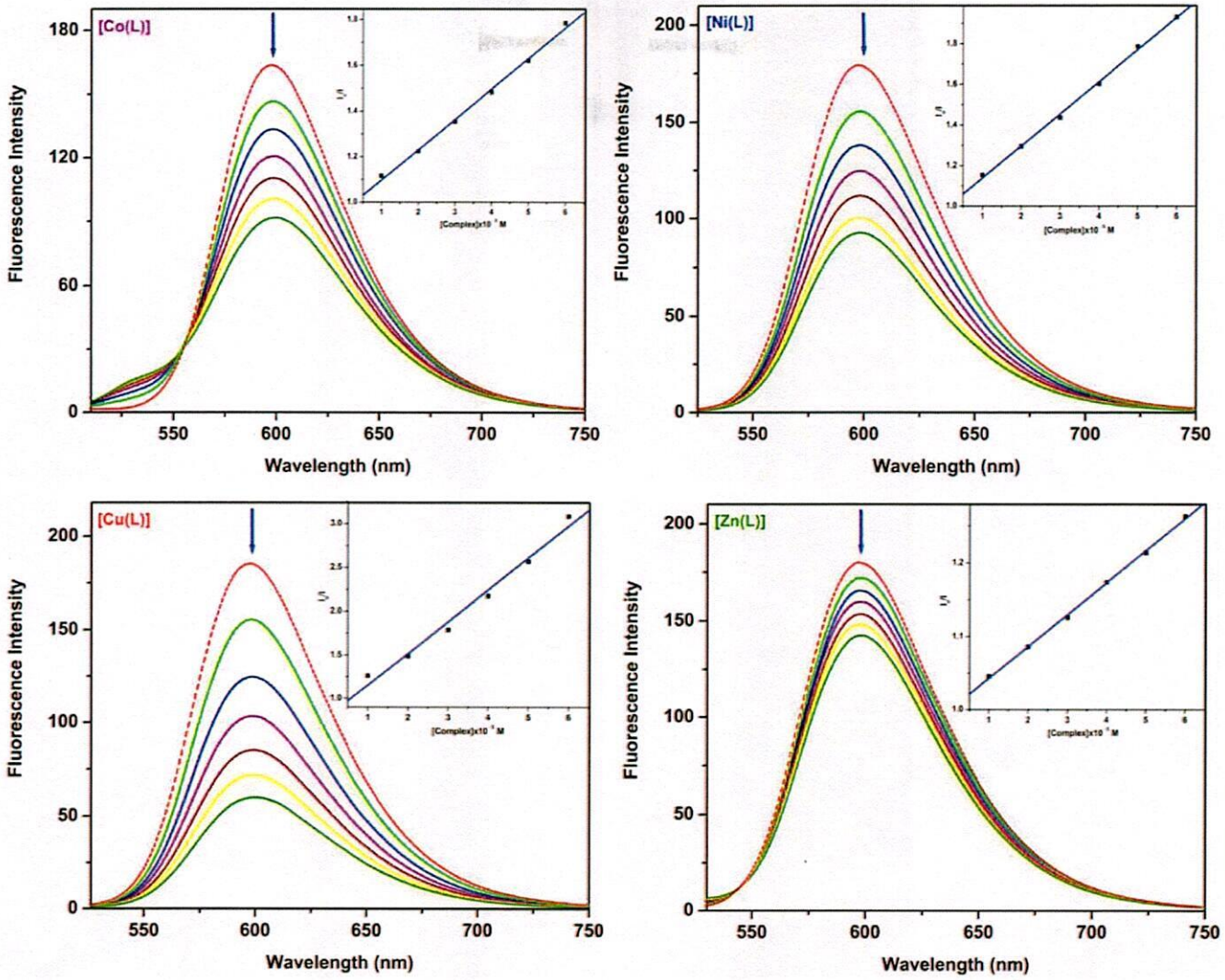


Fig. 6. Fluorescence quenching spectra of complexes, **1**–**4** in the absence (red curve) and presence (other curves) of EB bound to CT–DNA. Conditions: [EB] = 12.5 μM, [CT–DNA] = 125 μM, [complex] = 0–60 μM. The arrow (↓) shows the intensity changes with increasing concentrations of the complex. Inset: plot of I_0/I vs. [complex].

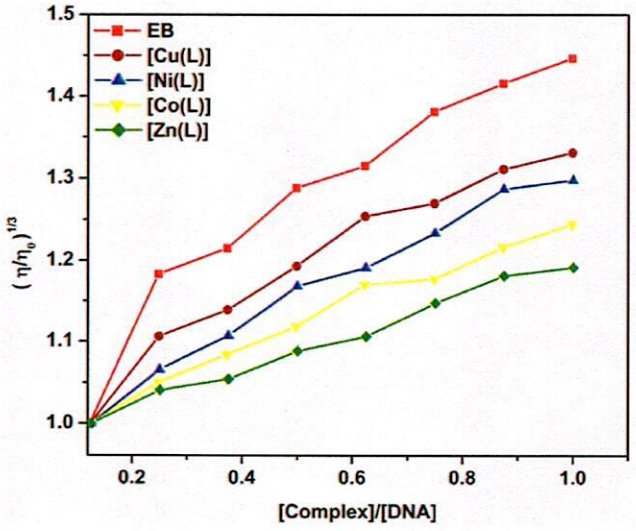
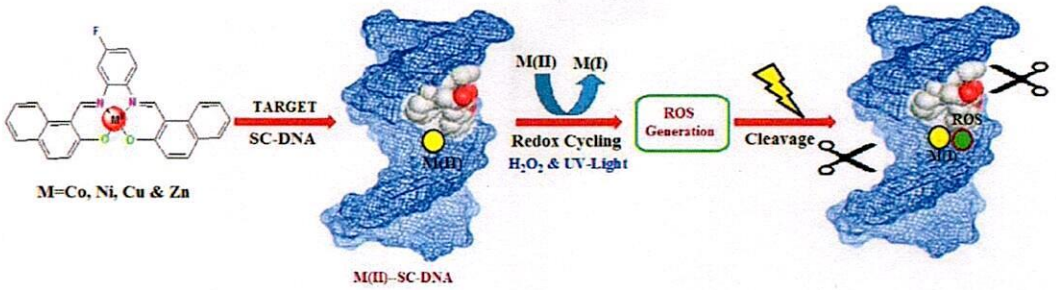


Fig. 7. Effect of increasing amounts of EB, complexes 1–4 on the relative specific viscosity of CT-DNA.



Scheme 2. The possible cleavage mechanism in association with H_2O_2 & UV-light of SC–DNA by **Co(II), Ni(II), Cu(II)** and **Zn(II)** complexes.

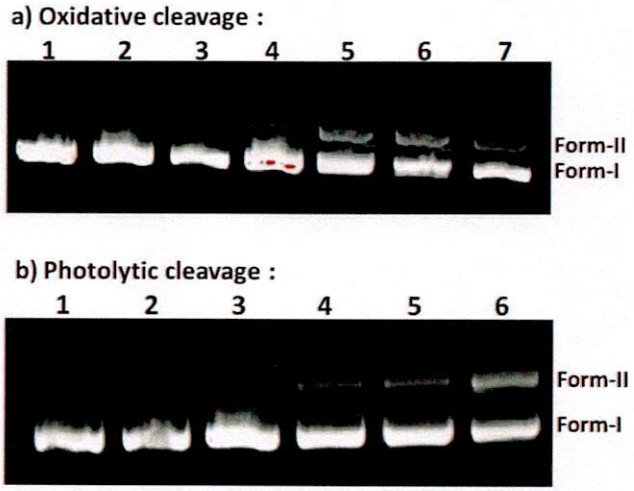


Fig. 8. Gel electrophoresis images of oxidative (**a**) and photolytic cleavages (**b**) of supercoiled pBR322 DNA (0.2 mg, 33.3 mM) in 5 mM Tris–HCl/NaCl buffer (pH = 7.2) by all compounds. In **oxidative Cleavage**, Lane 1, DNA control: Lane 2, DNA + H_2O_2 (1 mM): Lane 3, DNA + H_2O_2 (1 mM) + **H_2L** (20 mM): Lane 4, DNA + H_2O_2 (1 mM) + **Co(II)** complex (20 mM): Lane 5, DNA + H_2O_2 (1 mM) + **Ni(II)** complex (20 mM): Lane 6, DNA + H_2O_2 (1 mM) + **Cu(II)** complex (20 mM): Lane 7, DNA + H_2O_2 (1 mM) + **Zn(II)** complex (20 mM). In **photolytic cleavage**, Lane 1, DNA control: Lane 2, DNA + **H_2L** (20 mM): Lane 3, DNA + **Co(II)** complex (20 mM): Lane 4, DNA + **Ni(II)** complex (20 mM): Lane 5, DNA + **Cu(II)** complex (20 mM): Lane 6, DNA + **Zn(II)** complex (20 mM).

3.10. DNA cleavage activity

DNA cleavage experiments carried out by agarose gel electrophoresis method were very important to determine the cleavage efficiency of metal complexes against super coiled pBR322 DNA (SC–DNA) and are play a key role in design and invention of novel, efficient drugs, especially chemotherapeutic drugs targeted to DNA moiety. The H_2O_2 mediated cleavage (oxidative) and ultra violet light focused cleavages (photolytic) are employed to investigate the cleavage capacity of synthesized metal complex with SC–DNA via reactive oxygen species (ROS) involved mechanism (**Scheme 2**). The cleavage capacity was assessed by monitoring the conversion of fast moving form-I DNA (SC–DNA) in to slow moving form-II DNA (nicked DNA) and form-III DNA (linear DNA) which moves in between form-I and form-II DNA. The hydroxyl radical was generated from H_2O_2 and is responsible for the scission of DNA strands in oxidative cleavage [52] and in the photolytic cleavage, photo excitation of triplet state oxygen ($^3O_2$) takes place by gaining energy from UV–radiation (λ = 365 nm) and converts in to singlet oxygen ($^1O_2$) which is responsible for incision of DNA strands [53]. Fig. 8 depicts the oxidative cleavage method (**a**), and lane 1 (**control**), lane 2 (**DNA+ H_2O_2**) and lane 3 (**DNA+ H_2L**) showed no cleavage. Lane 4 (**DNA+ 1**), lane 5 (**DNA+ 2**), lane 6 (**DNA+ 3**) and lane 7 (**DNA+ 4**) showed efficient cleavage of SC-DNA in to form-II DNA by respected complexes. In photolytic cleavage method (**b**), no DNA cleavage was found in lane 1 (**control**) and lane 2 (**DNA+ H_2L**) where as Lane 3 (**DNA+ 1**), lane 4 (**DNA+ 2**), lane 5 (**DNA+ 3**) and lane 6 (**DNA+ 4**) showed DNA cleavage in to form-II DNA by respected complexes. From these investigations, it is concluded that, there is no cleavage was found by Schiff base ligand where as its metal complexes efficiently cleaved the SC–DNA (form-I) in to nicked form DNA (form-II).

3.11. Antibacterial and antifungal activities

Antibacterial and antifungal activity studies of ligand (**H_2L**) and its metal complexes were carried out by in vitro disc diffusion method. All compounds were screened against two Gram positive bacterial strains *Staphylococcus aureus (S. aureus), Bacillus amyloliquefaciens (B. amyloliquefaciens),* two Gram negative bacteria strains *Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli (E. coli)* and also against two fungal strains *Macrophomina phaseolina (M. phaseolina), Sclerotium rolfsii (S.*

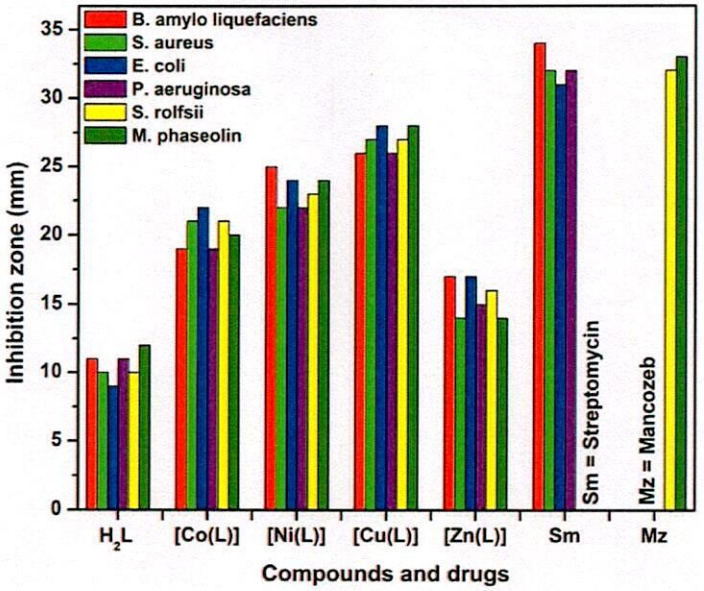


Fig. 9. Zone of inhibition (in mm) of the ligand, $\mathbf{H_2L}$ and its metal complexes (**1–4**) tested against bacterial and fungal strains.

Table 3
Antibacterial and antifungal activity results of ligand, $\mathbf{H_2L}$ and its metal complexes (**1–4**) at 1mg/mL concentration along with respective standard drugs at the same concentrations.

	Bacterium(mm) Gram-positive bacteria		Gram-negative bacteria		Fungi(mm)	
Compound	*Bacillus amyloliquefaciens*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Sclerotium rolfsii*	*Macrophomina phaseolina*
H_2L	11	11	9	10	8	10
[Co(L)] (1)	19	21	22	19	21	20
[Ni(L)] (2)	25	22	24	22	23	24
[Cu(L)] (3)	26	27	28	25	27	28
[Zn(L)] (4)	17	14	17	15	16	14
Streptomycin	34	32	31	32	-	-
Mancozeb	-	-	-	-	32	33

rolfsii). The antibacterial and antifungal results are presented in Fig. 9. The antimicrobial activity results reveals that the metal complexes show higher antibacterial and antifungal activity than the free ligand, which was confirmed by larger the sizes of inhibition zones (measured in mm, Table 3) of metal complexes against microorganisms than the free ligand inhibition zone. The structural changes, type of chelations, solubility nature, dipole moment, size and permeability nature may the reasons for the higher antimicrobial activity of metal complexes. Overtone's concept [54] and Tweedy's chelation theory [55] were given the explanation regarding the enhancement of potential antimicrobial activity of metal complexes against bacterial and fungal strains. Permeability nature is the key point of Overtone's concept and according to this concept, the lipid membrane around the cell favours the passage of only lipid soluble materials, which makes the liposolubility property is a key factor and controls the antimicrobial activity. On the other hand, while establishment of chelation, sharing of positive charge and delocalization of π-electron cloud occurs, due to this reduction in polar nature of metal ions observed. This process leads to increase in lipophilic nature of central metal atom intern raises its permeability capacity and potentially enters in to the cell, thus they have destroyed more aggressively [56]. In this investigation, the results concluded that, the Cu(II) complexes have shown greater antimicrobial activity than other complexes, which may be due to smaller the atomic radius and higher the electro negativity of Cu(II) ion than Co(II), Ni(II) and Zn(II) ions.

3.12. Antioxidant Activity

DPPH$^\bullet$ (2,2-diphenyl-2-picryl-hydrazyl) free radical scavenging technique was employed to investigate the antioxidant proficiency of synthesized metal complexes (**1–4**). Fig. 10 depicts the antioxidant activity of all metal complexes (**1–4**) along with standard, ascorbic acid (**AA**). In the process of antioxidant activity experiment, the solution of DPPH containing odd electron showed a strong absorption band at 517 nm in UV–vis spectroscopy. The stable DPPH$^\bullet$ radical has deep violet colour in its solution form, in combination with metal complex (antioxidant) the DPPH$^\bullet$ radical neutralized and converted in to 1,1-diphenyl-2-picryl-hydrazine, which was confirmed by turning of deep violet colour solution in to pale yellow colour and the antioxidant capacity can be measured by the decrease in its absorbance value at 517 nm. The following formula is

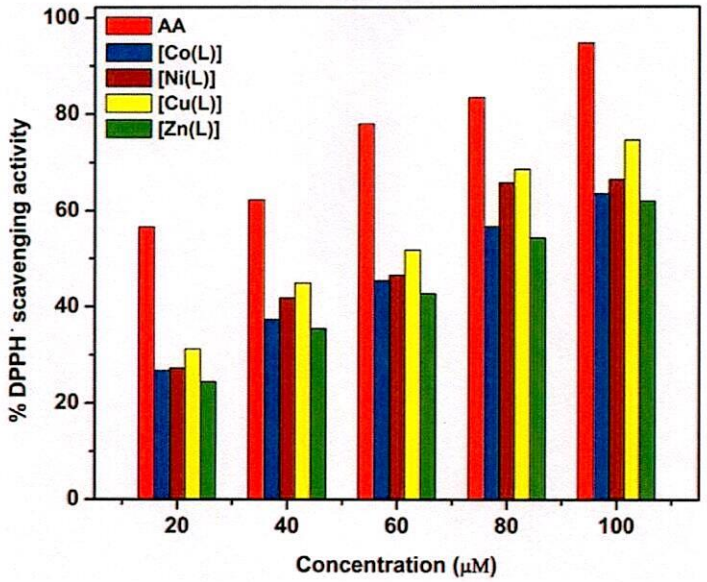


Fig. 10. Antioxidant activity of complexes, **1–4** by DPPH radical scavenging assay.

used to measure the percentage scavenging activity of DPPH• free radical.

$$\text{Percentage scavenging activity}(\%) = A_o - A_e / A_o \times 100$$

Where, A_o and A_e are absorbance of DPPH• in the absence and presence of antioxidant (metal complex) [30]. In the present study, it is observed that the free radical scavenging activity is increased by rising the metal complex concentration and the activity of complexes is expressed in terms of IC_{50} values (50 % inhibitory concentration) [33] and are compared with IC_{50} values of Ascorbic acid (AA), standard for antioxidant activity. Smaller the IC_{50} values indicates greater the antioxidant activity of metal complexes. The IC_{50} values of metal complexes found to be 3.43 μM (**1**) 3.04 μM (**2**) 2.62 μM (**3**) 3.66 μM (**4**) and 0.42 μM (AA) respectively, indicating the Cu(II) complex exhibits prominent antioxidant activity than Co(II), Ni(II) and Zn(II) complexes.

Conclusion

Four biologically active metal complexes (**1–4**) have been synthesized from the novel Schiff base ligand ($\mathbf{H_2L}$) and characterized by elemental, thermal and other spectroscopic techniques. The Co(II), Ni(II) and Cu(II) complexes are adopted a square planar geometry where as octahedral geometry is assigned to Zn(II) complex with 1:1 metal-ligand stoichiometry. The DNA interaction studies by UV-absorption, fluorescence emission and viscosity measurements showed that the metal complexes binding to CT-DNA via intercalative binding mode. Further, it is found that the Cu(II) complex showed higher DNA binding activity than Co(II), Ni(II) and Zn(II) complexes. In oxidative and photolytic DNA cleavage studies, the effective cleavage activity of metal complexes is shown against the pBR322 DNA. The antibacterial and antifungal activity studies concludes that all metal complexes showed greater antimicrobial activity than free Schiff base ligand, among all metal complexes, Cu(II) complex exhibit better activity than remaining metal complexes. The DPPH• radical scavenging activity results revealed that the Cu(II) complex showed enhanced antioxidant activity than Co(II), Ni(II) and Zn(II) complexes but lower than Ascorbic acid (**AA**), which is an antioxidant activity standard.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cdc.2020.100434.

References

[1] N. Raman, A. Selvan, S. Sudharsan, Metallation of ethylenediamine based Schiff base with biologically active Cu(II), Ni(II) and Zn(II) ions: Synthesis, spectroscopic characterization, electrochemical behaviour, DNA binding, photonuclease activity and in vitro antimicrobial efficacy, Spectrochim Acta A 79 (2011) 873–883.
[2] K.E. Erkkila, D.T. Odom, J.K. Barton, Recognition and Reaction of Metallointercalators with DNA, Chem. Rev 99 (1999) 2777–2796.
[3] B.L Fei, W.S. Xu, H.W Tao, W. Li, Y. Zhang, J.Y. Long, Q.B. Liu, B. Xia, W.Y. Sun, Effects of copper ions on DNA binding and cytotoxic activity of a chiral salicylidene Schiff base, J. Photochem. Photobiol. B: Biology 132 (2014) 36–44.
[4] P.K. Sasmal, A.K. Patra, A.R. Chakravarty, structure Synthesis, DNA binding and DNA cleavage activity of oxovanadium(IV) N-salicylidene-S-methyldithiocarbazate complexes of phenanthroline bases, J. Inorg. Biochem. 102 (2008) 1463–1472.
[5] Y. Gou, J. Li, B. Fan, B. Xu, M. Zhou, F. Yang, Structure and biological properties of mixed-ligand Cu(II) Schiff base complexes as potential anticancer agents, Eur. J. Med. Chem 134 (2017) 207–217.
[6] A.M. Pyle, T. Morii, J.K. Barton, Probing microstructures in double-helical DNA with chiral metal complexes: recognition of changes in base-pair propeller twisting in solution, J. Am. Chem. Soc. 112 (1990) 9432–9434.
[7] M. Howe-Grant, S.J. Lippard, Binding of platinum(II) intercalation reagents to deoxyribonucleic acid. Dependence on base-pair composition, nature of the intercalator, and ionic strength, Biochemistry 18 (1979) 5762–5769.
[8] S. Layeka, R. Gangulyb, D.D. Pathaka, Unprecedented formation of a μ; -oxobridged polymeric copper(II) complex: Evaluation of catalytic activity in synthesis of 5-substituted 1 *H* -tetrazoles, J. Organometal. Chem. 870 (2018) 16–22.
[9] Ayman A.Abdel Aziz, Hemmat A. Elbadawy, Spectral, electrochemical, thermal, DNA binding ability, antioxidant and antibacterial studies of novel Ru(III) Schiff base complexes, Spectrochimica Acta Part A: Mol. and Biomol. Spectr 124 (2014) 404–415.
[10] Z. Caoa, Y. Zhang, P. Song, Y. Caia, Q. Guoa, Z. Fanga, M. Penga, A novel zinc chelate complex containing both phosphorus and nitrogen for improving the flame retardancy of low density polyethylene, J. Analy. and Appl. Pyrol. 92 (2011) 339–346.
[11] Ayman A.Abdel Aziz, Ibrahim H.A. Badr, Ibrahim S.A. El-Sayed, Synthesis, spectroscopic, photoluminescence properties and biological evaluation of novel Zn(II) and Al(III) complexes of NOON tetradentate Schiff bases, Spectrochimica Acta Part A: Mol. and Biomol. Spectr 97 (2012) 388–396.
[12] H.P. Ebrahimi, J.S. Hadi, Z.A. Abdulnabi, Z. Bolandnazar, Spectroscopic, thermal analysis and DFT computational studies of salen-type Schiff base complexes Spectrochimica Acta Part A, Mol. and Biomol. Spectr 117 (2014) 485–492.
[13] D.S. Shankar, A. Rambabu, N. Vamsikrishna, N. Ganji, S. Daravath, Shivaraj, Three mononuclear Cu (II) complexes based on p-tolylmethanamine Schiff bases: In-vitro cytotoxicity, DNA binding ability, nuclease activity and antibacterial studies, Inorg. Chemi. Commu 98 (2018) 48–57.
[14] Abeer M.Farag Teoh Siang Guan Hasnah Osman A.M.S.Abdul Majid Muhammad Adnan Iqbal Mohamed B.Khadeer Ahamed, Synthesis of metal(II) [M 5 Cu, Mn, Zn] Schiff base complexes and their Pro-apoptotic activity in liver tumor cells via caspase activation, Med. Chem. Res 22 (2013) 4727–4736.
[15] D. Dreher, A.F. Junod, Role of Oxygen Free Radicals in Cancer Development, Eur. J. Cancer 32 (1996) 30–38.
[16] I. Wurtenberger, V. Follia, F. Lerch, C. Cwikla, N. Fahrner, C. Kalchschmidt, B. Flogel, B. Kircher, R. Gust, Fluorinated Fe(III), Salophene Complexes: Optimization of Tumor Cell Specific Activity and Utilization of Fluorine Labeling for in Vitro Analysis, J. Med. Chem. 58 (2015) 588–597.
[17] W. Chen, P. Guo, J. Song, W. Cao, J. Bian, The ortho hydroxy-amino group: Another choice for synthesizing novel antioxidants, Bioorg. & Med. Chem. Lett 16 (2006) 3582–3585.
[18] V.K. Gupta, A.K. Singh, S. Mehtab, B. Gupta, A cobalt(II)-selective PVC membrane based on a Schiff base complex of *N,N* _-bis(salicylidene)-3,4-diaminotoluene, Anal. Chimica Acta 566 (2006) 5–10.
[19] S.M. Pradeepa, H.S. Bhojya Naik, B. Vinay Kumar, K. Indira Priyadarsini, Atanu Barik, T.R. Ravikumar Naik, Cobalt(II), Nickel(II) and Copper(II) complexes of a tetradentate Schiff base as photosensitizers: Quantum yield of 1O2 generation and its promising role in anti-tumor activity. Spectrochimica Acta Part A: Mol. and Biomol, Spectr 101 (2013) 132–139.
[20] B.S. Garg, Deo Nandan Kumar, Spectral studies of complexes of nickel(II) with tetradentate schiff bases having N2O2 donor groups, Spectrochimica Acta Part A 59 (2003) 229–234.
[21] R.N. Patel, S.P. Rawat, M. Choudhary, V.P. Sondhiya, D.K. Patel, K.K. Shukla, D.K. Patel, Y. Singh, R. Pandey, Synthesis, structure and biological activities of mixed ligand copper(II) and nickel(II) complexes of N0-(1E)-[(5-bromo-2-hydroxyphenyl) methylidene]benzoylhydrazone, Inorg. Chimica Acta 392 (2012) 283–291.
[22] S. Tabassum, S. Amir, F. Arjmand, C. Pettinari, F. Marchetti, N. Masciocchi, G. Lupidi, R. Pettinari, Mixed-ligand Cu(II)evanillin, Schiff base complexes; effect of coligands on their DNA binding, DNA cleavage, SOD mimetic and anticancer activity, Eur. J. Med. Chem 60 (2013) 216–232.
[23] C. Marzano, M. Pellei, F. Tisato, C. Santini, Copper Complexes as Anticancer Agents, Antican. Age. Med. Chem 9 (2009) 185–211.
[24] S.O. Bahaffi, A.A.A. Aziz, M.M. El-Naggar, Synthesis, spectral characterization, DNA binding ability and antibacterial screening of copper(II) complexes of symmetrical NOON tetradentate Schiff bases bearing different bridges, J. Mol. Struct. 1020 (2012) 188–196.
[25] D.N. Kumar, B.S. Garg, Synthesis and spectroscopic studies of complexes of zinc(II) with N2O2 donor groups, Spectrochimica Acta Part A 64 (2006) 141–147.
[26] N. Raman, A. Selvan, S. Sudharsan, Metallation of ethylenediamine based Schiff base with biologically active Cu(II), Ni(II) and Zn(II) ions: Synthesis, spectroscopic characterization, electrochemical behaviour, DNA binding, photonuclease activity and in vitro antimicrobial efficacy, Spectrochimica Acta Part A 79 (2011) 873–883.
[27] J. Mendham, R.C. Denney, J.D. Barnes, M.J.K. Thomas, Vogel's Quantitative Chemical Analysis, 6th ed., (2000) (New York).
[28] A.M. Abu-Dief, I.F. Nassar, W.H. Elsayed, Magnetic NiFe2O4 nanoparticles: Efficient, heterogeneous and reusable catalyst for synthesis of acetyl ferrocenechalcones and their antitumour activity, Appl. Organomet. Chem 30 (2016) 917–923.
[29] J. Marmur, A procedure for the isolation of deoxyribonucleic acid from micro organisms, J. Mol. Biol. 3 (1961) 208–218.
[30] G. Ramesh, S. Daravath, N. Ganji, A. Rambabu, K. Venkateswarlu, Shivaraj, Facile synthesis, structural characterization, DNA binding, incision evaluation, antioxidant and antimicrobial activity studies of Cobalt(II), Nickle(II) and Copper(II) complexes of 3-amino-5-(4-fluorophenyl)isoxazole derivatives, J. Mol. Struct. 1202 (2020) 127338.
[31] G. Ramesh, M.P Kumar, A. Rambabu, N. Vamsikrishna, S. Daravath, Shivaraj, Synthesis, Spectral Characterization, DNA Binding, Cleavage and Biological Evaluation on Co(II), Ni(II) and Cu(II) Complexes of Substituted Isoxazole Schiff Bases, Asian J. Chem. Sci 4 (2018) 1–20.
[32] N. Ganji, A. Rambabu, N. Vamsikrishna, S. Daravath, Copper(II) Shivaraj, complexes with isoxazole Schiff bases: Synthesis, spectroscopic investigation, DNA binding and nuclease activities, antioxidant and antimicrobial studies, J. Mol. Struct. 1173 (2018) 173–182.
[33] K. Venkateswarlu, M.P Kumar, A. Rambabu, N. Vamsikrishna, S. Daravath, K. Rangan, Shivaraj, Crystal structure, DNA binding, cleavage, antioxidant and antibacterial studies of Cu(II), Ni(II) and Co(III) complexes with 2-((furan-2-yl)methylimino)methyl)-6- ethoxyphenol Schiff base, J. Mol. Struct. 1160 (2018) 198–207.
[34] S. Daravath, M.P. Kumar, A. Rambabu, N. Vamsikrishna, N. Ganji, Shivaraj, Design, synthesis, spectral characterization, DNA interaction and biological activity studies of copper(II), cobalt(II) and nickel(II) complexes of 6-amino benzothiazole derivatives, J. Mol. Struct. 1144 (2017) 147–158.
[35] H. Unver, Z. Hayvali, Synthesis, spectroscopic studies and structures of square-planar nickel(II) and copper(II) complexes derived from 2-{(Z)-[furan-2-ylmethyl]imino]methyl}-6-methoxyphenol, Spectrochim. Acta A 75 (2010) 782–788.

[36] Z. Chen, Y. Wu, D. Gu, F. Gan, Spectroscopic, and thermal studies of some new binuclear transition metal(II) complexes with hydrazone ligands containing acetoacetanilide and isoxazole, Spectrochim. Acta A 68 (2007) 918–926.
[37] A. Rambabu, M.P. Kumar, N. Ganji, S. Daravath, Shivaraj, DNA binding and cleavage, cytotoxicity and antimicrobial studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes of 1-((E)-(4- (trifluoromethoxy)phenylimino)methyl)naphthalen-2-ol Schiff base, J. Biomol. Struct. and Dyna. 38 (2020) 307–316.
[38] J.E. Kovxic, The C=N stretching frequency in the infrared spectra of Schiff's base complexes-I. Copper complexes of salicylidene anilines, Spectrochimica Acta 23 (1967) 133–187.
[39] S. Daravath, N. Vamsikrishna, N. Ganji, K. Venkateswarlu, Shivaraj, Synthesis, characterization, DNA binding ability, nuclease efficacy and biological evaluation studies of Co(II), Ni(II) and Cu(II) complexes with benzothiazole Schiffbase, Chemical Data Collections 17–18 (2018) 159–168.
[40] K. Venkateswarlu, N. Ganji, S. Daravath, K. Kanneboina, K. Rangan, Shivaraj, Crystal structure, DNA interactions, antioxidant and antitumor activity of thermally stable Cu(II), Ni(II) and Co(III) complexes of an N,O donor Schiff base ligand, Polyhedron 171 (2019) 86–97.
[41] M.P Kumar, N. Vamsikrishna, G. Ramesh, N.J.P. Subhashini, J.B Nanubolu, Cu Shivaraj, complexes with 4-amino-3,5-dimethyl isoxazole and substituted aromatic aldehyde Schiff bases: synthesis, crystal structure, antimicrobial activity, DNA binding and cleavagestudies, J. Coord. Chem 70 (2017) 1368–1388.
[42] F.K. Kneubuhl, Line shapes of slectron paramagnetic resonance signals produced by powders, glasses, and viscous liquids, J. Chem Phys 33 (1960) 1074.
[43] B.J. Hathaway, D.E. Billing, The electronic properties and stereochemistry of mononuclear complexes of the copper(II) ion, Coord. Chem. Rev 5 (1970) 143–207.
[44] C.J. Dhanaraj, M.S. Nair, Synthesis and characterization of metal(II) complexes of poly(3-nitrobenzylidene-1-naphthylamine- *co* -succinic anhydride), J. Eur. Polym. 45 (2009) 565–572.
[45] N. Vamsikrishna, M.P. Kumar, G. Ramesh, N. Ganji, S. Daravath, Shivaraj, DNA interactions and biocidal activity of metal complexes of benzothiazole Schiff bases: synthesis, characterization and validation, J. Chem. Sci. 129 (2017) 609–622.
[46] R.B. Nair, E.S. Tang, S.L. Kirkland, C.J. Murphy, Synthesis and DNA-Binding Properties of $[Ru(NH_3)_4dppz]^{2+}$, Inorg. Chem 37 (1998) 139–141.
[47] J.B. Lepecqc, C.J. Paoletti, A Fluorescent Complex between Ethidium Bromide and Nucleic Acids Physical-Chemical Characterization, J. Mol. Biol. 27 (1967) 87–106.
[48] N. Ganji, V.K. Chityala, M.P. Kumar, A. Rambabu, N. Vamsikrishna, S. Daravath, Shivaraj, DNA incision evaluation, binding investigation and biocidal screening of Cu(II), Ni(II) and Co(II) complexes with isoxazole Schiff bases, J. Photochem. Photobiol. B 175 (2017) 132–140.
[49] S. Satyanarayana, J.C. Dabrowiak, J.B. Chaires, Neither .DELTA.- nor .LAMBDA.-tris(phenanthroline)ruthenium(II) binds to DNA by classical intercalation, Biochemistry 31 (1992) 9319–9324.
[50] J.B. Lepecq, C.J. Paoletti, A fluorescent complex between ethidium bromide and nucleic acids: Physical–Chemical characterization, J. Mol. Biol. 27 (1967) 87–106.
[51] A. Rambabu, M.P. Kumar, S. Tejaswi, N. Vamsikrishna, Shivaraj, DNA interaction, antimicrobial studies of newly synthesized copper (II) complexes with 2-amino-6-(trifluoromethoxy)benzothiazole Schiff base ligands. J. Photochem. Photobiol. B 165 (2016) 147–156.
[52] G. Vaidyanathan, B.U. Nair, Oxidative cleavage of DNA by tridentate copper (II) complex, J. Inorg. Biochem. 93 (2003) 271–276.
[53] P.B. Merkel, D.R. Kearns, Remarkable solvent effects on the lifetime of 1.DELTA.g oxygen, J. Am. Chem. Soc. 94 (1972) 1029–1030.
[54] C.E. Overton, Studien uber die Narkose zugleich ein Beitrag zur allgemeinen Pharmakologie, Jena, Switzerland, Gustav Fischer (1901).
[55] B.G. Tweedy, Plant extracts with metal ions as potential antimicrobial agents, Phytopathalogy 55 (1964) 910–917.
[56] S. Daravath, A. Rambabu, D.S. Shankar, Shivaraj, Structure elucidation of copper(II), cobalt(II) and nickel(II) complexes of benzothiazole derivatives: Investigation of DNA binding, nuclease efficacy, free radical scavenging and biocidal properties, Chemical Data Collection 24 (2019) 100293.