SAPTHAGIRI COLLEGE OF ENGINEERING

Affiliated to VTU, Belagavi, Approved by AICTE, New Delhi
Accredited By NAAC with "A" Grade, Accredited by NBA for CSE,ISE,ECE,EEE & ME
ISO 9001-2015 & 14001-2015 Certified Institute

In Association With

International Conference on

"Global Convergence in Technology, Entrepreneurship, Computing and Value Engineering: Principles and Practices"

(ICGCP—2022)

24th - 26th June, 2022

Conference Proceedings

Jointly Organized by

Department of

Bio-Technology & Chemistry

Preface

Sapthagiri College of Engineering, Bengaluru was established in the year 2001 by Srinivasa Education and Charitable Trust with a vision to transform its students into competent, inspired and responsible professionals. It is one of the best Engineering Colleges in India.

It is our great honour and pleasure to publish the Proceedings of the **International Conference on Global Convergence in Technology and Entrepreneurship, Computing and Value Engineering, Principles and Practices - 2022 (ICGCP – 2022).** The conference was held on 24th and 26th June, 2022 in virtual mode. To encourage the young research minds and to bring all researchers, academics, scientists, industry experts, in common platform, the college organized this conference.

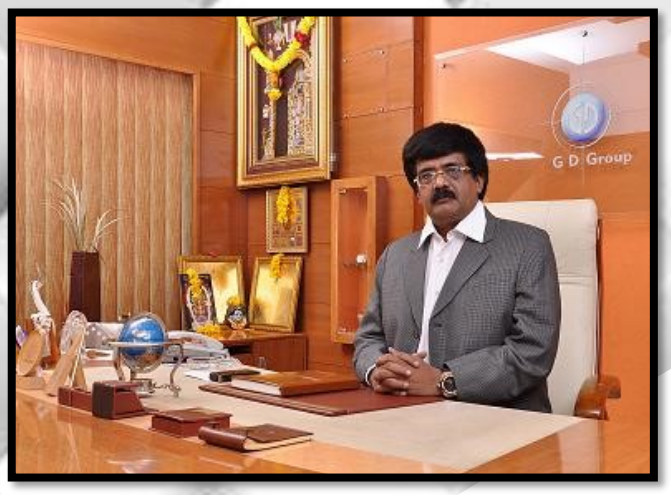
Present global scenario demands unprecedented actions and efforts across multiple convergences of social, economic and environment issues. Science, Technology and Innovations in the area of Internet of Things, Artificial Intelligence, Bio-Technology, Nano Materials and Renewable Energy must play a key role in achieving these goals. Also this is the era of start – ups, to achieve ambitious dream of Make in India concept. The conference covered all emerging areas of Science, Engineering and Technology.

The response to call for papers was excellent. More than 500 papers were received across the country, out of which 300 papers were selected for presentation and publication in the proceeding. These papers provided wide spectrum of research covering all the areas for which the conference was intended for.

We would like to express our gratitude and appreciation to the authors for their contributions. Many thanks go as well to all of the reviewers who helped us maintain the quality of the research papers included in the Proceedings. We also express our sincere thanks to the members of the organizing team for their hard work.

Conference Chair of ICGCP – 2022
Dr. Ramakrishna H
Principal,
Sapthagiri College of Engineering, Bengaluru.

Conference Co-Chair
Dr. Tulsidas.D
Prof. & Head, Department of Mechanical Engineering
Sapthagiri College of Engineering, Bengaluru



Message from Chairman

It gives me immense pleasure in congratulating the Chairman and team members of ICGCP- 2022, on successfully hosting the two days international conference at Sapthagiri college of Engineering. We are overwhelmed by the kind of response received by the research scholars across the country and I wish all of them a bright future and successful career. Also I would like to appreciate the contributions from the Principal, Heads of department, teaching and non-teaching faculties and other supporting staff of Sapthagiri college of Engineering for joining their hands in successful execution of the international conference.

Message from Managing Director

On this occasion, I express my heartiest congratulations to all the participants of ICGCP-2022 for publishing their research findings in the international conference. I hope that, the two days international conference has motivated faculties, research scholars and students to continue their research work. Also on behalf of management, I would like to extend my appreciation towards the sincere efforts of Principal, Heads of Department and Staff members of Sapthagiri College Engineering.

Message from Principal

At the outset I would like to congratulate the entire team of ICGCP-2022 for the successful conduction of international conference that witnessed an active participation of more than 350 research scholars across the state and also from outside Karnataka. On this occasion, I would like to thank our Chairman, Shri. G. Dayananda and executive director, Shri. G.D.Manoj for the magnanimous support extended in organizing the international conference. I would also like to congratulate all the faculties, research scholars and undergraduate students for publishing their research works in the conference and I hope that the two days interaction has motivated them to further pursue their research work and contribute to the society. Also I would like to appreciate the efforts of session chairs / reviewers / heads of department / technical support for their contributions in adding value to each session. Finally, I would like to congratulate the team ICGCP-2022 for bringing out the proceedings of international conference in a precise manner and for making it available for the researchers' community across the globe.

Editorial Board

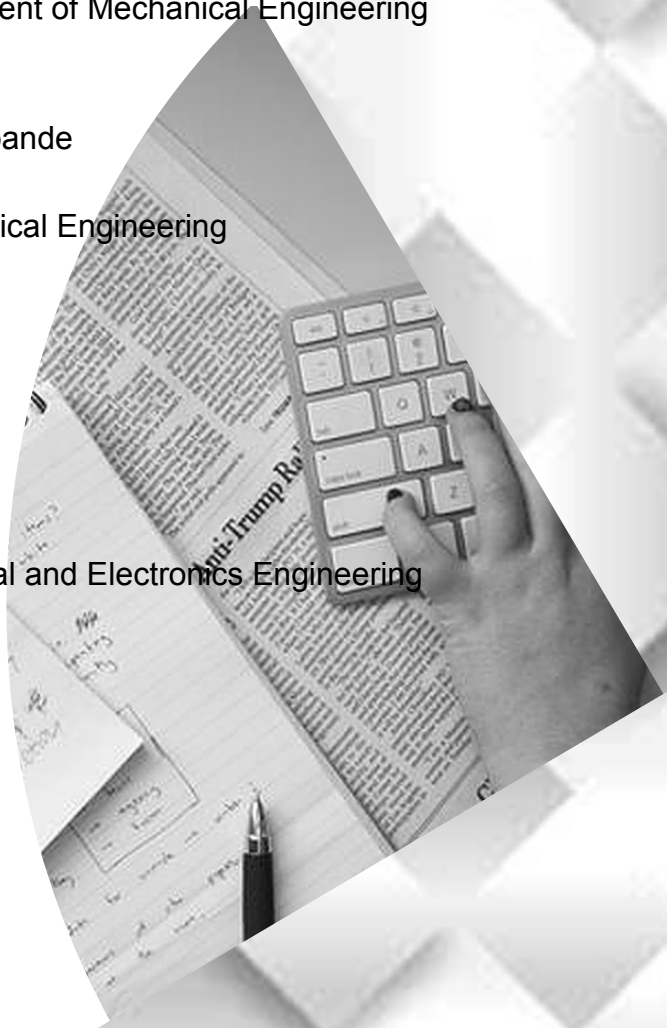
Conference Chair of ICGCP – 2022
Dr. Ramakrishna H
Principal

Conference Co-Chair
Dr. Tulsidas.D
Prof. & Head, Department of Mechanical Engineering

Coordinators:
Dr. Ragavendra Deshpande
Associate Professor
Department of Mechanical Engineering

Dr. Devaraja.C
Assistant Professor
Department of Physics

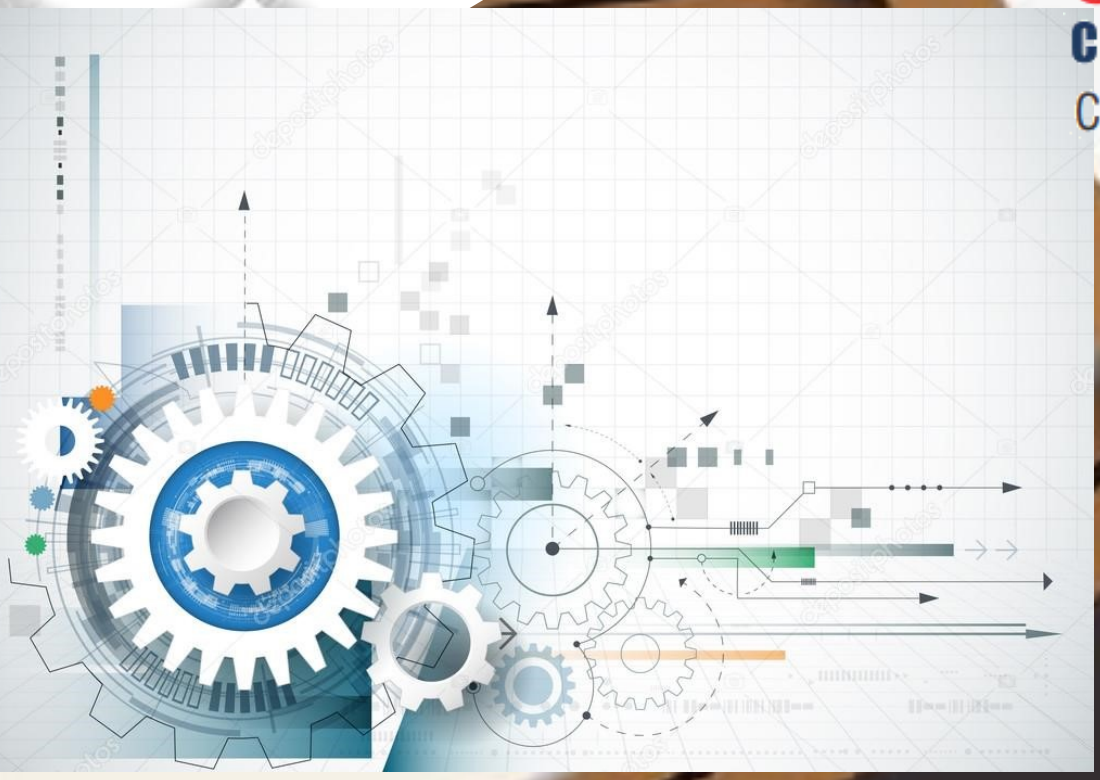
Mr. Nagaraja B S
Assistant Professor
Department of Electrical and Electronics Engineering



Glimpses of ICGCP - 2022

ICGCP-2022

SAPTHAGIRI
College of Engineering
Creating Tomorrow



sapthagiri.edu.in

Google Map

Sapthagiri College of Engineering

Affiliated to VTU, Belagavi, Approved by AICTE, New Delhi
(Accredited by NAAC with "A" Grade) (Accredited by NBA for ECE, CSE, ISE, ME, EEE)
& ISO 9001:2015 and ISO 14001:2015 Certified Institution
#14/5, Chikkasandra, Hesaraghatta Main Road, Bengaluru-560057

INDEX

Chemical Sciences Proceedings

Analysis of the Physico-Chemical parameters in the Groundwater of Hutridurga Hobli Kunigal Taluk, Karnataka, India

Krishne Gowda Y H[1*], Gurushantha K[2]

[1]*Department of Chemistry, Research centre, Sapthagiri College of Engineering*

Hesaraghatta Main Road, Bangaluru-560057, Karnataka, India.

[2]*Department of Chemistry, M S RamaiahInstitute of Technology*

MSR Nagar, Bengaluru-560054, Karnataka, India.

**Corresponding author: krishnegowdayyh@sapthagiri.edu.in*

Abstract: Thirty two groundwater samples of Hutridurga Hobli Kunigal Taluk, Tumkur District were analysed for the Physicochemical properties like pH, TDS, EC, Alkalinity, Total Hardness, Ca++, Mg++,$Cl^-$$Na^+$$K^+$following the standard guidelines of APHA (1998).

Key Words: Taluk, Hobli, Alkalinity, APHA, groundwater.

Introduction:

Water forms an important natural resource without which life becomes unmanageable.The entanglement between life and water is such that the first life forms were originated in water is the early seas and oceans formed in the beginning. Over 70% of the earth's surface is filled with water (1). Majority of the water is locked in the seas and oceans which are unfit for potability. Only About 1% of it is available as fresh water wherein majority of it is stored as groundwater in the Aquifers. Potable water is a vital element for good health and the socio-economic development of humans (2). Water chemistry is a function of the number of features namely the nature of recharge, hydrologic gradient and residence time in the aquifer (3). Groundwater refers to all the water occupying the voids, pores and fissures within the geological formations. The composition of groundwater obtained at different locations will have different chemical composition due to the possibility of extraction from different aquifers. Groundwater is a precious fresh-water resource, and its reservoir of the world is estimated to be approximately 5×10^{24} L (4). It is extracted through hand-dug wells, hand pumps operated shallow-wells and submersible pump, operated deep well or boreholes (5). Water contamination is a situation wherein unwanted substance which are detrimental to human health are present in water in considerable amounts. The cause for water contamination may be either Geogenic or anthropogenic or even both. The domestic, industrial, municipal and agricultural utility of groundwater is a function of it physical, chemical and bacterial characteristics (6) (7). The quality of water is superior to its available

quantity for the consumption (8) (9). Several disorders like the disruption of the immune system, reproductive disorders, damage of the nervous system and even cancer are caused by consumption of water both by the humans and animals depending on the type of pollutant dissolved and its degree of dissolution (10). India is a land of a huge population and this elevation in the population has also increased the demand for fresh water (11). Literature survey reveals that over 70% of the surface water resources namely rivers, streams, rivulets, ponds and lakes are already being severely contaminated. The industrial sector amounts to the 3% of the annual withdrawal of fresh water but is a major contributor of contaminants into fresh water. Cumulative data reveals that from 22 large cities in India, over 7267 MLD of domestic waste water is being generated (12). Continuation of this problem in the same magnitude paves way for a serious national catastrophe in the near future (fresh water crisis). So the concerned authorities need understand the intensity of the situation and deal with the problem meticulously to make sure that adequate amount of fresh water is available to each and every individual in the country now and also in the future.

Study Area:

Huttridurga hobli is located in the Kunigal (T) of Tumkur(dist) Karnataka India at 13.02 N longitude and 77.03 E longitude. The hobli is in the region of Deccan plateau. It is situated 773meter above the Sea level with an average rainfall of 680 mm.The study area is surrounded by Tiles, Brick, pharmaceutical industry and agriculture is the main occupation of the society.

Materials and Methods:

Groundwater samples from 32 sampling areas of Hutridurga Hobli were collected in pre-cleaned polythene containers. Parameters such as pH, Electrical Conductivity (EC) and Total dissolved Solids (TDS) were estimated on the field during the sampling. Total Hardness (TH), Calcium, Magnesium and Bicarbonate were estimated using Titremetry, Sodium and Potassium were estimated using Flame Photometer (Systronics FM 128). All the samples were analysed as per the procedures prescribed by the APHA (1998)

Results and Discussion:

pH: pH measures the alkalinity or acidity of water. pH values of the sample ranged from 6.91 to 8.03 , with an average value of pH 7.522 as show in fig.the pH values of all study area were within the permissible limits of WHO (1993) standards for drinking water.

ISBN: 979-88-35073-61-0

Electrical Conductance (EC): electrical Conductivity is the measure of the conductance of the water which is due to the total dissolved solids in water, in the present investigation the EC values varied between 300-1300 micro S/cm, average value of EC is 533.333 as shown in the fig.

Total Dissolved Solids (TDS): The TDS value in this investigation is high as 858 mg/L and a minimum value of 394 mg/L with an average TDS value of 504.833 mg/L as showed in table. In this finding all the bore well water samples were within the permissible limits of WHO (1993) standards for drinking water.

Total Hardness: Total Hardness is caused by the divalent metal ions and mainly due to the presence of Ca++ and Mg++ ions in the groundwater. The Hardness of water ranged b/n 238ppm to 480.1ppm with an average value of total hardness 289.633ppm. Study area were within the permissible limits of WHO (1993) standards for drinking water.

Calcium: In general, Calcium content in groundwater is greater than Mg. The Calcium value in this investigation is high as 90.53 and a minimum value of 41.2. with an average Calcium value of 58.76mg/L.The ground water samples within the permissible limits of WHO (1993) standards for drinking water.

Magnesium: The important bivalent ions in the determination of total hardness ismagnesium , the investigators recorded a minimum of 25.72 mg/L and a maximum of 68.5 mg/L with in average of 39.0533mg/L. All The ground water samples within the permissible limits of WHO (1993) standards for drinking water.

Chloride: Chloride originating from NaCl gets dissolved in water from rocks and soil, in the current study it fluctuated from a minimum of 73.2 mg/L to maximum of 254.7 mg/L and with an average of 165.18 mg/L, The few ground water samples within the permissible limits of WHO (1993) standards for drinking water.

Sodium and Potassium: Sodium occurs in nature in combined state and Potassium is an Alkaline Earth metal which dissolves in water mainly from agricultural leaching. Sodium levels in the groundwater samples varied from a minimum of 16.24 to a maximum of 55.55 mg/L with an average of 34.09 mg/L and Potassium levels in the groundwater samples varied from a minimum of 52.88 to a maximum of 70.76 mg/L with an average of 57.19mg/L , All The ground water samples within the permissible limits of WHO (1993) standards for drinking water.

ISBN: 979-88-35073-61-0

Sample No	pH	EC	TDS (ppm)	TH (ppm)	Ca^{2+} (ppm)	Mg^{2+} (ppm)	$HCO3^{-}$ (ppm)	Cl^{-} (ppm)	Na^{+} (ppm)	K^{+} (ppm)
1	7.32	300	840	356.4	47.64	28.58	121.2	137.72	18.91	52.88
2	7.07	1300	464	238	90.53	54.31	282.8	73.2	48.85	65.69
3	6.91	400	405	297.8	42.88	25.72	202	135.95	19.83	54.99
4	8.02	400	467	262	61.94	37.16	242.4	106.87	16.59	55.20
5	7.93	400	459	273.9	52.41	31.4	282.8	128	16.24	55.39
6	7.89	400	394	309.7	57.17	57.17	242.4	180.92	17.38	56.46
7	6.89	601	805	480.1	55.5	58.5	565	210	51.22	67.93
8	7.02	493	675	440	67.8	68.5	404	206	53.65	57.99
9	8.09	786	815	335.6	71.02	40.7	606	152	53.93	63.45
10	7.8	500	858	298.05	61.2	51.8	808	210	27.59	57.66
11	7.98	325	780	351.2	41.2	42.6	456.8	122	55.55	70.76
12	8.03	454	689	408.2	69.02	33.3	528.23	108	29.42	56.86
13	6.70	530	200	213	47.12	28.62	124.2	82.5	6.45	46.31
14	6.52	870	220	323.76	68.16	40.896	202.02	109	17.76	48.54
15	6.74	1550	430	221.52	54.528	32.716	80.8	123.6	10.58	45.74
16	6.15	620	180	357.84	71.568	42.94	242.4	143	51.51	47.97
17	6.16	940	130	315.24	71.568	42.94	161.6	128.8	22.92	47.62
18	7.20	1650	260	391.92	61.344	36.80	202.0	129	53.66	46.99
19	7.79	1410	140	323.76	64.752	38.85	727.2	165.8	50.87	47.49
20	8.2	1330	256	349.32	57.936	34.76	767.6	190	50.41	46.10
21	6.56	670	650	264.12	74.976	44.98	161.6	245.8	20.56	46.36
22	7.08	2840	452	502.68	85.2	51.12	80.8	165	61.75	47.08
23	6.90	1270	120	230.04	40.896	24.53	363.6	176.8	37.00	45.72
24	7.45	418	786	556.4	55.6	45.65	468.23	186.7	7.85	65.12
25	7.67	997	951	438.8	45.8	55.36	586.2	254.7	45.58	49.12
26	6.87	1100	690	498.9	49.64	48.12	328.21	198.6	56.52	58.38
27	7.12	1760	459	573.9	64.85	58.79	463.59	145.8	65.73	56.6
28	6.95	1364	767	609.7	73.85	38.96	321.01	232.87	52.92	57.9
29	8.19	1150	405	655.1	58.45	39.44	485.09	154.8	51.48	43.21
30	7.01	1250	858	567.8	69.08	45.25	125.45	276.9	38.48	68.95
31	6.32	1220	354	487.2	75.02	51.93	631.87	198.6	32.72	62.48
32	7.64	796	742	255.6	69.12	47.52	755.29	223.86	45.84	46.58
WHO (1993)	6.5-8.5		500	300 (BIS 1998)	75	50	500	200	200	12 (Excessive limit)

CONCLUSION:

From the above analysis it is evident that the groundwater some samples of Hutridurga hobli, kunigal taluk has got elevated concentrations of Chloride along with high values of TDS and Total Hardness. Water Softeners are suggested to remove excessive hardness from the groundwater to the respected authorities. Installation of Large scale 'Chloride removal units'

or Reverse osmosis unit are strictly recommended in Hutridurga hobli, kunigal taluk to free the groundwater from excessive concentration of Chloride and TDS for drinking water.

References:

1. Analysis of Wate Quality of Halena Block in Bharatpur Area. Sunil Kumar Tank, R. C. Chippa. Jaipur : s.n., 3rd March 2013, International Journal of Scientific and Research Publications.

2. Groundwater and Soil Vulnerability in the Cross River State. Udom TK, Akpan WZ, KoKo WS. 27, 2002, Euro J. Sci , Vol. 4, pp. 628-635.

3. Hydrogeochemical study of shallow carbonate aquifers, Rameshwaram Island, India. S. Krishna Kumar, N. Chandrashekar, P. Seralathan, Prince. S, Godson. N, S. Magesh. Rameshwaram : Springer, 2012, Environ Monit Assess, Vol. 184, pp. 4127-4138.

4. Groundwater: Characteristics, qualities, pollutions and treatments: An overview. Olumuyiwa I. Ojo, Fred A.O. Otieno and George M. Ochieng. 6, June 2012, Vol. 4, pp. 162-170.

5. OI, Ojo.Construction and maintainance of Borehole in Anambra state (. Department of Agricultural and Environmental Engineering, University of Ibadan. 2002. MSc seminar report.

6. (CGWB), Central Groundwater Board.Anual report on groundwater quality. New Delhi : Central groundwater boad, 2004.

7. Assessment if groundwater pollution in west Delhi, India using geostatistical approach. Adhikary P., Chandrashekaran H, Chakraborty D and Kamble K. 2010, Environmental Monitoring Assessment , Vol. 167, p. 599615.

8. Water pollution from major industries in Paradip area A case study. Patnaik K. N, Satyanarayan S. V and Poor R. S. 3, 2002, Indian Journal of Environmental Health, Vol. 44, p. 203211.

9. Assessment of surface water quality of the Ceyhan River basin, Turkey. Tanriverdi C. Alp A, Demirkiran A. R and Uckardes F. 2010, Environmental Monitoring Assessment , Vol. 167, pp. 175-184.

10. Analysis of Water Quality of Halena Block in Bharatpur Area. Sunil Kumar Tank, R. C Chippa. 3, Jaipur : International Journal of Scientific and Research Publications, 2013, Vol. 3.

11. Tyagi, P Buddhi D Chaudhary R and Sawhney, R.L.Degradation of groundwater quality in Industrial area of India-A review, In: Kumar, A (Ed). Ecology of Polluted Waters. New Delhi : APH Publishing Corporation, 2002. pp. 694-714.

12. Water quality in sustainable water management. Rao, S. M and Mamatha, P. 7, s.l. : Current Science, 2004, Vol. 87, pp. 942-947.

Assay of cephalosporins in bulk and pharmaceutical formulations

Veena K P[1], Dr. Roopa K P*[1]

[1]Department of chemistry, Rajeev Institute of Technology, Hassan, India

[*1]Department of chemistry, Sapthagiri college of engineering, Bengaluru 560057, India

veenkp@gmail.com[1]

*roopakp@sapthagiri.edu.in[*1]*

Abstract: A simple, accurate and sensitive spectrophotometric method is developed for the analysis of cephalosporins (ceftriaxone, cefotaxime , ceftazidime and cefepime) in bulk and pharmaceutical formulations. The method is based on the diazotization of cephalosporins in acidic medium and followed by coupling with 3-amino phenol to give orange red colored diazotized product at λ_{max} of 500nm.The optimum reaction conditions and other analytical parameters are evaluated. A study of the effect of commonly associated excipients does not interfere with the determinations. Statistical analysis of results indicates that the method is precise and accurate.

Keywords: ***Cephalosporins, Diazotization, 3-amino phenol, Diazocoupling reaction, spectrophotometry.***

INTRODUCTION

Cephalosporins are a class of β-lactum antibiotics discovered in 1950's and are produced by various species of the mold cephalosporium and from semi-synthetic processes. Cephalosporins are the broad spectrum of antibiotics is mainly used to control gram positive and gram negative bacterial infections. They are generally used in the treatment of upper respiratory and urinary tract infections. Cephalosporins are the second most important β-lactum after penicillin for treating infectious diseases. They are generally useful for the rare patient who is sensitive to penicillin although sensitivity to Cephalosporins is also sometimes found. Cephalosporins are derivatives of 7-aminocephalosporanic acid (7-ACA) , which composed of a β-lactum ring fused with dihydrothiazone ring , but differ in the nature of substituent at the 3- and/or 7-positions of cephem ring. Cephalosporins are traditionally divided into first, second, third & fourth agents based roughly on the time of their discovery and their antibacterial properties.

Several methods have been reported for the analysis of the cephalosporins. The methods include spectrophotometer [1, 2, 3, 6], Spectrofluorimetric [4, 5], Indirect polarographic [7], Atomic absorption and spectrophotometric [8], Colorimetric [9, 10], Voltametry and spectrophotometry [11, 12], Flow injection analysis [13, 14], kinetic spectrophotometric [15], Capillary electrophoresis [16], High performance liquid chromatography and mass spectrometry [17], and UV – Visible spectro photometric methods [18-20]. While each of these reported methods have advantages, majority of them are extensively time consuming, tedious and utilizes reagents which are expensive. Spectrophotometric methods are the most convenient techniques because of their inherent simplicity, high sensitivity, low cost and wide applicability in QC laboratories.

The aim of the present work is to develop simple and accurate method for the determination of cephalosporins in pharmaceutical formulations. The proposed method stands atop over the reported method with respect to simplicity, cost effectiveness and the method neither requires extraction nor prior separation of the drug.

MATERIALS & METHODS

Apparatus

A BL 198 Bio spectrophotometer (UV-VISIBLE) with 1.0cm matched quartz cells was used for electronic spectral measurements.

Reagents

Cephalosporins were received from pharmaceutical company. Cephalosporins (gift sample from strides Arco lab, Bangalore), Amino phenol (sigma, USA), Sodium nitrite (BDH), Hydrochloric acid (AR), Sulphamic acid (AR) were used for the experiment. All other chemicals and solvents used were of analytical reagent grade. Deionised water was used to prepare all solutions and in all experiments.

Standard solutions

3-amino phenol (0.3% AP): prepared by dissolving 0.3g of AP in water and diluting to 100mL with water.

Sodium nitrite (0.1%): prepared by dissolving 0.1g of sodium nitrite in 100mL distilled water.

Sulphamic acid (2%): freshly prepared by dissolving 2g of Sulphamic acid in 100mL distilled water. Aqueous solution of hydrochloric acid (1M) was used.

Standard procedure

Ceftriaxone (CEFT), Cefotaxime (CEFX), Cefepime (CEPM) and Ceftazidme (CEZD)

ISBN: 979-88-35073-61-0

Different aliquots i.e 0.5-4.0mL [400µg mL^{-1}] of CEFT, CEFX, CEPM and 0.4-2.4mL [600µg mL^{-1}] of CEZD were transferred into a series of 10 mL calibrated flasks.

To each flask containing the drugs CEFT, CEFX, CEPM , CEZD **,** A volume of 1.5 mL, 2.0 mL,1.5mL and 2.5mL of 0.1% sodium nitrite was added to each flask, followed by the addition of 0.5mL,0.5mL,0.5mL and 2.0 mL of 1M HCl , and the mixture is allowed to cool in an ice bath at a temperature of (-2 to -3^{0}C). The diazotization was carried out with constant stirring for about 10 min, 15 min, 20 min, and 20 min. After 10, 15, 20 , 20 min, the diazotized drug was treated with 1.5mL, 1 mL, 1mL, and 1mL of 2% Sulphamic acid followed by the addition of 2.0 mL, 1.5 mL, 1.5 mL and 1.0mL of 0.3% amino phenol and the mixture was allowed to stand in an ice bath for 10min, 10min, 15min, and 15min. After it attains room temperature the contents were diluted to the mark with distilled water and mix well. After 10min, the absorbance of the colored azo dye for each drug was measured at 505nm, 500 nm, 500 nm, 500 nm against the corresponding reagent blank.

The amount of drug was computed from the standard calibration graph [Fig.1] or regression equation.

Procedure for pharmaceutical preparations:

Various dosage forms, mainly tablet and powder for injection, were sourced from retail outlets and assessed for their content of the cephalosporins using the new AP method and official DMAB method for each cephalosporin. Stock solutions were made containing the equivalent of each cephalosporin to give 400 ppm & for CEZD 600 ppm , then diazotization of the dosage form was carried out as done for the pure cephalosporins.

The following brands were studied for cephalosporins.

For Ceftriaxone, monocef inj(250mg)

For Cefotaxime,Taxim inj(250mg)

For Ceftazidime,Fortum inj(500mg)

For cefepime,Cepime inj(500mg)

For the analysis of an injection, the powder for injection was transferred into a 100mL calibrated flask and 50mL of water was added and shaken thoroughly for about 30 min. The volume was made up to the mark with distilled water, mixes well and filtered using a quantitative filter paper. Appropriate aliquots of drug solution were taken and the proposed standard procedure was followed for the analysis the drug content. The same drug samples were also analyzed by reference method [6] for CEFX, CEFT, CEZD and [18] for CEPM and the results are given in Table 4.

RESULTS & DISCUSSION

Calibration graphs and analytical parameters

The calibration graphs for the studied drugs are obtained under optimum condition are shown in fig[1]. Good linear relationships were obtained over the concentration ranges given in Table [1]. The corresponding molar absorptivity values from Beer's law data and their sandell's sensitivity, slope, intercept, correlation coefficients, detection limit and quantitation limit of the method are given in Table [1].

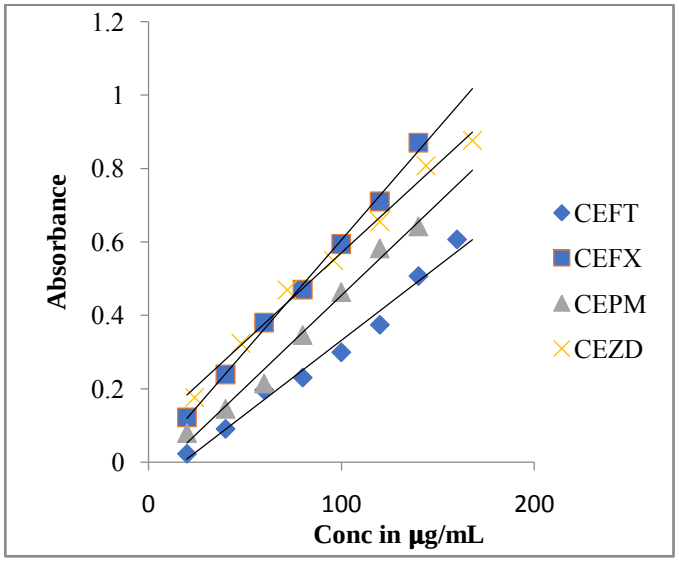


Fig.1. Beer's law curves of CEFT, CEFX, CEPM, CEZD.

Table 1. Optical characteristics of the studied cephalosporins

Parameter	CEFT	CEFX	CEPM	CEZD
Beer's law limit (µg mL^{-1})	20 -160	20 -140	20 -140	24 -168
Molar absorptivity (L mol^{-1} cm^{-2})	0.1585 X 10^4	0.2888 X 10^4	0.2069 X 10^4	0.3323 X 10^4
Sandell's sensitivity (µg cm^{-2})	0.349892	0.16532	0.230709	0.16448
Correlation coefficient [r]	0.9917	0.99832	0.99409	0.99586
Regression equation[Y*]				
Slope [b]	0.00416	0.00607	0.00502	0.00484
Intercept[a]	-0.08265	-0.00214	-0.04871	0.08586
Detection limit [DL] (µg mL^{-1})	1.07458	1.1061	0.54999	0.7773
Quantitation limit [QL] (µg mL^{-1})	3.2563	3.35189	1.66665	2.3557

*y = a + bx, where x is the concentration in µg mL^{-1}

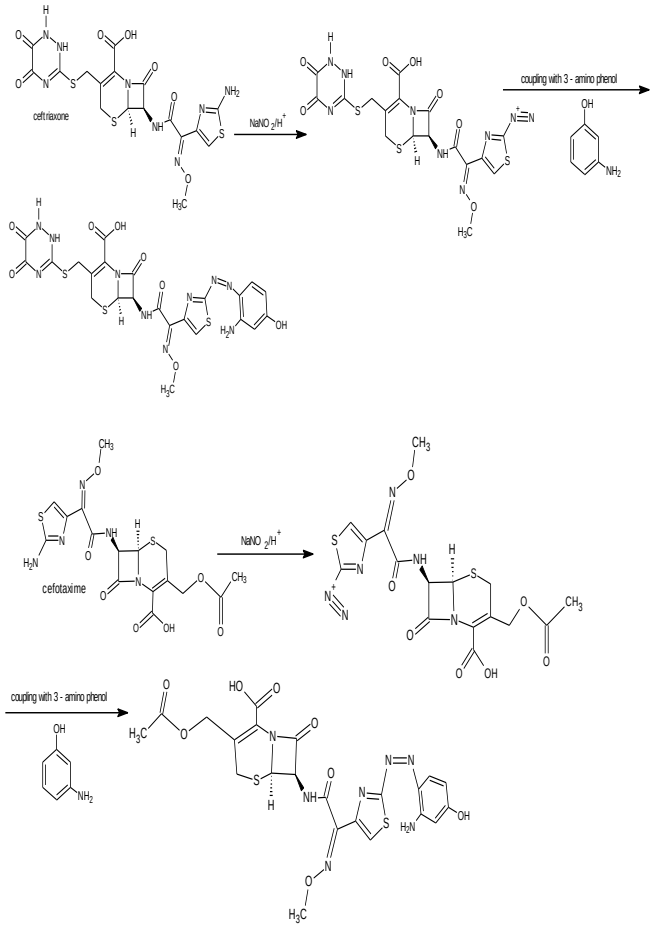
Quantification and reaction sequence

The proposed method is based on the diazo-coupling reaction of the studied drugs with 3-Aminophenol in an acidic

ISBN: 979-88-35073-61-0

medium to give an orange colored azo dye of λ_{max} 500nm for CEFX, CEPM, CEZD, and for CEFT at 505nm.

Two steps are involved in the reaction that produces the colored product. In the first step, the cephalosporins are treated with sodium nitrite solution in an acidic medium under cold condition; drugs undergo diazotization to give diazonium salt at a temperature of -2 to -3^{0}C. In the second step the diazonium salt is coupled with a coupling agent, 3-amino phenol, to form an azo dye in the acidic medium. The reaction pathway is presented in (scheme1).since ceftriaxone and cefepime are used as the model compound, since the other compounds were also behaved similar to it (scheme1).



Scheme 1. Reaction pathway of CEFT and CEFX with 3-AP.

Absorption spectra

The absorption spectra of the diazotized product with the drugs show the maximum absorption λ_{max} at 500nm CEFX, CEPM, CEZD, and for CEFT at 505nm. The blank solution showed negligible absorbance at λ_{max} in which drugs were examined. Stability of the color was studied and showed the result of 24 hours. The absorption spectra of the azo product and corresponding reagent blank are shown in Fig 2 & 3. The curves were found to be linear with good correlation coefficients.

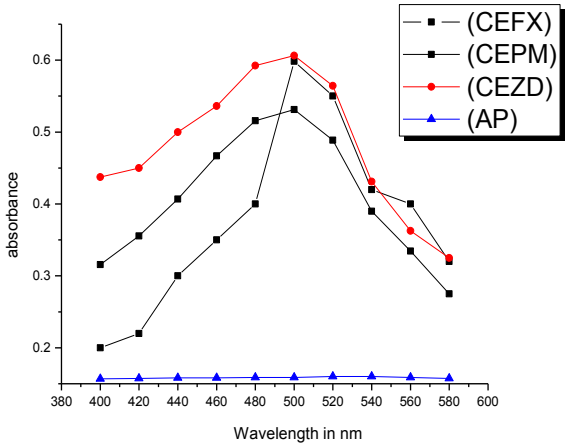


Fig 2. Absorption spectra of CEFX, CEPM, CEZD and blank with AP.

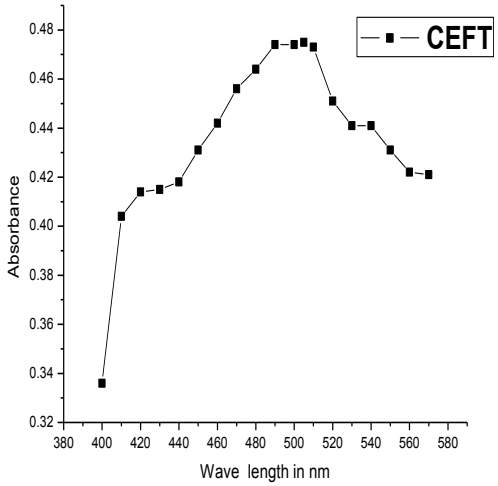


Fig 3. Absorption spectra of CEFT with AP

Optimization of experimental parameters

It was found that a 0.1% solution of sodium nitrite in the range of 1.0-3.0mL solution of 2% Sulphamic acid and 1.0-3.0mL solution of 0.3% 3-amino phenol were necessary to get the maximum color intensity. In this method, the excess of nitrite is removed by the addition of 2% Sulphamic acid solution. Addition of excess of Sulphamic acid has no effect on the absorbance values. In the AP method, dilution of the colored solution with different solvents like methanol, ethanol, acetic acid and acetonitrile have been tested. Results

ISBN: 979-88-35073-61-0

showed that water gives maximum intensity and stability of the color.

Order of addition of reagents

Reagents were added in the described sequence to achieve the maximum sensitivity of the color. Any changes in the order of addition of reagents affect the formation of an azo-dye and the sensitivity of the system.

Interference studies

In order to evaluate the suitability of the proposed method for the analysis of pharmaceutical preparations of the studied drugs, the interference of associated excipients and diluents in dosage forms was investigated. Under the diazotization reaction conditions used, other amines such as morphine, aniline, piperidine etc. give a positive reaction. However, the problem of interference does not arise in the analysis of commercially available cephalosporins. The method does not suffer any interference from common excipients such as starch, talc, magnesium stearate and lactose. Results are shown in Table 2.

Table 2. Recovery of the drug from solution in the presence of 100-fold concentrations of various additives used as excipients in the formulation

Excipient (Recovery± RSD[a], %	method A[b]	method B[c]	method C[d]	method D[e]
Lactose	99.7±0.3	99.7±0.3	99.9 ±0.3	98.9±0.2
Magnesium stearate	99.8 ±0.1	99.9 ±0.2	100.0 ±0.1	99.7±0.2
Talc	100±0.1	100±0.1	100 ± 0.1	100 ±0.2
Starch	99.8±0.2	99.8±0.2	99.9 ± 0.2	99.7±0.3

[a]Mean of three determinations, [b] CEFT concentration – 40 µg/mL (method A), [c] CEFX concentration – 40 µg/mL (method B), [d] CEPM con-centration – 20 µg/mL (method C), [e] CEZD concentration – 24 µg/mL (method D).

Accuracy and Precision studies

The precision of the proposed method was calculated in terms of intermediate precision (intra-day and inter-day) at three different concentration levels for CEFX, CEPM, CEZD, and CEFT in five replicates during the same day and for five consecutive days at the same concentration level. The analytical results obtained from the study are summarized in (Table 3). The low values of SD show excellent precision.

Table 3. Evaluation of accuracy and precision

Drug	[b]Amount taken (µg mL^{-1})	[a]Amount found (µg mL^{-1})	% Rec ± SD	% RSD	% RE
Ceftriaxone(CEFT)	20	19.90	99.5±0.0013	0.0065	0.5
	40	40.30	100.3±0.0011	0.0027	-0.75
	60	59.62	99.3±0.004	00067	0.63
Cefotaxime(CEFX)	20	20.05	100.2±0.0035	0.0174	-0.25
	40	39.84	99.6±0.0011	0.0029	0.4
	60	60.3	100.5±0.0039	0.0065	-0.56
Cefepime(CEPM)	20	20.37	100.1±0.0055	0.027	-1.85
	40	39.46	98.65±0.00058	0.00147	1.35
	80	79.52	99.4±0.0024	0.0030	0.6
Ceftazidime(CEZD)	24	23.8	99.16±0.0028	0.012	0.83
	48	48.08	100.1±0.0012	0.0024	-0.16
	72	71.94	99.99±0.00024	0.00034	0.08

RE relative error; SD. Standard deviation; RSD. Relative standard deviation.

*[a]Mean value of five determinations

Table 4. Analysis of studied drugs in pharmaceutical dosage forms

sample	Pharmaceutical formulation		[a]Proposed method		Reference method		t- value[b]	F -value[c]
		Amount taken (µg mL^{-1})	Amount found (µg mL^{-1})	% Rec ± SD		% Rec ± SD		
Ceftriaxone	Monocef inj 250mg	40	39.92	99.8 ±0.33		100.9±0.73	2.5	4.89
		60	60.1	100.1±0.41		99.70±0.64	2.2	2.43
Cefotaxime	Taxime inj 250 mg	40	39.6	99.0 ±0.41		99.36±0.93	0.84	5.0
Cefepime	Cepime inj 500 mg	20	20.12	100.6±0.64		98.95±1.079	2.85	2.06
Ceftazidime	Fortum inj500mg	24	23.94	99.75±0.67		100.99±0.95	2.41	2.01

a Average of five determinationS, b Tabulated value2.44, c Tabulated value 5.05

Application to formulations

The reproducibility of the method was checked with five replicate determinations for cephalosporins. The proposed methods were applied to the assay of cephalosporins in tablet

ISBN: 979-88-35073-61-0

and pharmaceutical dosage forms. The results obtained by the proposed methods and the official method [6 & 18] for the dosage forms were compared statistically by means of Student's *t*-test for accuracy and*F*-test for precision at a 95% confidence level. The calculated *t*- and *F*-values did not exceed the tabulated values (t = 2.44, F = 5.05) and indicated insignificant difference between the proposed method and the reported methods (Table 4), which shows the excellent agreement between the proposed methods and the official method

CONCLUSION

The present paper illustrated the evaluation of 3-Amino phenol as a good coupling agent, and inexpensive than MBTH or NEDA in the development of simple, fairly sensitive, rapid and economical with high degree of precision and reliable spectrophotometric method for the determination of cephalosporins in pure and pharmaceutical dosage forms. The developed methods are superior interims of simplicity and sensitivity than the previously published methods. Also the procedures do not involve critical reaction conditions such as extraction or tedious sample preparation steps. So this method is suitable for the assay of cephalosporins in pharmaceutical preparations.

REFERENCES

[1].Shazali, M.; Ali Ahmed; Abdalla, A.; Elbashir; Hassan ; Aboul-Enein. *Arab.J.Chem.In press, Corrected proof*, Available online.**2011**, 27.
[2]. Magda, M.; Ayad; Abdalla, A.; Shalaby; Hisham, E.; Abdellatef, A.; Heba ,M.; Elsaid. *J.Pharm.Biomed.Anal*.**1999**, 20, 557 - 564.
[3]. Abdel-Khalek, M.M.; Mahrous, M.S. *Talanta*. **1983,** 10, 792-794.
[4]. Marwa, S.;Moneeb. *Cairo University.***2013**, 2,139 - 150.
[5]. Abdel Fattah . M.; El Walily, Azza.; Abdel-Kader Gazy.; Saied, F.; Belal.; Essam , F.; Khamis. *J.Pharm.Biomed.Anal*. **1999**, 20, 643 - 653.
[6]. Olajire, A.; Adegoke; Monsurat, O.; Quadri. *Arab.J.Chem.In press, Corrected proof*, Available online.**2012**, 27.
[7]. Rodrigues, L.N.C.; Zanoni, M.V.B.; Fogg, A.G. *J.Pharm.Biomed.Anal,* **1999**, 21, 497-505.
[8]. Magda, M.; Ayad, Abdalla, A.; Shalaby, Hisham, E.; Abdellatef, A.; Heba ,M.; Elsaid. *J.Pharm.Biomed.Anal*. **1999**, 18, 975 - 983.
[9]. Irene Martínez-Martínez.; Silvia Montoro-García.; José Daniel Lozada-Ramírez.; Álvaro Sánchez-Ferrer.; Francisco García-Carmona. *Anal.Biochem.* **2007**,369, 210 - 217.
[10]. Tadayuki Tsukatani.; Tomoko Higuchi.; Hikaru Suenaga.; Tetsuyuki Akao.; Munetaka Ishiyama.; Takatoshi Ezoe.; Kiyoshi Matsumoto. *Anal.Biochem.***2009**, 393, 117 - 125.
[11]. Nafisur Rahman.; Manisha Singh.; Md. Nasrul Hoda. *Il Farmaco.* **2005**,60, 569 - 574.
[12]. Daniela, P.; Santos, Márcio, F.; Bergamini, Maria Valnice, B.; Zanoni. *Sensors and Actuators B: Chemical.* **2008**,133, 398-403.
[13]. El-Shahat, M.F.; Burham, N.; Abdel Azeem, F.M. *J. of Hazardous Mateir.***2010**, 177, 1054 – 1060
[14]. Al-Momani, I.F. *J.Pharm.Biomed.Anal*. **2001**, 25, 751 – 757.
[15]. Gamal, A.; Saleh, Salwa, R.; El-Shaboury, Fardous, A.; Mohamed, Azza, H.; Rageh. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* **2009**, 73, 946 – 954.
[16]. Genowefa Pajchel; Stefan Tyski. *J. Chromatogr. A.* **2000**, 895, 27 – 31.
[17]. Rocío Cazorla-Reyes.; Roberto Romero-González.; Antonia Garrido Frenich.; Manuel Angel Rodríguez Maresca.; José Luis Martínez Vidal. *J.Pharm.Biomed.Anal.***2014**, 89, 203-212.
[18]. Nawal, A.; El-Rabbat, Hanaa, M.; Abdel-Wadood1.; Mohammed Sayed,; Heba, S.; Mousa. *Bull. Pharm. Sci.***2012**, 35, 55 – 65.
[19]. El-Maali, N.A.; Ali, A. M.M.; Ghandour, M.A. *Electroanalysis(N.Y).* **1993**, 5, 599 – 604.
[20]..Misztal, G. *Pharmazie*. **1998**, 53, 723 – 724.

Optimisation studies on isolation of protease enzyme-catalytic activity on blood stain

Aviksha Sarika Gowda
Student, Department of Biotechnology, Sapthagiri college of Engineering, Bangalore 560057

Aruna Mohan
Student, Department of Biotechnology, Sapthagiri college of Engineering, Bangalore 560057

Chaitra U Kabbur
Student, Department of Biotechnology, Sapthagiri college of Engineering, Bangalore 560057

Rachana.G
Student, Department of Biotechnology, Sapthagiri college of Engineering, Bangalore 560057

Dr. J S S Allwin Ebinesar[1],
Associate Professor, Department of Biotechnology, Sapthagiri college of Engineering, Bangalore 560057

Ms Prakruthi
Laboratory head, Scientific and industrial research center (SIR),Bangalore 560022

Abstract— Proteases or proteolytic enzymes which consists of a very large and complex group of enzymes, have wide applications in different industries. Some of the efficient protease producers which are of great importance industrially were isolated from soil samples of different regions. 0.1ml of serially diluted soil samples were spread on Czpak dox agar, at 30C for 48 hrs. Total four bacterial colonies from garden soil showed clear zone around the colony indicating protease activity,of these, Mucor sp. which was identified morphologically in S-4 isolate produced high protease activity. Optimization studies were carried out for various phsiological parameters such as pH, temperature and inoculum size. Maximum protease activity was found at pH 5 and temperature 30C. Tyrosine was used as a standard to determine unknown concentration of crude protease. The protease whic was isolated from soil sample gave good result of washing with detergent. Therefore protease can be used with detergent. The above results indicate that these bacterial isolate can be use as biotechnological tool for industrial purpose.

1.INTRODUCTION

Proteases hydrolyze the peptide bonds in proteins and breaks it down into polypeptides or free amino acids. It includes proteinases, peptidases or proteolytic enzymes, breaks the peptide bonds between amino acids. A molecule of water is used during this reaction, and hence they are classified as hydrolases. Proteases are classified into three groups. If the pH optima is in the range of 2.0-5.0, they are classified as acid proteases and are produced by fungi. Neutral proteases perform best at pH 7 and are plant origins. Proteases which have maximum activity at pH range 8 and above fall into the category of alkaline proteases, produced from microorganisms. Free proteases are majorly used in dry cleaning ,detergents, meat processing, cheese making, silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and virulent wounds.

2. MATERIALS AND METHODS

2.1. Collection of soil

Mucor Species were isolated from the soil sample on Czapek Dox agar (CZA) medium. CZA medium composition is as follows. sucrose, (3g); sodium nitrate, (0.2g); dipotassium hydrogen phosphate, (0.1g); magnesium sulphate, (0.05g); potassium chloride, (0.05g); iron sulphate, (0.0010g); Agar, (2g) and pH 4.5 (g/ml Distilled water), followed by serial dilution for six times and spread plate method.

The isolated strains were screened for their protease production by plate assay. The screening medium is as follows. The clear zone was observed around the colony at regular intervals of time and measured the diameter of clear zone of hydrolysis. Mucor species were showed the best zone of clearance and has produced the high percentage of hydrolysis.

2.2. Thin layer chromatography (TLC)

The samples were periodically withdrawn at aseptic condition. The extract collected was filtered through Whatman filter. The clear extract was centrifuged at 5000 rpm for 15 min, supernatant were used as enzyme preparation. The prepared crude enzyme was used for TLC.

The protease production of mucor species was confirmed by subjecting the collected enzyme extract to thin layer chromatography (TLC). In this, the separation and identification of tyrosine (amino acid) is a hydrolysed product which is carried out by thin layer chromatography technique by using silica gel G and saturated phenol with water[9:1] used as a solvent system. The Rf values were calculated.

2.3. Molecular identification of fungal strain

The fungal strain of mucor species was identified morphologically and microscopically.

Mucor species usually produce chlamydospores. They form mold with irregular and non-septate hyphae. Initially they are white or yellow in colour along with a fluffy texture, Mucor mold colonies grow rapidly and turn into a dark colour as spores develop.

2.4. Optimization of fermentation Kinetics

Optimization studies of pH, temperature, inoculum size were conducted. Enzyme assay is performed by which the enzyme activity is measured via the amount of substrate consumed and, or the quantity of product formed over a fixed period of time.

Enzyme Activity (IU/ml) =

$$\frac{Micromoles\ of\ tyrosine\ liberated}{Molecular\ wt\ of\ tyrosine * volume\ of\ enzyme * time\ of\ incubation}$$

2.5. Purification of enzyme

Salt purification by ammonium sulphate method. Ammonium sulphate is the choice of salt as it is cheap and very soluble in water. The experiment is carried out at 4°C at 5000rpm for 15 min. After this the enzyme is separated from the crude extract.

2.6. Characterization studies

Characterization of enzyme is necessary to ensure the identify, specificity, purity, stability and activity of enzyme product. Characterization studies of pH, temperature, temperature stability, substrate specificity are carried out. Enzyme assay is carried out for this experiment.

2.7. Application

Chicken blood was collected from a meat shop. Different concentrations of detergents were prepared from different brands. Dialysed, crude enzyme and water for control was used. The blood was stained on tendercott cloth and over the period it's activity was checked. Then a contraction of dialysed enzyme and the most optimum concentration of detergent found was used to check it's activity.

3.Results and Discussion

Proteolytic enzymes (proteases) are enzymes that break down protein. These enzymes are made by animals, plants, fungi, and bacteria. Proteolytic enzymes break down proteins in the body or on the skin. This might help with digestion or with the breakdown of proteins involved in swelling and pain.

Fungi isolation was done by the serial dilution of soil. the isolated fungi was found to be Mucor, which was confirmed morphologically. Screening for protease was done by plate assay and followed by confirmation of the same by thin layer chromatography.

Protease was isolated by the microorganisms that were obtained from the soil sample. The fermentation kinetics were optimized. The pH, temperature and inoculum size were to be optimized to produce the enzyme in bulk.

For the first week the organism was grown in different ph with all the other physiochemical parameter constant and the OD values collected for six days. Enzyme activity was calculated and the optimum pH was found to be pH 5.

The optimization for temperature was obtained with organisms grown in ph 5 and temperature being 25,30,35,40 degree Celsius. Enzyme activity was calculated for the obtained OD for five days. Optimized temperature was found to be 30 degree Celsius. The same experiment was conducted for four days to obtain the optimum inoculum size, where the temperature was 30 degree Celsius and ph was 5 for the growth of the organism.

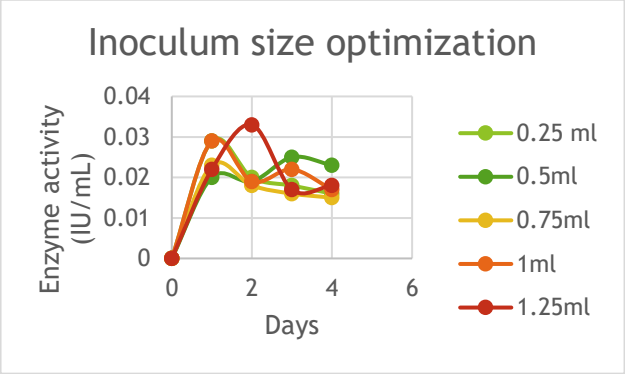


Figure 1 inoculum size optimization

Figure 1 shows the 1.25ml inoculum size has the highest enzyme activity thus it the the optimized inoculum size.

Partial purification by ammonium salt precipitation was done to obtained the pure enzyme from the crude that was obtained by filtering after fermentation. Characterization studies were conducted for the temperature stability which was found to be 1hr.

Protease enzyme have catalytic properties which is helps remove blood stains. Detergents are not capable of removing the blood stain from the cloths thus a blend of the enzyme into the detergents can make it successful. Distilled water and pure enzyme were also used directly to check their efficiency.

Four different detergents (tide,surf exel,ariel,wheel) were tested and the better result was found in Wheel.

ISBN: 979-88-35073-61-0

Protease enzyme was blended with Wheel in different percent concentrations to check for best results. A solution of detergent with 5%,10%,20%,50% of enzyme was prepared and used to wash the blood stain from the cloth. The test was conducted for 10 min and 30 min to obtain the best possible result. Stain was best removed by the 10% enzyme solution at 10min.

4.Conclusion

Fungal proteases have a major role in detergent, chemical, pharmaceutical,silk and other industries. Current studies has revealed that the Mucor sp. which was isolated from garden soil is a very good source for production of protease. Therefore we can conclude that this organism can be further studied for production of protease in large scale.

ACKNOWLEDGMENT

We sincerely thank Ms Prakruthi Mam and Mr Siddlingeshwara Sir for guiding us throughout the completion of our project at the Scientific and Industrial Research Center (SIRC). We also extend gratitude to our internal guide Dr. JSS Allwin Ebinesar, Associate Proffesor, Dept of Biotechnology, Sapthagiri College of Engineering, Bangalore.

REFERENCES

1.Abebe, Bizuye.,A,sago.,G,Admasu.,H,Getache w.,P,KassaandM,Amsaya.2014.Isolation , Optimization and characterization of protease producing bacteria from soil and water in Gondar town, Northwest Ethiopia. International Journal of Bacteriology, Virology and Immunology. Vol 1(3):020-024.

2.Balakrishnan, padmapriya., T,Rajeswari.,R, Nan ditaandF,Raj.2012.Production and Purificationof Alkaline Serine Protease from Marine Bacillus sp and its Application in Detergent Industry. European Journal of Applied Sciences.Vol 4(1):21-26.

3.Bharat,Pokhrel.,A,Pandeya.,S,Gurung., G, Bista., S ,Kandel.,R C,Kande and R T,Magar 2014.screening and optimization of extra cellular protease from bacteria isolated fromsewage.European Journal of Biotechnology and Biosciences.vol 2(1):46-49.

4.Asoodeh A, Lagzinan M, 2012. An extremely thermotolerant, alkalophilic substilisin-like protease from hyper thermophilic Bacillus sp MLA64. Int. J. Bio. Macro mole. 51, 960–67.

5.Banik RM, Prakash M, 2004. Laundry detergent compatibility of the alkaline protease from Bacillus cereus. Microbiol. Res. 159, 135–140.

6.Barrett AJ, Rawlings ND, Woessner JF, 1998. Handbook of proteolytic enzyme. Academic press Inc. london. Second edition (volume) 1.

Identification of fermentation mediating mechanism carried out by naturally available yeast (NAY) and commercially available yeast (CAY) under anaerobic conditions

Benita S Robin[1], Student, Department of Biotechnology, Sapthagiri college of Engineering, Bangalore 560057

Chinmay J[1], Student, Department of Biotechnology, Sapthagiri college of Engineering, Bangalore 560057

Chittoju Prerana[1], Student, Department of Biotechnology, Sapthagiri college of Engineering, Bangalore 560057

Vamshipriya N[1], Student, Department of Biotechnology, Sapthagiri college of Engineering, Bangalore 560057

Dr. J S S Allwin Ebinesar[1], Associate Professor, Department of Biotechnology, Sapthagiri college of Engineering, Bangalore 560057

Dr. Chethana K R[1], Assistant Professor, Department of Biotechnology, Sapthagiri college of Engineering, Bangalore 560057

Abstract— Fermentation, a biological process mediated by the microbial species during the conversion of sugars into alcohol with a by-product of carbon dioxide. Naturally, the *Saccharomyces cerevisiae* (Yeast), a eukaryotic cell majorly used in brewing industries for wine making. But the external addition of yeast species produces huge amount of cell mass which can able to reduce the production of Ethanol [1]. In this study, the product efficacy of wine was evaluated by using the different sources of fruits such as grape, pine apple and chilly in the aerobic and anaerobic conditions by using naturally available yeast (NAY) in the fruit and commercially available yeast (CAY) *S. cerevisiae*. In addition to that, the impact on production of wine yield was also analyzed in the presence and absence of sucrose.

Likewise, the quality of wine was evaluated by various physicochemical properties such as pH, Specific gravity, Total acidity, Alcohol percentage, Total sugars, COD are checked in regular intervals. The obtained result depicted that, the maximum product of wine was obtained from CAY at pH (3-4), Specific gravity (1.0), Total acidity (70g/l-1.05g/l), 15% of ethanol. In case of (NAY), the maximum product of wine was obtained at pH (3-4), Specific gravity (0.99), Total acidity (0.50g/l-90g/l), 11% of ethanol. Eventually, it was found that, the production of wine is more effective under anaerobic condition in pineapple medium mediated by the CAY [2].

Keywords— Fermentation, Saccharomyces cerevisiae (Yeast), wine, CAY, anaerobic.

1.INTRODUCTION

Wine is one of the most ancient beverages and comprised with a complex mixture of alcohol, sugars, aldehydes, tannins, pectins, vitamins, minerals and organic acids produced by the fermentation of simple sugars by the yeast, *Saccharomyces cerevisiae* [3]. In addition to that wines are undistilled alcoholic beverages made from various fruits which are nutritive, more tasty and mild stimulants.

Generally, the percentage of alcohol content present in the fermentable wine is around 5%to 13% of alcohol. The nutritive value of wine is increased due to release of amino acids and other nutrients by the metabolic activity endeavored by the yeast during fermentation.

The quality of fermented wine has been delineated by various parameters such as ionic strength of the wine, total acidity, relative density and ethanol production. *Ibegbulem et.al (2014)* stated that the value of total acidity of wine is lesser during anaerobic fermentation than the symbiotic process of aerobic and anaerobic fermentation process.

Sibylle Krieger- Weber (2009) reffered that, the *S. cerevisiae* and *Oenococcus oeni* are the major two microbial species involved in vinification process. The current taxonomy revealed that, there are 149 yeast genera and 1500 species can be isolated from the grape and its juices. Generally, the non *S. cerevisiae* yeast are commonly known as wild yeasts, because they are mostly present in grape vines, grape clusters and berry surfaces. *Imma Andorra et.al (2019).*

There is an abundance in exotic tropical fruits in India with the potential to be used by the food industry. Yeasts, especially different strains of *S. cerevisiae*, have long been used for the production of alcoholic beverages, solvents and other chemicals. Ethanol fermentation performed by yeast and some types of bacteria break the pyruvate down into ethanol and carbon dioxide [5]. This present study was aimed at determining the comparison of aerobic and anaerobic wine fermentation in the presence and absence of edible sugar(sucrose) and elucidating their physicochemical properties**.**

2. MATERIALS AND METHODS

2.1. Collection of fruits

In this study, the efficacy of wine production was enumerated by using three different fruits such as grapes, pineapple and chilli.

2.2. Preparation of wine under aerobic fermentation in the absence of edible sugar

A known amount (500gm) of crushed grapes, pineapple and chilli was added in 1lt of distilled water along with broken wheat(10gm), cloves, cardamom and cinnamon and flavoring agents. After that, the percentage of wine production was evaluated under aerobic fermentation by both CAY and NAY. Finally, the concentration of ethanol, glucose, sucrose, total acidity, pH, density, specific gravity and COD has been evaluated for an interval of every 5 days.

2.4. Determination of pH

The ionic strength of the fermentable wine was determined by using pH meter (Systronics India limited). Note: the digital pH meter should be calibrate before the measurement of pH of the wine samples.

2.5. Determination of relative density

The relative density of the wine was determined by measuring the ratio between the specific gravity of wine with respect to water. In this study, initially the empty weight of specific gravity bottle(W1) measured by using weighing balance. After that the weight of empty bottle plus water(W2) and empty bottle plus wine(W3) was measured. Finally, the relative density of the wine was calculated by using the below relationship.

Relative density = $\frac{W3-W1}{W2-W1}$

2.6. Determination of Total Acidity

In this experiment the total titrable acidity was evaluated by titrating the wine sample against 1N NaOH. Initially a known volume of sample 2ml was taken and distilled water 8ml is added to make up to 10ml and to that Bromothymol blue indicator (3 drops) was added and this was titrated against 1N NaOH solution. The conversion of color from yellow to blue indicates the end point containing organic acids.

2.7. Determination of ethanol production

Ethanol production was determined by Potassium dichromate method, a known amount (1ml) of each wine sample is taken and 2.5 ml of Chromic acid is added to each wine sample and kept for incubation for 15 mins at 80° Celsius in boiling water bath, once the incubation is completed it is allowed to cool and the OD is checked at 640nm.

3.Results and Discussion

Wines are unprocessed alcoholic alimentary beverages typically made from fruits such as grapes, banana, peaches, etc. The fruit juices endure through a process of ageing after the action of yeasts which leads to a major change in the composition and flavor. Besides ethanol, most wines contain different types of aldehydes, sugars, tannins, esters, vitamins, minerals and other flavoring compounds.

The impact on the production of wine by the NAY and CAY was analyzed by varying different

parameters such as pH, specific gravity, total titrable acidity, ethanol production. In this study we can observe a trend of initial increase and gradual decrease in the graph as in fig.1 (a), however there is a similar trend in the graph as in fig.1(b), this represents that the decrease in pH indicates the increase in the acidity of the wine.

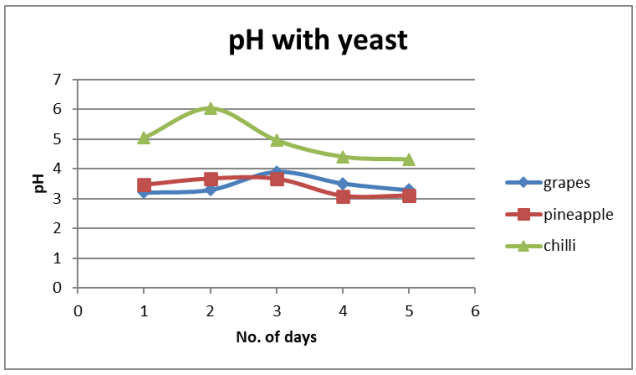


Fig 1. (a) pH with yeast

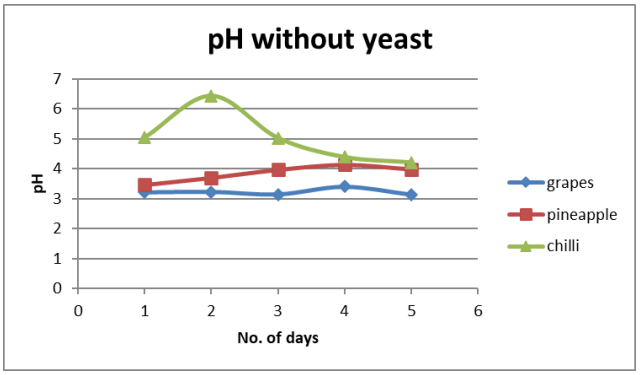


Fig 1. (b) pH without yeast

From the graph below fig 2. (a) and fig 2. (b) the observed results for specific gravity are gradually decreasing over the number of days as the yeasts starts converting the sugars into ethanol and carbon dioxide initially decreasing the density of the wine resulting in the decrease of specific gravity.

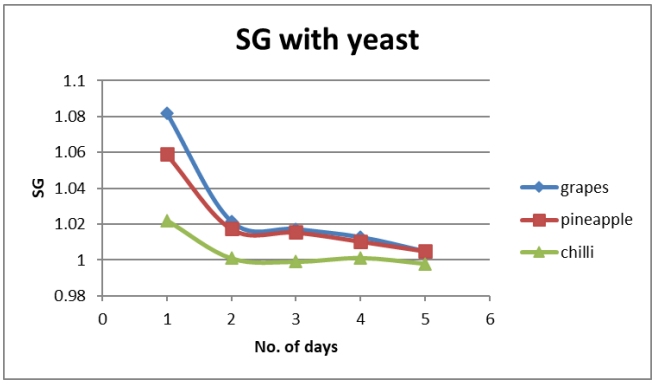


Fig 2. (a) Specific gravity of wine with yeast

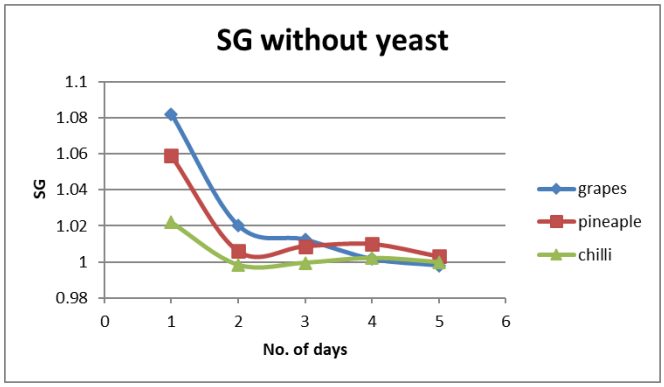


Fig 2. (b) Specific gravity of wine without yeast

From the obtained result fig3 (a) and (b) revealed the production of ethanol in the presence of CAY and NAY. The obtained result stated that the ethanol production was rapidly increased in the first two days in all the three substrates such as grape, pineapple and chilli fruit medium. After few days, the stability of ethanol is drastically decreased and reaches to zero on 15th day of fermentation. In addition to that the result delineated the maximum amount of wine in pineapple medium comparatively to grapes and

chilli substrate medium. It is mainly due to the demand created by the oxygen in the pineapple medium is very lesser than the demand created in grapes and chilli medium. It is clearly reflected in the fig 4. (a) and fig 4. (b).

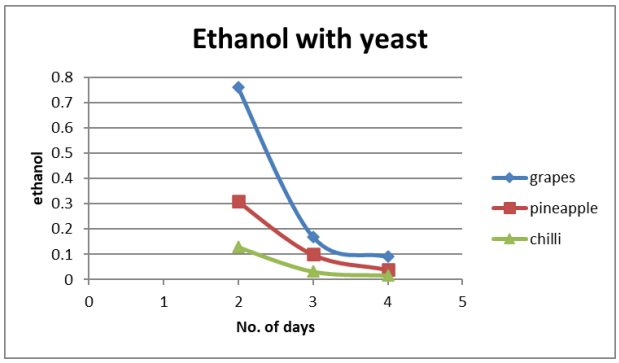


Fig 3. (a) Ethanol production of wine with yeast

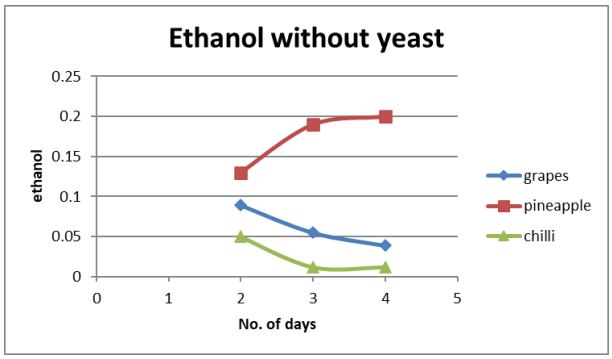


Fig 3. (b) Ethanol production of wine without yeast

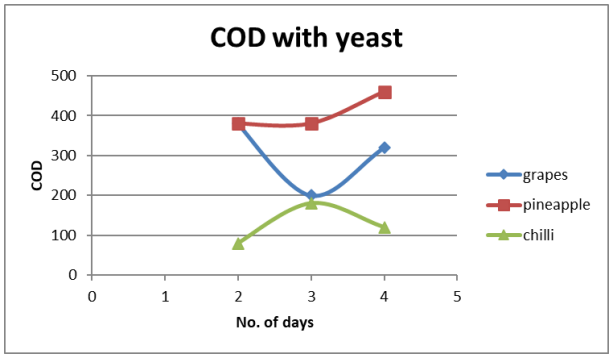


Fig 4. (a) COD with yeast

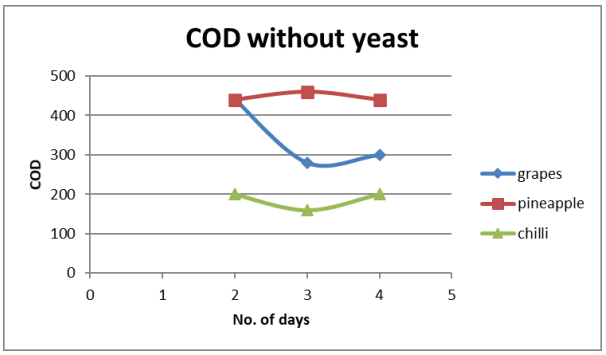


Fig 4. (b) COD without yeast

The fig 5. (a), (b) depicted the result on total acidity level in the three different wine fermentation medium. The maximum amount of total acidity changes in grape, pineapple and chilli was found to be 0.64, 0.75 and 0.08 g/l respectively. The total acidity concentration is gradually decreasing in the substrates grapes, pineapple and chilli. It is mainly due to the aerobic fermentation process. It can also be observed that reduction in volatile organic acids led to reduction in the total titrable acidity.

In case of fermentation carried out in the presence of CAY the results obtained were similar to the above explanation.

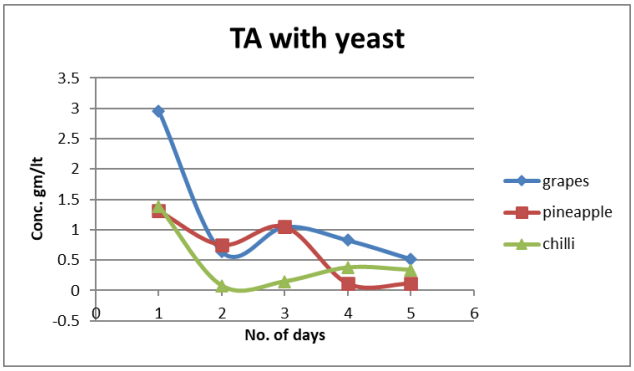


Fig 5. (a) TA of wine with yeast

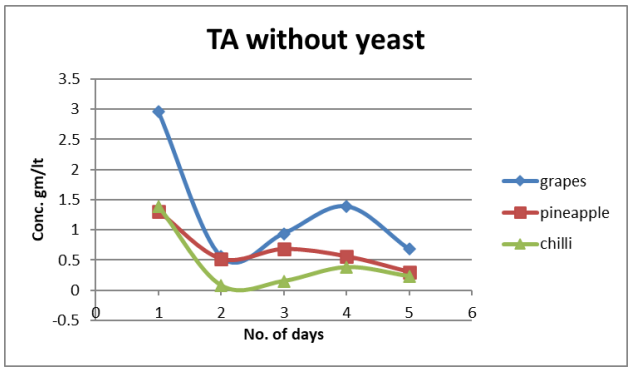


Fig 5. (b) TA of wine without yeast

Conclusion

A good quality wine produced from different fruits such as grapes, pineapple and chilli by varying different parameters in the presence of NAY(naturally available yeast) and CAY(commercially available yeast). The wine produced in the presence of CAY had a good flavor, color, taste and odor characteristics. Further research is required to comment on the anaerobic fermentation and to control the contamination caused by the aerobic microorganisms. From the present study different parameters analyzed will help to produce a good quality wine and, also can be improved during the large scale production.

ACKNOWLEDGMENT

We sincerely thank Dr. Chethana.K.R who guided us initially.

REFERENCES

[1]. Aakriti Guleria, "Production of grape wine by the use of yeast, Saccharomyces cerevisiae" (2014); Volume III, Issue 6, ISSN no: 2277-8160.

[2]. Jude lwouno, Chigozie Ofoedu, "Evaluation of Dealcoholized beverage from pineapple wine and sorghum beer" (2019); 33(5):1-10.

[3] Suhail Cholassery, Sabu K.R, Vandana Ranganathan, Rajesh B.R, "Analysis of physicochemical and sensory parameters of wine produced from Carica papaya"(2019); Vol.7(05),pp.74-78.

[4]. Michael Ukwuru, Jane Ijeoma R.K, "Properties of palm wine yeasts and its performance in wine making" (2013); Vol.12(19),pp.2670-2677.

[5]. Shrikant Baslingappa S, Nayansingh Thakor, "Fruit wine production:A review" (2014); Vol.2, Issue 3, pages 93-100.

[6]. Saranraj P, Srivasakthivelan .P, "Fermentation of fruit wine and its quality analysis: A review" (2017); Vol.1, Issue 2, ISSN Number(2208-6404).

[7]. Sibylle Krieger-Weber "Application of yeast and bacteria as starter cultures" (2009).

[8]. Imma Andorra "Wild yeast and lactic acid bacteria of wine" (2019).

[8].C.O.Ibegbulem,P.C.Chikezie,C.O.Nweke,C.E.Nwanyanwu,D.C.Belonwu "Effects of processing pineapple- based must into wines by anaerobic fermentation" (2014). American journal of food technology.

Comparison of 2D and 3D cell culture model using breast cancer cell lines

Harshitha P S, Nancy Yadav, Nayanika Aishwarya, Vrunda A Patel, Dr Shobha G
Department of Biotechnology
Sapthagiri College of Engineering, Bangalore, India
harshithapsharsha@gmail.com, nancyyadav2217@gmail.com,
naynikaaishwarya@gmail.com, vrundapatel202000@gmail.com,
shobhag@sapthagiri.edu.in

Abstract

Cancer is a vast spreading disease globally which holds second rank of death causing diseases. In India alone, around 148% rise of cancer is observed from 1990 to 2019. As the oncology drug market is hyping down the years, study models in in-vitro culturing plays a vital role in drug treatment and their cell signaling pathways. Two-dimensional models are simplest and cheapest methods to study primary functions of the experiments. Luminal A type of breast cancer cell line, MCF 7 was revived, cultured using 96 well plate and treated with drugs (cisplatin and camptothecin) for 48 hours. Cell viability is measured using MTT cell proliferation assay which reduces soluble tetrazolium into an insoluble formazan product by the mitochondria of viable cell.

Keyword: MCF 7 cell line, Cisplatin, Camptothecin, MTT assay, Cell viability.

1. Introduction

Cancer is a vast category of diseases that can occur in any organ or tissue of the body and spread to other organs when abnormal cells develop uncontrollably' The latter is known as metastasizing, and it is a leading cause of cancer-related death. Cancer is sometimes referred to as a neoplasm or a malignant tumour [1,2]. For the year 2020, India is expected to have 1,392,179 cancer patients, with the breast, lung, mouth, cervix uteri, and tongue being the top five cancer locations [3]. Cancer is caused mainly due to mutations of genes in which programmed cell death does not occur in normal cells.

1.1 Culture Techniques

As the cancer drug market is creating a new era of treatment, testing of these are drugs are

conducted rapidly. Any drug entering a market will undergo in-vitro study which is prior to in-vivo, pre-clinical and clinical trials. In vitro does not represent the actual environment of particular drug action but still this method holds a huge contribution in very first step of research. In-vitro studies occurs in a controlled environment, such as a test tubes or petri plates. Cell culture systems are critical tools for a wide range of in vitro research projects, both fundamental and clinical. The conventional favoured paradigm is static dish culture, which produces adherent two-dimensional (2D) cell monolayers [4]. To summarise, 2D culture models have been and will continue to be used to answer fundamental biological problems.

2D cells develop as a monolayer in a culture flask or a flat petri dish to a fixed plastic surface [4].

1.2 MCF 7 Cell Line

Breast cancer are regularly detected in women, where 1 out of 10 new cancer is diagnosed with breast cancer [6]. Some common causes of breast cancers are: age (>40), gender(female), obesity, alcohol, family history, radiation exposure, tobacco, hormone therapy for postmenopausal and many other mutations in BRCA 1, BRCA2, PALB-2. Invasive breast cancer can be caused by oestrogen, progesterone, and HER 2 that leads to ductal carcinoma or lobular carcinoma. From 84 cell lines of breast cancer available, MCF 7 is a widely used that belongs to a luminal A molecular subtype. It is positive towards oestrogen and progesterone receptors which express BRCA 1 wild type mutation causing Invasive ductal carcinoma type of tumour [5,7].

1.3 Drugs used for the Culturing Reference

1.3.1 Action of Cisplatin

Cisplatin is a platinum-based chemotherapy drug that is used to treat a variety of cancers including sarcomas, carcinomas, lymphomas, and germ cell tumours. Cisplatin is an anticancer drug that belongs to the alkylating agent class. Alkylating agents get their name from their capacity to add alkyl groups to a variety of electronegative groups in cells. Alkylating agents work through three mechanisms: 1) the attachment of alkyl groups to DNA bases, causing DNA to be fragmented by repair enzymes in their attempts to replace the alkylated bases, preventing DNA synthesis and RNA transcription from the affected DNA; 2) DNA damage via the formation of cross-links (bonds between atoms in the DNA),

preventing DNA from being separated for synthesis or transcription; and 3) the induction of nucleotide mispairing [8].

1.3.2 Action of Camptothecin

The Camptothecin (CPT) family of chemicals has been shown to be effective against a wide range of cancers. Human DNA topoisomerase I has been identified as their molecular target (topo I). CPT blocks the re-joining phase of topo-cleavage/relegation I's reaction, leading in the build-up of a covalent reaction intermediate, the cleavable complex. CPT kills cells primarily through S-phase-specific death caused by potentially deadly collisions between advancing replication forks and topo-I cleavable complexes [9].

2. Materials

2.1 Animal cell culture lab

A well-equipped animal cell culture laboratory with Liquid nitrogen tank, Refrigerator, Water bath, Incubator with 5% CO2, Biosafety cabinet, Inverted microscope, Hemocytometer, Centrifuge, weighing balance, Multimode microplate reader, T-25 flask, 96 wall plates, Spheroids culturing plates.

2.2 Chemicals

Dulbecco's minimum essential media (DMEM), Fetal bovine serum (FBS), Penicillin, Streptomycin, Dulbecco's phosphate-buffered saline (DPBS), Trypsin EDTA, Trypan blue dye, MTT dye, Isopropanol.

2.3 Cell line: MCF 7

2.4 Drugs: Cisplatin and Camptothecin

3. Methodology

3.1 Reviewing of cells

Transfer the MCF 7 cells from liquid nitrogen storage to -20°C, gradually thaw the cell to room temperature. Thawed cells are transferred to Eppendorf tubes and rinse the cryovial with 1ml of complete media. Centrifuge the cells for 5 minutes at 5000rpm and resuspend the cell pellet with 5ml of complete media in T-25 flask. MCF 7 are cultured in DMEM, which is supplemented with 10% FBS, 1% penicillin/streptomycin. Change the media alternatively till it attains 70-80 % confluency which is ready to seed. Maintain the constant temperature of 37°C in a humidified 5% CO2 atmosphere.

3.2 Seeding

As cells attains its confluency, discard the media and wash the cell monolayer with DPBS and trypsinize the cells using 0.025% Trypsin EDTA. Keep it in 5% CO2 Incubator for 1 minute, then add 2ml of media. The cells are collected and centrifuged for 5 min to obtain a pellet. Then supernatant is discarded and pellet is suspended in 1ml of fresh medium. Cells are counted using a hemocytometer by trypan blue exclusion method and then the total no of cells are counted and used for seeding. Cells are seeded in a 96-well plate at cell density of 10,000 cells per well. 100 µL of complete medium will be added to all the wells and they are allowed to grow for about 24hrs at 37°C in a humidified atmosphere with 5% CO_2.

3.3 Drug Treatment

Cisplatin and Camptothecin has its molecular weight of 301.1 g/mol and 348.85 g/mol respectively. Test compound will be weighed to get 10mM stock concentration. It will be dissolved in 1%DMSO and serially diluted in three-fold concentration to obtain 100uM to 0.001uM. The test wells are treated with drugs and are incubated for 48 hours at 37 C in a 5% CO2 atmosphere.

3.4 MTT Assay

After the incubation period, the spent media is replaced with fresh media and MTT reagent is added to all the wells at a final concentration of 0.5mg/ml of the total volume. Total volume of the assay is 100uL. The plate is wrapped with aluminium foil to avoid exposure to light and incubated further for 4 hours at 37 C in a 5% CO2 atmosphere. 100uL of the solubilizing solution (isopropanol) is added into each well after incubation. The MTT formazan crystal is dissolved using isopropanol by repeated pipetting. The absorbance is read in glomax multiplate reader at 570nm.

4. Result and Discussion

As cells are treated with different uM concentrations of drugs, viability of cells differs from higher concentration to lower concentration. MTT assay is a calorimetric method of determining the cell viability. Water soluble 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide is converted to water insoluble compound formazan by succinate dehydrogenase present in viable cells. Below line graph shows the concentration v/s viability.

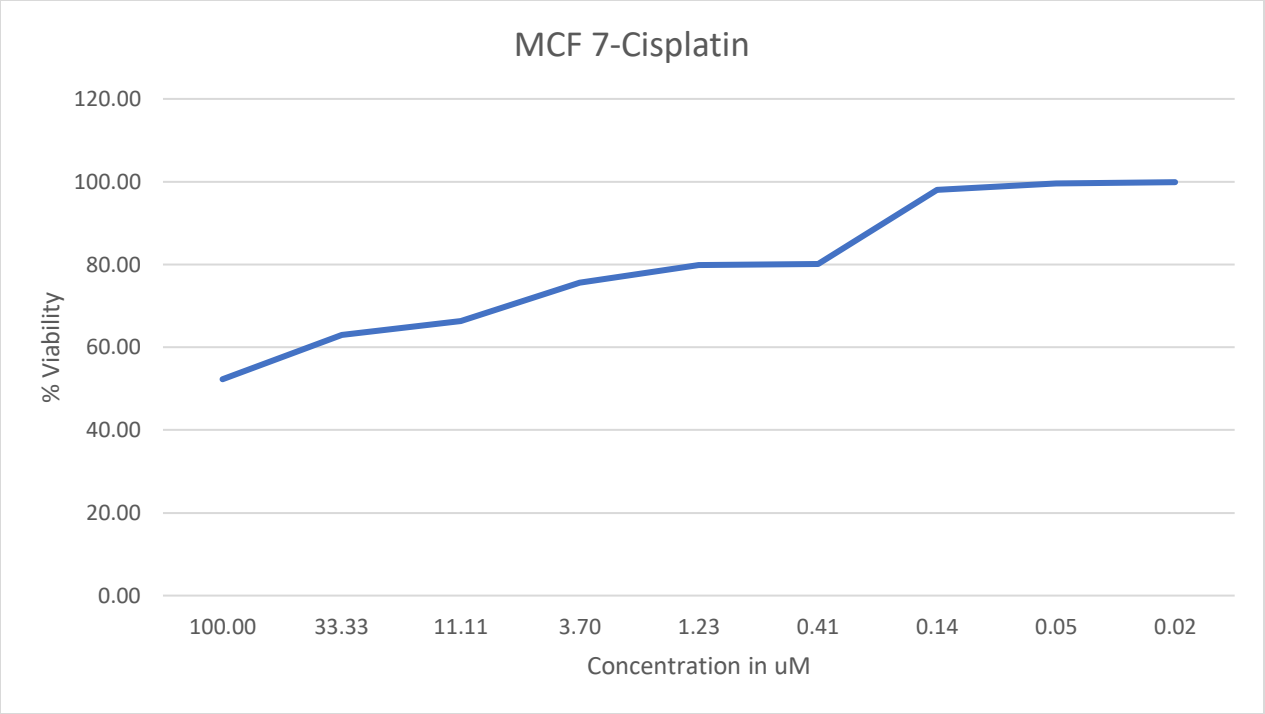


Fig 1: Line graph representing cell viability v/s concentration of Cisplatin on MCF 7 cell line

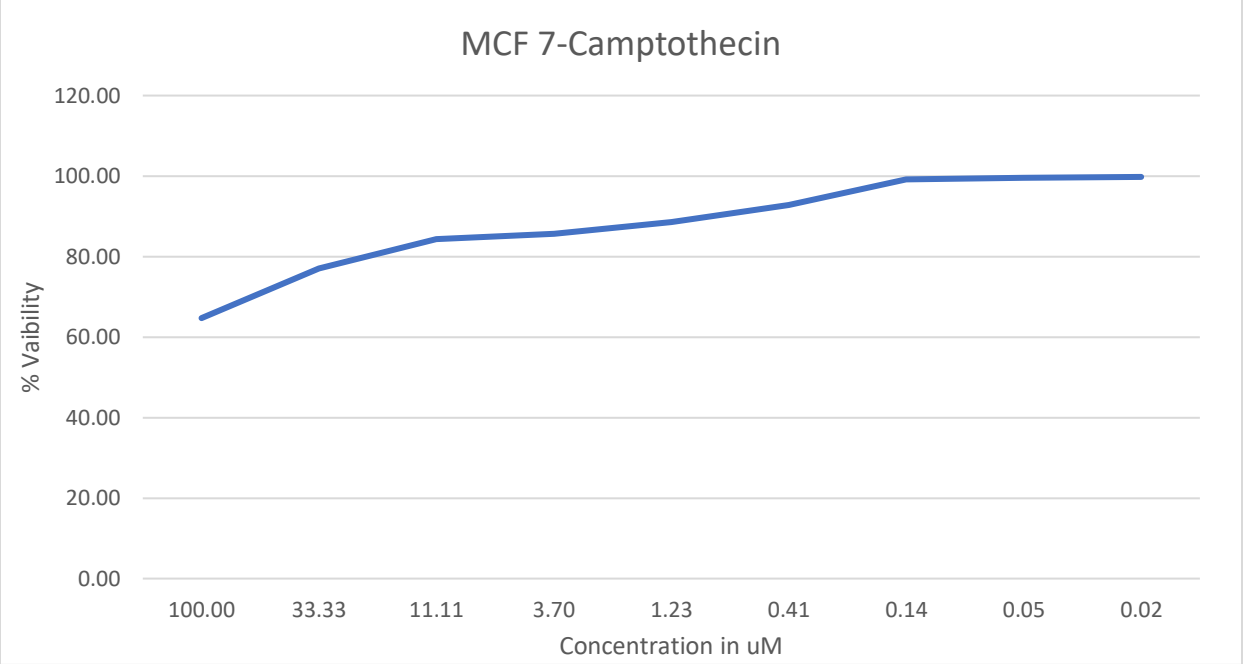


Fig 2: Line graph representing cell viability v/s concentration of Camptothecin on MCF 7 cell line

In Fig 1 Cisplatin shows 52.27% of cell viability at highest concentration i.e.,100uM and is increased as concentration decreases. In Fig 2 Camptothecin shows 64.71% of cell viability at 100Um and is also increased as concentration decreases.

5. CONCLUSION

Breast cancer cell line MCF 7 have a doubling time of 24 hours and a lag period of about 48 hours, these cells grew exponentially for 5 days. After culturing of 2D cells of MCF 7 and treated with two reference drugs with different concentration Cisplatin is more effective then camptothecin with the difference of 12.44%. Hence, by MTT assay we can determine the proliferation rate of the cells and can calculate cell cyto-toxicity.

6. ACKNOWLEDGMENT

We like to acknowledge our guide for helping us to carry out this project. We also like to acknowledge Department of Biotechnology for being supportive in every step of the project. We want to express our gratitude for Sapthagiri College of Engineering for providing us the necessary requirements. We also like to extend our thanks to Vipragen Bioscience Pvt. Ltd, where the project is been conducted and very blessed to receive their support in all aspects related to project.

7. REFERENCES

[1] WHO: World Health Statistics 2019: Monitoring Health for the SDGs,Geneva, Switzerland, World Health Organization, 2018.

[2] Parkin DM, “The evolution of the population-based cancer registry” 2006-Nat Rev Cancer; 6:603–612. - PubMed

[3] Jemal A, Ward EM, Johnson CJ, et al: “Annual report to the nation on the status of cancer” 1975-2014, featuring survival. J Natl Cancer Inst 109: djx030, 2017. - PMC – PubMed

[4] Ryan J. “Introduction to Animal Cell Culture” Technical Bulletin Corning. 2003:1–8. [Google Scholar]

[5] Done SJ,” Breast Cancer - Recent Advances in Biology, Imaging and Therapeutics.”, 2011, Rijeka, InTech, p IX, Google Scholar

[6] Fadi M. Alkabban , Troy Ferguson ¨Breast Cancer”, Publishing; 2022 Jan.2021 Aug 7;Bookshelf ID: NBK482286

[7] Gunduz M, Gunduz E," Breast Cancer – Carcinogenesis, Cell Growth and Signalling Pathways." 2011, Rijeka, Google Scholar

[8] Khalaila I, Bergamo A, et.al," The role of cisplatin and NAMI-A plasma-protein interactions in relation to combination therapy"2006 Jul; Int J Oncol, 29(1):261-8.

[9] Hsiang YH, Hertzberg R, Hecht S, Liu L. "Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I." . 1985 *J Biol Chem*;260:14873–14878. [PubMed] [Google Scholar]

Anti-Glycation Activity of Garcinia cambogia

Ruthwik K Shetty
Biotechnology
Sapthagiri College of Engineering
Bengaluru, India
ruthwikshetty1@gmail.com

Sharat S Tavarkhed
Biotechnology
Sapthagiri College of Engineering
Bengaluru, India
sharattavarakhed@gmail.com

Santhosh G K
Biotechnology
Sapthagiri College of Engineering
Bengaluru, India
santhu221100@gmail.com

Srirama P Bhat
Biotechnology
Sapthagiri College of Engineering
Bengaluru, India
srirambhat.315@gmail.com

Dr. Veena S More
Department of Biotechnology
Sapthagiri College of Engineering
Bengaluru, India
hodbt@sapthagiri.ac.in

***Abstract*—** The phenomenon of attaching carbohydrates like fructose and glucose to the proteins or lipid derivatives is called glycation. The reducing sugars' aldehyde and proteins' or nucleic acids' amino group forms a reversible product called Schiff's base, which is the first step of glycation reaction. The Schiff's base formed is not stable, hence it slowly starts to rearrange to form an amadori product. These amadori products undergo a series of dehydration and rearrangements over a long period of time to form an irreversible product called advanced glycosylation end products (AGEs).

Garcinia gummi-gutta or *Garcinia cambogia* with a common name of kudam puli, brindle berry and Malabar tamarind is a tropical fruit available in Indonesia. These fruits contain components like fats, tartaric acid, euxanthons, hydroxyl citric acid (HCA) and reducing sugars. Some AGEs related chronic diseases are due to the accumulation of glycation products. Diseases like cardiovascular disease (damages of fibrogen, collagen and endothelium), Alzheimer's disease (side products such as amyloid proteins of the reactions processing to AGEs).

The fruit rinds of *Garcinia cambogia* are subjected to extraction to obtain phytochemicals, and checked which phytochemical has the best anti-glycation activity, by carrying out Congo Red Assay.

Keywords—Anti-Glycation, Hydroxyl Citric Acid, Congo Red.

1. INTRODUCTION

Glycation is the process of attachment of sugar such as glucose, fructose, and their derivatives to a protein or lipid. L C Maillard, a food chemist was the first to describe the non-enzymatic reaction of reducing sugars with amino acids. Non-enzymatic protein glycation entails a variety of complicated processes, only a few of which are known with reasonable certainty. The creation of a Schiff base between the aldehyde of a reducing sugar like glucose and the amino group of a protein or nucleic acid initiates this process. Although glucose is frequently employed as a model sugar in non-enzymatic glycation processes, any reducing sugar with a free carbonyl moiety can form a Schiff base and other rearrangement products when reacting with amino acids in proteins. The creation of a Schiff base is quick and takes only a few hours to attain equilibrium. The Schiff base can rearrange over time to produce a more stable but still reversible Amadori product, although it takes several weeks to attain equilibrium. Amadori products can go through a series of poorly defined rearrangements and dehydrations over months to years, resulting in irreversible molecules known as advanced glycosylation end

products (AGEs). These end products are fluorescent, yellow-brown in color, and may cross-link proteins both intermolecularly and intramolecularly.

Unlike Schiff base and Amadori products, which are reversible and reach a steady-state level, AGEs are irreversibly bound and can accumulate over years. The amount of AGEs generated in vivo is determined by the sugar content and the protein's half-life. (Lee and Cerami, 1992).

Despite being a reversible reaction, glycation is a precursor to the Maillard or browning reaction, which leads in irreversible chemical changes, browning, fluorescence generation, and protein cross-linking during cooking. These identical processes occur in the body during normal ageing, although at a lower temperature and at a slower rate. Advanced glycation end products (AGEs) are irreversible adducts and cross-links in tissue protein (Lima and Baynes, 2013). In vivo, the primary AGEs, including methylglyoxal 3-deoxyglucosone, glyoxal, and, appears to be produced from hyperreactive intermediate carbonyl groups such as -dicarbonyls or oxoaldehydes. Pentosidine and N (carboxymethyl) lysine are two of the best chemically described AGEs in humans (CML). Because some AGEs, namely pentosidine, contain inherent fluorescence, the fluorescence of tissue and plasma can be employed as AGEs accumulation indicators. AGEs can come from external sources similar to tobacco smoke and food, in addition to endogenously produced products. Food preparation, particularly prolonged heating, hastens the formation of glyco-oxidation and lipo-oxidation products, and a large number of ingested AGEs are absorbed through food. Smokers and patients on high-AGEs diets show greater tissue and circulation AGEs levels, as well as higher inflammatory markers. Furthermore, evidence from animal studies reveals that exposure to high levels of exogenous AGEs contributes to complications in renal and vascular systems. (Goh and Cooper, 2008).

Garcinia gummi-gutta, also called *Garcinia cambogia*, Kudam Puli, Brindle berry, and Malabar tamarind is a tropical species of Garcinia native to Indonesia. Garcinia, a large diverse genus has approximately 240 species (Hart and Cock, 2016). They are small or medium-sized trees and shrubs up to 12m and have drooping branches and rounded crowns (Sudharani et al., 2018). In Indochinese Peninsula, the fruit rind is used as an agent for flavoring, a dietary supplement for weight loss, and food preservatives for a very long time (Hargunani et al., 2020).

The fruits of these plants contain components like camogin, euxanthons, fats, tartaric acid, reducing sugars, and Hydroxyl Citric Acid (HCA). These extracts are used in treatments for inflammatory bowel disease (IBD) referred to as Crohn's disease and ulcerative colitis, which are chronic inflammatory conditions that affect the gastrointestinal tract, and also in the treatment of purgatives, demulcent, astringent, and rheumatism. It is also hypothesized that these fruit extracts might possess protective action in an experimental model of colitis caused by a 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Madappa and Bopaiah, 2012; dosReis et al., 2009).

Few species have high antioxidant content which when rich in the diet decreases the incidence of a few chronic diseases. High levels prevent the development of cancer, cardiovascular disease, obesity, diabetes, and neural degeneration. Phenolic compounds are strong antioxidants (Hart and Cock, 2016). A study of phytochemicals studies indicates the existence of flavonoids, saponins, tannins, alkaloids, carbohydrates, phenolic compounds, and proteins (Hargunani et al., 2020; Dhanya and Benny, 2013). Among many phytochemicals in *G cambogia,* most of the interest has been on HCA as a result of its functional inhibition of citrate lyase (Hart and Cock, 2016). HCA is a derivative of citric acid which increases the availability of serotonin in the brain (Akshay et al., 2018). HCA also has the property of weight loss hence extensive research is being conducted on its regulatory effect on fatty acid synthesis, appetite, and lipogenesis. HCA in fruit is roughly 10-30% by weight and can be isolated in free form as mineral salt or as lactone (Semwal et al., 2015; Kolodziejczyk et al., 2009). These

lactones isolated are being used as drugs that target cancer and cardiovascular disease. (-)-HCA derivatives in combination drugs are helpful in weight loss and to correct abnormalities in lipid metabolism. HCA has four isomers namely; (+)-allo-HCA, (+)-HCA, (-)-allo-HCA, and (-)-HCA, which is the isomer found in Garcinia fruit and leaves (Bheemaiah and Kushalappa, 2019). HCA acts as an inhibitor of ATP- citrate lyase which can inhibit the endogenous lipogenic activity. Studies have shown that lipid profile in animal models can be improved by HCA which can promote the oxidation of fatty acids. It is also observed that in animals treated previously with a high-fat diet, the Garcinia extract may decrease insulin and leptin levels (Vasques et al., 2013).

Xanthocymol, Garcinol, and guttiferone are some of the polyisoprenylated benzophenone derivatives present in Garcinia species. These derivatives have anti-protozoal, anti-viral, antioxidant, anti-cancer, apoptotic, anti-bacterial, anti-inflammatory, anti-fungal, and anti-ulcer properties (Jacob et al., 2015). Garcinol is derived from a polyisoprenylated benzophenone present in *G cambogia* has anti-ulcer and antibiotic activities (Kolodziejczyk et al., 2009).

The dried fruit rind is employed in conventional remedy to treat oedema, intestinal parasites, piles, irregular menstruation, and constipation. It is being investigated for anti-cancer activity (Hargunani et al., 2020). Due to the presence of HCA in G gummi-gutta, it increases the level of glucose, total cholesterol, and LDL levels and by increasing immunological indicators it increases immunity (Jacob et al., 2015).

2. METHODOLOGY

A. Crude Mixing of Garcinia cambogia

Crude mixing was done using 70% ethanol (v/v) in two batches, where 45g of dried fruit rind was cut into small pieces and mixed in 200ml of 70% ethanol. This was taken in a conical flask and was kept in a shaker for 24 hours at 100rpm (Sripradha et al., 2015). The mixture was filtered and dried in the water bath.

B. Water Extract from *Garcinia cambogia*

10g of dried fruit rind was cooked with 3 volumes of water in the autoclave (10lb/in2) for 15 minutes (Edirisinghe et al., 2015). The extract was filtered and dried.

C. Petroleum Ether and Ethyl Acetate from Crude Mixture

The crude mixture was partitioned with petroleum ether and water (1:1 ratio) and ethyl acetate and water (1:1 ratio) in two different separating funnels. The extract phase was taken filtered and dried.

D. Chloroform Extract from Crude Mixture

The crude mixture was partitioned with chloroform and water (1:1 ratio). The extract phase was filtered and dried.

3. QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS (SHAIK ET AL., 2020)

For the detection of alkaloids, to a few mL filtrates, 1-2 drops of *Wagner's reagent* (Along the sides of the test tube) are added.

For the detection of flavonoids, to a few ml of plant extract, conc. H2SO4 is added.

For the detection of phenolic compounds, a few ml of plant extract is dissolved in 5mL distilled water, to that 3mL of 10% lead acetate sol.

4. ANTI-GLYCATION ASSAY

Phosphate buffer – pH – 7.4, 0.1 M was prepared. Glycation of BSA is carried out by dissolving phosphate buffer to yield a final concentration of 10mg/ml. To the solution, 1M D-ribose was added to make a stock solution and mix thoroughly. Plant extract of 100µg/ml was treated with the reaction mixture to yield a final volume of 0.5ml. Solutions are incubated at 37°C for 10 days. After incubation samples were treated with thioflavin for 10 minutes. Final volume made up to 1ml using phosphate buffer.

Detection by Congo red-binding assay, 0.2% Congo red assay in phosphate buffer with 10% v/v ethanol. 100µL Congo red solution + 150µL sample + 320µL phosphate buffer. Absorbance is taken at 480nm and 560nm.

5. RESULTS

The current study was done to determine the potential of *Garcinia cambogia* in the antiglycation of ribose. The potential of *Garcinia cambogia* extracts in the inhibition of glycation reaction based on amyloid aggregation.

	Crude Mixture	Water Extract	Ethyl Acetate Extract	Petroleum Extract	Chloroform extract
Phenolic Compounds	-	+	+	+	+
Flavonoids	+	+	+	+	+

Table1: Results of qualitative analysis of phytochemicals performed for different extracts of *Garcinia cambogia*.

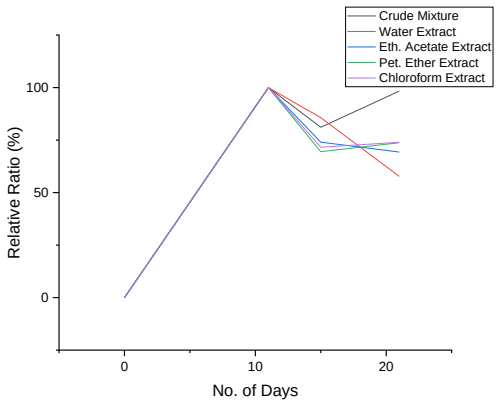


Figure1: Effect of different extracts on amyloid aggregation.

The qualitative analysis of phytochemicals provides the data that the plant extracts contain phenolic compounds and flavonoids, the detailed results are provided in Table1.

The congo red assay was performed to determine the formation of amyloid proteins. The intensity of congo red determines the binding of congo red to the aggregated proteins. The intensities of different extracts showed the inhibition of protein aggregation refers in Figure1. Here we can conclude that the water extract has the highest inhibition, reducing the glycation reaction by about 42.83%, followed by ethyl acetate extract which reduced to about 30.68%. Hence it can be concluded that the water extract has the highest inhibition property for the glycation reaction.

6. REFERENCES

- Akshay KR, Sudharani N, Back yard malabar tamarind (Garcinia Gummi- gutta): A miracle anti-obesity agent, 2018, Journal of Pharmacognosy and Phytochemistry.
- Shehwaz Anwar.Protective,Effects of Ginger Extract against Glycation and Oxidative Stress-Induced Health Complications: An In Vitro Study, 2020, Processes mdpi.
- E. A. H. E. Edirisinghe, Extraction, crystallization, preservtion, pelletizing and quantification of hydroxy citric acid from *Garcinia cambogia*, 2015, Research gate, Volume 2 Issue 4.
- H. N. Fernando, Synthesis, Characterization and Antimicrobial Activity of Garcinol Capped Silver Nanoparticles, 2019, J. Microbiol. Biotechnol.
- Su-Yen Goh and Mark E. Cooper, The Role of Advanced Glycation End Products in Progression and Complications of Diabetes ,2008, jcem.endojournals,.
- Priya Hargunani, *Garcinia cambogia*: an ancient fruit rind with recently discovered therapeutic activity, 2020, Journal of Medical Pharmaceutical and Allied Sciences.
- C. Hart1, I. E. Cock, An examination of the antimicrobial and anticancer properties of *Garcinia cambogia* fruit pericarp extracts, 2016, BEMS Reports, Vol 2, Issue 2.
- Kokkaiah Irulandi, Evaluation of Antioxidant Properties and Antimicrobial

Activity of *Garcinia cambogia*, 2016, Journal of Advanced Applied Scientific Research.
- Kokkaiah Irulandi, GC-MS analysis and phytochemical studies of methanolic fruits extract of *Garcinia cambogia* Hort. Ex Boerl and Ziziphus trinervia Roth, 2016, Journal of Advanced Applied Scientific Research.
- Kizhakedathil Moni Philip Jacob, Evaluation of Antibacterial and Antioxidant Activity of Garcinia gummigutta, 2015, International Journal of Drug Development and Research.
- Annette T. Leeasb and Anthony Cerami, Role of Glycation in Aging, 1992, the Rockefeller University Laboratory of Medical Biochemistry New York, New York 10021.
- M Lima and J W Baynes, Glycation, 2013, Elsevier.
- Hang Ma, The hydrolyzable gallotannin, penta-O-galloyl-β-D-glucopyranoside, inhibits the formation of advanced glycation endproducts by protecting protein structure, 2015, ReasearchGate.
- Madappa M.B1, A.K.Bopaiah, Preliminary phytochemical analysis of leaf of Garcinia gummigutta from Western Ghats, 2012, Journal of Pharmacy and Biological Sciences.
- Antioxidant, antiglycation and cytotoxicity evaluation of selected medicinal plants of the Mascarene Island, 2012, BMC Complementary and Alternative Medicine.
- Milena Masullo, Direct Interaction of Garcinol and Related Polyisoprenylated Benzophenones of *Garcinia cambogia* Fruits with the Transcription Factor STAT-1 as a Likely Mechanism of Their Inhibitory Effect on Cytokine Signaling Pathways ,2014, American Chemical Society and American Society of Pharmacognosy .
- HN Murthy, Garcinia gummi-gutta (L.) Robs: A promising feedstock for biodiesel production, 2017, International Journal of Green Energy.
- Basavaraj S, Simultaneous extraction of four different bioactive compounds from Garcinia indica and their enrichment using Aqueous Two-Phase Systems ,2019,Elseiver.
- Nguyen Hoai Nguyen, Antioxidant and Antimicrobial Activities of the Extracts from Different Garcinia Species, 2021, Evidence-Based Complementary and Alternative Medicine.
- Mahesh Mansing Patil1, Lipids and Fatty Acid Profiling of Major Indian Garcinia Fruit: A Comparative Study and its Nutritional Impact, 2016, Springer.
- Junaid R Shaikh and MK Patil, Qualitative tests for preliminary phytochemical screening: An overview, 2020, ResearchGate, International Journal of Chemical Studies.
- Ramalingam Sripradha, Efficacy of *Garcinia cambogia* on Body Weight, Inflammation and Glucose Tolerance in High Fat Fed Male Wistar Rats, 2015, Journal of Clinical and Diagnostic Research.
- Vishma B. L., Prashantha Naik, Sujayraj R. S, Studies on genotoxic, geno-protective and cyto-protective effects of Garcinia gummigutta extract against mitomycin C ,2017, Journal of Pharmaceutical and Biological Sciences .
- Sudharani N, Anti-microbial activity of garcinia gummigutta fruit rind, 2018, Journal of Pharmacognosy and Phytochemistry.

Isolation, Identification and Optimization of Debittering enzyme from microbial source.

Dikshitha S, Pooja S Yadav, Preethi HK, Sahana M and Dr Veena S More
Department of Biotechnology
Sapthagiri College of Engineering,
Bangalore,India
dikshithagowda32@gmail.com, poojayadav74861@gmail.com, preethihkgowda153@gmail.com,
msahana.harsha.20@gmail.com, veenasmore@gmail.com

Abstract

Bitterness is a major issue faced by many of the industry especially wine industry, food industry and pharmaceutical industry. Due to unpleasant taste of the bitter compound many of the consumers don't accept the product creating a huge barrier for many of the industries. Commercialization of citrus fruit juice is always obstruct by the development of bitterness in juice. Naringin is one of the bitter compound present in citrus fruits which are widely used in industries. This naringin can be broken down into acceptable and tasteless compounds by the action of enzyme Naringinase. This work is mainly focused on isolating the enzyme naringinase from the microbes present in the spoiled citrus fruits. The cultured microbes are further sub cultured n number of times to increase the enzyme production in microbes. Further enzymatic assay is carried out to determine the activity of the enzyme followed by strain improvement methods and enzyme characterization.

Keywords: Naringin, Naringinase, Naringenin, enzyme assay, Spoiled lemon and orange source, citrus fruit, Bitterness.

1. Introduction

The fruit juice industry has to come up with the varieties of raw materials that have many excellent attributes. Main aim of these fruit juice industries is about processing the fruits at the least possible price by maintaining the organoleptic quality and stability of the finished product [1]. Mainly the fruit juice industry use citrus fruits for it's medicinal properties such as antioxidant agents, anti-kidney stones and gall formation and anti-genotoxic effects [2]. But the major limitation in citrus fruits is bitterness property for their commercial acceptance [1][3]. Generally Citrus is a genus of

flowering tree and shrub in the family of rue, Rutaceae. Plants in the genus produce citrus fruits, including important crops such as oranges, lemons, grapefruits, pomelos, and limes [4]. Citrus fruits are commonly known as agrumes, which means "**sour fruits**". These citrus fruits are native to the tropical and subtropical regions of Asia. In 2015– 2016, the total production of citrus fruits was more than 124 million tons [5]. Citrus fruit juice is rich in vitamins, flavonoids, terpenoids and etc.; it plays an important role in health improvement as well as it reduces the risk of disease. Its flavor, essence and therapeutic values are the driving force for the overall development of juice economy [6].

The juice obtained after squeezing the citrus fruit contains Naringin, Limonin, and tannin which develop the bitterness [6]. The enzyme Naringinase degrades the bitterness present in citrus fruits and also its enzymes remove bitterness from edible substances. Conventional methods used to reduce the bitterness in citrus juices involve not only the removal of the bitter compounds, but also it removes bioactive components from the juice [7].

Naringinase is a debittering enzyme that catalyzes a hydrolytic reaction, chemically α-rhamnopyranosidase, expressed as α-L-rhamnosidase (E.C. 3.2.1.40) and β-D-glucosidase (3.2.1.21) acts as an active centers [8][9]. Naringinase enzyme breaks the compound Naringin that gives bitter taste for citrus fruit juices. Mechanism of action firstly includes rhamnosidase which breaks the Naringin into prunin and rhamnose, lastly glycosidase breaks prunin into glucose and naringenin which is flavorless, which is also found in various citrus [1][3]. Naringinase enzyme breaks rhamnosidase into Naringin which is a bitter flavonone glycoside responsible for the bitterness in citrus fruits, and it's a aglycone naringenin. Naringinase occurs widely in nature and it has important applications in the food, wine, bakeries and pharmaceutical industrial sector, steroid biotransformation, antibiotics, flavonoids, or glycolipids and deglycosylation of glycopeptide and Glycoside compounds like naringin, hesperidin, ter-phenyl glycosides, rutin, quercitrin, and, it has substrates namely terminal α-rhamnose and βglucose. For its medicinal properties such as improvement of the signaling pathway, anti-inflammatory, anticancer by inhibition of proliferation and promotion of cell apoptosis in tumor cells like breast cancer (TNBC) cells, against the liver diseases, human cervical cancer (SiHa)

cells and bladder cancer cell having a high commercial importance, it has beneficial effects in various neuronal diseases like Alzheimer's disease, Parkinson's disease and Epilepsy [2]. Many microorganisms produce Naringinase mainly fungal strains produces more Naringinase than bacterial strains. The fungal strains namely Aspergillus niger, Lasiodiplodia theobromae, A. oryzae, A. usamii, A. flavus, Cochiobolus miyabeanus, Coniothyrium diplodiella, Penicillium decumbens, Rhizopus nigricans and Rhizoctonia solani, and bacterial strains are Burkholderia cenocepacia, Clostridium stercorarium, Lactobacillus acidophilus, S. paucimobilis, and Thermomicrobium roseum and Bacillus species. Fungi while producing Naringinase enzyme takes a lengthy production time with the low growth rate, but the bacteria have a varity of beneficial advantages from an industry point of view but it is not stable as fungi, so they are poorly noticed [2].

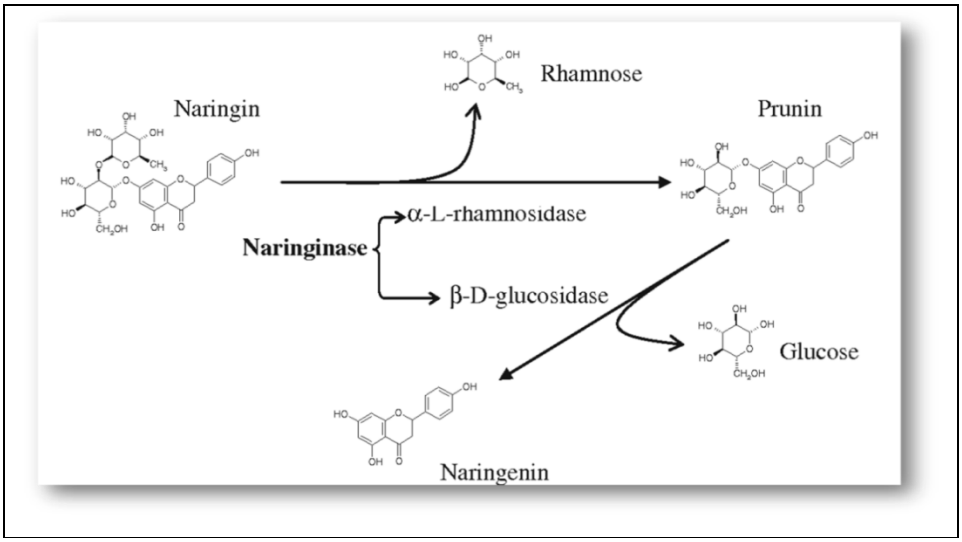


Fig1: Hydrolysis of α- L- rhamnosidase and β- D- glucosidase on naringin molecule and produce prunin, rhamnose, naringenin, and glucose

2. MATERIALS AND METHODS

2.1. Source

Spoiled lemon and orange were taken as source for microbes for the present study. These fruits were naturally spoiled in home for a week.

2.2. Chemicals

Naringin(pure,97%) and naringenin(pure,95%) was bought from Mudiyappa Scientific chemicals , Rajaji nagar Bangalore Karnataka .other chemicals required for media and other purpose were obtained from the Department of Bio technology, Sapthagiri college of Engineering, Bangalore.

2.3. Collection of samples

The fresh lemon and orange was brought and allowed to decay for two weeks, and taken as the source of microorganism [10].

2.4. Isolation of Naringinase producing bacteria

The steps followed to isolate the Naringinase producing bacteria are: primarily a small quantity of source (I.e., spoiled lemon and orange) were taken by using sterile loop and 1-2ml of distilled water was added and grinded using pestle and motor. On the other side medium was prepared to isolate the bacteria for the production of extracellular Naringinase by simple agar plate assay. Prepared a modified minimal Davis medium [MDN] of 100ml in 250ml of conical flask with a composition (g/100ml) of K2HPO4-0.7, KH2PO4- 0.2g, Sodium Citrate:0.05g, MgSo4: 0.01g, (NH4)2SO4: 0.1g, Naringin: 0.1g (0.1%) and Agar:1.5g. And adjust PH 7.0±0.2 later media is poured into two petri dishes labelled as lemon and orange. Then allowed to solidify, later the sample was added on to the media using spread plate method and incubated @ 28±2°C for 48 hrs. After 48hrs of incubation bacterial colonies are spotted on the agar plate. Continued with the sub culture with different concentrations of naringin. The same procedure was followed for the isolation of fungus.

After many rounds of subculture the concentration of Naringin was fixed to 1.5% for both bacteria and fungi [11].

2.5. Screening of microorganisms

Naringinase producing organism was screened by iodine vapours. The plates were exposed to iodine vapour for 15-20mins without lid. After 20mins zone of clearance around the microbial colonies producing naringinase enzyme was observed [11].

Fig.1: zone of clearance observed in microbes obtained from spoiled lemon source

Fig.2: zone of clearance observed in microbes obtained from spoiled orange source.

ISBN: 978-81-953973-3-8

2.6. Staining of bacteria

Gram's straining method was carried out to check the strains of bacteria. As a result the microbes cultured from spoiled orange and lemon samples were found to be gram positive cocci.

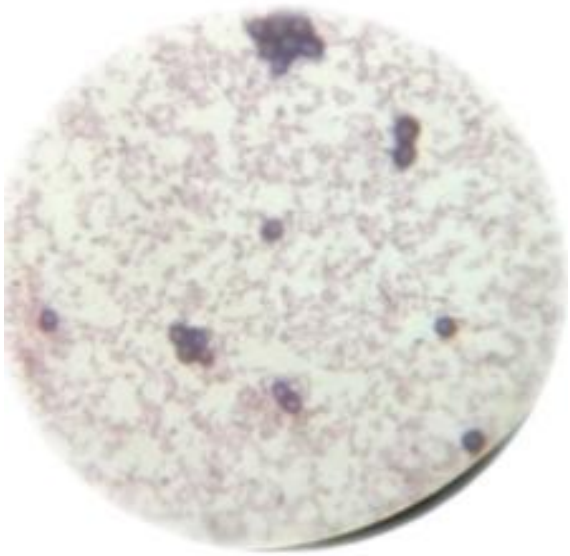


Fig.3: gram positive cocci (orange)

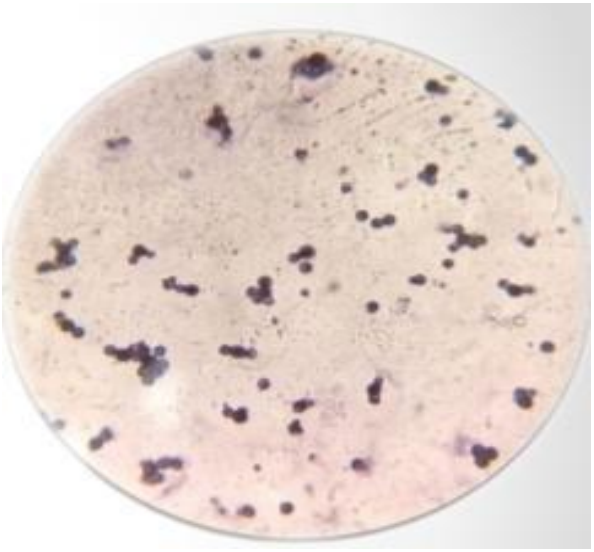


Fig.4: gram positive cocci (lemon)

2.7. Extraction of protein

In order to extract the enzyme from the broth media, a small amount of inoculated media containing the cells and enzyme was centrifuged at 10,000 rpm for 15 minutes and the supernatant was collected. The supernatant was further treated with chilled acetone in the ratio of 1:4 (supernatant:acetone) and kept in ice for a time of 1 hour then further it is centrifuged at 14,000 rpm for 15 minutes and the pellets obtained is dissolved in required buffer and used for further analysis.

2.8. Enzymatic Assay

An enzymatic assay was carried out to check the presence and activity of enzyme naringinase. The pellet dissolved in buffer is taken as the enzyme sample and a specified amount of naringin as a substrate is added. 0.4ml of enzyme sample followed by 1.6ml (80µg/ml) of naringin is added and incubated for 30 minutes at 50°C for the

reaction to take place. After the incubation period 1.6ml of 0.5N NaOH is added and then 2ml of 1% FeCl3 is added and left for 10 minutes at room temperature, after which the OD was measured at 422nm.
In blank instead of enzyme sample 0.4 ml of buffer is taken [12].

2.9. Naringin standard

A stock solution of naringin was prepared with acetate buffer of pH4 (0.1N) from the stock solution of naringin varying concentration of naringin was further added to different test tubes (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml). Then 2ml of buffer was added to each test tubes followed by 1ml of NaOH (0.5N) and then 1ml of Fecl3 (1%) was added to all the test tubes. Finally the OD was measured at 422nm [12].

3. Result and Discussion

Microbes were first cultured in the media with 0.1% Naringin concentration, later the concentration of naringin was further increased in the order of .5%, 1%, 1.5% and 2%. The microbes started adopting for the media provided and showed increase in their growth. Later the concentration of Naringin in the media provided was fixed to 1.5%. Many rounds of sub-culture were done to increase the growth of the microbes. Later the microbial sample obtained from the spoiled lemon source was only considered for further estimation. The cultured broth samples were collected centrifuged and the protein was extracted using chilled acetone. The extracted protein was considered as the enzyme sample and used for carrying out enzyme assay. After taking the readings of Optical Density (OD) at 422nm, they were used to find out the concentration of left out naringin which was added during the enzyme assay. As a result it was observed that there was decrease in the concentration of the naringin when compared to the blank sample were the enzyme sample was not added.

4. Conclusion

After carrying out the work and observing the results obtained, we like to conclude that, the microbial samples obtained from the spoiled lemon source were observed to utilize the naringin provided in the media as the carbon source. The protein extracted from this microbial source was able to decrease the concentration of the naringin. By this we like to conclude that the protein extracted may contain the enzyme which is responsible for degrading the naringin compound, such as Naringinase.

5. Acknowledgment

We like to acknowledge our guide for helping us to carry out this project. We also like to acknowledge every staff of Department of Biotechnology for cooperating with us. We want to express our gratitude for Sapthagiri College of Engineering for providing us the necessary requirements. We also like to extend our thanks to those who provided us required chemicals for our project.

6. Reference

1. Munish Puri, Uttam Chand Banerje, “ Production, purification, and characterization of the debittering enzyme Naringinase,” Elsevier Biochemical Engineering Research and Process Development Centre, Institute of Microbial Technology, Sector 39-A, Chandigarh 160036, India, 18 (2000) 207–217
2. Patil, Satish V., et al. "A novel screening method for potential naringinase-producing microorganisms." *Biotechnology and applied biochemistry* 66.3 (2019): 323-327.
3. Thammawat, Kanokpan, et al. "Isolation, preliminary enzyme characterization and optimization of culture parameters for production of naringinase isolated from Aspergillus niger BCC 25166." *Agriculture and Natural Resources* 42.1 (2008): 61-72.
4. Wu, Guohong Albert, et al. "Genomics of the origin and evolution of Citrus." *Nature* 554.7692 (2018): 311-316.
5. Gupta, Arun Kumar, et al. "Isolation and characterization of naringinase enzyme and its application in debittering of Pomelo juice (Citrus grandis): A comparative study with macroporous resin." *Journal of Food Processing and Preservation* 45.5 (2021): e15380.
6. Kundu, Debajyoti, Sandipan Karmakar, and Rintu Banerjee. "Simultaneous debittering and clarification of enzyme mediated mixed citrus juice production." *Applied Food Research* 2.1 (2022): 100031.
7. Busto, María D., et al. "Enzymatic debittering on antioxidant capacity of grapefruit juice." *Processing and impact on antioxidants in beverages*. Academic Press, 2014. 195-202.

8. Srikantha, Keerthini, Ranganathan Kapilan, and Vasantharuba Seevaratnam. "Characterization of best naringinase producing fungus isolated from the citrus fruits." *Int. J. Biol. Res* 4 (2016): 83.
9. Caraveo, Leonela, et al. "A simple plate-assay for screening extracellular naringinase produced by streptomycetes." *Journal of Microbiological methods* 102 (2014): 8-11.
10. .sinthuja karuppaija., Ranganathan kapilan., vasantharuba seevartnam., (2016). Characterization of best Naringinase producing fungus isolated from Palmyra (Barrasus flabellifer) fruit pulp, 4(2), 97-101.
11. Satish v patil., sunflower H koli., Bhavana V Mohite., Rahul patil., Robin R Patil., Hemant P Borase., Vikas S Patil. A Novel screening method for potential Naringinase producing Microorganisms.
12. Kabyashree phukan and David kardong (2020) Isolation of Naringinase producing soil bacteria from psidium guajava L. And terminalia chebula retz and its enzymatic activity, AIMS molecular science, 7(3): 292-304

Synthesis Characterization and Antimicrobial Studies on 5-(2-phenyl-2, 3-dihydro-1H- benzimidazole-yl) quinolin-8-ol and Transition Metal Complexes as a Biologically Active Pharmacophore.

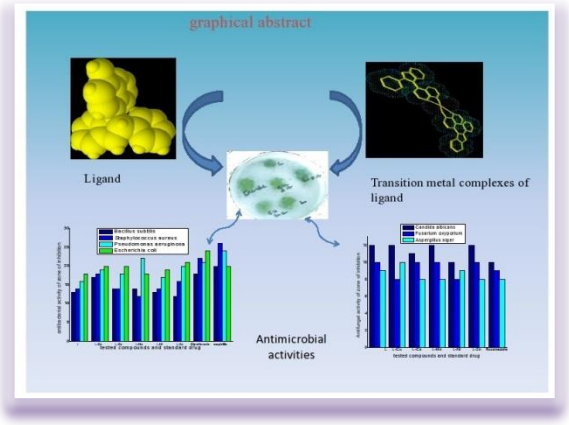
Anusuya A.M [1], **B.S Krishna** [1 a)],

1, 1 a), *Department of Chemistry,*
Sapthagiri College of Engineering
#14/5 Chikkasandra, Hesaraghatta Road, Bengaluru-560057
[1)] *Corresponding author: anusuyaam@sapthagiri.edu.in*

Abstract:

5-(2-phenyl-2,3dihydro-1H-benzimidazol-yl) quinoline-8-ol ligand and transition metals Copper, cobalt, nickel, zinc and manganese complexes prepared by condensation method. The synthesized ligand and its complexes characterized by various physicochemical and spectral characterization methods like IR, NMR, MASS, Uv-Visible and elemental analysis. The biological activity of the ligand and complexes screened for the antibacterial and antifungal studies for the selected bacterial and fungal strains by using cup plate disc diffusion method, inhibition zone of the tested compounds measured and tabulated the values, the synthesized compounds screened for antifungal activity of selected fungal strains..

Keywords: ***Chelating agent, alkylation, pharmacophore, antimicrobial, cup plate disc diffusion method, benzimidazole, gram positive bacteria, gram negative bacteria, fungal species*.**



I.INTRODUCTION

The foremost prominence of the researcher is to explore on biologically active and clinically significant moieties in the budding field of research, benzimidazole and it derivatives are the pleasing group of pharmacophore to meet the target of various microbial diseases due to their structural diversity(Salahuddin et al., 2017; Trotsko et al., 2020).anatomically benzimidazole is a fused structure of Benzene and imidazole containing chemically two different nitrogen reveals diverse action and biological activity helps to characterize such compounds in the study of microbial activity(Gürsoy et al., 2020). 8-hydroxyquinoline is the another part of reactant in the synthesis of ligand which is a versatile structural molecule acts as a good chelating agent complexes with biologically significant transition metal complexes plays an important role in the study of microbial activity and being used in various industrial, pharmaceutical, agricultural, metallurgical and biological applications(Martins et al., 2004). The coupling of benzene and 1,2-phenylenediammine is the key reaction step involved in getting the benzimidazole (Bildstein et al., 1999),the cyclisation and bond formation favored under acidic condition maintained in the synthetic procedure, the purity and the product formation confirmed by thin layer

II. MATERIALS AND METHODS:

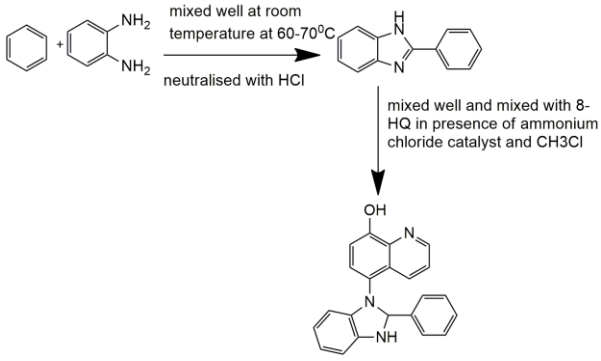
All the chemicals and reagents used were analytical grade procured from commercial suppliers and used without purification, IR spectra were recorded on FT-IR spectrometer KBr disc, UV-Visible spectra recorded by using UV-Visible double beam spectrometer software 6.89.9, 1H NMR were recorded in bruker (600MHz) by using DMSOd6 as a solvent, Mass spectra were recorded using maldi mass spectrometer IISER pune, melting points were determined by open capillary method, elemental analysis by using CHN analyzer & metal analyzed by standard laboratory method.

III. Experimental procedure:

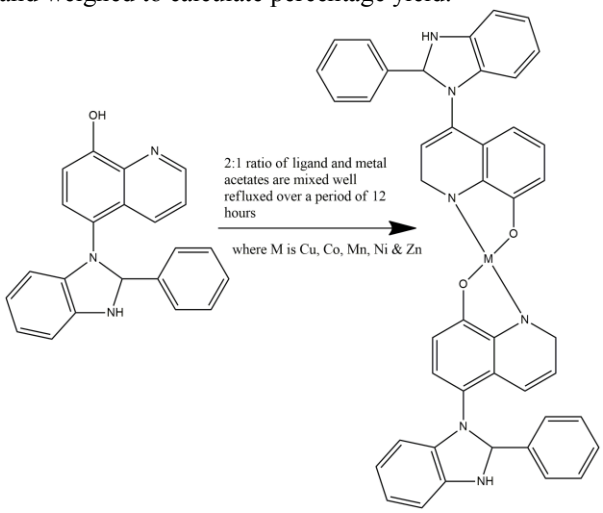
Synthesis of ligand 5-(2- phenyl-2, 3-dihydro-1H-benzimidazol-yl) quinoline-8-ol:

Equimolar ratio of 0.02mole of 1,2-phenylene diamine and 0.02 mole benzene are allowed to mix continuously at room temperature for one hour and neutralized with 5M HCl and this reacting mixture treated with 0.02mole of 8-hydroxyquinoline in presence of catalytical amount of ammonium chloride and kept at room temperature nearly for

12-18 hours. The formation and quality of the phenyl substituted benzimidazole derivatised 8-hydroxyquinoline compounds confirmed by TLC which is used with 1:1 ethyl acetate and hexane as an eluent, final product is recrystalised repeatedly by using 95% ethanol or acetone and then dried under reduced vacuum to calculate for the reaction yield(Arora, Pragi; Arora, Varun; Lamba, H. S.; Wadhwa, 2012; Imidazole Benzimidazole Synth., 1997).



Synthesis of copper, cobalt, nickel, zinc and manganese transition metal complexes: The synthesized phenyl substituted benzimidazole derivatised 8-hydroxy quinoline ligand mixed with metal acetates of copper, cobalt, nickel and zinc in the ratio of 2:1 mixed at room temperature subjected for condensation for a period of 12 hours, the precipitate formation confirmed through TLC, separated and recrystalised by repeatedly washing with ethanol, dried and weighed to calculate percentage yield.



IV: RESULTS AND DISCUSSION

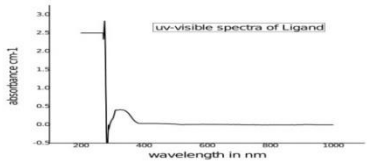


Fig.1: Uv-visible spectra of ligand 1

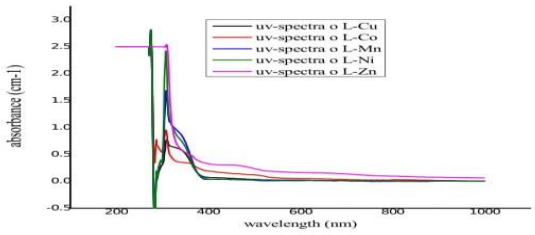


fig. 2: Uv-visible spectra of copper, cobalt, manganese, nickel and zinc

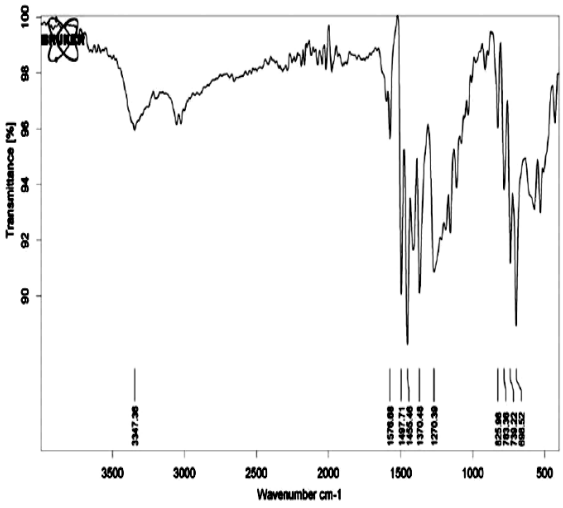


fig.3: IR spectra of ligand

v:Table1 physicochemical characterization of ligand and complexes elemental analysis

TABLE1:ELEMENTAL ANALYSIS OF LIGAND AND COMPLEXES							
Ligand & complexes	Formula weight	Yield percentage	Elemental analysis				
Ligand			C Calculate (found)	N calculated (found)	O calculated (found)	H calculated (found)	M calculated (found)
L-$C_{22}H_{17}N_3O$	339.39	76	77.86 77.84	12.38 12.36	4.71 4.76	5.05 5.01	
L-Cu $C_{48}H_{34}CuN_6O_2$	734.34	82	78.51 78.43	3.81 3.80	4.36 4.07	4.67 4.63	8.56 3.94
L-Co $C_{48}H_{34}CoN_6O_2$	729.20	80	79.00 78.99	3.84 3.83	4.39 4.38	4.70 4.66	8.08 3.70
L- Mn $C_{44}H_{34}MnN_6O_2$	733.21	78	72.03 72.67	11.45 10.82	4.36 4.12	4.67 5.32	7.49 3.40
L-Ni $C_{44}H_{34}N_6NiO_2$	736.21	69	71.66 71.71	11.40 11.77	4.34 4.30	4.65 4.61	7.96 3.80
L-Zn $C_{44}H_{34}N_6O_2Zn$	744.16	74	71.02 70.95	11.29 11.28	4.30 4.30	4.61 4.56	8.79 4.03

Sl no	Formula	Cationic adduct	Charge state	m/z
1	C45H34ZnN6O2	H+/-	+1	721.2816
2	C45H34ZnN6O2	Na+/-	+1	743.26354
3	C45H34ZnN6O2	K+/-	+1	759.23748
4	C45H34ZnN6O2	CH3OHH+/-	+1	753.30782
5	C45H34ZnN6O2	NH4+/-	+1	738.30815
Anionic adduct				
6	C45H34ZnN6O2	-/H+	-1	719.26705
7	C45H34ZnN6O2	Cl-/-	-1	755.24373
8	C45H34ZnN6O2	-2(H2OH+)	-2	356.11932
9	C45H34ZnN6O2	Br-/-	-1	769.19321

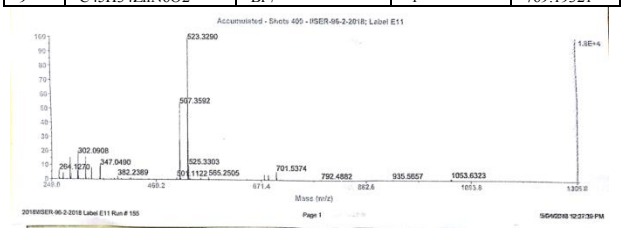


Fig.4: Mass spectra of L-Zinc complex

Spectral characterization of the ligand: 6-[(2-phenyl-2, 3-dihydro-1H-benzimidazol-1-yl)quinolin-8-ol: M.P218^{0}C-224^{0}C: colorless, Amorphous crystalline solid: Chemical formula: C23H16N2O: molecular weight: 339.39g/mole: IR (KBr pellets cm-1), 3307.36 cm-1(OH),1670.00(C=N), 1407.71cm-1(CH), 1370.45cm-1(C6H5), 825 cm-1(C-N), 783.36cm-1(C=C),739.23cm-1(C-C). UV-Visible: 290-310nm (n-Π*). Mass: m/z; 339.39(100%), 340.14(24.0%), 341.14(3.2%).

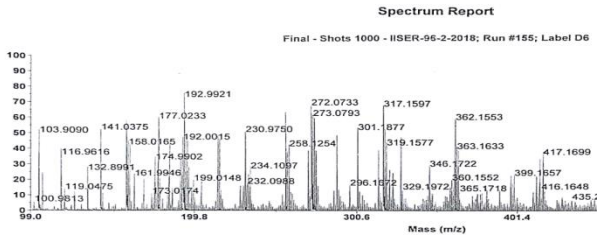


Fig.5: mass spectra of ligand

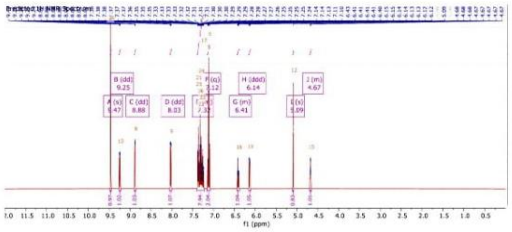


Fig. 6: NMR spectra of ligand

ANTIMICROBIAL STUDIES OF LIGAND & COMPLEXES:

Validation and evaluation of the biological action of synthesized compounds are the prime importance for the biologically active pharmacophore, research on new antimicrobial agents and its pharmacological actions of small molecules major source of importance in the synthetic chemistry and heterocyclic chemistry, even today majority of antimicrobial agents are the natural source but the major issues lies in their systematic research and their biological application for the benefit of mankind, so in this view there is a vast importance for the synthesis

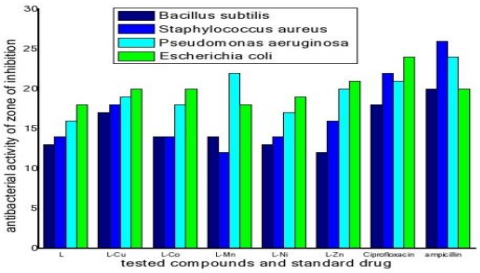
Antibacterial activity of ligands and complexes

Tested compounds	Gram positive bacteria				Gram negative bacteria			
	Bacillus subtilis		Staphylococcus aureus		Pseudomonas aeruginosa		Escherichia Coli	
	50mg/mL^{-1}	100 mg/mL^{-1}	50mg/mL^{-1}	100mg/mL-1	50mg/mL-1	100mg/mL-1	50mg/mL-1	100 mg/mL^{-1}
L	10	14	14	20	16	18	16	20
L-Cu	12	16	15	22	19	22	14	22
L-Co	12	18	12	18	18	20	18	23
L-Mn	10	13	12	16	10	16	12	18
L-Ni	13	17	14	20	17	22	16	21
L-Zn	12	15	16	23	20	23	15	18
Ciprofloxacin	18	20	22	21	21	22	20	20
ampicillin	20	24	26	22	24	20	18	19

of innovative and potential pharmacophore followed by simple convenient well known procedure. The synthesized ligand and copper, cobalt, manganese, nickel and zinc complexes

Screened for preliminary antimicrobial studies, gram-ve and gram +ve bacterial cultures used for antibacterial studies and selected fungal cultures Used for antifungal studies (Gudasi et al., 2008).

The antibacterial and antifungal activities were carried out by the cup plate disc diffusion method and the typical procedure is refereed, molten agar prepared and kept at 450C and then poured in to petridishes allowed to solidify. Holes of 6mm diameter were punched using sterile cork borer and then all the holes were completely filled with the prepared solution of the tested compounds of the concentration 50 and 100 mgmL-1 in DMSO solution followed by 24hrs of incubation at 370C.The diameter of the inhibition zone measured(in terms of millimeter) for all the compounds and compared with the standard drug ampicillin of the same concentration referred in the procedure under standard sets of condition as reported in the table2(Dharmaraj et al., 2001; Gürsoy et al., 2020; Hernández-Hernández et al., 2020; Penta, 2016; Riswan Ahamed et al., 2014; Saini et al., 2013).



Antifungal activity of ligand and complexes

Tested compounds	Candida albicans		Fusarium oxyporium		Aspergillus niger	
	50mg/mL^{-1}	100mg/mL^{-1}	50mg/mL^{-1}	100mg/mL^{-1}	50mg/mL-1	100 mg/mL^{-1}
L	10	12	12	14	9	12
L-Cu	12	14	8	10	10	14
L-Co	11	12	10	12	8	13
L-Mn	12	14	10	11	8	11
L-Ni	10	13	8	10	9	12
L-Zn	12	15	10	12	8	13
fluconazole	20	22	20	24	18	24

Antifungal studies: antifungal activity of the ligand and complexes was carried out against Candida albicans, Fusarium oxyporium, aspergillus Niger by the typical cup plate disc diffusion method, potato dextrose agar medium prepared desired for the experiment. The plates were incubated at 37^0C for about 48hrs, the diameter of the inhibition zone measured in millimeter and compared with the standard drug fluconazole of the same concentration as referred in the procedure under identical condition.(Afrin Dalia et al., 2018; Gaál et al., 2018; Gaikwad et al., 2016; Gänzle, 2014; Malathi et al., 2009; Noble and Anderson, 2013; Oshin and Thapa, 2015; Sunita et al., 2017)

ACKNOWLEDGMENT: our sincere thanks to IISER Hyderabad for providing spectral characterization and Sapthagiri institute of medical science, biochemistry department for providing bacterial and fungal strains.

REFERENCE:

1.S.Afrin Dalia, , B.Farhana Afsan, , B.Md Saddam Hossain, , M. Nuruzzaman Khan, , B.Md Kudrat-E-Zahan, , B.Md Mahasin Ali, , C. Md Kudrat-E-Zahan, , F. Afsan, , M.Saddam Hossain, , C.Zakaria, , M. Kudrat-E-Zahan, , M.Mahasin Ali, , 2018. A short review on chemistry of schiff base metal complexes and their catalytic application. ~ 2859 ~ Int. J. Chem. Stud.

2. Y.K. Al-Majedy, , A.A.H. Kadhum, , A.A.Al-Amiery, , A.B.Mohamad, , 2017. Coumarins: The Antimicrobial agents. Syst. Rev. Pharm. 8, 62–70. https://doi.org/10.5530/srp.2017.1.11.

3.Y. Álvarez-Gallego, , S.P. Nunes, , A.E.Lozano, , J.G.de la Campa, , de Abajo, J., 2007. Synthesis and Properties of Novel Polyimides Bearing Sulfonated Benzimidazole Pendant Groups. Macromol. Rapid Commun. 28, 616–622. https://doi.org/10.1002/marc.200600700.

4.Arora, Pragi; Arora, Varun; Lamba, H. S.; Wadhwa, D., "IMPORTANCE OF HETEROCYCLIC CHEMISTRY: A REVIEW | INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH," INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH | IJPSR, Sep. 01, 2012. https://doi.org/10.13040/IJPSR.0975-8232.3(9).2947-54 (accessed Jun. 08, 2022).

5.M. Baumann, I. R. Baxendale, S. V. Ley, and N. Nikbin, "BJOC - An overview of the key routes to the best selling 5-membered ring heterocyclic pharmaceuticals," BJOC - An overview of the key routes to the best selling 5-membered ring heterocyclic pharmaceuticals. https://doi.org/10.3762/bjoc.7.57 (accessed Jun. 08, 2022).

6.B. Bildstein, , M. Malaun, , H. Kopacka, , Ongania, K.-H., K.Wurst, , 1999. N-Heterocyclic carbenes with N-ferrocenyl-N′-methyl-substitution: synthesis, reactivity, structure and electrochemistry. J. Organomet. Chem. 572, 177–187. https://doi.org/10.1016/S0022-328X(98)00943-7.

7.B.Blom, , M. Stoelzel, , M.Driess, , 2013. New Vistas in N-Heterocyclic Silylene (NHSi) Transition-Metal Coordination Chemistry: Syntheses, Structures and Reactivity towards Activation of Small Molecules. Chem. - A Eur. J. 19, 40–62. https://doi.org/10.1002/chem.201203072.

8. N. Dharmaraj, P. Viswanathamurthi, and K. Natarajan, "Ruthenium(II) complexes containing bidentate Schiff bases and their antifungal activity - Transition Metal Chemistry," SpringerLink, Feb. 01, 2001. https://doi.org/10.1023/A:1007132408648 (accessed Jun. 08, 2022).

9.A.Gaál, , V.G.Mihucz, , S.Bősze, , I. Szabó, , M.Baranyi, , P.Horváth, , Streli, C., Szoboszlai, , 2018. Comparative in vitro investigation of anticancer copper chelating agents. Microchem. J. https://doi.org/10.1016/j.microc.2016.12.007

10.V.K.Gaikwad, , Urmila, &, M.Yadav, , 2016. Metal Complexes of Schiff Bases. Sch. Res. J.

11. M.G. Gänzle, , 2014. Enzymatic and bacterial conversions during sourdough fermentation. Food Microbiol.37,2–1 https://doi.org/10.1016/j.fm.2013.04.007

12. K.B.Gudasi, M.S.Patil, , R.S.Vadavi, , 2008. Synthesis, characterization of copper(II), cobalt(II), nickel(II), zinc(II) and cadmium(II) complexes of [7-hydroxy-4-methyl-8- coumarinyl]glycine and a comparitive study of their microbial activities. Eur. J. Med. Chem. https://doi.org/10.1016/j.ejmech.2008.01.028.

13. E.Gürsoy, , E.D.Dincel, , L.Naesens, , N.Ulusoy Güzeldemirci, , 2020. Design and synthesis of novel Imidazo[2,1-b]thiazole derivatives as potent antiviral and antimycobacterial agents. Bioorg. Chem. 95, 103496. https://doi.org/10.1016/j.bioorg.2019.103496

14.J.G.Hernández-Hernández,,Aguilar,C.A.H.,Thangarasu, P., Hernández-Trujillo, J., 2020. Photochemical and antibacterial properties of ruthenium complex of N, N'-bis(benzimidazole-2yl-ethyl)ethylenediamine under visible light: Experimental and theoretical studies. J. Mol. Struct. 1203,1273-77. https://doi.org/10.1016/j.molstruc.2019.127377

15. S.S.Jawoor, S.A.Patil, , M.Kumbar, , P.B. Ramawadgi, 2018. Green synthesis of nano sized transition metal complexes containing heterocyclic Schiff base: Structural and morphology characterization and bioactivity study. J. Mol.Struct.1164,378–385. https://doi.org/10.1016/j.molstruc.2018.03.084

16. Z.E.Koc, H. Bingol, A.O.Saf, , E.Torlak, , A.Coskun, 2010. Synthesis of novel tripodal-benzimidazole from 2,4,6-tris(p-formylphenoxy)-1,3,5-triazine: Structural, electrochemical and antimicrobial studies. J. Hazard. Mater.183,251–255. https://doi.org/10.1016/j.jhazmat.2010.07.018

17. M.Malathi, P.S. Mohan, R.J.Butcher, C.K.Venil, , 2009. Benzimidazole quinoline derivatives — An effective green fluorescent dye for bacterial imaging. Can. J. Chem. 87, 1692–1703. https://doi.org/10.1139/V09-139

18. M. A. Malik, O. A. Dar, P. Gull, M. Y. Wani, and A. A. Hashmi, "Heterocyclic Schiff base transition metal complexes in antimicrobial and anticancer chemotherapy - MedChemComm (RSC Publishing)," Heterocyclic Schiff base transition metal complexes in antimicrobial and anticancer chemotherapy - MedChemComm (RSC Publishing), Dec. 06, 2017. https://doi.org/10.1039/C7MD00526A (accessed Jun. 08, 2022).

19.A.O.Martins, E.L.da Silva, E.Carasek, , N.S.Gonçalves, , Laranjeira, M.C.M., de Fávere, V.T., 2004. Chelating resin from functionalization of chitosan with complexing agent 8-hydroxyquinoline: application for metal ions on line preconcentration system. Anal. Chim. Acta 521, 157–162. https://doi.org/10.1016/j.aca.2004.06.033

20. A.Noble,J.C.Anderson, 2013. Nitro-Mannich Reaction. Chem.Rev.113,2887–2939.

https://doi.org/10.1021/cr300272t

21. S.Oshin, A.Thapa, 2015. Schiff base metal complexes of Ni, Pd and Cu. J. Chem. Pharm. Res.

22. S.Penta, 2016. Antimicrobial Agents, in: Advances in Structure and Activity Relationship of Coumarin Derivatives. Elsevier, pp. 9–45. https://doi.org/10.1016/B978-0-12-803797-3.00002-3

23. M.G Rabbani,., H.M.El-Kaderi, , 2012. keSynthesis and Characterization of Porous Benzimidazole-Linked Polymers and Their Performance in Small Gas Storage and Selective Upta. Chem. Mater. 24, 1511–1517. https://doi.org/10.1021/cm300407h

24. N. Raman, A. Kulandaisamy, C. Thangaraja, P. Manisankar, S. Viswanathan, and C. Vedhi, "Synthesis, structural characterisation and electrochemical and antibacterial studies of Schiff base copper complexes - Transition Metal Chemistry," SpringerLink, Mar. 01, 2004. https://doi.org/10.1023/B:TMCH.0000019409.50574.0a (accessed Jun. 08, 2022).

25. M. A. Riswan Ahamed, R. S. Azarudeen, N. M. Kani, and Hindawi, "Antimicrobial Applications of Transition Metal Complexes of Benzothiazole Based Terpolymer: Synthesis, Characterization, and Effect on Bacterial and Fungal Strains," Antimicrobial Applications of Transition Metal Complexes of Benzothiazole Based Terpolymer: Synthesis, Characterization, and Effect on Bacterial and Fungal Strains, Sep. 14, 2014. https://doi.org/10.1155/2014/764085 (accessed Jun. 08, 2022).

26. M. J. Saif and K. R. Flower, "A general method for the preparation of N-heterocyclic carbene–silver(I) complexes in water - Transition Metal Chemistry," Springer Link, Nov. 06, 2012. https://doi.org/10.1007/s11243-012-9667-3 (accessed Jun. 08, 2022).

27. M.S.Saini, A. Kumar, J.Dwivedi, , R.Singh, , 2013. a Review : Biological Significances of Heterocyclic Compounds . Int. J. Pharma Sci. Res.

28. R. P. Saini, V. Kumar, A. K. Gupta, and G. K. Gupta, "Synthesis, characterization, and antibacterial activity of a novel heterocyclic Schiff's base and its metal complexes of first transition series - Medicinal Chemistry Research," Springer Link, Jun. 15, 2013. https://doi.org/10.1007/s00044-013-0657-6 (accessed Jun. 08, 2022).

29.Salahuddin, M.Shaharyar, , A.Mazumder, , 2017. Benzimidazoles: A biologically active compounds. Arab. J.Chem.10,S157–S173. https://doi.org/10.1016/j.arabjc.2012.07.017

30. M. J. Sever and J. J. Wilker, "Visible absorption spectra of metal–catecholate and metal–tironate complexes - Dalton Transactions (RSC Publishing)," Visible absorption spectra of metal–catecholate and metal–tironate complexes - Dalton Transactions (RSC Publishing), Feb. 27, 2004. https://doi.org/10.1039/b315811j (accessed Jun. 08, 2022).

31. R.A.Shastri, I.N.Witwit, Z.Y.Motaweq, H.M.Mubark, , S.Yadav, B.Narasimhan, , S.M Lim, K. Ramasamy, , M.Vasudevan, M., Shah, S.A.A., Mathur, A., Maleki, B., Keshvari, H., Mohammadi, A., Tahlan, S., Kumar, S., Narasimhan, B., Patel, R.B., Desai, P.S., Desai, K.R., Chikhalia, K.H., 2013. Synthesis and biological screening of some novel 2-(2-benzisoxazol-3-yl) ethyl)-1Hbenzimidazoles. Indian J. Chem. - Sect. B Org. Med. Chem. 52, 160–163. https://doi.org/10.1016/j.ejbas.2017.11.001

32. M. Sunita, B. Anupama, B. Ushaiah, C.Gyana Kumari, , 2017. Synthesis, characterization, DNA binding and cleavage studies of mixed-ligand copper (II) complexes. Arab. J.Chem. https://doi.org/10.1016/j.arabjc.2014.01.017.

33.M. B. Swami, A. H. Jadhav, S. R. Mathpati, H. G. Ghuge, and S. G. Patil, "Eco-friendly highly efficient solvent free synthesis of benzimidazole derivatives over sulfonic acid functionalized graphene oxide in ambient condition - Research on Chemical Intermediates," SpringerLink, Sep. 20, 2016. https://doi.org/10.1007/s11164-016-2745-y (accessed Jun. 08, 2022).

34. P.Tempest, Ma, V. Thomas, S., Hua, Z., Kelly, M.G., Hulme, C., 2001. Two-step solution-phase synthesis of novel benzimidazoles utilizing a UDC (Ugi/de-Boc/cyclize) strategy. Tetrahedron Lett. 42, 4959–4962. https://doi.org/10.1016/S0040-4039(01)00919-4.

35.N.Trotsko, J.Golus, , P.Kazimierczak, A.Paneth, A.Przekora, G.Ginalska, , M.Wujec, , 2020. Bioorganic Chemistry Design , synthesis and antimycobacterial activity of thiazolidine-2 , 4-dione- based thiosemicarbazone derivatives. Bioorg. Chem. 97, 103676. https://doi.org/10.1016/j.bioorg.2020.103676.

36. J.Zhang, Xu, , L.Wong, W.-Y., 2018. Energy materials based on metal Schiff base complexes. Coord. Chem. Rev. 355, 180–198. https://doi.org/10.1016/j.ccr.2017.08.007.

A Novel Separation Method for Isolating Dissolved and Suspended Solids in Dairy Waste

Bhagyashree.R[1], Varsha.K[1], Allwin Ebinesar J S S[1*]

(1) Students, Department of biotechnology, Sapthagiri college of Engineering, Banglore-560057

(1*) Associate Professor, Department of biotechnology, Sapthagiri college of Engineering, Banglore-560057

Email id : bhagyabillav@gmail.com, harshithapsharsha@gmail.com, varshagowda353@gmail.com

Corresponding Author: allwinjss@sapthagiri.edu.in

ABSTRACT

Dairy plants consist of huge amount of clean water. Around 80% of clean water gets convert into waste water which leads to the environmental problem. Discharging dairy waste water without treatment even can cause public health problem. Moringa oleifera (MO) pods have been employed as an inexpensive and effective sorbent for the removal of organics, and coagulant for water treatment. It is a non-toxic natural organic polymer. Seeds of the plant species Moringa oleifera contain natural polyelectrolyte which can be used as coagulants to clarify turbid waters [1]. The objectives of this work was to Study the effect of Moringa olifera dosage, study the effect of MO on different concentration of dairy effluents and study the stability of MO on dairy effluent. From the experiment conducted it was found that the optimum dosage for 1% of dairy effluent is 0.4g and pH remained unchanged.

1. INTRODUCTION

Due to high pollution load of dairy waste water, the milk processing industries discharging untreated/partially treated wastewater cause serious environmental problem. Many coagulants are widely used in conventional water treatment processes, based on their chemical characteristics. The most common used coagulant in water and wastewater treatment are the inorganics that are trivalent aluminum and iron salts, despite the performance and cost effectiveness of these coagulants proven, there is still certain amount of residual aluminum content after treatment. It has recently been the subject of discussion, due to be evidence that alzheimer's disease may be linked to aluminum present in water intended for human consumption. [2] In recent years, there has been considerable interest in the development of natural coagulant such as Moringa oleifera. By using natural coagulants, considerable savings in chemicals and sludge handling cost may be achieved, MO seed kernels are biological coagulant consisting of significant quantities of low molecular weight water-soluble proteins, which in solution carry an overall positive charge. Turbidity removal is one of the important step in a water treatment process,which is generally achieved using coagulants.Dairy Wastewater treatment methods include precipitation,coagulation, sedimentation,filtration, membrane process, electrochemical techniques, ion exchange,

biological process, and chemical reactions. Each method has its own merits and limitations in applications because of their cost. A large number of cheaper materials including industrial and agricultural wastes have been used to remove different pollutants from the industrial effluents for their safe disposal into the biosphere [3]. If the particulates are removed and the sludge that is generated is proven to be non-hazardous by analysis, then this sludge may be used as a fertilizer and/or soil conditioner after stabilization [4].

2. MATERIALS AND METHODS

2.1 Sample preparation

The dairy effluent of different concentration was prepared by diluting the milk with water

2.2 Preparation of coagulant:

Moringa oleifera seeds were dried at 40 0C temp and the seed coat was removed manully, Seeds were powered and sieved through a 150 mm sieve. This MO seed powder was used for the coagulation study.

2.3 Turbidity measurement

Turbidity was measured based on Nepholometric method. This method is based on a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered light, the higher turbidity. Formazin polumer was used as the primary standard reference suspension is defined as 4000 NTU. Turbidity meter calibration followed the manufacturer's operating instruction. Calibrated the instrument by using distilled water taking in standard test tube by adjusting zero reading. Fixed the range to be used. For reading measurement, water sample for which the turbidity was to be measured was placed in the turbidity meter and the reading was noted. The instrument of turbidity meter should be warmed by starting switch before 15-20 minutes.

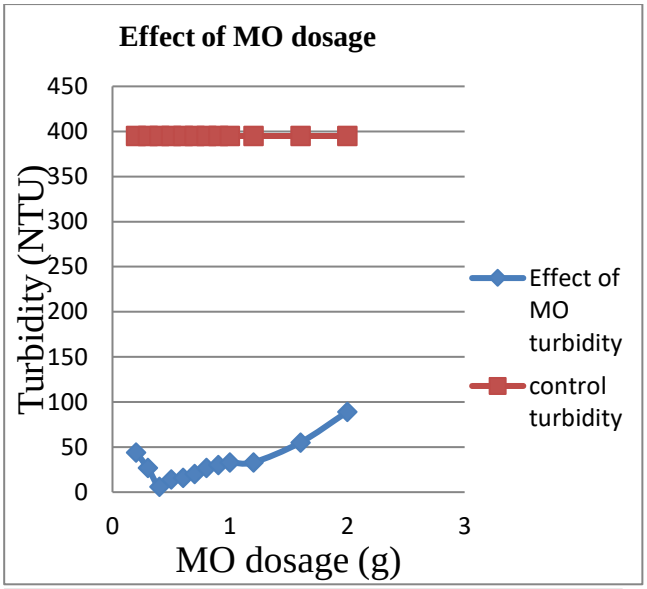
2.4 Protein estimation

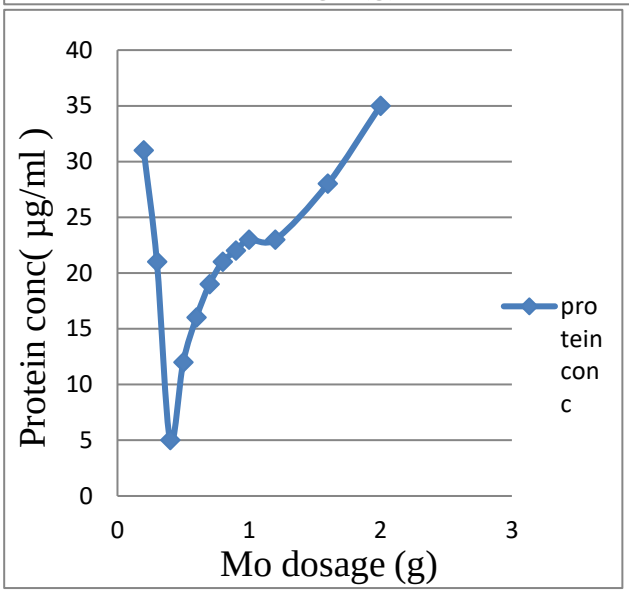
The concentration of protein present in the sample after treatment with MO was estimated using lowry's method of protein estimation. In this method the peptide nitrogen with copper ions under alkaline conditions and subsequent reduction of Folin-ciocalteay phosphomolybdicphosphotungstic acid to heteropolymolybdenum blue by copper catalyzed oxidation of aromatic acids. The blue colour developed is measured at 660nm using uv spectrophotometer.

Fig 1: schematic representation of coagulant preparation [5]

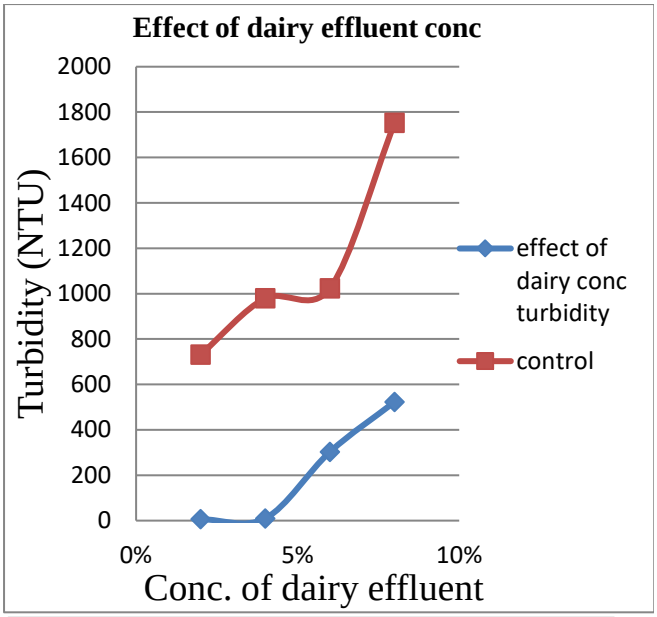
3. EXPERIMENTAL PROCEDURE

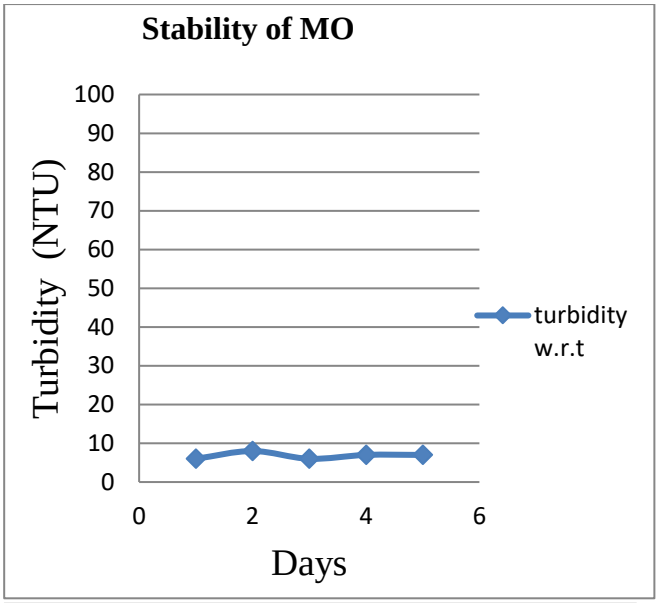
In this experiment MO seed powder was used as the coagulant for the treatment of dairy effluent. since the coagulation is dependent on the type of coagulant used, dairy concentration, dosage of coagulant and stability of coagulant we carried out three different experiments to determine the same.The effect of MO dosage was carried out by keeping 1% dairy effluent as constant and by varying the dosage of MO from 0.2 to 2g and the turbidity and protein concentration was observed for different dosage of MO. This experiment was carried out to determine the optimum dosage of MO that is used for coagulation and it was found to be 0.4g

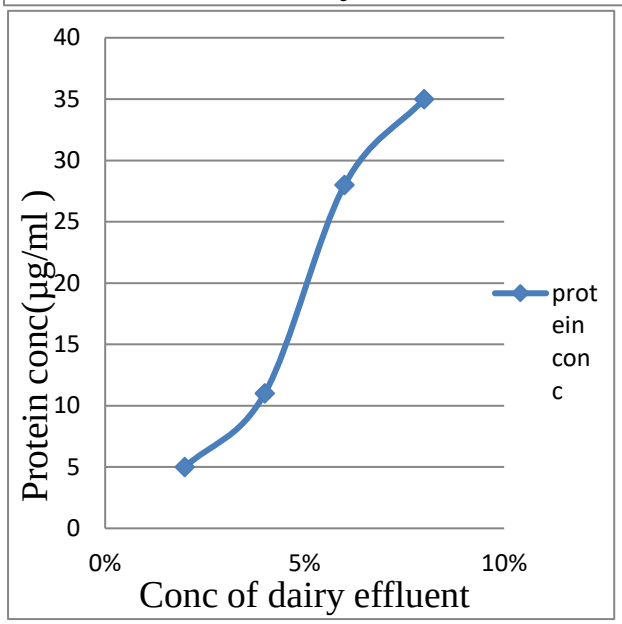


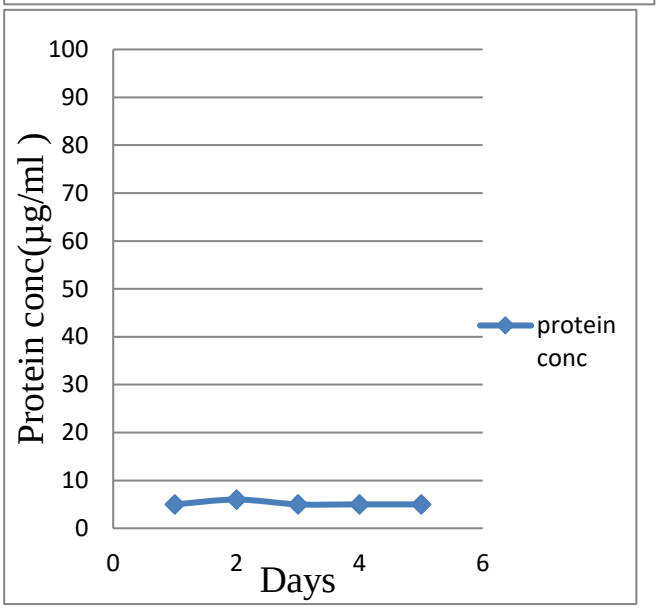


In the effect of dairy effluent concentration experiment the dosage of MO was constant (0.4g) and the dairy effluent concentration was varied, this was done to check until what concentration of dairy effluent the optimum dosage was effective. From the obtained results we found that optimum dosage (0.4g) was effective up till 2% of dairy effluent concentration by obtaining a low turbidity of 6NTU.









The stability of MO was checked to observe if the coagulant properties changed with respect to days.This experiment was done for 5 days and there was no difference in turbidity and protein concentration of the treated water.

For the effect of pH experiment the turbidity and protein concentration was measured by varying the pH from 8-3 without considering the natural coagulants and was found that at pH4 the turbidity was 33NTU which was found to be greater than the turbidity obtained after treating with natural coagulant i.e.6NTU.For all the 3 experiment that was carried out using natural coagulant there was no change in pH of the treated dairy water which is considered to

be one of the major advantage of using MO as coagulant

4. MECHANISM OF ACTION

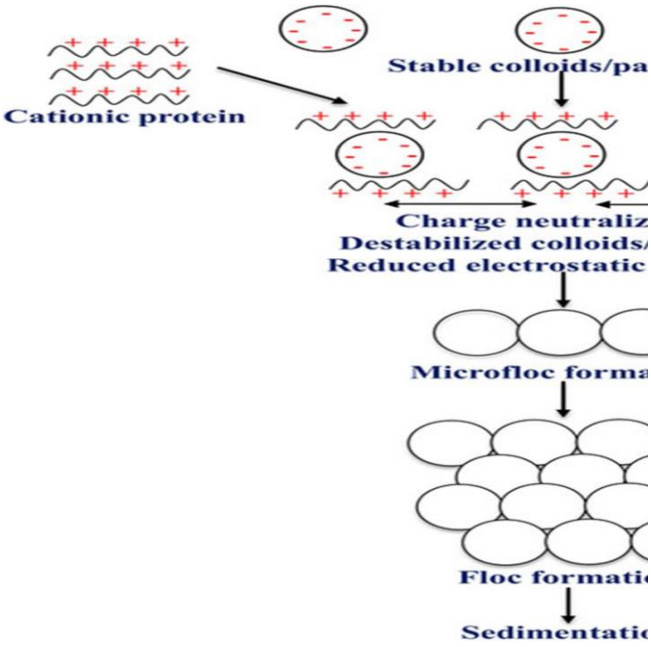


Fig 2: Coagulation mechanism for MO cationic proteins [5]

5. CONCLUSION

Dairy wastewater treatment using MO seed flour is a highly interesting alternative method in developing countries, specifically for small wastewater treatment plants. Of the several advantages of using MO seed flour, high cost-efficiency and low toxicity are the most important. Using MO as natural coagulant the lowest turbidity and protein concentration was attained without change in pH, therefore we can say that natural coagulant as an added advantage over other chemical methods hence by improving the application of MO seed we can replace the chemical coagulant in dairy waste water treatment.

6. REFERENCES

[1] Parmar, Kokila, Dabhi, Yogesh, Patel, Rinku and Prajapati, Sarju. Effectiveness of Moringa oleifera as natural coagulant aid for waste water treatment of dairy industry. Asian J. Environ. Sci., 7 (2):167-171, 2012.

[2] Mehtab hakim khan, shivshant chandrakant hugar, nitin premsukh khatmode. Removal of dairy waste water characteristics by using natural and chemical coagulant. Asian Journal of Convergence in Technology Volume III, Issue I Issn No.:2350-1146

[3]Akhtar M., Moosa, Hasany S, Bhanger M.I, Iqbal S. Sorption potential of Moringa oleifera pods for the removal of organic pollutants from aqueous solutions. Journal of Hazardous Materials, 141(3): 546–556, 2007.

[4] Bhuptawat H, Folkard G. K, Chaudhari S. Innovative physico-chemical treatment of wastewater incorporating Moringa oleifera seed coagulant. J. Hazardous Materials, 142(1-2) : 477–482. 2007.

[5] Deborah L. Villaseñor-Basultoa, Pablo D. Astudillo-Sáncheza, Jorge del Real-Olverab, Erick R. Bandalac. Wastewater treatment using Moringa oleifera Lam seeds: A review. Journal

of Water Process Engineering, pp. 151–164, 2018 .

[6] Dr. Anila George 1, Jensy Roshan. F2, Dr. Jude Emmanuel3 .Moringa oleifera- A Herbal Coagulant for Wastewater Treatment International Journal of Science and Research (IJSR) Volume 5 Issue 7, 2016

[7] Anselme ndabigengesere i, k. subba narasiah and Brian g. Talbot .Active agents and mechanism of coagulation of turbid waters using moringa oleifera , Water Research, volume 29, pp. 703-710, 1995

[8] M.Maheswari, Prashanth.S, Arunachalam.J, Jayaprakash.E, Manigandan.P.M. An experimental study on treatment of dairy effluents using natural coagulants to reduce turbidity, International Journal of Applied Environmental Sciences, Volume 11, pp. 1469-1486 , 2016