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A
Compendium of Research Articles by Prospective Researchers
Volume-VI
2023-24

Khandesh College Education Society's
Post Graduate College of Science, Arts & Commerce, Jalgaon

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Compendium of ResearchArticles by Prospective Researchers

2023-24

Volume-VI

Under the

Prospective Researchers' Scheme (PRS)

Recognized by Govt. of Maharashtra vide G. R. No. NGC 2010/247/10 & Affiliated to K. B. C.North Maharashtra University, Jalgaon

Khandesh College Education Society's

Post Graduate College of Science, Arts and Commerce, Jalgaon

Accredited 'B+' Grade by NAAC with CGPA 2.52 in 1st cycle

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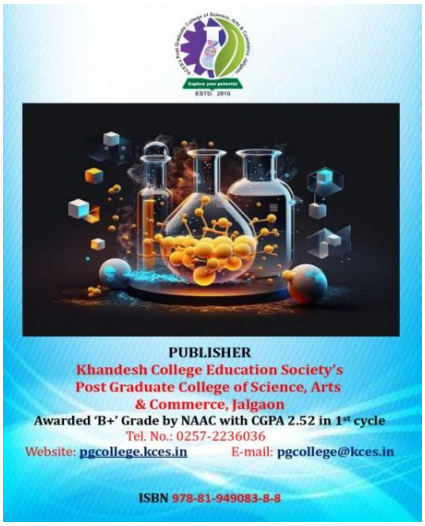

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दिनांक 20/02/2025

FOREWORD

It is a matter of great pleasure that KCE Society's Post Graduate College of Science, Arts & Commerce, Jalgaon is doing a commendable job in inculcating the scientific attitude amongst the students by publishing a **'Compendium of Research Articles by Prospective Researchers'** (Volume VI) with an ISBN number under the Researchers' Promotion Scheme for the post graduate students.

It is an appropriate platform for the students of the college for creation of innovative ideas on awareness of various research activities. Such a venture will go a long way to create a conducive research environment amongst the student community. I am sure that this volume touches upon latest research in the field of science and technology which is encouraging for all those aspirants and interested in science education and research.

It is really appreciable that the content of the volume is creative, academic and inspiring reflected by scholarly articles by the budding researchers. I take this opportunity to congratulate the principal, coordinator, supervisors and students for their contribution for this compendium.

My best wishes to 'A Compendium of Research Articles by Prospective Researchers' (Volume VI).

KHANDESH COLLEGE EDUCATION SOCIETY ★ JALGAON

(Mr. N. G. Bendale)
President, KCE Society, Jalgaon

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PREFACE

It is a matter pride for me that our college is publishing the **compendium of Volume VI** containing full length research articles prepared by the students under the guidance of our expert teachers. This compendium is outcomes of the research projects completed by the students during 2023-24.

The Research plays important role in the overall development of the nation. To understand the research methodology and basic concepts of research, the College has initiated a unique scheme **'Prospective Researchers' Scheme' (PRS)** for prospective researchers since 2018-19. This is a golden opportunity for the students to experience research culture at college level. This year **76** students from **4** departments completed **18** research projects under the guidance of **9** teachers. The uniqueness of the scheme is that, the research projects are evaluated by the experts in their field from various colleges and university departments. The best research projects are ranked and awarded cash prizes.

I have seen the interest shown by the Co-ordinator of the PRS scheme and his fellow colleagues as well as the immense curiosity, anxiety and interest shown by the students. The multi-disciplinary nature of all the research topics is a welcome attitudinal change. I am hopeful that the industry shall look to this attempt to hunt the young talent. As a principal of the college, I, hereby, express my firm commitment for such activity on sustainable basis for the years ahead.

(Dr. K. B. Mahajan)
Principal
Date: 18th February 2025

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FOREWORD

18th February 2025

The KCE Society's Post graduate College of Science, Arts and Commerce, Jalgaon has been working with the aim of providing thrust to the research activities to be carried out to inculcate the research attitude amongst the students. The teachers and co-ordinator of PRS scheme works with a specific perspective of encouraging the prospective researcher students of the college in conducting research work, viz; writing basic research project reports and research articles through which they gain orientation to the regulatory requirements and looking after the Ethical requirements.

I am really happy to see the wide spectrum of topics, under different disciplines; prospective researchers have chosen to work on. Index of the volume indicates that students from the department of Chemistry, Microbiology and Biotechnology have contributed to this volume by undertaking the eighteen research projects, like green synthesis, antibacterial & antifungal activities of microorganisms, synthesis of Bio-plastic by using potato starch, production of Bio-fertilizer using plant growth promoting *Rhizobacteria* and development and evolution of a formulation for the treatment and prevention of inflammatory Bowel disease etc.

I take this opportunity to congratulate the principal, co-ordinator of PRS scheme, supervisors and researchers of the project for realizing this wonderful compendium of **Volume VI**. This year the college is publishing compendium of Volume VI on sustainable basis, it is only possible due to continuous motivation of Hon'ble Shri Nadkumar Bendale, President of KCE society.

(Dr. V. S. ZOPE)

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From the Desk of Editor

I am pleased to present to you the Fifth edition of ***A Compendium of Research Articles by a Prospective Researchers*** under the activity of "Prospective Researchers' Scheme" for the year 2023-24. This volume presents the eighteen articles from students and teachers of four Science Departments of college. **'Prospective Researchers' Scheme** is uniqueresearch activities of the college in which students undertake researchprojects under the supervision of teachers.

The outcome of this scheme is reflected in the publication of "A compendium of research articles of prospective researchers" with ISBN number. At the beginning of an academic year,potential students are identified and small research projects are assigned to them. The innovation and feasibility of research proposal is scrutinized followed by undertaking of projects in the stipulated time. The students are provided with research assistance to complete the research projects within stipulated time period. After completion, projects are evaluated by external experts and best performers are felicitated with cash prizes. This year eighteen research projects were completed from four Departments of Organic Chemistry, Analytical chemistry, Microbiology and Biotechnology. The research papers based on their articles is published in a separate volume as "*A Compendium of Research Articles by a Prospective Researchers Volume VI"* with ISBN. I thank all the committee members, faculty wise project guides & students for helping me in this endeavor.

I would like to thank the Principal Dr. K. B. Mahajan, Academic Advisor Dr V. S. Zope and our Hon"ble President Shri. N. G. Bendale, KCE Society, Jalgaon, provided help and encouragement to compile the new edition. This volume is result of continuous efforts of teachers & students of this college.

I am very happy to handover this volume to you all.

Dr. Ravindra M. Patil
Chief Editor
18th Feb 2025

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CHEMICAL SCIENCES

ORGANIC CHEMISTRY

Preparation of Ag-Incorporated Co-Succinate Metal Organic Framework For Detection of Hg(II) Heavy Metal Ions

Mitesh C. Ahire, Gauvrav P. Patil, Mayur J. Patil and Ravindra M. Patil*
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Abstract

We prepared an Ag-Incorporated Co-Succinate metal–organic framework by the solvothermal method. The synthesized Ag@Co-Succinate metal–organic framework was characterized through various techniques such as X-ray diffraction (XRD), Fourier-transform infrared (FT-IR) analyses to explain its structure. We also detected (Hg) heavy metal ions from contaminated water bodies and effectively it can be controlled by detection using electrogravimetric workstation.

Keywords: Ag@Co-Succinate metal–organic framework**,** (Hg) heavy metal ions

Introduction

The development of industrial manufacturing has released large amounts of toxic Heavy Metal Ions (e.g., Cr, As, Pb, U, Hg, Cd, and Ni, etc.) into the natural environment, posing serious threats to the surface and subsurface environments [1-3].

This persistence can lead to the bioaccumulation of heavy metals in living organisms, posing severe health risks to humans and wildlife. The development of advanced and sensitive detection methods for HMIs is of paramount importance to mitigate these concerns. Electrochemical sensors, with their high sensitivity and selectivity, have emerged as a promising avenue for HMI detection [4].

MOFs as electrode modifiers enhance HMI detection due to high surface area, tunable pores, and functionalizable structures [5-8]. MOFs offer a versatile and promising platform for HMI detection, contributing to environmental protection, public health, and safety by providing highly sensitive, selective, and efficient detection methods. Researchers continue to explore and develop new MOF-based materials and sensor designs to address the challenges of HMIs contamination. Various approaches have been explored before to inculcate the conductivity of the MOFs [9-10]. The incorporation of metal nanoparticles into an MOF array is one of the most prominent approaches to tune the conductivity of the MOF. MOFs modified with specific metal nanoparticles have shown great promise in HMI detection. These modified MOFs leverage the properties of both the MOFs and the incorporated metal nanoparticles to enhance the selectivity, sensitivity, and versatility of HMIs sensors. When managed appropriately, cross-reactivity in electrochemical detection can offer several positive advantages: enhanced sensitivity reduced false negatives, multiplexed detection, etc.

Even though cross-reactivity has numerous benefits, it should be carefully managed, validated, and controlled to avoid issues such as false positives, loss of specificity,and inaccurate quantification [11].

MOF also has may applications such as catalyst, gas and energy storage, imaging, gas molecular separation, sensing, biosensors, contrast agents for magnetic resonance imaging and etc [12].

Pioneered in the late 1990s ("Design and synthesis of an exceptionally stable and highly porous Metal-organic framework") by Prof. Omar Yaghi at UC Berkeley, MOFs have become a rapidly growing research field [13]. After that many research teams works on MOFs to explore their various applications. Yu-Ri Lee, et al. studied Synthesis of Metal-organic frameworks. Starting from the conventional solvothermal/hydrothermal synthesis, microwave-assisted, sonochemical, electrochemical, mechanochemical, ionothermal, drygel conversion, and microfluidic synthesis methods[14]. Mohadeseh Safaei, et al. gives brief information about MOFs in different fields such as the detection of toxic substances from gas and liquid, a variety of sensors, catalysts, storage of clean energies and environmental applications [15].

We are prepared Ag-incorporated Co-succinate MOF for detection of Hg(II) heavy metal ions from contaminated water bodies and effectively it can be controlled by detection using electrogravimetric workstation

Materials and Methods

All reagents used were of laboratory grade. Melting points were determined in open capillaries. The purity of compound was checked by TLC.

Synthesis of Co-Succinate and Ag@Co-Succinate

The solvothermal method was used to synthesize Co-succinate. 50 mL N,N-dimethylformamide (DMF) and 2.5 mL Deionized water, approximately 1.5 mmol of the 1.7 g succinic acid ligand was dissolved. The mixture was swirled for 15 min. After that, the mixture was stirred for further half hour while adding 1 mmol of $Co(NO_3)_2$. The reaction mixture was heated at 120^0C for 20 hr. Purple crystals of Co-MOF was produced as by-product, which were separated and cleaned using 5:0.25 DMF and DI mixture. The resulting goods were subsequently heated at 60^0C to dry them out.

Moreover, 0.5 g of $Ag(NO)_3$ salt in proportion to 1:2 Co-Succinate 1 gm was used to modify each metal. The mixture was placed in 10 mL of DMF and annealed for 3 hr at 60 ◦C. The brown-colored precipitate was dried using a vacuum filter and dried overnight at room temperature. The process of synthesis of Co-Succinate and Ag@Co-Succinate

Result and discussion

MOF Characterization-

The XRD pattern in Figure 1a shows the Co-Succinate material's crystal lattice diffraction peaks at 2θ angles of 11.056^0 and 13.16^0. These peaks are consistent with X-ray diffraction by the Co-Succinate crystal lattice indicating the synthesized Co-Succinate material's reliability and consistency. The observed sharp diffraction peaks at 2θ angles of 38.15^0, 44.33^0, 64.5^0, and 77.43^0, and their similarity to the JCPDS (Joint Committee on Powder Diffraction Standards) card No. 04-0783, confirm the presence of Ag nanoparticles in the Co-Succinate [25,26]. The excessive peaks suggest the extensive presence of silver in the Ag@Co-Succinate without affecting the Co-Succinate's phase. This means that the active sites for organic linkers remained unbonded, allowing specific HMIs to form a bond with these sites, as further explained by spectroscopic analysis.

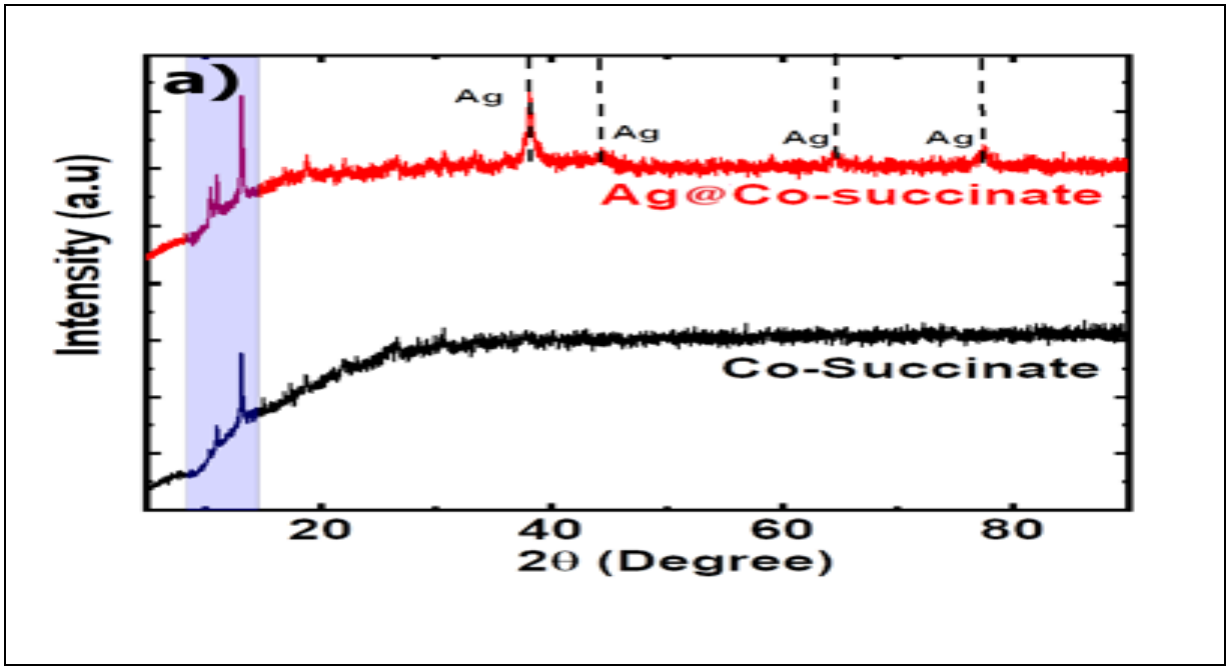

Figure 1. (a) X-ray diffraction patterns of Co-Succinate and Ag@Co-Succinate,

The FTIR spectra of Co-Succinate and Ag@Co-Succinate in the range of 600 cm^{-1} to 2000 cm^{-1} are shown in Figure 1b. The peaks observed in Co-Succinate MOF at 650 cm^{-1} and 780 cm^{-1} are attributed to CH_3-metal groups [27], formed due to Co attachment to a methanoic group. The appearance of an additional peak for Ag/La-TMA at the spectral band of 680 cm^{-1} to 750 cm^{-1} indicates the formation of a CH_3-Ag bond. The presence of C=O stretching in both Co-Succinate and guest Ag@Co-Succinate confirms the existence of the carboxylic group. An extra peak is observed for Ag/La-TMA in the same spectral range of 1500 cm^{-1}, signifying bond formation between Ag and the carboxylic group. Other peaks at

1300 cm^{-1} and 1580 cm^{-1} are attributed to C=O, C-O, and O-H bending vibrations of carboxylate ligands. These functional groups not involved in bond formation with guest metals may create an active site for HMIs accumulation.

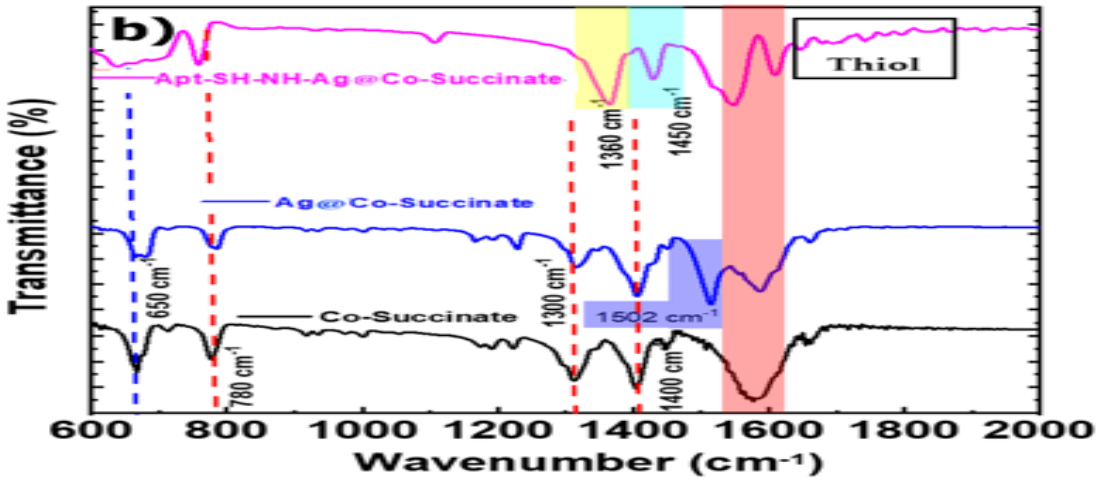

Figure-1(b) FTIR spectra of Co-Succinate, Ag@Co-Succinate.

Electrochemical sensing studies:

Responses of Ag/La-TMA ranging from 1nM to 10 nM d), the repeatable electrochemical behavior of Ag/La-TMA for 5nM Hg^{2+} e), peak current Vs no. of experiments f)

Fig 2-a) The electrochemical cell arrangement, pristine as well as metals modified La-TMA was dropcasted on GCE and utilized as a working electrode. Fig 2-b) shows the DPV response of Ag-Co Succinate towards Hg^{2+} ranging from 1nM to 10nM. The presence of Hg^{2+} improves the charge transfer capabilities of Ag-Cosuccinate, as evidenced by a steady and dramatic increase in the current responses, as shown in Fig 2-b) when Hg^{2+} concentration is raised. The detection limit is 1nM which is far below the Maximum Concentration level suggested by US-EPA[2]. Calibration plots (Fig 2-c)) show the linearity of the obtained analytical current responses. Sensitivity is found to be 5.2µA/M with R^2= 0.9539. At a concentration of 5nM Hg^{2+}, five independent experimental trials yielded consistent findings. Fig 2-d) showed the repeatable differential current overlapping signals. This confirmed the repeatable current output after a repeatable sort of experiment at a low molar concentration of Hg^{2+} as shown in Fig 2-e.

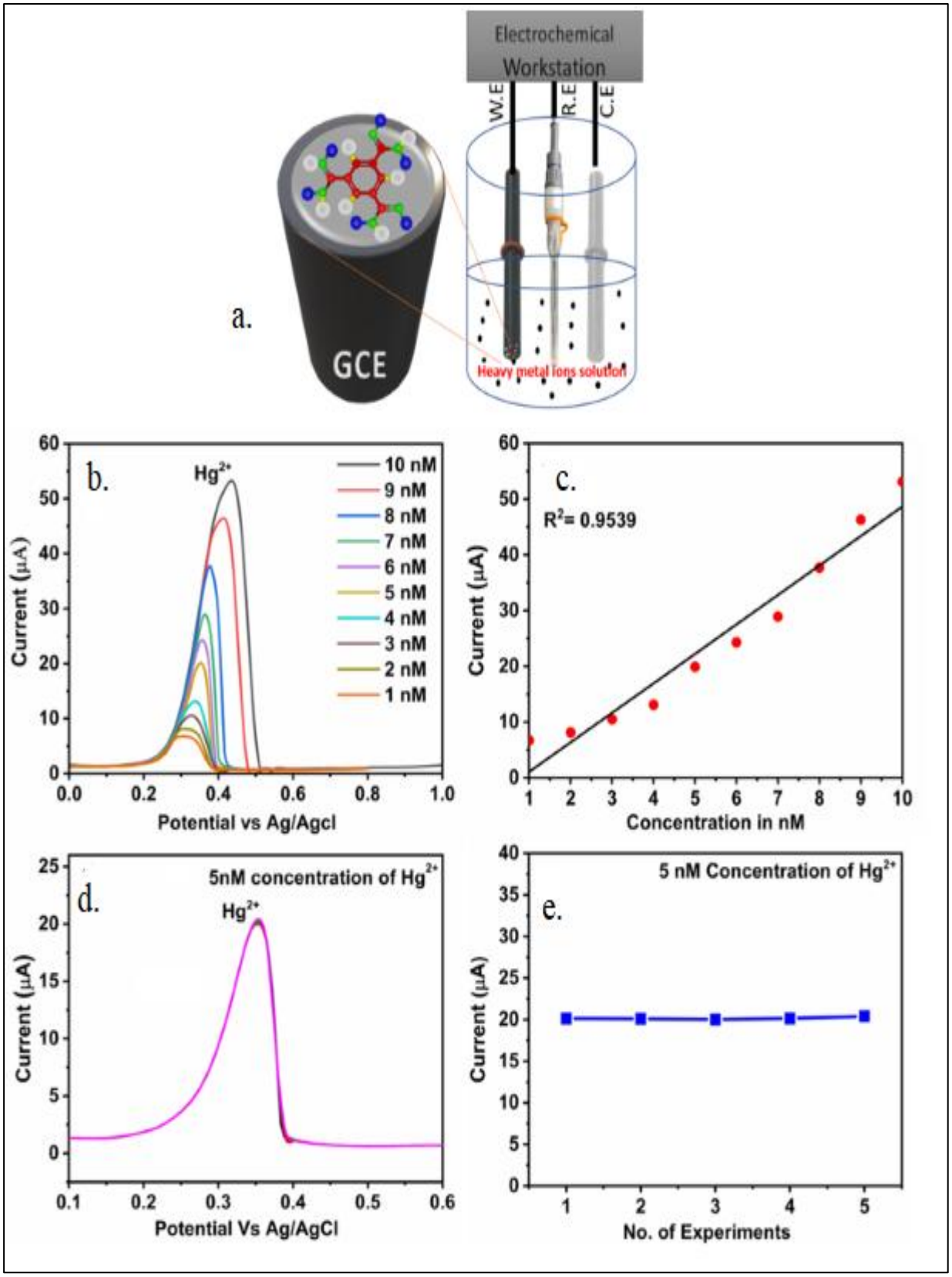

Figure 2- Electrochemical cell arrangement for Ag-Co succinate, a), DPV behavior of Ag-Co succinate for Hg heavy metal ions b), Linear calibration plot for DPV

Conclusion

By performing this experiment, we prepared an Ag-Incorporated Co-Succinate metal–organic framework by the solvothermal method. The synthesized Ag@Co-Succinate metal–organic framework was characterized through various techniques such as X-ray diffraction (XRD), Fourier-transform infrared (FT-IR), analyses to explain its structure. We also concluded that we detected (Hg) heavy metal ions from contaminated water bodies and effectively it can be controlled by detection using electrogravimetric workstation

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Biosynthesis & Characterization of Silver Nanoparticles and Its Application as a Nano Organic Catalyst for One Pot Synthesis of Pyranopyrazole

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Abstract

Various parts of the colocasia leaves extract Cannonball tree (*Couroupita guianensis*) have been reported to contain oils, keto steroids, couroupitine, glycosides, indirubin, isatin and phenolic substances. We report the biosynthesis of silver nanoparticles (AgNPs) using colocasia leaves extract. Colocasia leaves extract as a reducing agent converts silver ions to AgNPs in a rapid and ecofriendly manner. Overall, this environmentally friendly method of biological silver nanoparticles production provides rates of synthesis faster than or comparable to those of chemical methods. Further, the study also assessed the catalytic activity of silver nanoparticles for the synthesis of Pyranopyrazole derivatives.

Keywords: silver nanoparticles (AgNPs); colocasia leaves extract, Pyranopyrazole.

Introduction

Nanoparticles have unique electronic, Optical, mechanical, magnetic and chemical properties when compared to larger matter. Nanotechnology is an emerging technology which is an incorporation of different aspects of science and technology that includes disciplines such as electrical engineering, mechanical engineering, biology, physics, chemistry, and material science[1-4]. Promising new technologies based on nanotechnology are being utilized to improve diverse aspects of medical treatments like diagnostics, imaging, and gene and drug delivery.In present situation, silver nanoparticles (AgNPs) are in great use in the medicinal[5], pharmaceutical[6], agricultural industry[7] and in water purification. These nanoparticles can be synthesized either chemically or biologically. But the chemical process for synthesis of silver nanoparticles is more elaborate and leaves behind toxic effect that adversely affects the ecosystem. On the other hand, biological synthesis of silver nanoparticles is less time consuming, less costly, and more ecofriendly; therefore, in recent time, scientists are looking forward to the possible biological methods for the synthesis of silver nanoparticles.

In organic synthesis, the product yield and reaction time are extremely important. The increase in reaction steps results in a decrease in final product yield and increase in total reaction time. Multicomponent reactions help to solve this problem. By novel developing multicomponent reaction strategies, synthesis of the desired product in the one-pot method is possible thereby increases the product yield and reducing reaction time required for the reaction.

In this study, we used colocasia leaves extracts due to its availability throughout the year and

most importantly Colocasia contain some microcrystals made up of calcium oxalate which are used by plant for storing calcium[7].

In the present study, the green synthesis of silver nanoparticles from the colocasia leaves extract has been carried out. However, the characterization of silver nanoparticles has yet to be completed. The antimicrobial activity of synthesized AgNPs against Gram Negative bacteria E.Coli and Proteus Vulgaris was determined. The silver nanoparticles applied as catalyst for the synthesis of the pyranopyrazole derivtives, via the reaction of various aromatic aldehydes, malanonitrile, ethyl acetoacetate and hydrazine hydrate at room temperature and using ethanol as green solvent and considering green chemistry approach. The synthesised compounds were characterised by FTIR technique.

Further, the study also assessed potential environmental applications of biosynthesized AgNPs in photocatalytic degradation of harmful Methylene blue dye.

Material and methods

All reagents used were of laboratory grade. Melting points were determined in open capillaries. The purity of compound was checked by TLC.

Preparation of colocasia leaves extract-

Freshly collected colocasia leaves (50 gm) were thoroughly washed and ground in mortar pestle. The paste obtained was then re-suspended in 50 mL of distilled water and then filtered with clean muslin cloth at ambient temperature and centrifugation was carried out at 10,000 rpm for 5 minutes in a desktop centrifuge to obtain clear solution of *colocasia* extract.

Preparation of 1mM Aqueous Solution of Silver Nitrate:

0.017gm of Silver Nitrate ($AgNO_3$) was added to the 100 ml of distilled water and the solution was stirred well continuously until the silver nitrate is dissolved. This 1mM Silver Nitrate solution stored in brown bottle at 4°C for further use for the synthesis of Silver Nanoparticles.

Synthesis of AgNPs: For the synthesis of Ag-NPs, 0.5 mL (1%) of *colocasia* extract was added drop by drop to 50 mL of 1mM aqueous silver nitrate solution. The reaction mixture was stirred with a magnetic stirrer at 200 rpm until (~30min) the reaction mixture became yellowish brown. Formation of yellowish brown color indicates the synthesis of silver nanoparticles. The pH of reaction was maintained at basic condition throughout the experiment and the concentration of the *colocasia* extract was increased sequentially from 1% to 5% (V/V) to observe the effect of concentration of reducing agent on the characteristics of Ag-NP Schematic representation of synthesis of nanoparticles as shown in **figure1.**

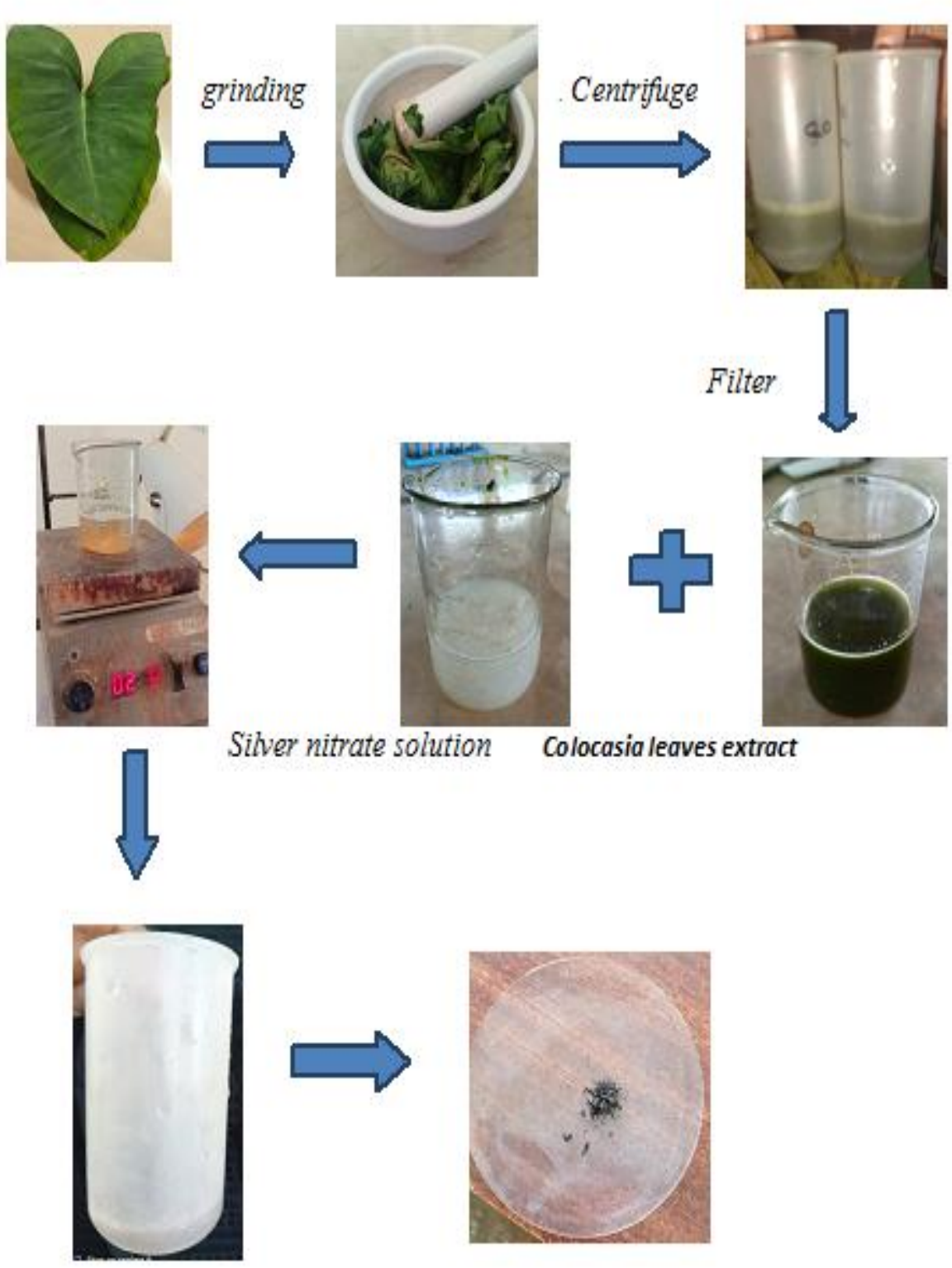


Figure 1-Schematic representation of synthesis of nanoparticles

Result and discussion

Optimized reaction conditions:

a) Effect of catalyst:

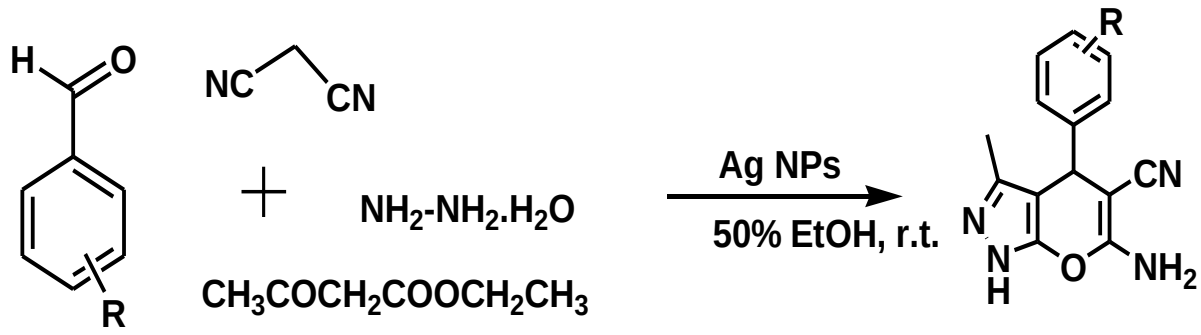
Initially we performed reaction without catalyst the yield of product only about 60%. The time required was also more to complete the reaction. To optimize the reaction condition; we performed the model reaction with different amount of silver nanoparticles catalyst loaded as shown in **table-1.**

Table 1: Optimized amount of catalyst loaded

Entry	Catalyst (mg)	Time(Min)	Yield (%)
1.	0	30	60
2.	5	25	72
3.	**10**	**10**	**93**
4.	15	10	93

It was found that, the 10 mg catalyst is sufficient to push the reaction forward. Hence the reaction was perform with 10 mg catalyst by optimized the reaction condition.

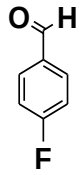
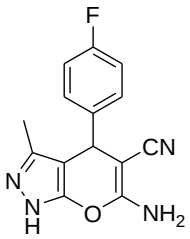
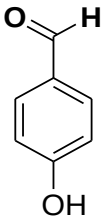
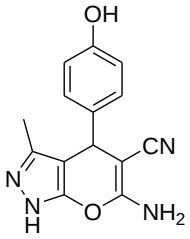
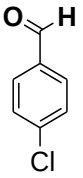
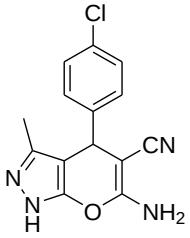
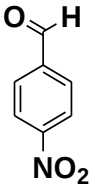
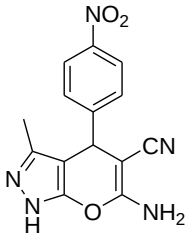
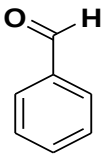
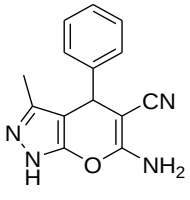
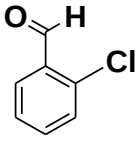
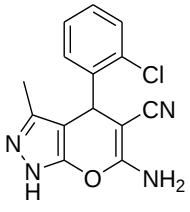
After the study of above optimized reaction condition were explored for the synthesis of series of pyranopyrazole derivatives from various substituted benzaldehyde, malononitrite, hydrazine hydrate and ethylacetoacetate using calcium carbonate powder as catalyst as shown in **scheme-2** and the results are summarized in **Table-2**.



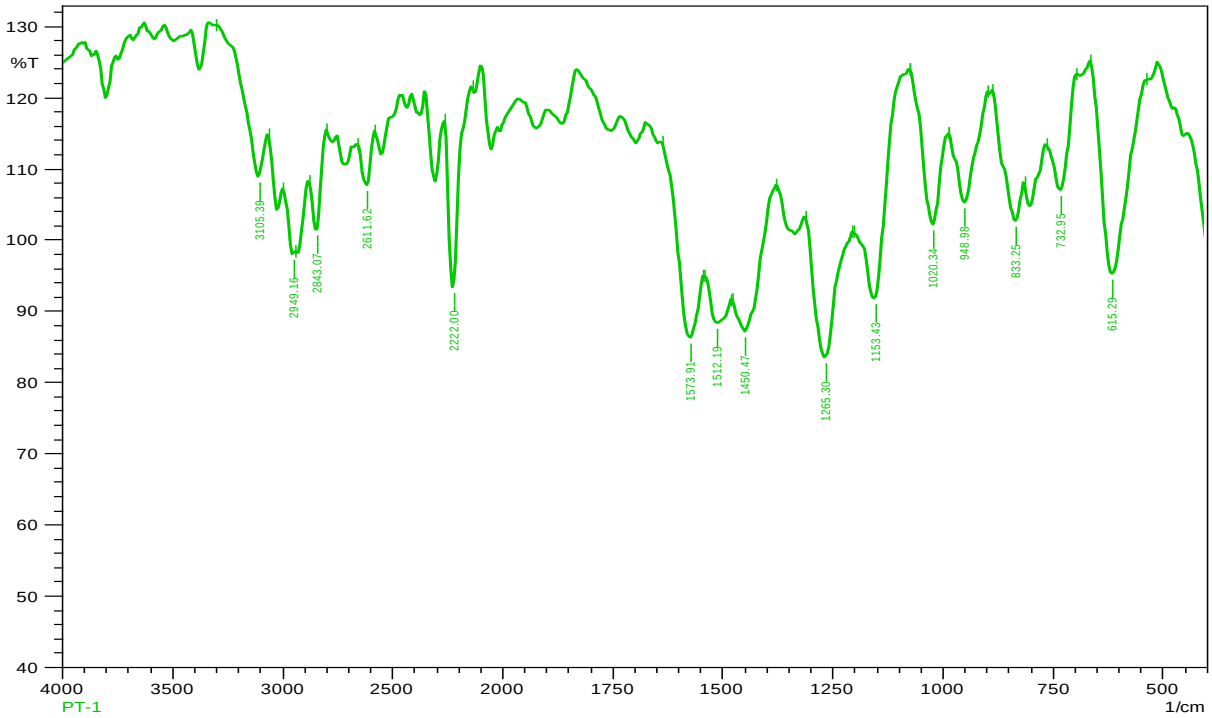
R=H, 4-OMe,4-Me,4-Cl,etc

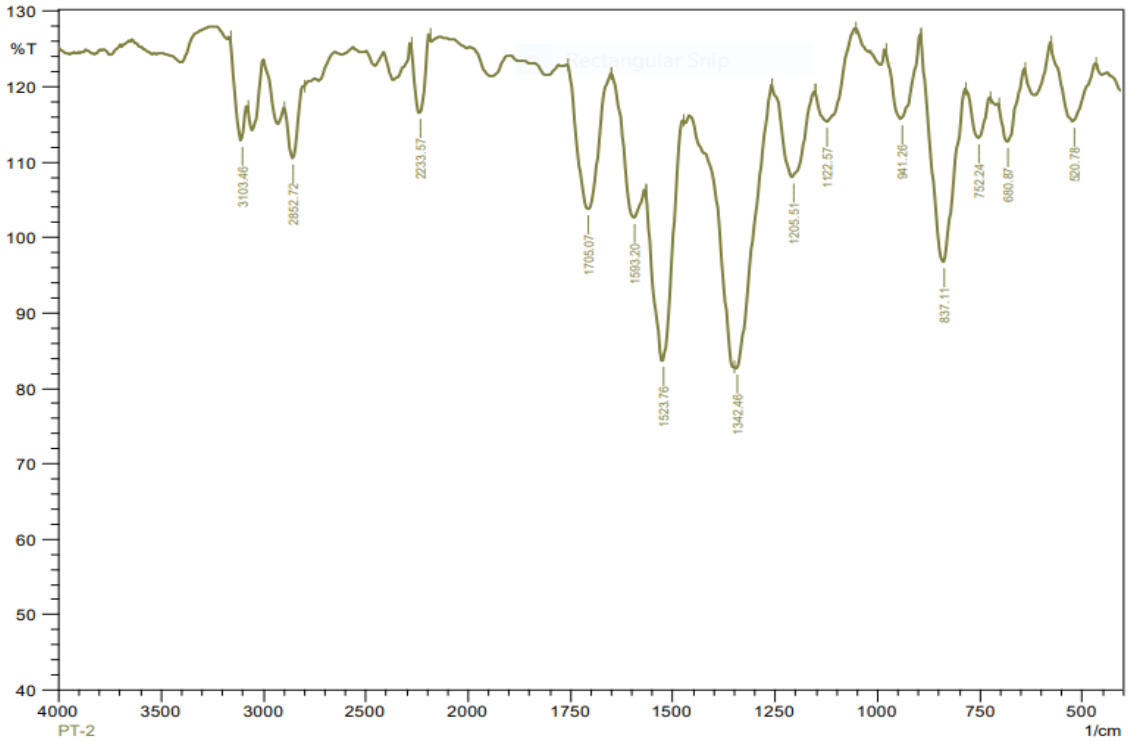
Scheme 2: Synthesis of pyranopyrazole derivatives by using calcium carbonate as catalyst

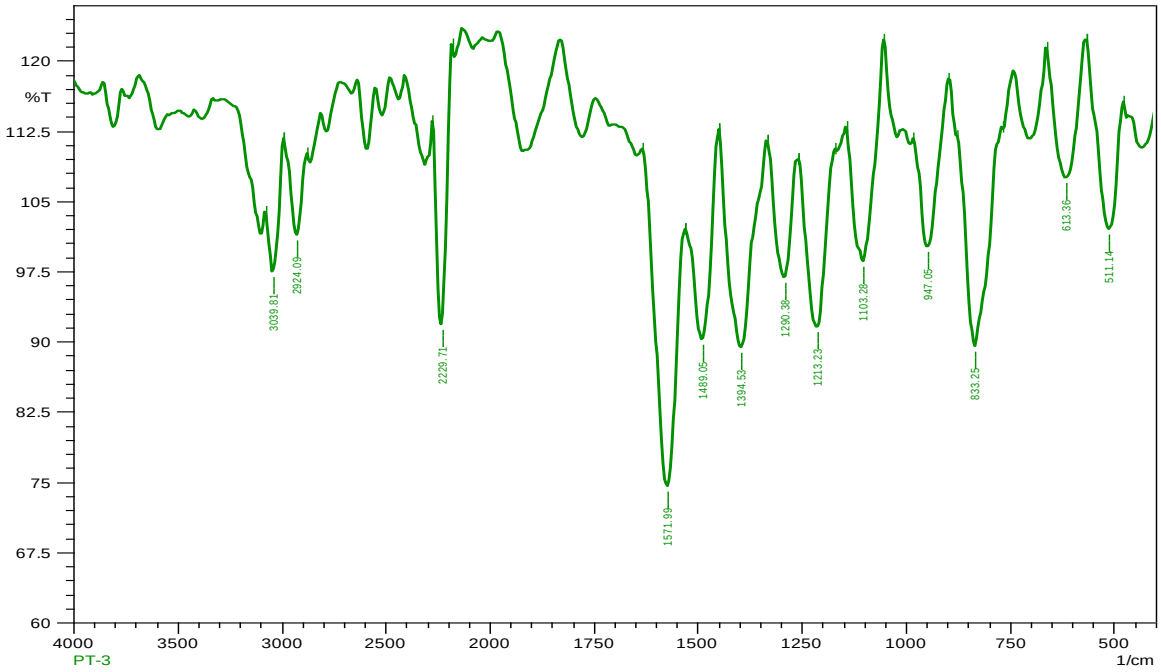
Table-2: Synthesis of pyranopyrazole derivatives

Entry	Starting compounds	Product	Time (min)	Yield (%)	Melting point(^{0}c) Obs.	Melting Point (^{0}C)Lit.
1.			10	94	235	Present work
2.			12	88	210	Present work
3.			10	95	250	Present work
4.			120	93	228	Present work
5.			150	85	230	228-234
6.			150	93	234	234-235

IR Spectra of compounds (1 to 3)







IR Spectral data of compounds (1-6)

1) Cream white IR (cm^{-1}): 3333.1(-N-H); 2210.9 (-CN), 1646.5(C=N), 1589.2(C=C), 1330 (C-N),

2) White solid, IR (cm^{-1}): 3300.9(-N-H); 2205.6(-CN), 1631(C=N), 1594.74(C=C), 1311 (C-N), 790.57(C-Cl)

3) White solid, IR (cm^{-1}): 3339.9(-N-H); 2235.99 (-CN), 1603.3(C=N), 1598.74(C=C), 1344 (C-N),

4) Brown solid, IR (cm^{-1}): 3349.0(-N-H); 2211.3(-CN), 1638(C=N), 1589.2(C=C), 1311 (C-N), 1346.86(NO_2)

5) Pale Yellow solid, IR (cm^{-1}): 3338.0(-N-H); 2227.(-CN), 1661(C=N), 1589.2(C=C), 1311 (C-N),

6) White solid, IR (cm^{-1}): 32909.9(-N-H); 2205.99 (-CN), 1623.3(C=N), 1578.74(C=C), 1304 (C-N),

Conclusion

In conclusion, the silver nanoparticles are prepared using colocosia leaves extract by biosynthesis method. We have developed a simple and efficient method for one-pot synthesis of pyranopyarzoles derivatives from various substituted benzaldehyde, malononitrite, hydrazine hydrate and ethylacetoacetate in the presence of catalytic amount of using the silver nanoparticles powder as catalyst as reusable catalyst at room temperature and using ethanol as green solvent and considering green chemistry approach. The high catalytic activity of the silver nanoparticles was accounted due its Lewis acid sites. The advantages of procedure include simplicity of operation, wide substrate scope, good yields, no chromatographic separation

technique, an easy recovery of the catalyst and recyclability of catalyst.

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Efficient One Pot Synthesis of Imidazole Derivatives by Using Cu-Catalyst

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Abstract:

Imidazole and derivative are one of the most vital & universal heterocycles in medicinal chemistry. Imidazole is a five membered, heterocyclic ring, which contains two hetero (Nitrogen) atoms & two double bonds. There are so many compounds which contains Imidazole ring & exhibit different types of pharmacological & biological activities like metronidazole, nitrosoimidazole as bactericidal, 1-vinyl imidazole as fungicidal, megazol as trypanocidal, imidazole-2-one as antileishmanial & other antimicrobial, antimalarial, anticancer activities, uses & applications.

Keywords: Cu Nanoparticles, Imidazole, Heterocyclic compounds, Green chemistry.

Introduction:

Green chemistry is basically environmentally begin chemical synthesis and is useful to reduce environment pollution .Green chemistry efficiently utilized row material eliminated waste and avoids the use of toxic or hazardous reagent and solvent in the manufacture and application of chemical product.[1] Multicomponent reaction [MCR] in which three or more reactant are combined in one process have become an efficient and powerful tool for the construction of complex molecule.[2] In recent years MCRs have attracted extensive effort by researches in modern synthesis chemistry because they increase the efficiency by combining several operational step without the isolation of in0termediate or changing the reaction condition the development and application of MCR are how an integral part of the work of anymajor medical units[3] . Multicomponent reactions (MCR) play an important role in combinatorial chemistry because of the ability to synthesize target compound with greater efficiency and atom economy generating structural complexity in a single step from three or more reactant moreover, MCR offer the advantages of simplicity and synthetic efficiency overconventional chemical reactions[4].

Imidazole is a class of heterocyclic compounds that contain nitrogen are currently under intensive focus due to their wide range of application.[5] Study of imidazole units it is veryimportant due to their potent biological activity.[6] Imidazole with nitrogen at 1 & 3 position in the ring have been known from over 160 years it was reported by Henrich debus 1858 by reaction of glycol

formaldehyde and ammonia which afforded the low yield.[7] Imidazole class of hetero cycle being the core fragment of different natural products and biological system compound containing imidazole moiety have many pharmacological proprieties & play important roles in biochemical process[8] .Imidazole is probably the most well-known heterocyclic which is common & important feature of variety of naturals products andmedicinal agent. [9]

Green method using leaf extract of *ixora coccinea* has been used for the first time for the synthesis of copper oxide nanoparticle *ixora coccinea* Linn(Rubiaceae) commonly known as jungle of geranium & red ixora is an evergreen shrub found throughout India depending the medical condition the flower leaves, roots & the stems are used to treat various ailment in the Indian traditional system of medicine the *Ayurveda* and also in folk medicine phytochemical studies indicate that the plant contain important phytochemical such as lupeol, ursolic acid, oleanolic acid. Pharmacological studies suggest that the plant process ant oxidative, antibacterial ,gastro protective, hepatoprotective ,thus leading scientific support to the plant ethno medicinal uses .the present is an effort in this direction so the synthesis of copper oxide nanoparticle has been reported so that *ixora coccinea* could also be taken as potential candidate plant specimen for the synthesis of metal as well as oxide nanoparticle.[10] Due to the peculiar structural characteristic of imidazole scaffold with a worthy electron rich feature it is advantageous for imidazole group to combine with various receptor and enzymes in biological system ,through diverse weak interactions ,thereby showing a variety of biological activities. At present , a legion of imidazole containing with high medical potential as a clinical drug havebeen widely used to treat diverse type of illness such as antimicrobial [11,] anticancer n- heterocyclic carbine [12] meanwhile substituted imidazole are extensively used as antibacterial[13] antitumor, antifungal antiviral, antiparasite[14] the potency and wide applicability of the imidazole pharmacophore can be attributed to its hydrogen bond donar-acceptor capability as well as its high affinity for metals (e.g. Zn, Fe, Mg)[15] and also as pesticide[16] recent advances in a green chemistry and organometallic catalysis have extended the application of imidazole as ionic liquid[17].

Result and discussion:

Optimized reaction conditions:

Initially we performed reaction without catalyst the yield of product is only about 50% , the time required was also more (greater than 3 hrs) to complete the reaction but we used CuNPs as a

catalyst the time was reduced.

To investigate the role of CuNP, reaction is carried out in presence of CuNP catalyst. It was observed the derivative of imidazole formation timing was decreases in presence of CuNP. Hence, by using CuNP catalyst we performed the model reaction with different mole% of CuNP to optimized reaction condition as shown in **table-1**.

Table 1: Optimized amount of catalyst loaded

Entry	Catalyst(mole %)	Time (min)	Yield(%)
1	5	55	83
2	10	46	92
3	**15**	**35**	**94**
4	20	25	89

Here we find 15 mole % catalyst is sufficient to push the reaction. Hence the reaction can perform with 15 mole % as catalyst by optimized the reaction condition.

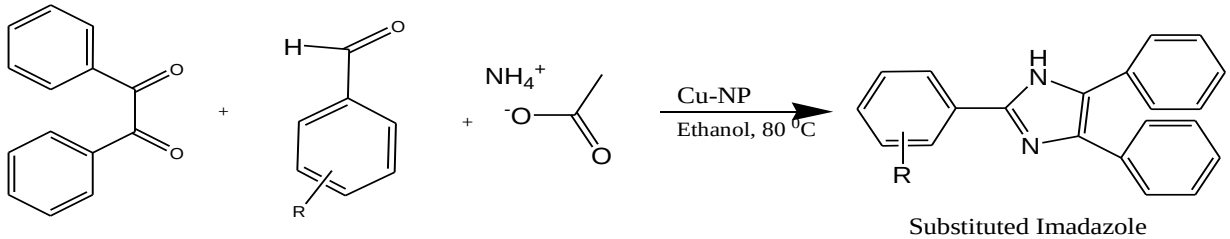
Synthesis of biologically active imidazole compounds we use readily available, inexpensive and environment friendly reagent. The reaction of various substituted aromatic aldehyde with benzil and ammonium acetate carried out using CuNP as catalyst as shown in **scheme-1** and the results are summarized in **Table-2**.

Preparation of ixora coccinea leaves extract:-

Firstly, 60 gm of fresh *ixora coccinea* leaves were washed with running tap water and then by distilled water. The leaves were crushed with mortar and pestle. After that mashed fresh leaves was mixed with 200 ml of distilled water in a 250 ml of round bottom flask and kept in water bath at 80^{o}C for 45 min. Then it was filtered off using normal filter paper and then again filtered using what Mann no.1. The resulting leaf extract was stored at refrigerator for further use.

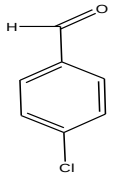
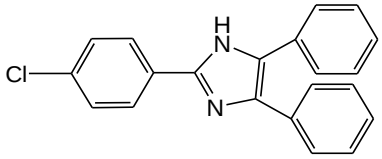
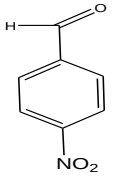
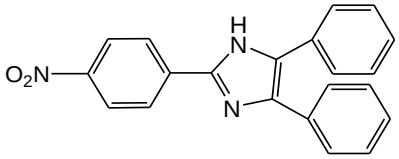
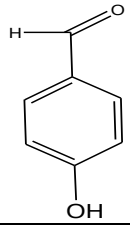
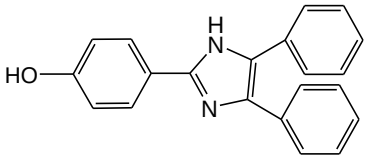
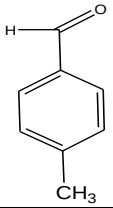
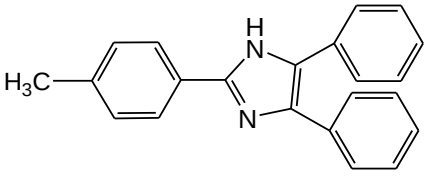
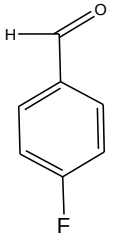
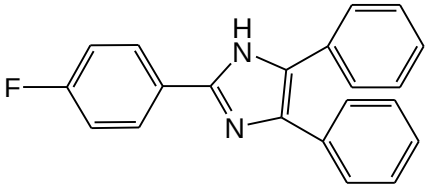
Green synthesis of copper nanoparticles:-

In a typical synthesis of copper nanoparticles, 30 ml of fresh leaf extract was added to 270 ml of 0.01M $CuSO_4.5H_2O$ aqueous solution and the mixture was kept at 80^{o}C with constant stirring on a magnetic stirrer for about 3 hrs. The suspension produced was centrifuged at 3000 rpm for 30 min and the supernant liquid was decanted off and residue was washed with 10 ml of distilled water. Centrifugation-decantation-washing processes were repeatedly done three times to remove impurities. The obtained precipitate was dried in an oven at 70 ^{o}C for 30 min. The as synthesized copper nanoparticles were then subjected for further characterization.



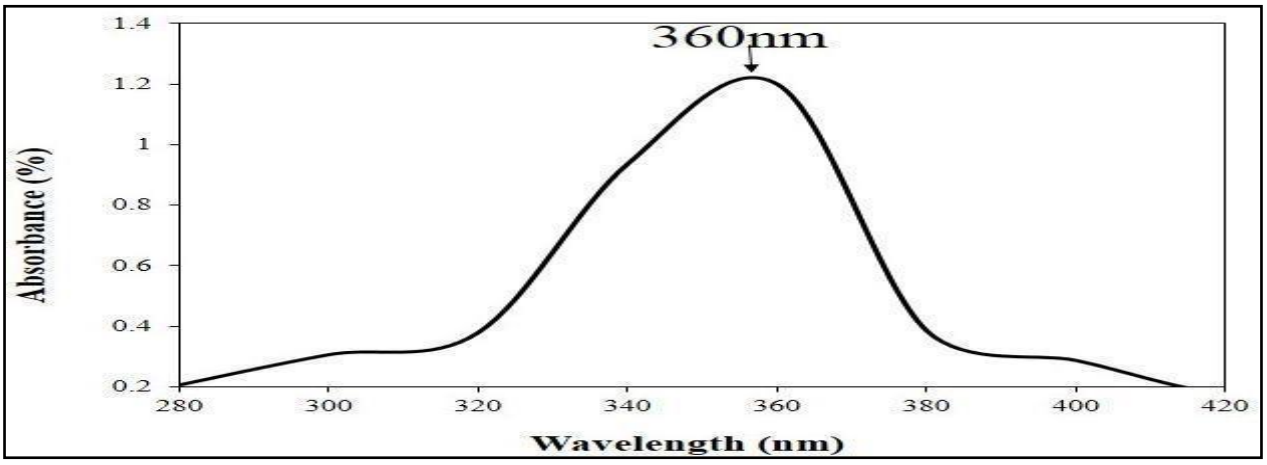
Scheme 1: Synthesis of Substituted Imadazole

Table-2: Synthesis of Imidazole:

Sr. No.	Substituted benzaldehydes	Product	Time (hr.min)	Yield (%)	Melting Point (°C)
1.			2 hr 15 min	90	256
2.			1 hr 40 min	82	238
3.			1 hr	80	254
4.			35 min	94	256
5.			2 hr 10 min	85	256

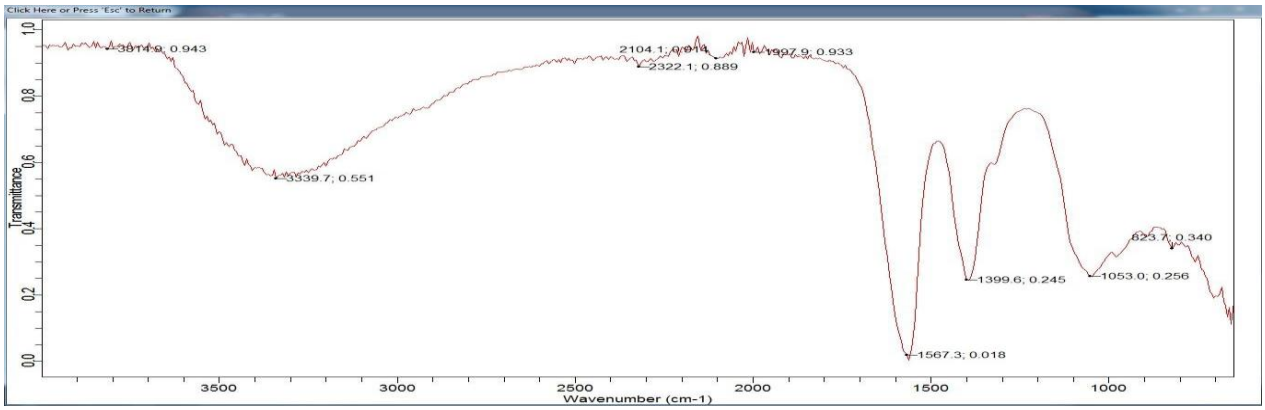
Characterization :

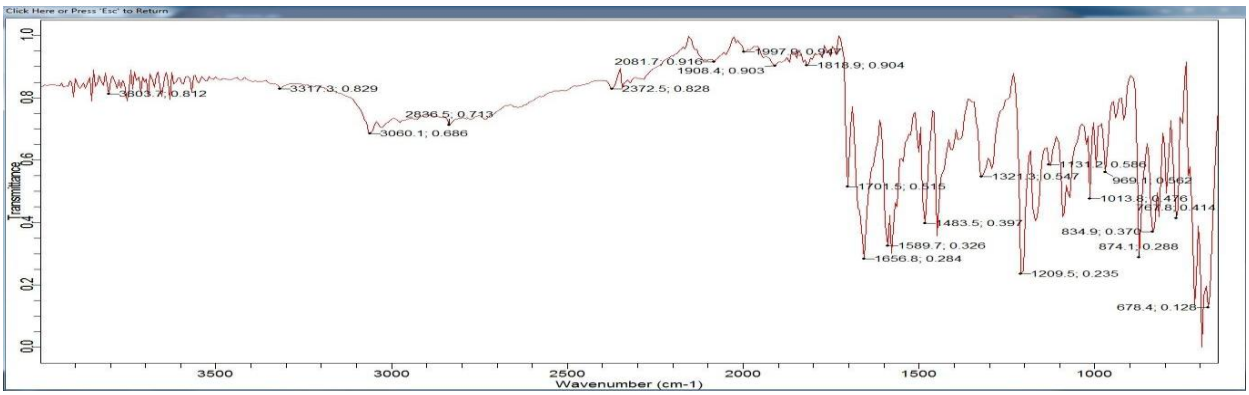
UV – Visible spectra of green synthesized copper nanoparticles after 3 hr.



Reference λ_{max} = 364nm

IR Spectra of compound (1&2)





Spectral data of compounds (1-4)

1.Sky Blue Solid,IR ZnSe(cm^{-1}): 3339.5 cm^{-1} (O-H), 1567 cm^{-1} (C=C), 1053 cm^{-1}(C-O)

2.Pale Yellow Solid,IR ZnSe(cm^{-1}):2836.5(N-H),1209.5(C-N),678.40(C-Cl), 3060.1 cm^{-1} (C-H)

3.Yellow Mustured Solid,IR ZnSe(cm^{-1}):2858.5(N-H),1209.5(C-N), 1159.2(C-F), 3065.7(C□C)

4. Yellow Solid, IR ZnSe(cm^{-1}): 3513.0 (N-H), 1209.5 (C-N), 3311.7 (O-H), 1656.8 (C-H)

Conclusion:-

Imidazole is the easily form by the reaction of aromatic aldehyde with contain electron withdrawing as a substituent. The quality of product is good with sufficient quality. The structure of synthesize compound, were confirmed & characterized with the help of analytical data such as IR spectra. In summary, under the in-depth research and application in imidazole based medicinal chemistry and the progress in other disciplines –such as cell biology, molecular biology, pharmacology and organic superior pharmacokinetic characteristics, effective pathologic probes and diagnostic agents would be used in clinics. This could make remarkable contributions for the protection of mankind's health. Additionally, we hope this project would build a full foundation and reference source which would open up new thoughts for researchers to focus on in imidazole –based medicinal molecule design and synthesis chemistry.

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Synthesis of Thiazine & Oxazine by Using Chalcones

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Abstract

Heterocyclic compounds play a pivotal role in medicinal chemistry due to their diverse biological activities. Among these, thiazines and oxazines represent significant classes of heterocycles with promising pharmacological properties. This review highlights the synthesis of thiazine and oxazine derivatives utilizing chalcone as a key precursor. Chalcones, known for their facile synthesis and versatile reactivity, serve as valuable starting materials for the construction of various heterocyclic scaffolds.

Keywords: Thiazine, oxazine, chalcone, heterocyclic compounds, synthesis, cyclization reactions.

Introduction

Chalcones either natural or synthetic and their heterocyclics are known to exhibit various biological activities. They have been reported to possess antioxidant, antimicrobial, antileishmanial, anti-inflammatory, antitumour and antibacterial activity. The presence of a reactive, unsaturated keto function in chalcones is found to be responsible for their antimicrobial activity, which may be altered depending on the type and position of substituent on the rings. In the present communication we report the reaction of 3-acetylpyridine with different aromatic aldehydes (2a-g) to form chalcones [1-2] (3a-g) in the presence of alkali. The resulting chalcones after purification and characterization by physical and spectral methods have been successfully converted into substituted pyrimidines [3-5]

The presence of a reactive α, βunsaturated keto function in chalcones is found to be responsible for their antimicrobial activity, which may be altered depending on the type and position of substituent on the aromatic rings. In the present communication we report the reaction of 2- acetyl pyridine with different aromatic aldehydes to form chalcones [4-9] oxazine heterocycles has shown that they possess varied biological properties such as antibacterial [13], analgesic[14] antitubercular [15], anticancer [16], and anticoagulant [17]. Thiazine is a six membered heterocyclic which contains two hetero atoms (N and S) [18]. Thiazine is fairly basic diuretics supplement it reduces water and increase vascularity, so it also use as anabolic agent in

medicine [19] . The ability of thiazine to exhibit antibacterial [20-21], anti-inflammatory [22] and used as cannabinoid receptor agonist [23]. Pyrazole is an important class of compounds and attracted widespread attention due to their pharmacological properties [24], being reported to have a large spectrum of biological effects, especially antibacterial [25], antifungal [26] and antiinflammatory properties [27-28].

Chalcone, an open-chain flavonoid also known as chalconoid, is unsaturated ketone containing the reactive keto ethylenic group –COCH=CH- that serves as the basic center for a number of significant biologically active molecules. Chalcones, one of the main families of naturally derived chemicals present in an extensive number of foods such as vegetables, spices, fruits, tea, and soybean products, have received a lot of study due to their interesting pharmacological properties [12]

Experimental Section- Synthesis of "Chalcones" by Strirring method

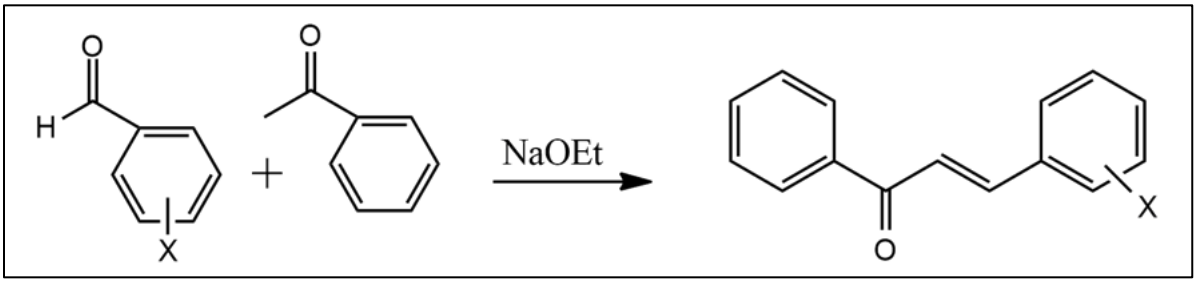
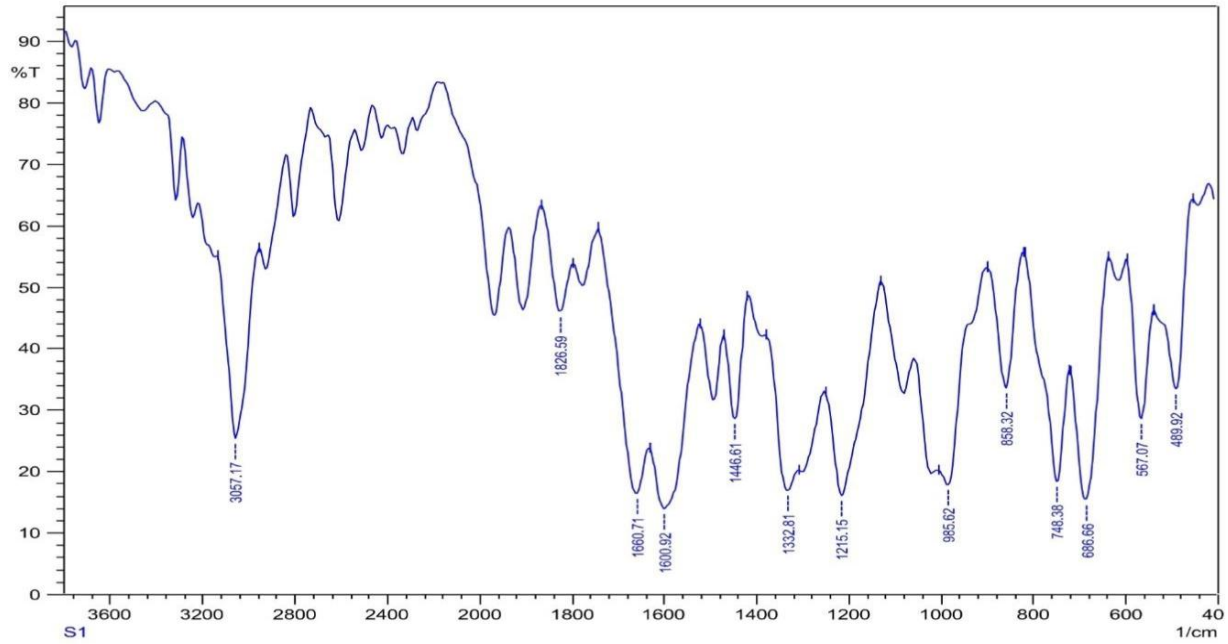


Figure 1 : Reaction of synthesis of Chalcones**Results and Discussion**

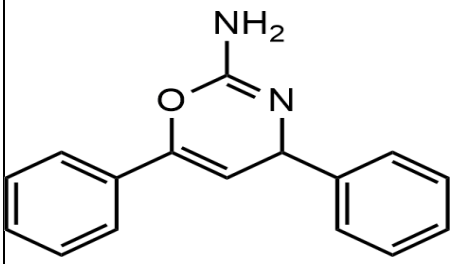
Compound	***Name***	***% Yield***	***Melting Point***
A	1,3Diphenylprop2enone	91.04%	57^0C
B	3(2chlorophenyl)1phenyl prop 2enone	85.68%	52^0C
C	3(3chlorophenyl)1phenyl prop 2enone	83.44%	85^0C
D	3(3nitrophenyl)1phenylprop 2enon	82.29%	145^0C
E	4,6Diphenyl4H1,3oxazine2 amine	72.00%	50^0C
F	4(2chloro phenyl)4H 6 phenyl1,3oxazine2amine	73.68%	55^0C
G	4(3chloropheny)4H6phenyl 1,3thiazine2amine	80.38%	90^0C
H	4(3nitrophenyl)4H6phenyl 1,3 thiazine 2 amine	80.99%	132^0C

Characterization

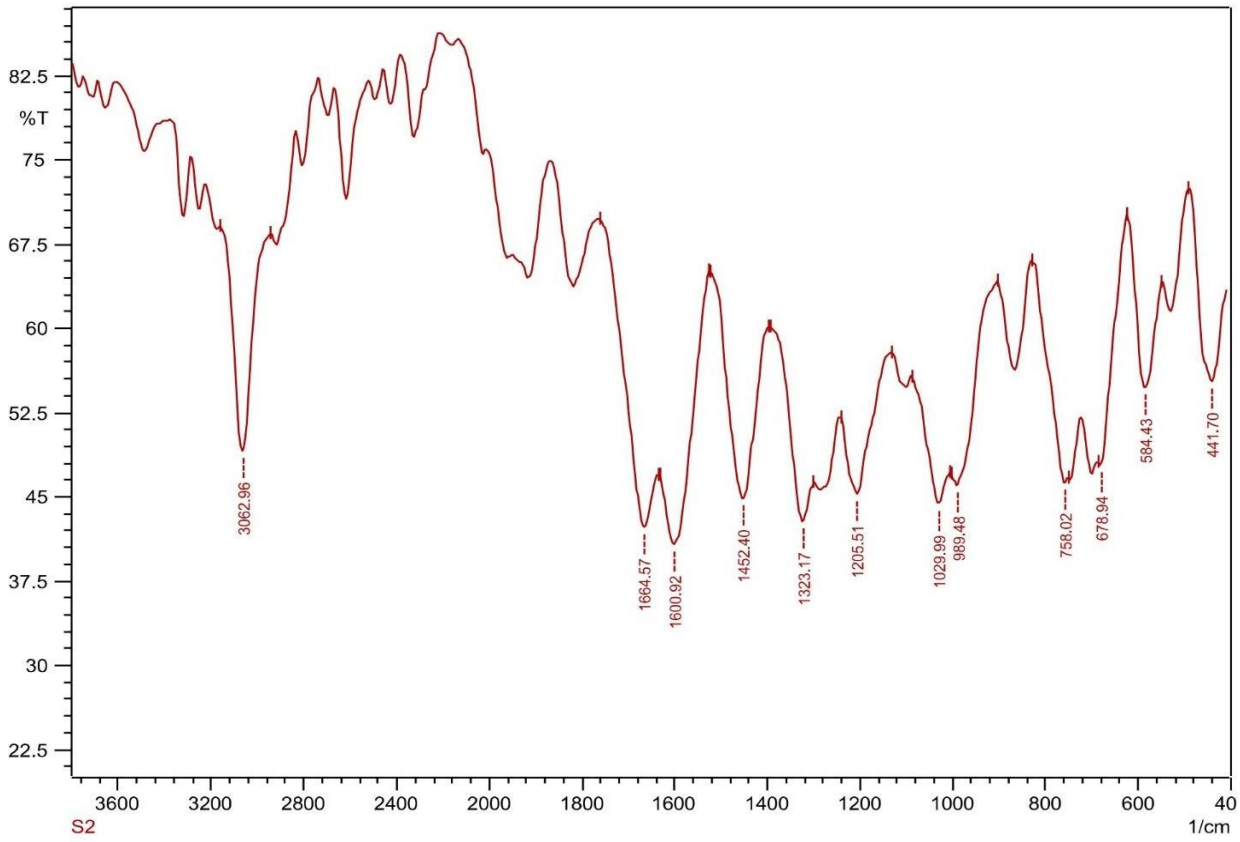
IR Of compound 'E'



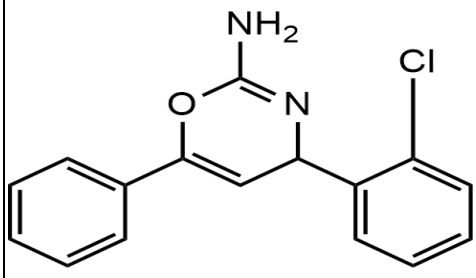
C=Cstreatching	1600.92cm^{-1}
N-Hstreatching	3057.18cm^{-1}
C=Nstreatching	1660.71cm^{-1}
C-Ostreatching	1215.15cm^{-1}

COMPOUND'E'		 4,6,diphenyl 4H 1,3 oxazine 2 amine

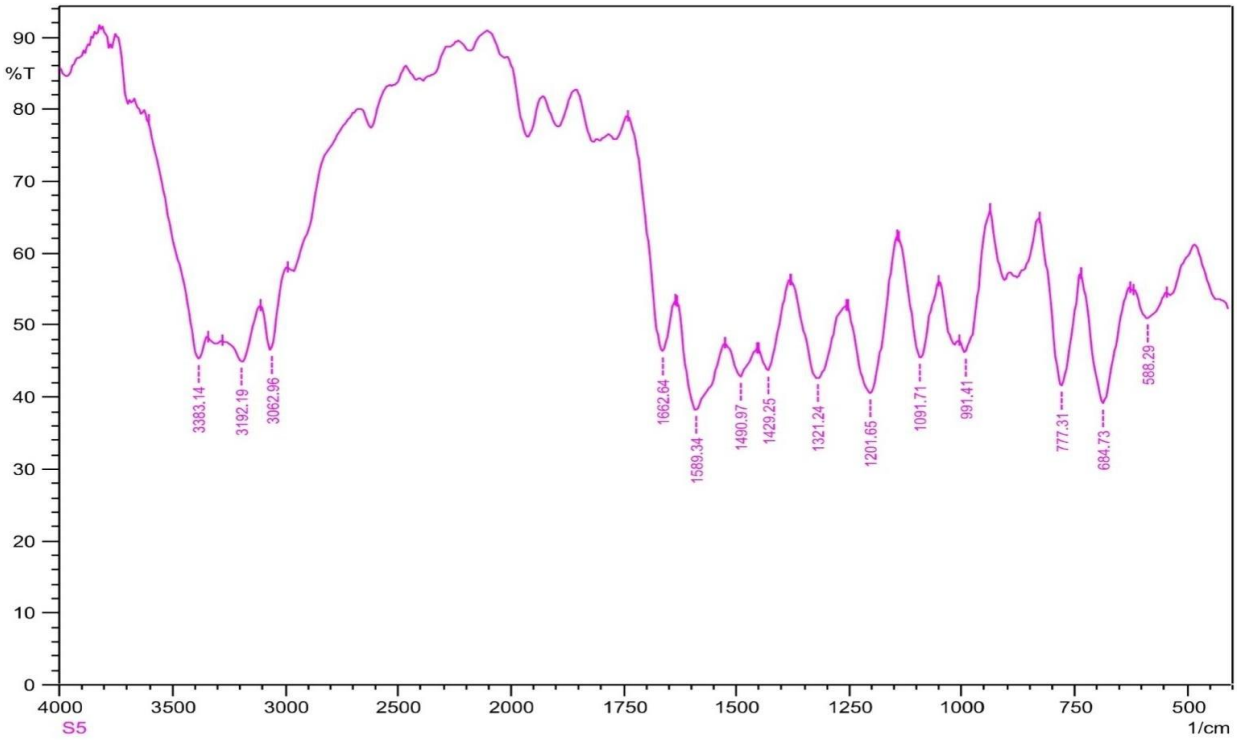
IR. of compound 'F'



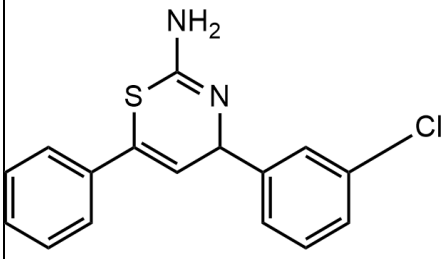
C=Cstreatching	1600.92cm^{-1}
N-Hstreatching	3062.96cm^{-1}
C=Nstreatching	1664.57cm^{-1}
C-Ostreatching	1205.51cm^{-1}
Ortho-Clstreatching	758cm^{-1}

Compound 'F'		 4(2chlorophenyl) 4H6phenyl1,3oxazine2amine

IR. of compound 'G'



C=Cstreatching	1589.34cm^{-1}
N-Hstreatching	3062.96cm^{-1}
C=Nstreatching	1662.64cm^{-1}
C-Sstreatching	684.76cm^{-1}
meta-Clstreatching	777.31cm^{-1}

COMPOUN D 'G'		 4(3nitrophenyl)4H6phenyl1,3thiazine2amin e

Conclusion

The synthesis of thiazine and oxazine derivatives using chalcone as a key starting material has been successfully demonstrated. This method offers a convenient and efficient route to access these important heterocyclic compounds. The versatility of chalcone as a precursor

allows for the facile introduction of various substituents, thereby enabling the synthesis of diverse thiazine and oxazine derivatives. The structural characterization of the synthesized compounds confirmed their identity, paving the way for further exploration of their biological and chemical properties. These newly synthesized thiazine and oxazine derivatives hold promise for applications in drug discovery and agrochemical development. Future studies may focus on optimizing the synthetic protocol and exploring the pharmacological activities of these compounds to unlock their full potential in various fields.

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"Synthesis of Cyanuric Chloride Derivative and its Antibacterial Activity"

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Abstract:

A new and effective one-pot method for the synthesis of 6-chloro-N^2,N^4-diphenyl-1,3,5-triazine-2,4-diamine derivatives is described in this paper. By using acetone solvent from a mixture of cyanuric chloride and substituted Aniline, a series of 6-chloro-N^2,N^4-diphenyl-1,3,5-triazine-2,4-diamine derivatives was synthesized in high yield under mild conditions and simple workup. Furthermore, the method was performed under mild conditions characterized by simplified pathways and workup, minimized energy, and fewer reaction steps, compared with the previous methods. The proposed method, which is a simpler alternative than the published methods, is applicable for the synthesis of other 6-chloro-N^2,N^4-diphenyl-1,3,5-triazine-2,4-diamine derivatives.

Keywords: Cyanuric chloride, 1,3,5-triazine-2,4-diamine, catalyst, promoter, activator,

Introduction:

Due to the development of resistance towards antibiotic there is a constant need for the development of new antibacterial agents. There are many molecules containing ring such as imidazole, triazole, pyrazole, benzimidazole, benzotriazole, oxypurine, pyrimidinyl, naphtyl, 1,3-dioxolane, thiosemicarbazone, pyridine, furan, thiophene which have shown antibacterial activity. [1] Use of various pharmacophore that are not comma to micro-organisms or against which resistance has not been observed should be focused to develop novel antibacterial agents. S-triazine derivatives in agrochemical and medicinal properties have been subjected toinvestigation. It is found that substituted s-triazine derivatives are an important Class of compounds having antibacterial anticancer, antitumor, antiviral, antifungal and antimalarial activities.[2]

In the reaction cyanuric chloride with ammonia or with amines one two or all of the chlorine atoms may be replaced by amino groups depending upon the temperature of the reaction.[3] A few simple aliphatic and aromatic[4] melamines have long been known but recent attention has been directed to more complex derivatives.[5-6] The s-triazine based chalcones and their derivatives demonstrate a range of biological activities and in general have been studied extensively because of their wide range of biological activity. They are found to be effective as local anesthetics [7,] antibacterial [8,] antimalarial[9], antiprotozoal [10-11] antitubercular [12,] anticancer [13] and antifungal agents [14.] These diverse properties of chalcones have prompted us to synthesize them in order to study their biological activities. The study of pyrazoline derivatives has been a

developing field within the realm of heterocyclic chemistry for the past several decades because of their ready accessibility through synthesis, wide range of chemical reactivity and broad spectrum of biological activity.[15]

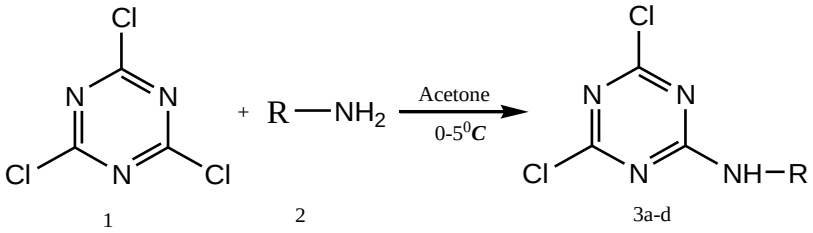
Material and Methods

All reagents used were of laboratory grade. Melting points were determined in open capillaries. The purity of compound was checked by TLC.

Experimental Section:

Scheme 1: -Cyanuric chloride (0.01 mole) was added to acetone (25 ml) at 0–5^0 C, then Aniline (0.01 mole) was added drop wise with constant stirring for 3 hrs. Sodium carbonate solution (10%) was added slowly to neutralize HCl evolved during the reaction. Finally, the contents were poured into crushed ice. The solid separated, was filtered, washed with water, dried and recrystallized from ethanol to give compound. Yield 85.59%, M.P 198^0 C.

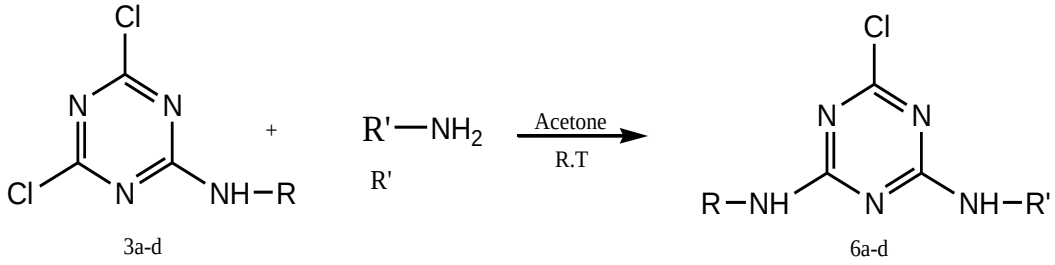
Scheme 1: -



1 2 3a-d

Scheme 2:-Compound 3 (0.01 mole) was added to acetone (30 ml) at room temperature, then Aniline 2 (0.01mole) was added dropwise with constant stirring for 3 hrs. Sodium carbonate solution (10%) was added slowly to neutralize HCl evolved during the reaction. Finally, the contents were poured into crushed ice. The solid separated, was filtered, washed with water, dried and recrystallized from ethanol to give compound 4. Yield 78.87%, M.P 176^0 C.

Scheme 2:-



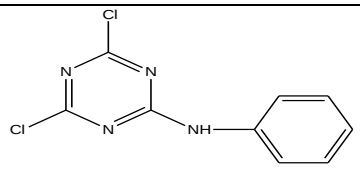
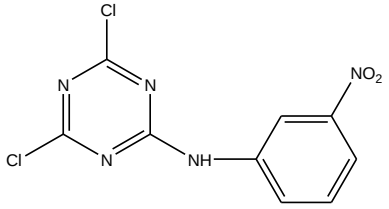
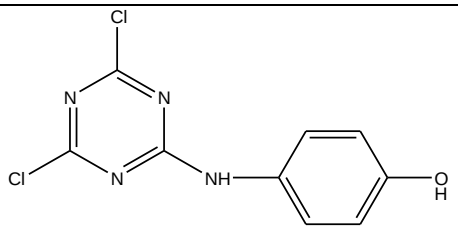
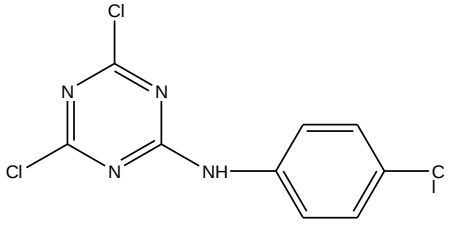
3a-d R' 6a-d

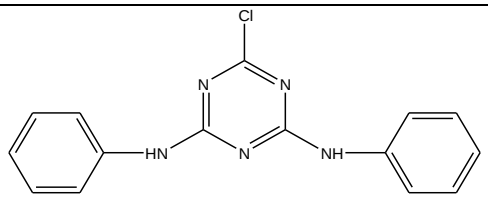
Result and Discussion-

In the series 1- The reaction the cyanuric chloride With Substituted amine Groups at 0-5^{0}c temperature with constants stirring to give the monosubstituted cyanuric chloride derivatives to acidic form then neutralize the derivatives to the appropriated base to give different cyanuric chloride derivatives. First the acetone is used to 8 ml but the reaction is not presided then acetone is used to 10 ml but reaction time is more. Then I used to 12ml acetone to reaction react are increase and reaction time also less in 3hr.

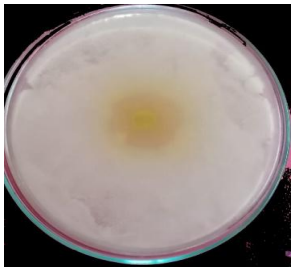
In the series 2-The Product of series 1st react With Substituted amine Groups at room temperature with constants stirring to give the Disubstituted cyanuric chloride derivatives to acidic foam then neutralize the derivatives to the appropriated base to give different cyanuric chloride derivatives.In the second series First the acetone is used to 12 ml but reaction is not presided then I used to 20ml acetone but reaction is presided but reaction time is 5hr. then used to the 25 ml of acetone the reaction reduces to 1hr but reaction time is more to I training to reaction time is less to the acetone are used to the 30ml then reaction time is 3hr.

The Physicochemical data and IR synthesized compounds are shown in following.

product	**Structure**	**R**	**M.P** ^{0}C	**Yield (%)**	**Colour**
3a		Aniline	180	98.16	White
3b		m-Nitroaniline	194	84.56	White
3c		4-Aminophenol	210	85.51	Faint Pink
3d		4-Chloroaniline	174	80.42	White

6a		Aniline	210	84.26	White

Antibacterial Activity: -



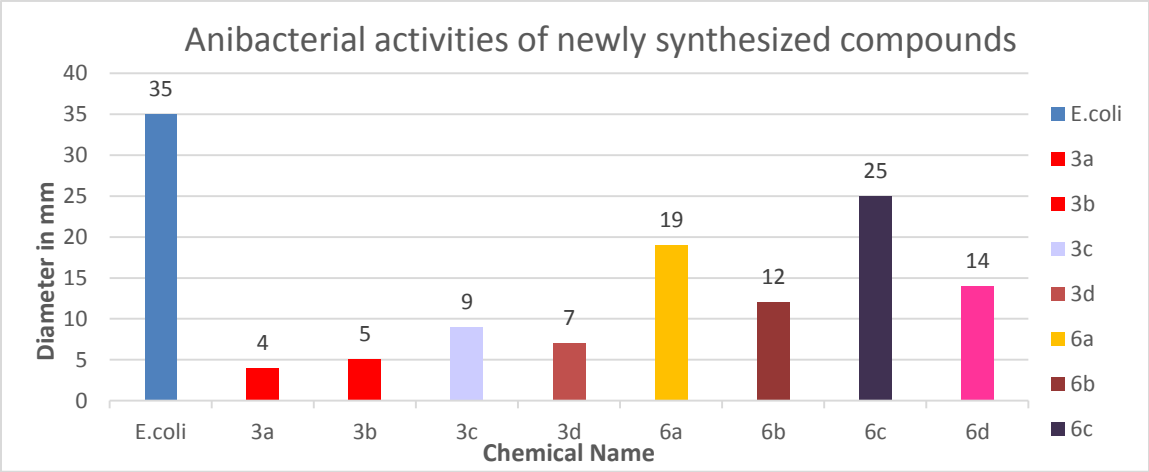
E. coli

Product

The Cyanuric chloride evaluated their antibacterial activity against strain of E.coli.

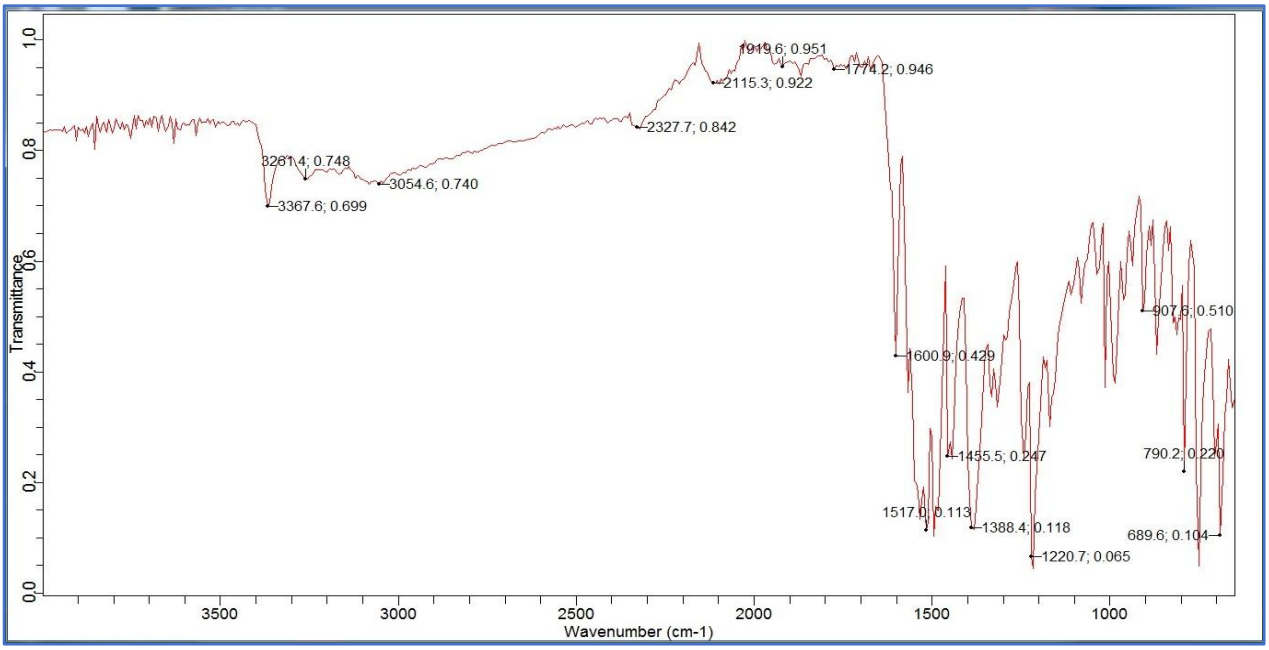
Antibiotic: - Azithromycin, Organism: - E-coli (35.mm)

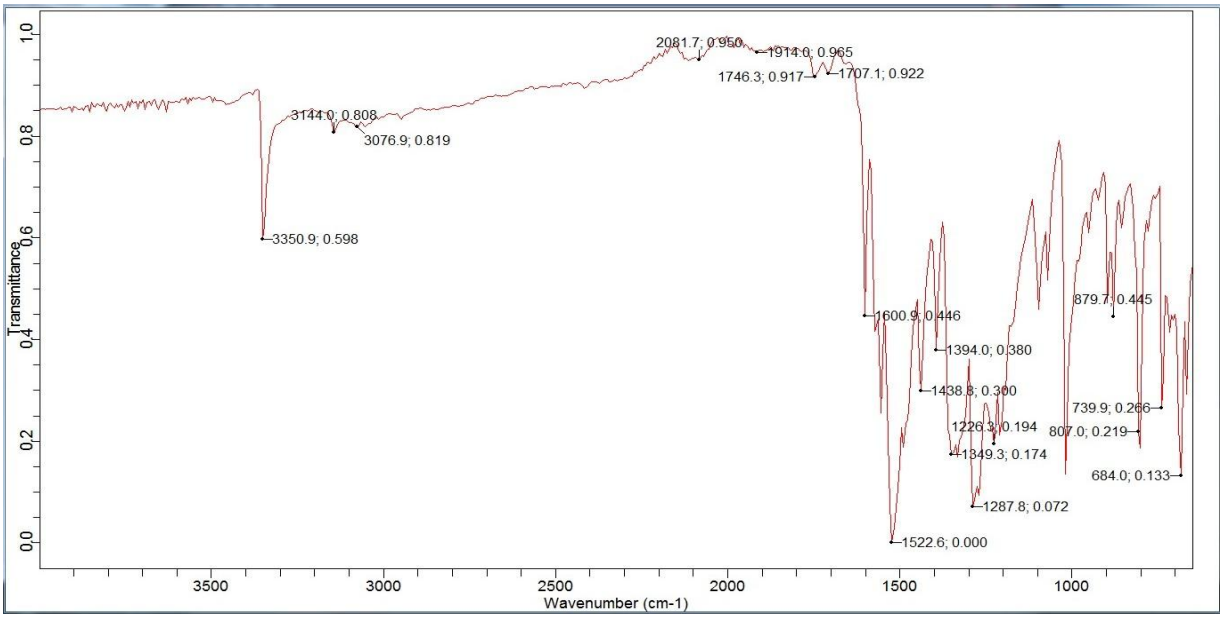
Almost all the synthesize compounds were found to be active Against both Gram-positive and Gram-negative bacteria.



Characterization

FT-IR Spectra of Compound: -3a & 3b





Colour of product: - White (solid)

IR Spectral data: -

3a) Colour: White (solid)- 3367.cm^{-1} N-H, 1600 cm^{-1} C=C, 1620 cm^{-1} C=N, 689 cm^{-1} C-Cl 3054 cm^{-1} C=C-H .

3b) Colour: White (solid)-3350.cm^{-1} N-H, 1600 cm^{-1} C=C, 1650 cm^{-1} C=N, 684 cm^{-1} C-Cl, 3144 cm^{-1} C=C-H , 1394 cm^{-1} NO_2 Stretching

Conclusion: -

A new and effective one-pot method for the synthesis of 6-chloro-N^2,N^4-diphenyl-1,3,5-triazine-

2,4-diamine derivatives is described in this paper. By using acetone solvent from a mixture of cyanuric chloride and substituted Aniline, a series of 6-chloro-N^2,N^4-diphenyl-1,3,5-triazine-2,4-diamine derivatives was synthesized in high yield under mild conditions and simple workup. Furthermore, the method was performed under mild conditions characterized by simplified pathways and workup, minimized energy, and fewer reaction steps, compared with the previous methods. The proposed method, which is a simpler alternative than the published methods, is applicable for the synthesis of other 6-chloro-N^2,N^4-diphenyl-1,3,5-triazine-2,4-diamine derivatives.

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ANALYTICAL CHEMISTRY

Synthesis of Bioplastic by Using Potato Starch

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Abstract: Plastic is very much indestructible even after a long period of time in the environment as it is mainly a derivative of strongly bonded long chain petrochemical-based material. Now-a-days, plastic waste has become an environmental hazard. However, a starch based bio plastic can be a solution of this problem. Starch is a natural biopolymer having mainly two types of polymer glucose. In this research work, a biodegradable plastic is developed from potato starch. The advantages of starch based plastic are its abundance and low cost. However, most of the starch based materials exhibit very poor physic-mechanical properties like low tensile tear strength, high stiffness, elongation at break and poor moisture. Stability improvement of starch based bio plastic properties is being attempted through starch modification, reinforcement (both organic and inorganic), processing conditions and use of compatibilizers to develop substitutes for conventional plastics.

Keyword: Potato starch, Bioplastic, Ecosystem, Acetic acid, Glycerol,
Biodegradab

Introduction:

Bioplastic are biodegradable plastics which are made from biomass. Based on the definition, biodegradable plastic is capable to break down or decomposed through action by bacteria or other living organism.[1] The first plastic was made by using plant through the internachemical synthesis. One of the first synthetic plastics was made from cellulose which is a substance come from plants and trees. Plastics are the most widely used polymer in daily life.[2]The plastic waste problem is increasing until today. Regarding on the environmental issue, plastic waste is responsible in polluting the ocean which can affect not only marine and aquatic organisms but also human beings.[3] The main advantage of starch-based biodegradable plastic are its abundance of raw materials and easy manufacturing process with low cost. During the photosynthesis process, starch is produced in the plants as the reserve food supply.[4]

Most of the starch-based plastics show very poor physical properties such as tensile strength, stiffness and elongation at break and poor moisture stability, which are not suitable for being used as the replacement of plastic product.[5] It is important to enhance the physical and mechanical properties of starch-based plastic in order to use them as the alternative of plastic. In this project, works main focus is to develop the manufacturing process of starch based biodegradable plastic with optimal physical and mechanical properties. [6] Glycerin is used to

control the moisture content in the biodegradable plastic. [7]The whole word, even the ocean, is full of plastic wastes. In addition, the plastic industry has some disadvantages related to economics and environmental problem. [8]The first disadvantage related to the environment is the shrinking of the landfill capacity because of the increasing of the plastic waste amount in the landfill areas. [9] Bio-based bioplastics are derived from renewable resources containing starch, protein, and cellulose. [10] Among the renewable resources, starch is a potentially useful material for bioplastic because it is inexpensive andeasily available.[11-13]

In many countries, bioplastic is mostly used as cutlery, diapers, packing material etc. in many industrial areas. [14] The benefit of using bioplastic is its capability to undergo decomposition in a short period of time without any side effects on the environment. It is ecofriendly plastic and made from inexpensive raw material. Additionally, the manufacturing cost of bioplastic is less compared to petroleum-based plastic. [15]Starch can easily decompose by microorganisms in soil without polluting the ecosystem. These properties led starch to be the second major raw material that can be used in plastic industries for the production of bioplastic. Currently bioplastic shows the main implementation in packaging material, however, in the future, it may use in different fields also such as pipe fittings, insulation material construction material, insulation material, etc..[15-16]

Plastic materials developed from petrochemicals would cause serious environmental impacts due to their non-degradable nature. With the imminent fossil fuel crisis, the search for alternatives is essential in reducing mankind's dependencies in non renewable resources due to the alarming rate of petroleum prices and environmental impacts associated with the products. [17] Biodegradable plastics are of particular interest for non- hardwearing applications such as packaging and agricultural films where biodegradability provides new end-of-life management options that are not applicable to non-degradable plastics such as anaerobic degradation and composting. The motivation for the development of biodegradable plastic is that it will easily disintegrate and biodegrade when inadvertently emitted to the environment.[18]Oceans are also full of plastic waste. the damaging of the marine ecosystem is the second disadvantage. The third disadvantage is that the waste management options are inadequate.

Recycling proportion of plastics is very low. On the other hand, toxic emissions such as carbon dioxide and methane are generated because of plastic incineration. These greenhouse gases (GHG) affect the worldwide climate change negatively.[19] Plastic's non-degradability or

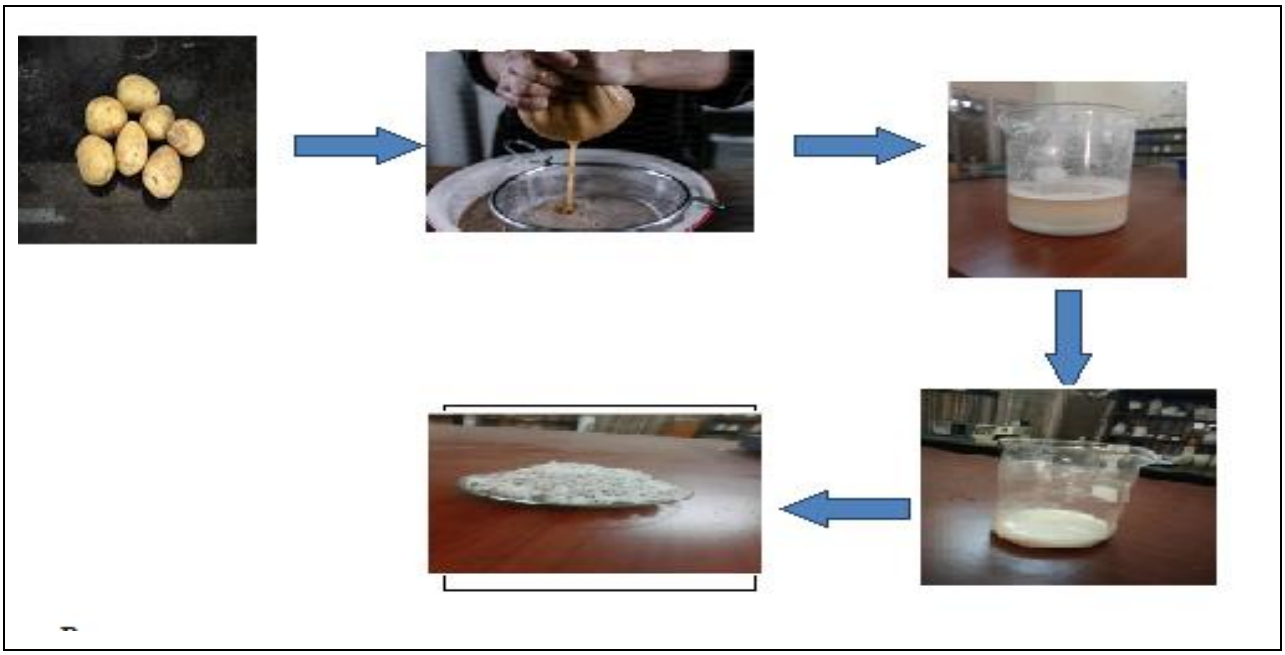
durability is the fourth disadvantage. It was known that plastics are not biodegradable and it can remain in the environment for hundreds of years. [20] In addition, it is expected that fossil fuel will become more expensive and the supply will become more volatile.[9]The economic problem is the increasing fossil fuel prices. These environmental/economic disadvantages and social concerns have led to the development of the green materials, such as bioplastics, in recent years.[21] Today, bioplastics are considered as a promising alternative to plastics.[22]

Material:

Tuber potato (*Solanum tuberosum*) was purchased from market in order extract potato starch from it. Distilled water was used, Acetic acid, glycerin having the purity of 98%.

Method: Extraction of starch :

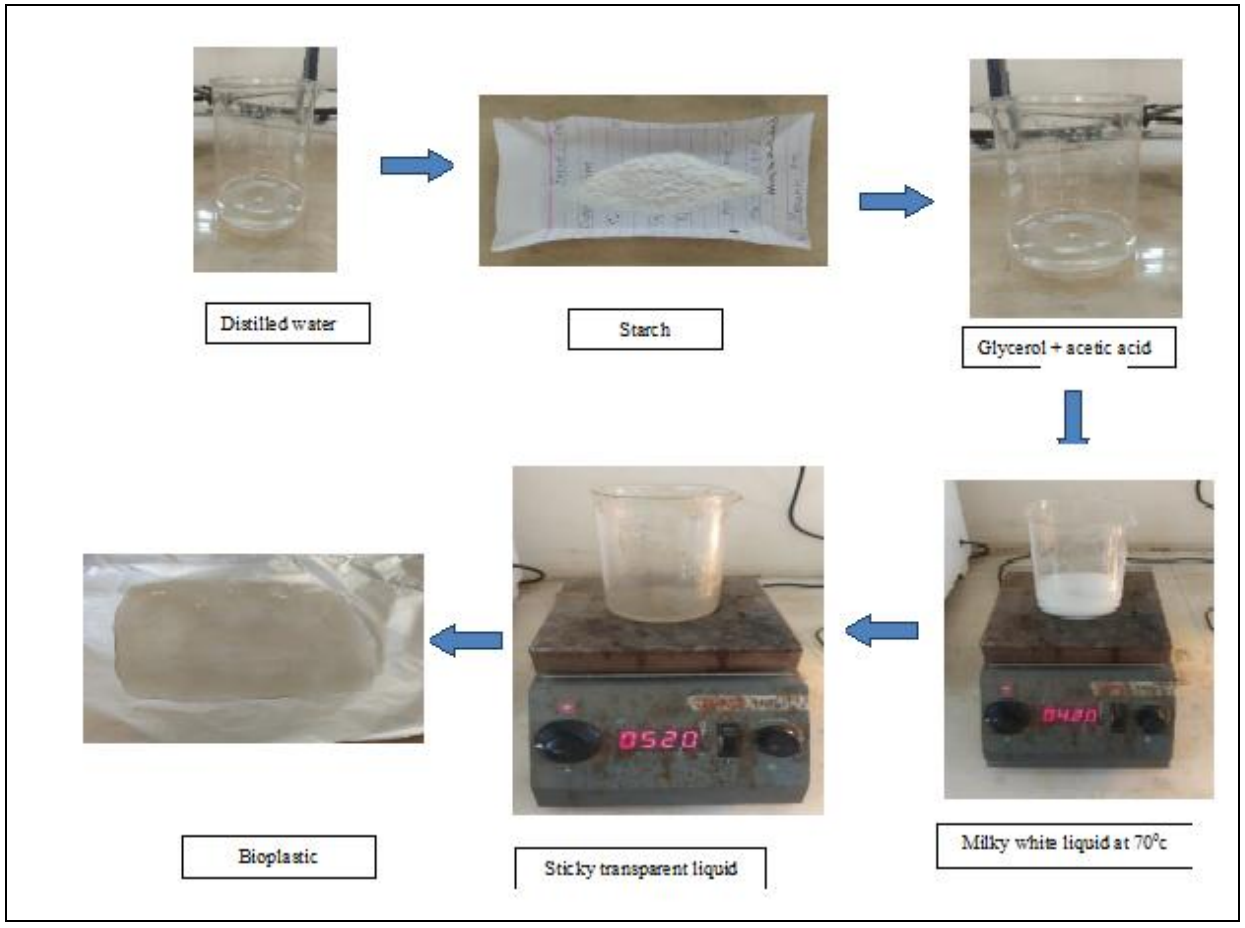
The first process was extraction process of potato starch. involves crushing of potato into a paste and then soaking in a bowl filled with water. The mixture is squeezed. Properly and the paste has to be crushed after that the chess cloth was to get ad possible squeezed hard in order much water out This process was repeated several. Extracted water then needed to be settled for about 20 "minutes. and by this time the potato starch settled down at the bottom. of the pot. The excess water then poured out of the pot and the starch was left to become dry hard.



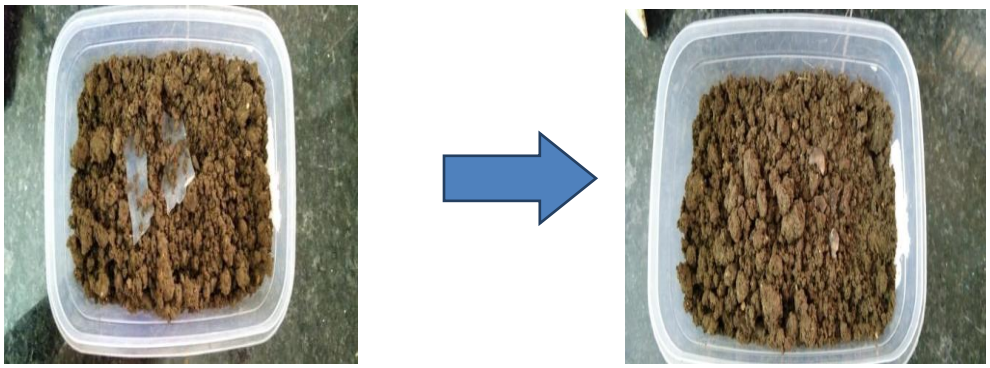
Preparation of Bioplastic

An amount of 40 ml of distilled water was measured using 100 ml measuring cylinder and poured into the 100 ml beaker. Secondly 10gm of potato starch was measured using weight

balance and placed in the beaker with distilled water. The mixture was stirred and mixed together using glass rod. After that some of glycerol and some of acetic acid poured into the beaker. The mixture was stirred again and the hot plate was turned on and set at 700c when the mixture was well mixed. The beaker was placed on the preheated hot plate. The mixture was stirred continuously. Then a milky white liquid appeared in the beaker when it becomes sticky and almost transfer the hot plate was turned off and mixed was spread on aluminium foil. Lastly the mixture was left for four days to cool room temperature.



Biodegradability Test



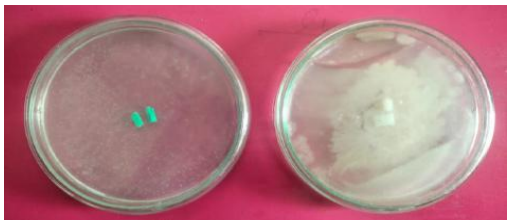
The biodegradability test of bioplastic was determined after 50 days.

- The biodegradability behaviour in reducing the mass of bioplastic, the initial mass of potato starch-based bioplastic was 2.150 gm on day 0 and reduced to 0.530gm on day 50 with the optimum of weight loss of 90%.

Water Absorption Test

Potato starch bioplastic of 16.71gm with the maximum rate of water absorption 55.88% on day 50.

Microbial Test



The bioplastic sample is showing degradation after 48 hrs of inoculation. The other plastic which was used as a control for the comparison do not show any change in their size & weight.

Solubility Test

The results of solubility test of bioplastics are presented in Table. The results of the study revealed that the material was insoluble in water which makes it more eligible to be a bioplastic material. It was also insoluble in acetone (polar solvent), ethyl alcohol (non-polar solvent), acetic acid (polar solvent) and partially soluble in ammonia (polar solvent) and completely soluble in sulphuric acid (strongly acid solvent). The insolubility of synthesized bioplastic film in water showed that it can be further studied to replace, use and throw plastic bottles.

Sr. No.	Solvent	Solubility Test		
		Insoluble	Partially Soluble	Completely Soluble
1	Ammonia	-	+	-
2	Acetic acid	+	-	-
3	Acetone	+	-	-
4	Sulphuric acid	-	-	+
5	Ethyl alcohol	+	-	-
6	Water	-	+	-

+ = Positive - = Negative

Result & Discussion:

Sr. No	Test	Percentage
1	Biodegradability Test	90%
2	Water Absorption Test	55.88%

Microbial Test

Sr. No.	Test	Bioplastic	Plastic
1	Length at an inoculation time	1 cm	1 cm
2	After 48 hours	0.6 cm	No chnage
3	Texture at inoculation time	Hard	Hard
4	After 48 hours	Soft	Hard

Solubility Test

Sr. No	Solvent	Percentage
1	Ammonia	50%
2	Acetic acid	0.7%
3	Acetone	12%
4	Sulphuric acid	98%
5	Ethyl alcohol	10%
6	Water	35%

Conclusion:

We have prepared the potato starch-based bioplastic. Then biodegradability test, water absorption test, microbial assay & solubility test of bioplastic have been determined. From the result obtained, it can be concluded that potato-starch-based bioplastic is able to degrade by 90%

within 50 days and in water absorption test, the potato starch-based bioplastic absorbed water by 55.88 % in 50 days. Also, in solubility test, the bioplastic is completely soluble only in the sulphuric acid. By performing microbial test, we can conclude that bioplastic can be degrading fastly and completely with the help of micro-organism (E-coli) by the degradation method.

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Extraction of Oil from Orange Peels and Study of an Its Antimicrobial, Antifungal and Phytochemical Activity

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Abstract:

The orange peel which is considered as a waste can be used for the extraction of essential oil which has many applications ranging from food flavoring agent to cosmetic. Orange oil can be extracted by various conventional methods like steam distillation, solvent exaction. This paper focuses on improved Solvent extraction method. This method enhances the oil yield.

Keywords: Orange peel, Extraction, Soxhlet extraction, HPLC, Essential oil, Limonene.

Introduction:

Sweet Orange (*Citrus Sinensis*) is the most commonly cultivated fruit tree in various districts of Maharashtra. Orange trees are widely cultivated for its taste and natural value, which is peeled, cut, eaten whole or processed to extract orange juice and also for the fragrant peel. Orange essential oil is produced by cells within the rind of an orange fruit [1]. In many developing countries today, microorganisms are regarded as the main cause of diseases mortality. Despite advances in diverse antimicrobials made by pharmaceutical endeavors, the protection from antitoxins has increased overall in many bacterial microorganisms [2]. It has been reported that a significant number of the world's population depends on traditional medicine for primary healthcare [3]. Over the years, essential oils and other plant extracts have stirred up curiosity as sources of natural products and have thus, been screened for their potential uses as alternatives for the treatment of many infectious diseases [4]. It has been severally hypothesized and empirical data have shown that the antibacterial potential of agents from natural sources, such as essential oils from plant could serve as means of combating multidrug resistance challenges [5-8]. The citrus plant belongs to the Rutaceae family, which also includes about seventeen different species and is spread throughout the following three climate zones: tropical, subtropical and temperate. Around 140 counties annually produced 70 million tons of citrus. Pakistan is the 12th-largest producer of citrus, with a yearly production of over 1,816,000 tons [9]. Peels and bagasse, which make up 40-50% of the total fruit mass, are produced in large quantities during the production of citrus juice [10].

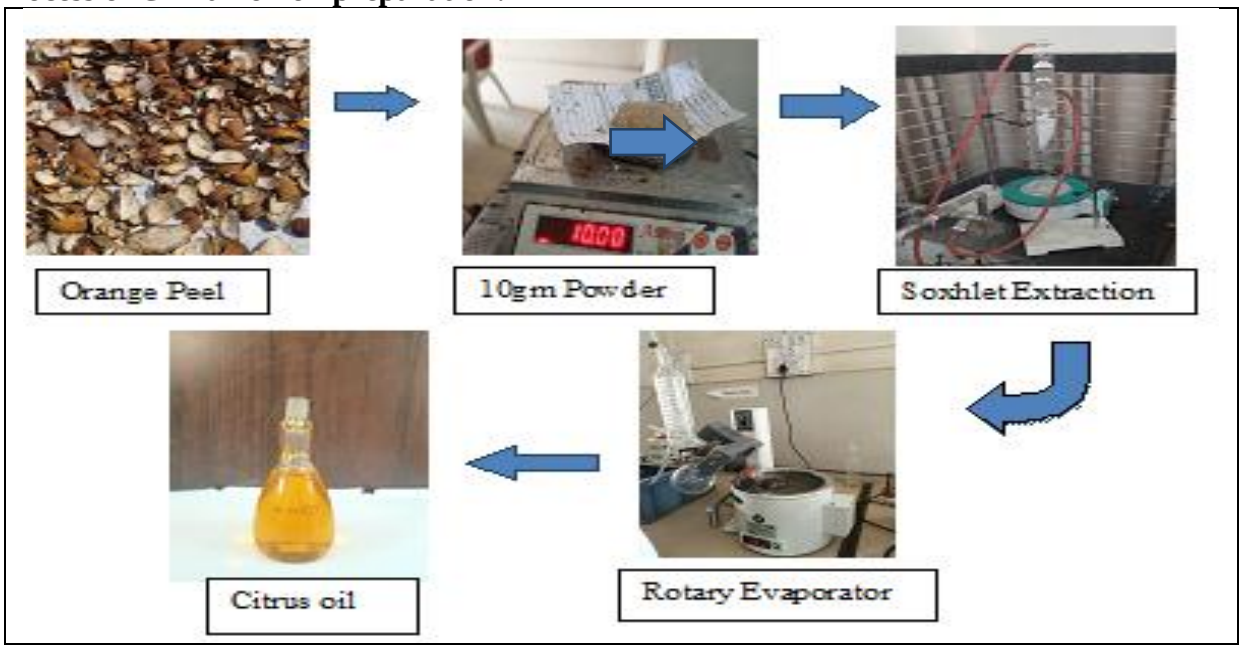
Material:

Materials use for study were Beakers, Test tubes, Measuring cylinder, Funnel, Whatmann filter paper, Ethanol, Dichloromethane, Hydrochloric acid, Mayer's reagent, Pyridine, Sodium nitroprusside, 1% Ferric chloride, Lead acetate, Chloroform, Conc. Sulfuric acid, dil NaOH, Ammonia, Nutrient Agar, Petri plates, L-shaped spreader, Dropper, Well puncher, Orange peel, Heating Mentle, Round Bottom flask, Rotatory Evaporator, HPLC, UV spectrophotometer.

Method:

After collection, the peels were cleaned and washed with water. The peels were then dried in the shade until removal of all moisture content and powdered with the help of pestle and mortar instead of using an electric grinder which cause the loss of useful components due to rotation at high speed. The oil was extracted by using the solvent extraction method. For these purpose, the soxhlet apparatus was used with solvent petroleum ethanol. The 200 ml of petroleum ethanol was measured and pour into a flask. The 10gm powder of citrus peel was weighed and dropped into an extractor by the thimble. The Soxhlet apparatus set for 4hrs. at 60^{O}C. For oil extraction, a rotary evaporator was used to separate the oil from the solvent.

- **Process of Cinnamon oil preparation:**



Experimental section:

Preparation of Test sample:-

5 ml of Citrus oil transferred to conical flask and 50 ml ethanol were added to flask. The mixture was stored for 24 hr. After 24 hr Has filter the sample filtrate is used for test

1.Detection of Alkaloids. To 5 ml of extract, dilute hydrochloric acid was added and filtered. The filtrate was used to perform the following test for the detection of alkaloids. **Mayer's test:** Filtrates were treated with Mayer's reagent consisting of potassium mercuric iodide. Formation of a yellow-coloured precipitate confirms the presence of alkaloids.	
2. Detection of glycosides. **Legal's test** Test sample extracts (1 ml each) were added to individual test tubes and 1 ml pyridine and 1 ml sodium nitroprusside added to each test tube. The development of pink to red colour confirms the glycosides.	
3.Detection of saponin Froth Test: Extracts were diluted with distilled water to 20 ml and vigorously shaken in a conical flask for 15 min. The formation of foam indicates the presence of saponin.	
4. Detection of phenols: **Ferric Chloride Test**: Extracts were treated with 3-4 drops of 1% ferric chloride solution. The formation of bluish black colour confirms the presence of phenols.	
5. Detection of flavonoids: **Lead Acetate test:** To the test sample, 12-15 drops of lead acetate solution were added. Formation of yellow colour precipitate confirms the presence of flavonoids.	
6..Detection of terpenoids: **Salkowski test :**Test sample extract (5 ml) was transferred to separate test tubes. 2 ml of chloroform and 3 ml of concentrated sulfuric acid were added to each to form a layer. The formation of a reddish-brown colouration at the interface shows positive results for the presence of terpenoids.	

7. Detection of coumarin: About 0.5 g moistened dry powder of each test sample was taken in separate test tubes. The test tubes were covered with Filter paper and presoaked in dilute NaOH. The tubes were kept in a water bath. The filter papers were then exposed to UV light and observed for colour development. The appearance of yellowish-green fluorescence indicated the absent of coumarin. Ammonia and 2 ml of 2N HCl were added to 2 ml of aqueous extract. There is no appearance of pinkish-red colour later turn in to bluish-violet confirms the absence of anthocyanin	

Antimicrobial Activity:-

1. **Disc Diffusion Agar Method.**

The microbial suspension was streaked onto the petri dishes containing NA. The essential oil (20 µL) was added to soak the filter paper discs with 6 mm in diameter, and the disc's were then placed on the inoculated plates, followed by incubation at 37°C for 24 hrs. The inhibition zone diameter around filter paper disc's was measured in millimeters.

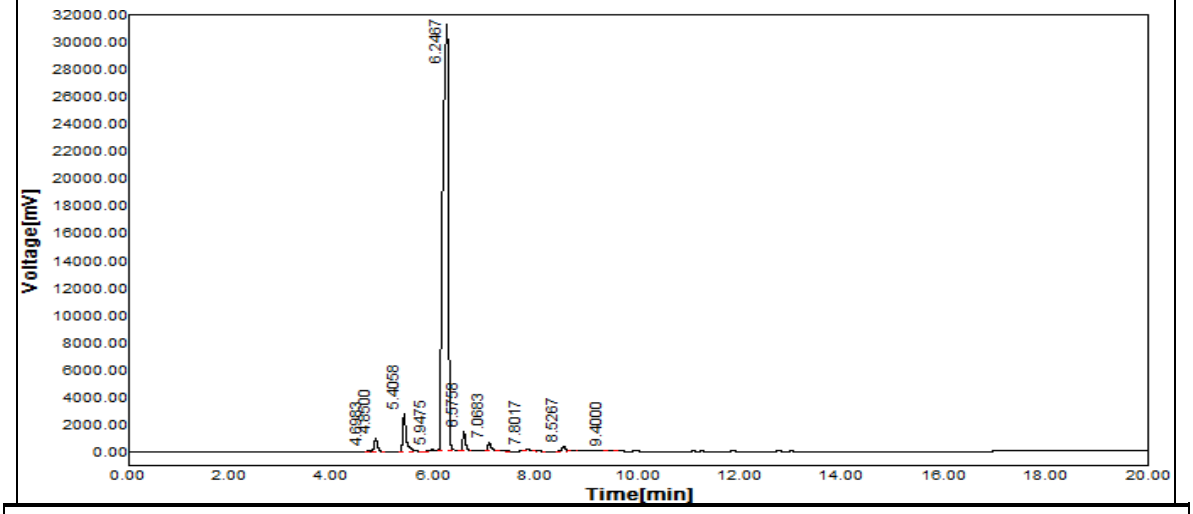
2. **Well Diffusion Agar Method.**

In this assay, the microbial suspension was spread onto NA medium in petri dishes using a L-shaped spreader. Afterwards, several wells (6 mm in diameter) were created on the medium surface and loaded with the essential oil (20µL). The petri dishes were kept at a constant temperature of 37°C for 24 hrs, and the diameter of the inhibition zones around the well was measured and expressed in millimeters.

Characterization:

HPLC Data:-

ANALYSIS REPORT
Post:
Name:
Analysis
Sample Name: Citrus Sinesis
Sample ID:
File: 0362.RAW
Date: 2024-04-03 PM 01:20:16
Channel: 1. GC A
Chromatogram



Result

No.	Name	RT[min]	Area[mV*s]	Height[mV]	Area%
1		4.6683	223.5718	63.2724	0.08
2		4.8500	3625.8723	641.5547	1.47
3	Limonene	5.4058	12866.7141	2713.6841	4.82
4		5.6475	533.1006	66.0001	0.20
5	Citrus Sinensis	6.2467	237830.5250	31113.1113	86.05
6		6.5758	5364.7466	1448.7364	2.01
7	Myrcine	7.0683	3078.1402	645.7558	1.15
8	Octanol	7.8017	870.2687	134.5216	0.33
6		8.5267	2003.8008	370.1761	0.75
10		6.4000	377.3435	56.5747	0.14
Sum			267077.1000	37586.3867	

Result and Discussion:

1. Phytochemicals test:-

Phytochemical Test.	Ethanolic Extract.
Alkaloids	+
Glycosides	+
Saponin	+
Phenols	+
Flavanoids	+
Terpenoids	+
Coumarin	-

1. Antimicrobial Activity:-

Sr. No.	Microbial strain	Zone of inhibition Disc
1.	E-coli	2.2 mm
2.	Bacillus	1.8 mm

2. Sensory evaluation of Citrus oil

Citrus oil Property	Rating
Appearance	
Colour	Brownish yellow
Aroma	Tangy smell
Density	0.778 g/cm^3
Solubility	Insoluble in water
Specific gravity	0.843 g/cm^3

Conclusion

Citrus oil has good flavor & aroma. And presence phytochemicals in oil. Extraction of Citrus oil is easy process it does not require expertise and automated lab equipment. Citrus oil contains many compounds or chemicals. Citrus fruits are the widely available and maximum consumed fruits. It has so many medicinal properties and very less toxic effect. In the present study essential oil was extracted by steam distillation method, it is the cheapest and easiest method. Many of the volatile chemicals are detected, which were naturally derived from fruit peel of *Citrus sinensis*. The oil extracted from *C.sinensis* fruit peels through the Soxhlet apparatus was brownish yellow in colour with tangy smell.

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Formulation and Evaluation of Herbal Hand Santizer and Herbal Hand Wash

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Abstract: This research is based on the superiority of Herbal Hand Sanitizer using *Azadirachta Indica, Ocimum Tenuiflorum, Mentha Pipereta, Aloe vera, Rose water* and camphor and Herbal Handwash using *Reetha, Ocimum Tenuiflorum, aloe vera and rose water*. Majorly this research has focused on Hand Hygiene. Since hands are the primary mode of transmission of various disease causing germs. With the help of proper methods of washing hands and sanitization during daily lifestyle as well as during Patient care can inhibit or reduce the chances of infection. Goal of this research is to prepare environment friendly biodegradable Herbal Hand sanitizer and Herbal Handwash which shows effective antibacterial and are inexpensive.

Keyword: Azadirachta Indica, Ocimum Tenuiflorum, Mentha Pipereta, Aloe vera,

Introduction:

Hand hygiene plays a vital role in maintaining a good health. Since, hands are becoming main reasons behind spreading the disease [1, 2]. Hence, hand hygiene is a vital principle and exercise in prevention, control and reduction of health acquired infections1.Proper method of washing and drying hands stops the chain of transmission of deadly pathogens (from contaminated surface or site). Since, chemical used in hand sanitizer and handwash shows side effects like redness or rashes on hands. So, Use of herbal hand sanitizer and herbal handwash becomes must in recent circumstances. It was found that microbes had slowly developed resistance against various antibiotics. Since many decades Plants were the chief source of treating various diseases. Hence, this research highlights the use of various plants with antimicrobial activities [2]. The main reason of present study was to prepare herbal hand sanitizer incorporating the leaves extract of azadirachta indica (Neem) [3], ocimum tenuiflorum (Tulsi), mentha (Mint), aloe barbandis (Aloe Vera), syzygium aromaticum (clove), camphor, rose water. This is best herbal combination which gives multidimensional activities; and to evaluate these formulations for their antimicrobial efficacy and safety of hands [4]. The formulation was evaluated against the specified microorganism (Bacteria: E-coli, staphylococcus aureous) by culture sensitivity test [5].The significance was found to be more in comparison with standard references. Handwash made from reetha powder as a foaming agent, tulsi as main ingredient, rose water as a fragrant and aloe vera as smoothening agent is an excellent herbal

combination with multidimensional activities. Personal as well as hand hygiene is important to prevent many communicable diseases. Hospital and community acquired infections are considered a serious public health problem worldwide [6]. The centers for disease control and prevention, the world health organization, promote hand hygiene as the most important measure in the prevention of hospital and community acquired infections. As transmitting modes of microbes, hands are considered to be the primary route for the causative microbes and infections to the individuals [5, 6]. Applying both washing hands with soap and hand antiseptics are the most important hygiene methods which significantly increase the chance of maintaining the hands clean and aseptic. Staphylococcus aureus, Staphylococcus epidermidis, and Enterococcus faecalis conserved as resident floras and commonly colonized the different *place* of body such as superficial layers of skin that consists of *S.* aureus, Escherichia coli, and Pseudomonas aeruginosa [7].

To reduce infections in the community, alcohol based hand sanitizers are recommended as a component of hand hygiene [8]. Hand hygiene generally refers to different methods of eliminating or killing microorganisms which may be present on hands, by either hand washing or sanitizing (Pittet et al., 2006,Zapka et al., 2017) [9, 10]. Furthermore it was explained well that hand washing removes body's own fatty acids from the skin, which may cause cracked skin that formed an entry portal for pathogens, using of hand washing, hand sanitizers were introduced to prevent those pathogenic micro-organisms as well as to protect and improve skin condition [11, 12]. In fact, despite a label them of reducing "germs and pathogenic bacteria" by 99.9%, some studies have observed an apparent increase in the concentration of bacteria in handprints impressed on agar plates after cleansing [13]. Though the concept of hand sanitization has been in place right from the start of the hand hygiene campaign by semmelweis [2, 14], many hand sanitizers are available in the market with different effectiveness against pathogenic microbes some of them in the market as antimicrobial hand sanitizers are not effective in reducing bacterial counts on hands. Most of the early reports focused on the role of hand washing as an] infection control measure [15]. This changed by 2000s, when the centers for disease control and Prevention (CDC) issued a guideline recommending that alcohol-based hand rub (ABHR) be routinely used for decontaminating hands [16] which are often composed of alcohol, ethanol, isopropanol or propanol [17, 18]. It has been recommended that concentration range of 60% to 95%.

Material and Method:

The plants *Azadirachta indica* (Neem), *Ocimum tenuiflorum* (Tulsi), *Mentha* (Mint), *Aloe Barbandis* (Aloe Vera), *Syzygium Aromaticum* (Clove) were collected the sample.

Preparation of Extracts:

The leaves were taken from collected plants such as *Azadirachta indica* (Neem), *Ocimum tenuiflorum* (Tulsi), *Mentha* (Mint), *Aloe Barbandis* (Aloe Vera), *Syzygium Aromaticum* (Clove) and separately powdered. 5grams of leaf powder of each plant were collected and soaked in 200ml of ethanol seperately and kept for maceration for about 3-4 days. After maceration the prepared extract was collected as well as filtered and used further for making hand sanitizer and handwash.

Preparation of Hand Sanitizer:

The following formula was used to prepare hand sanitizer. In this formulation the hand sanitizer was prepared using 5ml ethanoic extract filtrate of each plant. To this filtrate 10gm of camphor and 4-5 drops of rose water is added and the volume is made up to 50ml with alcohol.

Table 1: Formula for Hand Sanitizer.

Sr. No.	Ingredients	Quantity
1	Neem Extract	5 ml
2	Tulsi Extract	5 ml
3	Mint Extract	5 ml
4	Clove Extract	5 ml
5	Aloe vera Extract	5 ml
6	Camphor	10 ml
7	Rose water	5 ml
8	Alcohol	20 ml

Preparations of Handwash:

The leaves were taken collected plants such as tenuiflorum (tulsi), alove barbandis (aloeVera), reetha powder and separately powdered. 5 grams of leaf powder of each plant were collected and soaked in 100 ml of ethanol seperately and kept for maceration for about 3 – 4 days. After maceration the prepared extract was collected as well as filtered and used further for making hand wash.

The following formulas were used to prepare hand sanitizer. In this formulation the hand sanitizer was prepared using 5ml ethanoic extract filtrate of each plant. To this filtrate 10gm of camphor and 4-5 drops of rose water is added and the volume is made up to 50ml with alcohol.

Table 2: Formula for Hand Wash.

Sr.No.	Ingredients	Quantity
1	Reetha Powder	10 ml
2	Tulsi	5 ml
3	Aloe vera	5 ml
4	Rose Water	5 ml
5	Purified Water	Upto 50 ml

Process of Hand Sanitizer

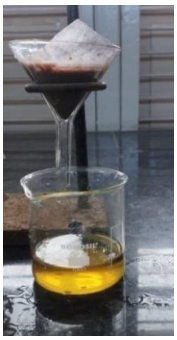
5 gm of leaf powder of each plant (Neem, tulsi, Mint, Clove, Reetha)

soaked in 100 ml of ethanol separately for about 3-4 days .

Filtration

Hand sanitizer

Process of Hand Sanitizer



5 gm of leaf powder of each plant soaked in 100 ml of ethanol Tulsi,Reetha **)**

Soaked in 100 ml of ethanol separately for **about 3-4 days.**

Filtration

Handwash

- **Evaluation of the Prepared Formulations of Hand Sanitizer:**

1. **Organoleptic Properties:**

Determination of clarity.color and odor was done. Appearance as well as color was tested by naked eyes against white background, while the odor was smelled.

2. **Physical Properties:**

a) p^H: The p^H of the hand sanitizer was in between 7 to 7.5 .

b) Viscosity: The viscosity of was determined by using digital brookfield viscometer.

c) Skin Irritancy Test: 10 healthy volunteers were selected. The herbal hand sanitizer was applied on palm of 5 healthy volunteers and time was noted. Same as hand sanitizer was applied on palm of 5 irritancy, redness, dryness and itching like parameters were checked.

d) Antimicrobial testing of the prepared formulations:

The prepared hand sanitizer was exposed to antimicrobial screening by agar well diffusion method. Organisms used were E coli. 1ml of sanitizer was mixed with 5ml DMSO, while 1ml of was mixed with 5ml of sterile water; and it is further used for evaluating the antimicrobial activities. The plates were incubated at 370C for 24 hours and the zones of inhibition were recorded.

- **Evaluation of the Prepared Formulations of Hand Wash:**

1. **Organoleptic Properties:** Determination of clarity.color and odor was done. Appearance as well as color was tested by naked eyes against white background, while the odor was smelled.

2. **Physical Properties:**

a) p^H: The p^H of the hand wash was in between 7 to 7.5 .

b) Viscosity: The viscosity of was determined by using digital brookfield viscometer.

c) Skin Irritancy Test: 10 healthy volunteers were selected. The herbal hand wash was applied on palm of 5 healthy volunteers and time was noted. Same as hand wash was applied on palm of 5 irritancy, redness, dryness and itching like parameters were checked.

d) Foam Height: For handwash foam height was 5 cm.

e) Antimicrobial testing of the prepared formulations: In this particular evaluation test, the antibacterial of herbal handwash were found to be safe as well as according to this information, we can say that the herbal handwash shows to some extent lesser activity than pure antibiotic Gentamycin. But, definitely the formulated herbal handwash has anti-microbial activity against bacterial species like E coli.

Result of hand sanitizer:

The present study was carried out to formulate *Azadirachta indica (Neem), Ocimum tenuiflorum (Tulsi), Mentha (Mint), Aloe Barbandis (Aloe Vera), Syzygium Aromaticum* (Clove) and Reetha Powder extracts based hand sanitizer. The formulation was prepared by using herbal ingredients and excipients that are compatible with any similar hand cleansing formulations. It was organoleptically evaluated to ensure product stability and performed Invitro antimicrobial test to demonstrate its efficacy against infectious bacteria which are collected from volunteers.

Result of hand wash:

The present study was carried out to formulate *Azadirachta indica (Neem), Ocimum tenuiflorum (Tulsi), Mentha (Mint), Aloe Barbandis (Aloe Vera), Syzygium Aromaticum* (Clove) and Reetha Powder extracts based handwash. The formulation was prepared by using herbal ingredients and excipients that are compatible with any similar hand cleansing formulations. It was organoleptically evaluated to ensure product stability and performed Invitro antimicrobial test to demonstrate its efficacy against infectious bacteria which are collected from volunteer.

Conclusion:

Most of the people do not even know the importance of using herbal hand sanitizer and herbal handwash. Since, hands are the primary mode of spreading various infections. proper methods of washing and drying hands can prevent infection. Hence, it was proved that the herbal hand sanitizer and herbal handwash are very effective against various bacteria, viruses and microbes and must in recent circumstances. As compared to chemically prepared hand sanitizer and handwash, herbal hand sanitizer and herbal handwash is very effective, environment friendly, biodegradable and inexpensive. Herbal handwash is much better than the plain soap due to their ingredients and effectiveness on our skin of hands as well as suitable for all type of skin. Mostly herbal handwash protect us from many daily encounter bacteria. The result suggest that the constituents of the various extracts of ocimum tenuiflorum, aloe barbandis and their combination are capable of giving superficial anti-microbial, antibiotic as well as antibacterial activity and they are able to inhibit the many pathogens than commercially available antiseptic soap.

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Analysis of Clove Oil and Extraction of Eugenol

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Abstract

Clove is one of most ancient and precious spice in the world. Clove oil is well-known for its physiological and pharmacological benefits. It is also known as the oldest remedy for relieving tooth pain. The aroma profile of the spice oil plays an important role in consumer acceptance. Hence, analysis of volatile compounds becomes crucial as it facilitates the authentication of the namely, conventional distillation apparatus and Clevenger's apparatus. Also, the clove oil was extracted from two different physical forms i.e. from whole clove buds and powdered clove buds.

Keywords: Clevenger's Apparatus, Antioxidant, Eugenol, Marker Compounds.

Introduction

Clove (*Eugenia caryophyllata Thunb*) is widely cultivated in Madagascar, Sri Lanka, Indonesia and the south of China [6]. Clove (*Syzygium aromaticum*), Is Indonesian native species, is an aromatic flower buds. Java and Manado are parts of the largest clove productions in Indonesia. Beside used as cooking ingredients directly, clove can be proceed furthermore as medicinal properties [1]. Three types of essential oil are available from clove species: clove bud oil, clove steam oil and clove leave oil. Each has different chemical composition and flavor. Clove bud oil the most expensive and the best quality product, Contains eugenol (80% -90%), eugenyl acetate (15%-17%), β- caryophyllene (5-12%). Essential oils are complex mixtures, made up of terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes. They originate from the plant secondary metabolism and are responsible for their characteristics aroma [2]. Aroma chemicals present in nature leaves and flowers have been widely used in aroma therapy since ancient times, suggesting that they have some beneficial health effects in addition to their pleasant odor. Until recently, aroma chemicals have been studied mainly from the aspects of flavor and fragrance chemistry [3]. Clove bud oils have biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and are used traditionally as flavoring agent and antimicrobial material in food. For example, clove oil was effective against L. monocytogenes and S. Enteritidis in tryptone soya broth and cheese. The high levels of eugenol contained in clove essential oil give it strong biological activity and antimicrobial activity. This phenolic compound can denature proteins and reacts with cell membrane phospholipids changing their

permeability. Clove oil also has several therapeutic effects, including antiphlogistic, antivomiting, analgesic, antispasmodic, anticarminative, kidney reinforcement, antiseptic, HCMV extracorporeal restraining effect. In Korea, clove oil has been successfully used for asthma and various allergic disorders by oral administration[6]. Different methods can be used to extract essential oil from aromatic plants, such as hydro distillation, Steam distillation, Soxhlet, Microwave, and super critical fluids extraction. However, the disadvantages of these processes are that the extracts are constantly heated and this can damage thermolabile compounds and initiate the formation of artifact [2]. Since the clove oil has been used widely as pharmaceuticals, flavoring and antimicrobial agents in food industry, it is necessary to find the most suitable method for the improvement of the quality of clove oil. The aim of this work is to compare clove oils obtained by the supercritical CO2 extraction with other three traditional methods, in which extraction of clove oil from clove buds with supercritical CO2 was first investigated intensively. Compositions of clove oil were analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) [6].

Anti Cancer Activity- To stay protected from cancer eat more cloves, as the eugenol in clove possesses strong anticarcinogenic properties and helps control lung cancer, breast cancer, and Ovarian Cancer at its early stage. Clove also reduced the abnormal crowding of cells in particular Regions of Lung tissue and checked the growth of pre-malignant cells by more than 85 per cent. In Another in vitro study, Trusted Source, researchers found that clove oil stopped the growth of Several Cancer cell lines, including but not limited to breast, cervical, and colon cancer. Clove Extract also Increased cell death and disrupted cell division in a colon cancer cell line.

Anti fungal activity- The present study indicates that clove oil and eugenol have considerable Antifungal activity against clinically relevant fungi, including fluconazole-resistant Strains, Deserving further investigation for clinical application in the treatment of fungal infections. Studies Have shown that clove essential oil is both fast and effective in killing fungal infections.

Anti diabetic Activity- Cloves also help keep your blood sugar levels in check and are known To promote insulin production, further controlling diabetes. Research shows that the compounds in cloves may help keep blood sugar under control. As little as one teaspoon of the super-star Spice Is enough to reap benefits [8].

Materials and Methods

Materials use for study were Beakers, Test Tubes, Measuring cylinder, Funnel, Whatman filter paper, Ethanol, Dichloromethane, Hydrochloric acid, 1% Ferric chloride, Chloroform, Concentrated sulfuric acid, dil NaOH, Ammonia, Nutrient Agar, Petri plates, L-shaped spreader, Dropper, well puncher, clove bud purchased at from Jalgaon in October 2023 . The basic ingredient of essential clove bud oil in this research is isopropyl alcohol, Clove bud, and water. Tools that used in this research is soxhletation kit (including Soxhlet, round bottom flask, condenser, and isomantle), Simple steam distillation, grinder, Heating Mentle, Round Bottom flask, Rotatory Evaporator, GC [gas chromatography] [6]

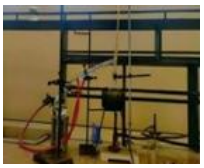
Soxhlet Extractor

First we wash clove with the help of water & dry it in sunlight. Then we make granular powder of clove bud using grinder measure 30 gm of clove powder & fill clove powder in cone shaped extraction thimble Made of filter paper. Packed extraction thimble over all with the help of cotton stringe To avoid floating of clove powder in chamber. But the extraction thimble in to the extraction chamber of Soxhlet apparatus secondly we measure the 250 ml of ethanol using measuring cylinder and pour 250ml of ethanol in to the round bottom flask of soxhlet apparatus. Then we start the heating using heating mantle. After starting heating to round bottom flask. We observe that When ethanol get boiled the fumes of ethanol pass through solvent vapour tube connected to extraction chamber vapours are converted in to liquid due to water condenser droplets when droplets are start that time consider as starting point of extraction. Due to droplet when extraction chamber is fully filled with the extract. Then all the extract were driven in to round bottom flask of solvent through siphon tube these process is repeated many times at the when extraction is started for 6-8 h. [6]

Steam Distillation

The plant (60 g of dried and ground clove buds) in 500 ml flask was submitted to simple steam distillation for 8–10 h. The volatile distillate was collected until no oil drop out. The distillate was saturated with sodium chloride and added with some ethanol. Then, the ethanol layer and simple steam layer were separated by funnel. After dehydrated by anhydrous sodium sulphate, the ethanol layer was further heated in 60°C water bath to make oil to be concentrated and the ethanol to be recovered. The oil was weighed to analysis.[6].

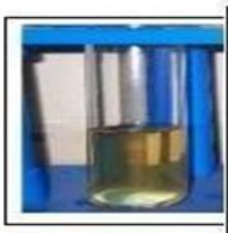




1. Clove 2. 60 gm powder 3. Soxhlet Extraction 4. Steam Distillation 5. Extracted Clove Oil

EXPERIMENTAL SECTION

Phytochemical Test :

Detection of steroid: Salkowski Reaction: To 2 ml of extracts was Jenadded 2 ml of chloroform and 2ml of concentrated H_2SO_4 shake 'well. Chloroform layer **present** greenish yellow fluorescence.	
Detection of carbohydrates : Reduction of Fehling's Solution: 10 ml of Fehling solution (copper sulphate in alkaline condition) were added to the concentrated extracts and heated on a steam bath. Brick-red precipitates indicated the **presence** of carbohydrate.	
Colour test for clove oil: Permanganate test: the solution is originally purple, and when it reacts with a double bond, or eugenol, it loses the purple colour and forms a precipitate **presence** in a colour test of clove oil.	
Detection of alkaloids: Wagner's Test: To 2-3 ml filtrate with few drops Wagner's reagent gives reddish brown precipitate **present** in alkaloids.	

Antimicrobial Activity

1. **Disc Diffusion Agar Method.**

The microbial suspension was streaked onto the petri dishes containing NA. The essential oil (20 mL) was added to soak the filter paper discs with 6 mm in diameter, and the disc's were

then placed on the inoculated plates, followed by incubation at 37°C for 24 hrs. The inhibition zone diameter around filter paper disc's was measured in millimeters. [7].

2. **Well Diffusion Agar Method.**

In this assay, the microbial suspension was spread onto NA medium in petri dishes using a L-shaped spreader. Afterwards, several wells (6 mm in diameter) were created on the medium surface and loaded with the essential oil (20mL). The petri dishes were kept at a constant temperature of 37°C for 24 hrs., and the diameter of the inhibition zones around the well was measured and expressed in millimeters.

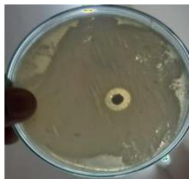



Figure-1: **Bacillus**

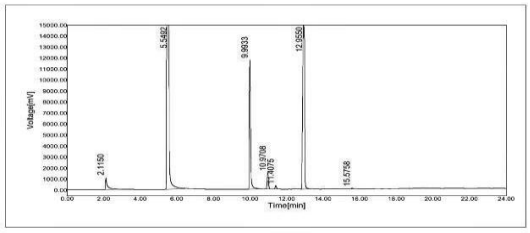

Figure-2: **E.coli**

Preparation of derivative

E.coli plate Dissolve 0.5 gm of phenyl hydrazine hydrochloride and 0.8 gm of Sodium acetate in 8 ml of water. Add 0.2 gm of given compound dissolved in alcohol. Shake and warm the content on water bath for 10 to 15 min. Cool the content and filter the solid derivative. Recrystallize from dil. alcohol, record yield MP of the recrystallise product.

***Analysis of Clove Oil in Gas* Chromatography**

Chromatogram



Result

No.	Name	RT[min]	Area[mV*s]	Height[mV]	Area%
1	a-(2 propenyl phenol)	2.1150	7115.3867	1132.7545	1.88
2	Isolendene	5.5492	182401.6500	23015.4746	48.19
3	a-Cubebene	9.9933	55827.3250	11766.1416	14.75
4	B-Caryophyllene	10.9708	7555.2656	1494.3488	2.00
5	Eugenyl acetate	11.4075	1606.7947	304.1261	0.42
6	a-Humalene	12.9550	123531.1250	18960.1641	32.63
7	Eugenol	15.5758	499.2298	88.7423	0.13
Sum	-		378536.7750	56761.7500	

Results and Discussion

Phytochemical Test

TEST	Ethanoic Extract
Glycosides	+
Carbohydrate	+
Permanganate	+
Proteins.	-
Steroids	+

+ (positive result)

- (negative result)

Antimicrobial Activity

Sr. No.	Microbial strain	Zone of inhibition Disc	Zone of inhibition Well
1.	E-coli	6.9 mm	6.4 mm
2.	Bacillus	4.5 mm	3.9 mm

Eugenol

[Eugenol melting point is -7.5°C]

[Eugenol boiling point is 254°C]

Characterization

Sensory evaluation of clove oil

Clove oil Property	Rating
Appearance	
Colour	Dark brown colour
Aroma	Strong sweet & spicy fragrance.
State	Slightly viscous liquid
Clarity	Thick dark colour no clarity
preservation temperature	4°C
Eugenol.	
Colour	Pale yellow
Aroma	Strong spicy & sweetaroma.
State	Liquid
Clarity	No transparency
Preservation	4°C

Conclusion

Clove oil has good flavor & aroma, and presence phytochemicals in oil. Extraction of clove oil is easy process it does not require expertise and automated lab equipment. Clove oil contains many compounds or chemicals. Mostly clove is highly present in clove oil. Extraction of eugenol is done using laboratory methods eugenol is aromatic aldehyde which has several pharmaceutical or medicinal applications.

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Formulation and Evaluation of Herbal Antibacterial Face pack

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Abstract

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than synthetic ones. Herbal formulations have growing demand in the world market. The objective of this work is to formulate and evaluate cosmetic preparation polyherbal face pack made from herbal ingredients. Karoline, tragacanth, orange peel powder, neem powder, chandan powder, aloe juice powder, turmeric powder, fullers earth and *cicer arientinum* powder were procured from the local market in dried, powdered and then passed through sieve no 80, mixed thoroughly prepared and evaluated for its organoleptic, physicochemical and microscopically characters.

Keyword: Cosmetics, Face pack, Natural, Herbal, Antibacterial, Organoleptic

Introduction:

Everybody wants to get fair and charming skin. Now a day, Acne, black head, pimples, dark circle are common among youngsters and person who suffers from it. According to Ayurveda, Skin problems are normally due to impurities in blood. Accumulated toxins in the blood during improper food and lifestyle are causing skin related diseases. Various herbs and medicines are described in Ayurveda for blood purification. The herbal paste which is applied on face to treat acne, pimple, scars, marks and pigments are known as "Mukha Lepa" in Ayurveda. The process of smearing this herbal mix on face is known as "Mukha lepana". [1] The process of smearing this herbal mix on face is known as "Mukha lepana". This beauty therapy is popular as facial. The smooth powder which is used for facial application is "face pack". A good herbal face pack must supply necessary nutrients to skin. It should penetrate the subcutaneous tissues in order to deliver the required nutrients. Different types of skin need different types of herbal face packs. [2] The main advantage of using herbal cosmetic is that it is pure and does not have any side effects on the human body. People have rough skin and when they don't take sufficient care, then the skin turns dark due to overexposure to the sun, other pollutants etc. In this article we have formulated herbal face pack to whiten, lighten and brighten the skin naturally for men and women. This face pack has natural skin lightening property and can be easily prepared at home. Face packs with natural constituents are rich in vital vitamins that are essential for the health and glow of the skin. [3]

Acne vulgaris is an extremely common disorder of the skin (pilocebaceous unit) that affects virtually all individuals at least once during life. The incidence of acne peaks of teenage, but substantial numbers of men and women between 20-30 years of age are also affected by the disorder.[4] Even today, people especially in rural areas, and hilly regions go for the natural remedies like plants extracts for various cosmetics purposes like neem, aloe vera, Tulsi, orange peel, rose etc. Herbal cosmetics are the products which are used to purify and beautify the skin. The main advantage of using herbal cosmetic is that it is pure and does not have any side effects on the human body. People have rough skin and when they don't take sufficient care, then the skin turns dark due to overexposure to the sun, other pollutants etc. [5]

The herbal face packs do contain some vital vitamins that are required for the health and glow of our skin. These substances also prove to be beneficial for our skin in many ways. Herbal facial packs are less complicated and pretty simple to use. They help us in looking after skin and also prove its worthiness by increasing the circulation of the blood within the veins of the face. Ayurvedic face packs increase skin shine and are best ayurveda treatment to increment reasonableness. Face packs are useful for preventive, promotive and healing of any skin issues. The fundamental significant spices utilized for reasonableness of skin are as underne. Effects of the facial packs are generally temporary and for the regular glow it should be used 2-3 times a week. [3]

In ancient times, women were very conscious about their beauty and took special care of their specific skin types every type of skin is specific for the requirement of skin pack. Nowadays different types of packs are available separately for the oily. Normal and dry skin. Face packs are used to increase the fairness and smoothness of the skin. It reduces wrinkles, pimples, acne and dark circles of the skin. Face packs which are recommended for oily skin prone to acne, blackheads, usually control

the rate of sebum discharge from sebaceous glands and fight the harmful bacteria present inside acne lesion.[6]

Material and Methods:

Formulation of Herbal Face pack:

All the required herbal powders for the face pack preparation were accurately weighed individually by using digital balance. The quantity and compositions are listed in table.

Sr. No.	Ingredient	Quantity (In gm)

1	Turmeric	0.2 gm.
2	Red Sandalwood	3 gm.
3	Ashwagandha	2 gm.
4	Aloe Vera	1.8 gm.
5	Hibiscus	3 gm.
6	Rose Water	Sufficient quantity

Procedure:

Take prepared face pack powder in a bowl as per the requirement. Add water or rose water to mix it well and apply over the facial skin. Cover the acne and blemishes spots. Kept as it is for complete drying for 30 to 40 min and then wash the face with cold water.

Material and Method:

The face pack prepared accordingly the particle size and their binding property mixed thoroughly in plastic bag which shown in the table.

Sr. No.	**Ingredient**	**Face Pack 1**	**Face Pack 2**
		Qty in gm	**Qty in gm**
1	Orange Peel Powder	8	10
2	Neem Powder	12	09
3	Sandalwood Powder	20	18
4	Aloe Vera Powder	05	10
5	Turmeric Powder	10	07
6	Fullers Earth	15	10
7	Cicer Arientinum Powder	15	20
8	Liquor ice	5	06

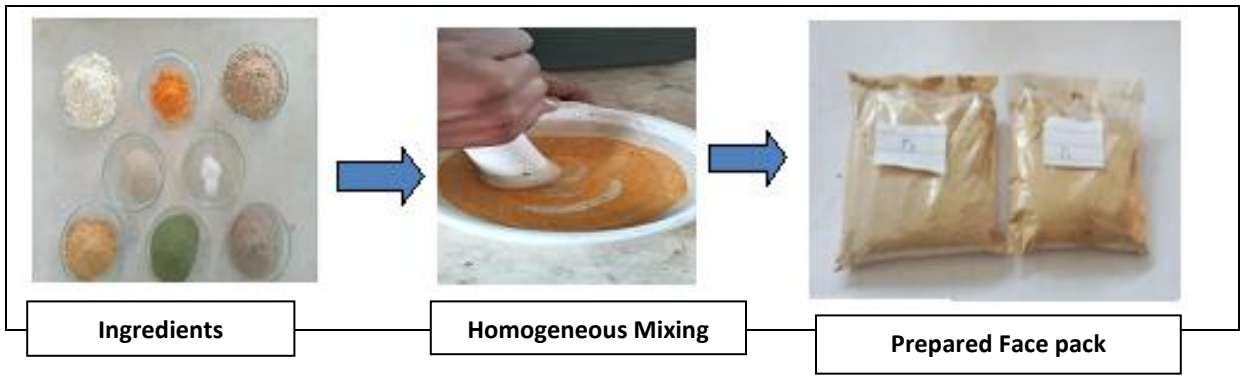
Process of Facepack preparation:

i)Weight accurately all herbal powder such as orange-peel powder, neem powder, Chandan powder aloe juice powder.

ii) Weigh accurately kaolin, Multani mitti, turmeric and tragacanth powder and triturate them together to form a uniform mixture.

iii) Mix them together to form a uniform mixture with the help of mortar and pestle.

iv)In this mixture add previously prepared herbal drug and triturate to obtain a uniform drug powder of face pack.



Procedure of Development of Formulations Face Pack Application [14, 15, 17]

Various formulation batches were prepared according to the Table. Take prepared face pack powder in a bowl as per the requirement, add water or rose water to mix it well and apply over the facial skin.Cover the acne and blemishes spots. Kept as it is for complete drying for 30 to 40 min and then wash the face with cold water.

Evaluation and Formulation:

Following evaluation parameters were performed to ensure superiority of prepared face pack.

1) Physical evaluation-

Physical parameters such as colour, odour, appearance and texture were checked visually.

2) Determination of moisture content-

Weigh about 1.5 gm. of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100^{o} C or 105^{o} C, until two consecutive weighing do not differ by more than 0.5 mg. Cool in desiccators and weigh. The loss in weight is usually recorded as moisture. [18]

3) Total ash-

Place about 2-4g of the ground air-dried material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600°C until it is white, indicating the absence of carbon. Cool in a desiccators and weigh. If carbon-free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrate R. Dry on a water-bath, then on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and then weigh without delay. Calculate the content of total ash in mg per g of air-dried material.

4) Water-soluble ash-

To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ash less filter-paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per g of air-dried material [19].

5) Particle size-

Particle size is a parameter, which affects various properties like spread ability, grittiness etc., particle size was determined by sieving method by using I.P. Standard sieves by mechanical shaking for 10 min.

6) pH-

pH of 1% aqueous solution of the formulation was measured by using a calibrated digital pH meter at constant [19].

7) Microbial Assay-

The antibacterial activities of different formulations were determined by modified agar well diffusion method. In this method, nutrient agar plates were seeded with 0.2 ml of 24 h broth culture of Escherichia coli. The agar plates were allowed to solidify. A sterile 8 mm borer was used to cut wells of equidistance in each of the plates. 0.5 ml of formulations, herbal extracts were introduced into the wells at randomly. The plates were incubated at 37°C for 24 hours. The antibacterial activities were evaluated by measuring the zones of inhibition (in mm). The results of evaluation are shown in Table.

Face pack 1

Standard (Azithromycin)

Face pack 2

8) Wash ability

This is the common method for checking the washability of the formulation. The formulation was applied on the skin and then ease and extent of washing with water were checked manually by using 1 liter of water is used to remove all content of the formulation were applied on the surface [19].

Result and Discussion:

Sr. No.	Evaluation Parameters	Observation	
		Face Pack 1	**Face Pack 2**
Organoleptic Evaluation			
1	Nature	Powder	Powder
2	Odour	Pleasant	Pleasant
3	Colour	Yellowish	Yellowish
4	Texture	Fine	Fine
Physiochemical Evaluation			
Ash Value			
5	Total Ash	2.5 %	2.7 %
6	Water Soluble Ash	1.2	1.3
7	PH	6.97	6.39
8	Moisture Content	2.6% w/w	2.8% w/w
General Powder Characters			
9	Particle Size	28-32 µm	25-30 µm
10	Washability	Easily washable	Easily washable
11	Grittiness	Nil	Nil
12	Nature of face after wash	Soft and Cl	Soft and Clean

Microbial Assay:

Sr. No.	Bacteria	Zone of Inhibition of Face Pack (mm)		
		Face Pack 1	**Face Pack 2**	**Standard (Azithromycin)**
1	EscherichiaColi(E. coli)	22 mm	6 mm	30 mm

Conclusion:

The herbal face pack was successfully formulated which was as par the synthetic face pack available in market. All ingredients used to formulate face packs were commonly used across Asia and were safe and effective. Several tests were performed to evaluate and compare the property of formulation. Formulation shows good antimicrobial activity when compared with

azithromycin. Herbal face packs have potential to glowing effect on skin. Hence, from this result we can conclude that natural formulation is safe, harmless and effective for skin.

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MICROBIOLOGY

Antimicrobial Activity of Turmeric Extract and Its Phytochemical Analysis

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Abstract

The plant *Curcuma longa* is the medical plant belongs to the Zingiberaceae family. Turmeric powder derived from the rhizome of *Curcuma longa*, is commonly used as a spice, food preservative and food-coloring agent. It also has a long history of therapeutic uses. The methanolic extracts of turmeric was prepared and tested against common pathogens. In present study Antibacterial activity of *Curcuma longa* was established against pathogens *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* and *Bacillus subtilis.* The qualitative phytochemical analysis of Curcuma longa rhizome extract was performed for detection of carbohydrates, protein, glycosides, steroids, tannins, saponins, flavonoids. The phytochemical analysis reveals the presence of carbohydrate's, glycosides, steroids, saponins, flavonoids, tannins and protein.

Keywords - Antimicrobial activity, phytochemicals, turmeric

Introduction

From ancient times in India, turmeric is used as spice, derived from the dried rhizomes Curcuma longa, a member of the ginger family.(Adebisi et al, 2021, Mahajan ,1999). The herb is known as golden spice of India Turmeric is used in our century as home remedy to cure a number of ailments, including anti-inflammatory , anti-neoplastic , anti- oxidant , anti- coagulant and anti-viral activities. The active compound in turmeric, curcumin, possesses potent antimicrobial properties against various pathogens, including bacteria, fungi, and viruses (Barisi et al, 2021) Curcumin has been shown to inhibit the growth of a wide range of microorganisms, including *Staphylococcus aureus*, *Escherichia coli, Candida albicans*, and even antibiotic-resistant strains (Ikpeama et al 2014).

Materials and method

Collection of ground part of *curcuma longa*

The Turmeric sample was collected from local market areas of Jalgaon.

Preparation of Turmeric extracts

50 g of dry powder was packed in Soxhlet apparatus for extraction of bioactive molecules from the rhizome by the use of solvent methanol. The extract was concentrated (Sahne et al, 2016). The concentrated extract transferred from the extraction chamber in to the reservoir. The extract is then dried and ready for studies.

Qualitative analysis of phytochemicals

The extracts were subjected to phytochemical tests for determination of plant secondary metabolites such as tannins, saponins, steroid, alkaloids and glycosides.

Test of Carbohydrates

For testing the presence of carbohydrates, the extract (1mL) and few drops of concentrated sulphuric acid was added. The formation of a violet ring confirmed the presence of carbohydrates in given samples.

Test for Glycosides

The extracts were measure (2mL), treated with chloroform (3mL) and 10% ammonia solution. The development of a pink colour indicates the presence of glycosides

Test for Saponins

For testing the presence of saponins, (20mL) water was added to extract (1mL) kept in graduate tube. The tube was shaken for 15 min to check for the presence of foamy confirms the presence of saponins.

Test for steroids

The presence of steroids was checked by adding (1mL) of extract and (1mL) chloroform in test tube, and a few drops of concentrated sulphuric acid was added to observe change in colour. Solution turned red, indicate presence of steroids.

Test for Protein

A screering test for protein was performed by adding an equal volume of biuret reagent solution and each extract (2mL) to test tube. The appearance of a bluish violet colour indicated the presence of protein.

Test for Tannin

For determining the presence the tannin, a small quantity of extract (1mL) was added to water and then heated in water bath. To this heated mixture. A few drops of 0.1% w/v ferric chloride solution were added; the appearance of a dark green colour indicated the presence of tannins.

Results and discussions

Methanolic extraction of turmeric powder was carried out using Soxhlet extraction apparatus, From 50 g of turmeric 8.5g extract was obtained.

Antimicrobial activity of extract

The antibacterial activity of curcumin extract at 1000µl/well against Gram-positive (*S. aureus and B. subtilis)* and Gram-negative (*E. coli, and P aeruginosa) and Candida* was evaluated. The maximum zone of inhibition was seen against *P aeruginosa* and minimum zone of inhibition was seen *B. subtilis (*Kumar et al 2020).

Table1. Antibacterial activity of *Curcuma longa rhizome* extract

Extract/ Test organism	***B.subtilis***	***E.coli***	***Psudomonas aeruginosa***	***S. aureus***	***Candida***
Methanol (Zone diameter in mm)	9	14	17	13	12

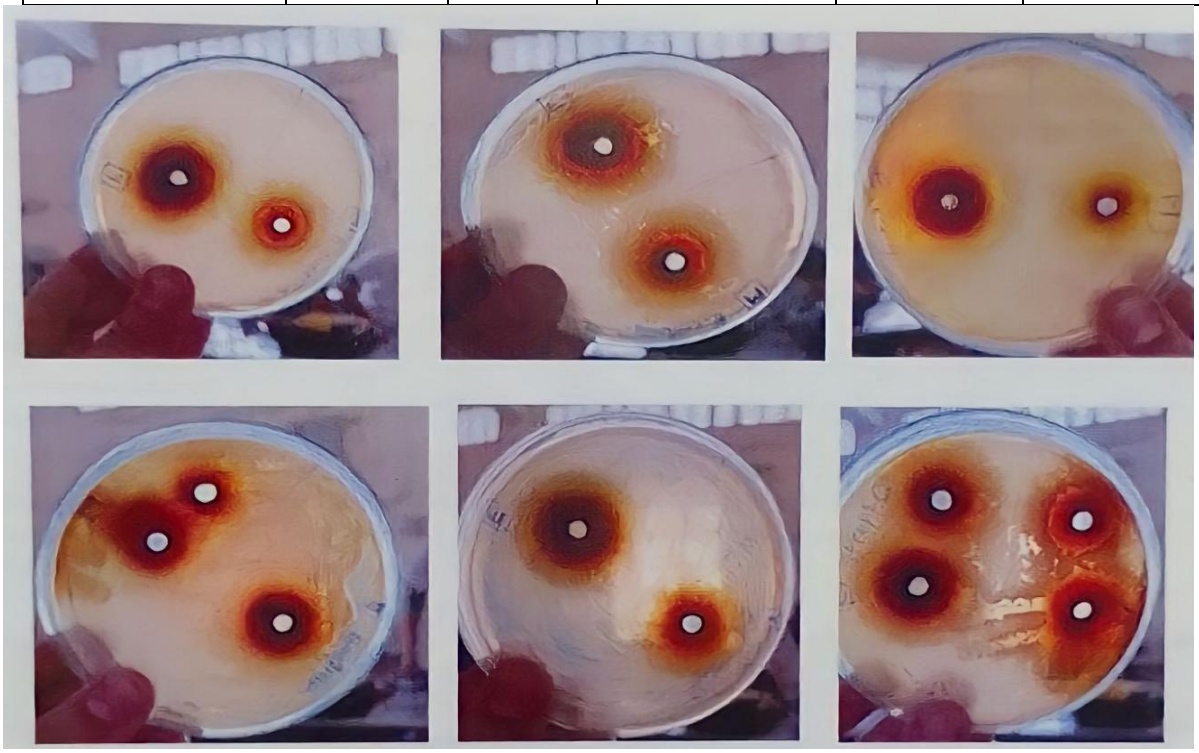

Fig. 1. Antimicrobial Activity of extract of turmeric against the test microorganisms.

E-coli, S-aureus, Streptococcus, Candida, Bascillus,Pseudomonas.

Table 2. Qualitative analysis of phytochemical tests

Test	Methanolic extract	Contents		Observation	Result
		1	2		
Carbohydrate	1ml	2-3 drops of concentrated sulphuric acid	---	Violet colour appear	positive
Glycoside	2ml	Chloroform 3ml	10 % of ammonium solution	The pink colour was not observed	Negative
saponins	1ml	20ml distilled water		A foam layer was obtain on the top of test tube	positive
Steroid	1ml	Chloroform 10ml	2-3 drops of sulphuric acidl	The red coloured layer was not observed	Negative
Protein	2ml	2ml buret reagent		appearance of bluish violet colour was observed	Positive
Tannin	1ml	10ml distilled water	2 drops of 5 % $FeCl_3$	Greenish ppt	Positive

CONCLUSION

In this study, the antibacterial activity of turmeric extract in methanol for their inhibitory effect on bacterial strains was evaluated and phytochemical analysis done. Antibacterial activity of Curcuma longa was established against microbes *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa and Bacillus subtilis.* The methanolic extract of *C. longa rhizome* is most effective against *P aeruginosa.*The phyatochemical analysis reveals the presence of carbohydrates, glycosides, steroids, saponins, flavonoids,tannins and protein.

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Efficient Production of Biofertilizer Using Sugarcane Waste

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Abstract

PGPR are beneficial microorganisms that colonize rhizosphere and help in promoting plant growth, protecting from biotic and abiotic stresses and significantly increasing soil fertility. The present work was carried out to find the effect of plant growth promoting activity by wide variety of mechanisms like phosphate solubilisation, Siderophores production, biological nitrogen fixation, and production of phytohormone. Sugarcane waste based media for the growth of Rhizobacteria production was optimize and tested. The isolated organism was able to produce significant Siderophore, IAA and was also able to solubilise phosphate. The PGPR treated seeds shown more plant growth and germination rate as compare to non treated seeds.

Keywords: PGPR, Rhizobium, Biofertilizer etc.

Introduction

A Biofertilizer is a substance which contains living organisms which when applied to seeds plant surfaces or soil colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant.(Khanafari et al, 2013, Dutta et al, 2011) A huge amount of sugarcane waste generated daily from sugar mills, so there is a need to manage such type of sugarcane waste disposals. (Dimas,2007). In past 25 years population pressure, urbanization, industrialization and mechanized agricultural activities have increased, due to which organic waste materials have been accumulated in the environment. Nine million tons per day sugarcane wastes are generated with no dearth of manpower. Sugarcane waste based media for the growth of Rhizobacteria production will help to solve the problem of eco-friendly waste disposal.

Sugarcane Wastes

The sugarcane wastes especially bagasse and trash were collected from the Jalgaon, sugarcane farm, Maharashtra, India. All types of sugarcane waste materials were chopped in to small pieces (3-4 cm) & kept in shade for 15 days on 30°C for the removal of extra moisture content before using for the biofertilizer media preparation. Rhizosphere Soil and Root nodules from leguminous plant (Fenugreek Plant) were collected in sterile polythene bags.

Extraction and isolation plant growth promoting bacteria from leguminous plant

Isolation was done on Yeast Extract Mannitol Agar plate by streaking the crushed root nodules on YEM gar plates and incubated at 25°C (Aneja, 2007).

Media preparation by using sugarcane wastes

Saccharification

Take 5 gm of Sugar Cane molasses was diluted upto 100 ml distilled Water then add 5 ml HCl acid placed in boiling water bath at 90°C for 1hr. After Cooling at room temperature the medium was neutralized with lime Cao and left to stand overnight.

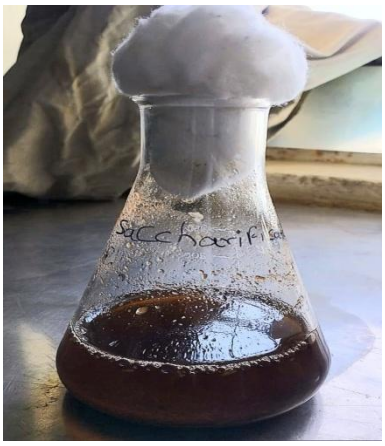

Fig 1. Saccharification of Sugar Cane molasses

Plant Growth promoting Activity of Isolates

Auxin test

Take a 1ml Culture & add in 1 ml Salkowski reagent observed for pink color formation

Siderophores

Iron free Succinate medium was prepared and its pH was adjusted to (7.0) with NaOH and sterilized. Bacterial culture was inoculated in the succinate medium with single colony transfer and incubated at 28°C for 48 hours at 120 rpm on shaker. After incubation, the cultures were centrifuged at 7000 rpm for 15 minute Loop for suspension was spotted inoculated and incubated at 28°c for 2-3 days observe blue to purple color formation (Gholami et al, 2009).

Phosphate Solubilisation

Pikovaskaya's agar medium containing calcium phosphate as the inorganic form of phosphate was used in assay. Loop ful of bacterial culture was spot inoculated at the canter of the plates and kept for incubation at 28°C for 4-5 hrs. Appearance of transparent halo zone around the bacterial colony indicated the phosphate solubilising activity of the bacteria(Gupta et al,2015)

Pot Assay

Preparation of Bacterial inoculums for seed treatment

Bacterial strains were cultured in 250 ml conical flasks containing sugarcane based media and incubated on orbital shaker at 120 rpm for 72 h at 28 C. Bacterial cells were collected via centrifugation at 10000 rpm for 1 min at 4°C, and each pellet was washed twice with sterile DW. The seeds were surface sterilized in $HgCl_2$ for 1 min and washed three times in SDW. Dry seeds were immersed in each bacterial suspension, and the preparation was stirred frequently for 5 min.

Effect of bacterial seed treatment on germination and vigour index

In order to determine the effect of the isolates on germination and seedling vigour, seeds inoculated with isolate were incubated in seed pot containing sterile soil. As a control treatment, seeds treated with water instead of bacterial suspensions were also planted. Root and shoot length were measured.

Results

Root nodule associated bacteria were isolated from the nodules of *Fenugreek* plants, 13 isolates were obtained. The isolates exhibited phosphate Solubilization and auxin production.

The treated seeds after sowing have shown significance increases in shoot length as compared to non treated seeds.

Fig.2. Shoot growth of Rhizobium

Plant height was taken at 3 week of the plant growth. Root elongation, shoot length and number of leaves germinated were recorded.

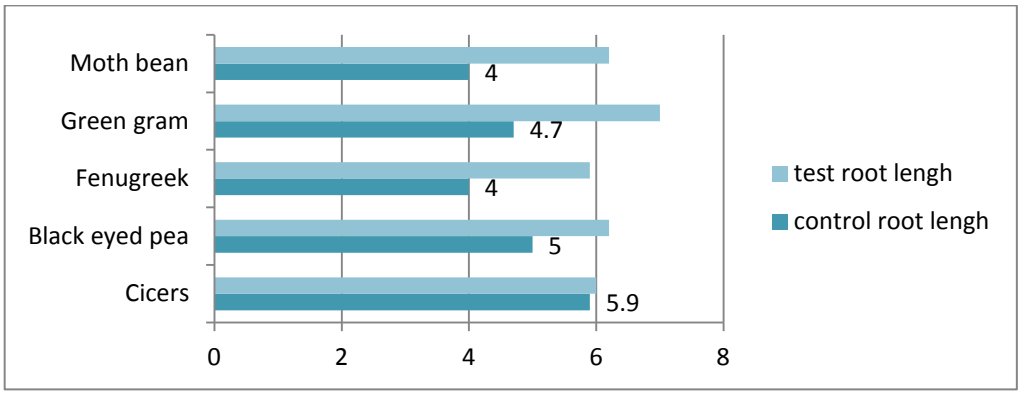

Fig3. Comparative graph of root length of control and test(in cm)

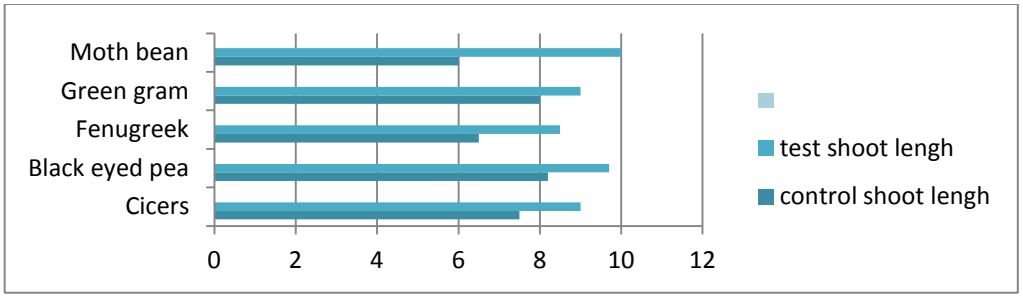

Fig4. Comparative graph of shoot lengh of control and test(in cm)

Table 1. plant gowth promoting activity of phosphate solubilizing bacteria on seeds.

Sr. No.	Test Sample	No. of seed grown	No. of seed germinated	Root length mean (cm)	Shoot length mean (cm)
1	Control seed + sterile D/W + Soil	5	4	5.95	4.8
2	Test - Coated seed + Sterile D/W + Soil	5	3	3.36	3.2

Initially the best growth was recorded for the plant growing in control but after some days the wheat seeds treated with PSM 3 inoculums shows highest root length and shoot length as compare with the inoculums of the PSM 1 and PSM 2 in the plant growth promoting activity of PSB inoculums on wheat seeds

Conclusion

Biofertilizer prepared from sugarcane waste can provide required nutrients to the crops in the sufficient quantity. Reduce volumes of sugarcane can be converted into useful biofertilizer

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Production of Bio-Fertilizer Using Plant Growth Promoting Rhizobacteria

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Abstract

The Institution of microorganism that Colonize root or rhizosphere soil and beneficial to crops is referred as Plant growth promoting Rhizobacteria (PGPR). These soil microorganisms colonize plant root and benefit the crop through their growth and cultivation. The present study focus on PGPR activity of isolates. PGPR strainswere isolated using Ashby's media. The strains exhibited phosphate solubilizing activity, greater Auxin production and Siderophore production. The seeds treated with isolate shown more plant growth as compare to non-treated seeds.

Keywords - Ashby's media, Auxin, biofertilizer etc.

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Introduction

The term plant growth promoting bacteria refers to bacteria that colonize the roots of plant (rhizosphere) that enhances plant growth (Ahmad et al, 2006).The utilization of plant growth promoting rhizobacteria (PGPR) in agriculture is continuously increasing as it offers an effective tool to replace the use of chemical fertilizers,pesticides and other harmful supplements (Andy,2020) Azotobacter is group of gram negative free-living,nitrogen fixing aerobic bacteria inhabiting in the soil.Biofertilizers are widely used to accelerate those microbial processes which augment the availability of nutrients that can be easily assimilated by the plants.(Bhattacharyya et al, 2011) They improve soil fertility by fixing the atmospheric nitrogen and solubilising insoluble phosphates and produce plant growth promoting substances in the soil(Bowen, 1999). Plant bacterial interaction in the rhizosphere is the determinants of plant health and soil fertility.

Material and Method

Isolation of microorganism

Soil Samples were collected from wheat plant root soil from, Jalgaon. The growth of plant promoting bacteria the 1 g soil was enriched in Ashby's Broth and Incubated on rotary shaker at 25°C for 48hrs Enriched culture was serially diluted and spread plated on ASHBY'S agar plates and incubated at 25 □ for 24- 48 hrs. Large gummy colonies of rhizobia will appear plates. The azotobactor colonies appear flat, soft, milky and mycoid (Gupta et at 2005).

Morphological characterization of bacterial isolates

The isolated bacteria were identified based on morphology growth condition gram staining and biochemical tests.

Phosphate Solubilisation

Pikovaskaya's agar medium containing calcium phosphate as the inorganic form of phosphate was used in assay. Loopful of bacterial culture was spot inoculated at the canter of the plates and kept for incubation at 28°C for 4-5 hrs. Appearance of transparent halo zone around the bacterial colony indicated the phosphate solubilising activity of the bacteria.

Auxin Production

The culture of the isolate were grown in Luria Bertaini(LB) broth amended with 500 ml of tryptophan at 27□ for48 hrs at 120 rpm at shaking incubator After the broth was centrifugation at 500 rpm for 15 min and supernatant assayed a IAA production. The supernatant 1 ml and added to 1 ml Salkowskireagent . The observed in pink colour so auxins production.

Siderophore Production

The cultures of the isolated were spot inoculated on CAS agar plates, positive result indicated by the Zone around the spot.

Pot Assay Techniques

Selected seed were collected (Cicers, fenugreek, green gram, black eyed, moth bean). After that seed is soaked in respective broth culture at overnight 24 hrs for bacteria growth (ASHBY'S broth,). The soaked seed sown in sterile soil and observed for 10 days. Observed it's positive result it indicates the growth stem and root length.

Result and discussion

Isolation of Rhizospheric organisms

The organism was enriched in Ashby's broth Gummy, soft, milky colonies were obtained on the plates were selected for further characterization. and were named AB1, AB2, AB3, AB4, AB5 respectively.

Fig. 1. Isolated colony

Biofertilizer production

Mass production of isolate was done and Plant Growth Promotingactivity was determined by pot assay.

Fig. 2. Pot Assay

Siderophore production was determined on CAS medium following the method of the bacterial strain (24 hrs old culture) spotted separately on CAS medium plate and were incubate at 25□ for 48 hrs formation of blue to yellow zone around the colonies showed production of siderophore.

Pot Assay Plant Growth Promoting of the strain detected by treating seedlingd and sowing into pot. After then 10 days we measured the shoot and root length of plants.

Conclusion

The isolated organism was able to produce IAA. Siderophore production activity was shown by isolated organism. The isolate after treated with seeds shown more plant growth as compare to non-treated seeds. The germination rate of treated seeds was also better as compare to non-treated seeds.

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Screening and Characterization of Protease Producing Microbes from Soil Samples of Jalgaon District

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Abstract

This project dissertation embodies the findings of the study undertaken to isolate proteases producing microbes from various soil samples from Jalgaon District. Microbial diversity analysis revealed the abundance of bacterial strains. Twenty nine protease producing bacterial strains were isolated through screening on skim milk and casein agar media. Three strains were selected for enzyme studies. The protease enzyme was partially purified using ammonium sulphate precipitation and dialysis method. The enzymes exhibited maximum protease activity at pH 9.0 and temperature 37°C. The specific activity of the strain SN9 and SN15was found to be 230.46 U/mg and 283.91 U/mg, respectively. The biochemical characterization of these proteases indicated that enzymes showed enhanced activity in the presence of temperature and were found stable at various pH. The enzymes also exhibited the blood stain removal activity. The leads obtained in the present study will help in exploring the potential of these proteases in various industrial applications.

Keyword: Proteases, skim milk and casein etc

Introduction

Proteases/ proteinases refer to a group of enzymes whose catalytic function is to hydrolyze peptide bonds of proteins, belonging to the class of hydrolases (E.C.3.4.21.14) (Jabalia *et al.*, 2014; Patil and Kurhekar, 2020 and Chakraborty and Karmakar, 2020). Protease, a hydrolytic enzyme has attracted much attention because of its wide applications in detergent, leather, food, pharmaceutical, agricultural industries they also have important role in bioremediation of proteinaceous waste (Thakur *et al.*, 2018). The protease contributes more than 60% of the worldwide production of industrial enzymes (Maitig *et al*., 2018). Proteases are ubiquitous in biology where they have a biochemical and/or physiological involvement in many aspects of cell and organism function, including nutrition, protein turnover, growth, adaptation, regulation, sporulation and germination, disease, and death (Moo-Young, 2011 and Moni, 2022). Due to their stability in unusual conditions, they are considered as the most important group of enzymes

used for commercial purposes. Haloalkaline proteases are one of such commercial enzymes used in various industries due to their high specific activity and stability under extreme conditions. However, proteases obtained from plants and animals are not able to meet industrial demands and therefore microbial proteases have been given greater importance. Moreover, microbial proteases have greater desired characteristics for biotechnological applications, and known for ease of genetic manipulation of microorganisms as well as their rapid growth (Zanoelo *et al.*, 2016). Furthermore, higher yield, cost-effectiveness industrial level production of proteases is possible by using microbial sources (Ward, 2011).

Material and method

Sample collection, and isolation of proteolytic bacteria

Collection of Soil samples

The soil samples were collected from different places, including garden, domestic waste, sewage-contaminated site. Soil samples were collected from 5-10 cm depth. The soil samples were filtered through 2 mm sieve and then used.

Screening of proteases producing microorganisms

The organisms will be screened by using Skimmed Milk Agar and Casein Agar. Proteiolytic activity of the bacteria was be noted by the zone of hydrolysis. Colonies showing higher zones were screen selected for further studies. (Chakraborty and Karmakar, 2020; Masi et al., 2021; Kotb et al, 2023).

Identification of proteolytic bacteria

Protease production

The positive isolates were further screened quantitatively to assess their protease production potential. Submerged fermentation was carried out using Skim milk broth inoculated with inoculum and incubates at RT on rotary shaker at 120 rpm for 72 h.

Preparation of crude enzyme

The overnight grown bacterial culture was inoculated in the protease production medium. The flasks were incubated at RT˚C in on rotary shaker at 120rpm up to 72 h. The crude extracellular enzyme was collected after centrifugation at 10,000 g for 10 min and assayed for protease activity at different pH and temperature.

Protease assay

The protease assay was performed using casein as substrate. Protease activity was assayed by incubating casein stock with 1 ml of crude enzyme at RT for 10 min. After incubation 1ml of 5% trichloroacetic acid was added to stop the reaction and the mixture was allowed to stand for 15 min. Enzyme blanks were prepared by mixing DW, trichloroacetic acid and enzyme Then add distilled water and Reagent 'C' leaves for 10 min Add FC reagent and kept for 30 min at dark place, OD was taken at 660 nm.

Partial Purification of Protease Enzyme

Enzyme supernatant was subjected to protein fractionation by differential ammonium sulphate precipitation (Sawney et al, 1999).

Effect of pH on enzyme activity

The effect of variable pH on the production of Protease was analysed by varying the pH 4 to 9. Isolated were grown in the production medium for 48 hours incubation period incubating at 37°C

Effect of Temperature on enzyme activity

The effect of variable temperature on the production of Protease was analysed by growing the selected bacterial isolate in the production medium by varying Incubation temperature (30, 35, 40, 45, 50 and 55°C).

De-staining of blood stained cloth

Application of protease enzyme from isolated organism as a detergent additive was studied; three white cotton clothes were stained with blood and dried in oven. The stained cloth was treated with

1. Blood stained cloth dipped in beaker containing only distilled water.
2. Blood stained cloth dipped in beaker containing 2ml enzyme and distilled water.

   The flasks were incubated at room temperature for 2-3 hours. After incubation, cloth pieces were taken out, rinsed with water and dried and examine for the de-staining property.

Results and Discussion

Primary screening was performed for collected samples. Protease activity of isolated bacteria was detected by the colonies that had formed a clear zone around the growth were considered as protease positive isolates. Total 29 isolate exhibiting proteiolytic activity were obtained and named SN1 to SN29. Isolate SN9 and SN15 were selected for further studies as it exhibite largest zone on agar plates.

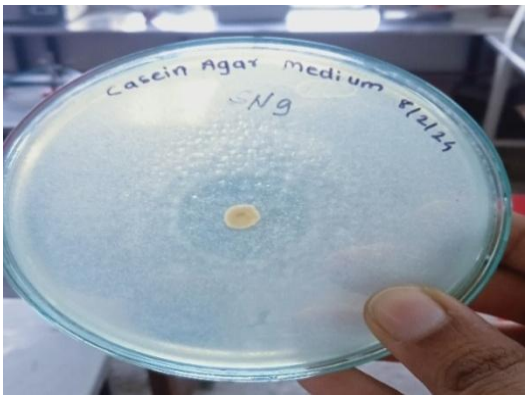

Fig 1. Zone of Clearance on Casein agar plate

Purification of protease enzyme

Protease enzyme purification is done by using the ammonium sulphate precipitation, Centrifugation and dialysis technique. The protein content was precipitated at 80% of salt. Further the precipitated enzyme was dialysis to remove ammonium sulphate.

Table 1. Fold Purification of enzyme

Isolate Enzyme Activity	Before Dialysis ($\mu g/ml$)	After Dialysis ($\mu g/ml$)
SN9	528.556	649.45
SN15	230.46	283.91

Effect of pH on enzyme activity

The protease activity was evaluated at different pH to deduce the nature of the protease enzyme. The maximum protease activity was observed at pH 9.0.

Effect of temperature on enzyme activity

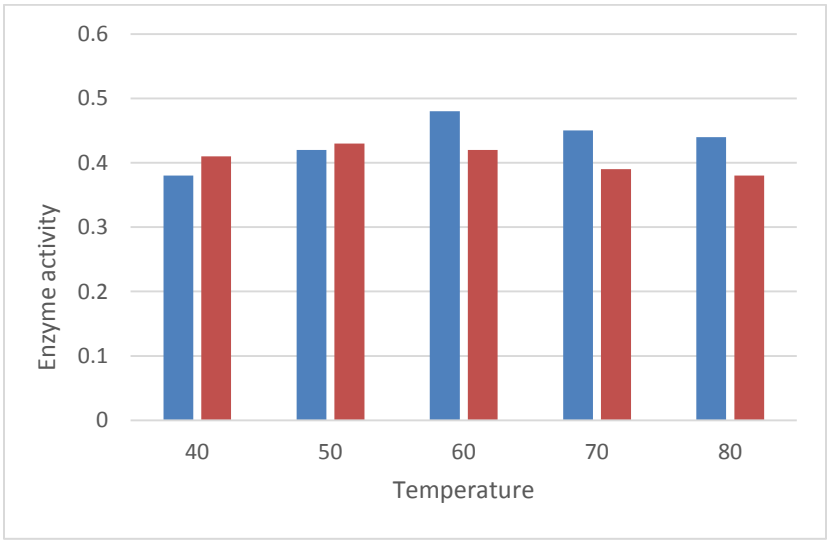

Fig.2. Effect of temperature on enzyme activity

The strains were active over a broad range of temperature, however, the maximum activity was observed at 60˚C assay temperature.

De-staining of blood stained cloth

The enzyme is exhibited the minor effect on cloth pieces, and successfully removes stains on cloth

. 

Fig 3. De-staining of blood stained cloth

Conclusion

From the present study it can be concluded that protease producing organism was Screened and isolated. Morphological and biochemical characteristics of organism reveal that both isolates belongs to Bacillus spp and it is able to produce protease enzyme. The isolate shows maximum protease production at pH 9 and up to temperature 60℃. The isolates also exhibited De-staining of blood stained cloth at significant level.

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Screening and characterization of the L-asparginase producing microorganisms from Jalgaon

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Abstract

L-Asparaginase, an enzyme with significant pharmaceutical and industrial applications, is the focus of this study aimed at isolating, screening, and optimizing its producers from soil samples. A comprehensive soil sampling strategy was employed to collect diverse microbial populations. Subsequently, isolation techniques were utilized to obtain potential L-Asparaginase- producing strains, followed by screening assays to identify high-producing isolates. Further, optimization of culture conditions, including pH, temperature, carbon, and nitrogen sources, was carried out to enhance enzyme production. The study underscores the importance of soil as a rich source of L-Asparaginase produce and provides insights into optimizing production for potential biotechnological applications.

Keywords: L-Asparaginase, enzyme etc.

Introduction

L-asparginase (asparagine amidohydrolases) constitutes the most biotechnologically and bio-medically important group of therapeutic enzymes accounting for about 40% of total worldwide enzyme.(Vimal et al, 2017) Asparginase (EC 3.5.1.1) is an enzyme that catalyzes the hydrolysis of asparagine to aspartic acid. Asparginase are naturally occurring enzymes expressed and produced by animal tissues, bacteria, plantsL-Asparginase is a critical enzyme of medical importance as an anti-cancer agent for the treatment of infectious disease, auto immune disease and many other medical relevance applications (Ganapathy, 2009).

Material and Methods

Isolation of L-asparaginase producing organisms

Soil samples are collected from different plantations and composting sites in Jalgaon.

L-Asparginase producing strains were initially screened by rapid plate assay method, based on their capability to from a pink zone around colonies on agar plate of modified M-9 medium. The medium was supplemented with 0.005% phenol red dye and the pH was adjusted to 6.2 using 1N HCl. Plates were then incubated at 37^0C for 24-48hrs. A set of tube was also run as a control

without L-asparagine. The strain having potential for L-Asparaginase production were selected on the basis of pink zone formation and retained for further screening (Moorthy et al, 2010, Raj et al,2016).

Production of L-asparginase

The 24-hour old inoculum is added to sterile M-9 basal medium and incubated at 37°C for 48 hours (about 2 days) at the end of fermentation period; the crude enzyme is prepared by centrifugation at 1000xg for 20 minutes in a cold centrifuge. The cell free supernatant is taken as the crude enzyme and an enzyme assay is performed. (Castro et al, 2021).

Optimization of Environmental parameters for the L-asparginase production

Effect of pH on L-Asparginase production: -

The effect of pH on L-asparginase production is studied by growing the isolates in sterile M-9 basal medium of different pH (6,7 & 8) maintained by using phosphate buffer

Effect of incubation temperature on L-Asparginase Production

The effect of incubation temperature on L-asparginase production is studied by growing at 5 different incubation temperature (25°C – 45°C) for 48 hours and assayed for enzyme activity.

Estimation of L-Asparginase activity

At the end of a 48-hour incubation period, the crud enzyme is prepared by centrifugation at 10000 rpm for 20 minutes. Cell-free supernatant is taken as a crude enzyme. The reaction is started by adding 0.05 ml of 0.01m L-asparagine to 0.5ml of 0.05 m Tris-HCl buffer (pH 7.0) and incubated for 30 minutes at 30±2°C. The reaction is stopped by the addition of 0.1 ml of 15% Trichloroacetic acid solution and centrifuged in a cooling centrifuge at 10000 rpm for 10 min. The supernatant is collected and 0.1 ml distilled water. The mixture is incubated for 10 min for colour development. The optical density (OD) is read at 450 nm.

Asparaginase Assay

The enzyme assay mixture consisted at 900µL of freshly prepaid L-Asparagine (20mM) in 50 mM Tris-HCl buffer (pH 8.0), 50mMKCl. And 100µL of crude extract of the enzyme. The reaction mixture was incubated at 37℃ for 30 min. The reaction was stop by adding 100µL of 15% trichloroacetic acid. The reaction mixture was centrifuged at 10,000xg for 5min at 4℃ to remove the precipitates. The ammonia release into determined using colorimetric technique by adding 100µL Nessler's reagent into the sample containing 100µL supernatant and 100µL distilled water. The contents in the sample were vortexed and incubated at room temperature for

10 min and OD was measured at 425nm. The ammonia produced in the reaction was determined based on the standard curve obtained with ammonium sulphate. One unit of L-Asparaginase activity is defined as the amount of the enzyme that liberates 1µmol of ammonia per min 37℃.

Biochemical Test

Protein Assay

BSA, casein and tyrosine stock were used to determine the absorbance of protein activity.

Screening of bacteria

The selected isolates were further screened for L-Asparginase activity in M-9 basal medium. The bacterial growth was observed 24 hours after the incubation period 37°C. L-asparginase producing isolates were identified by a pink coloured colony on modified M9 agar medium with phenol red as an indicator. The microbial with the pink colored colony was selected for further studies. The twelve isolates showed positive result for screening.

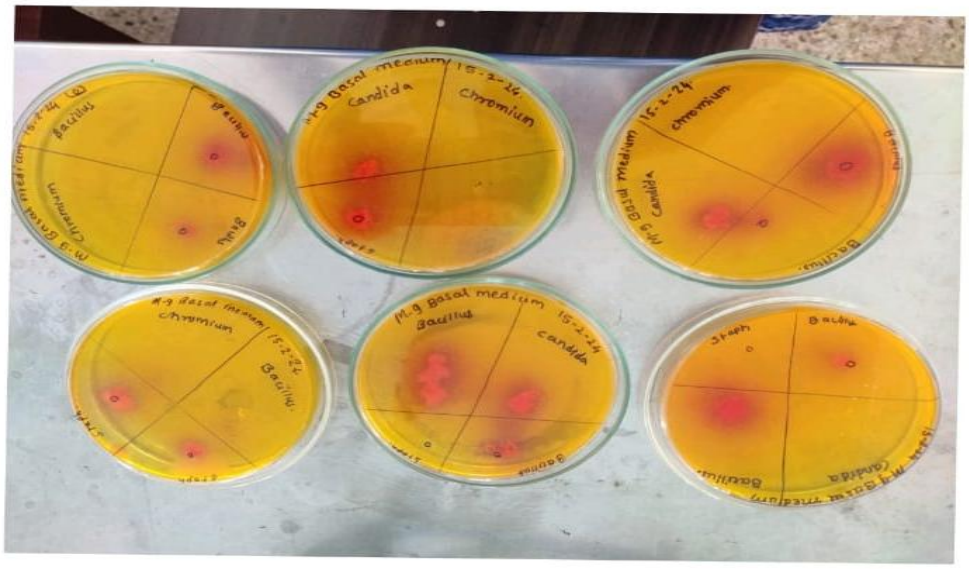


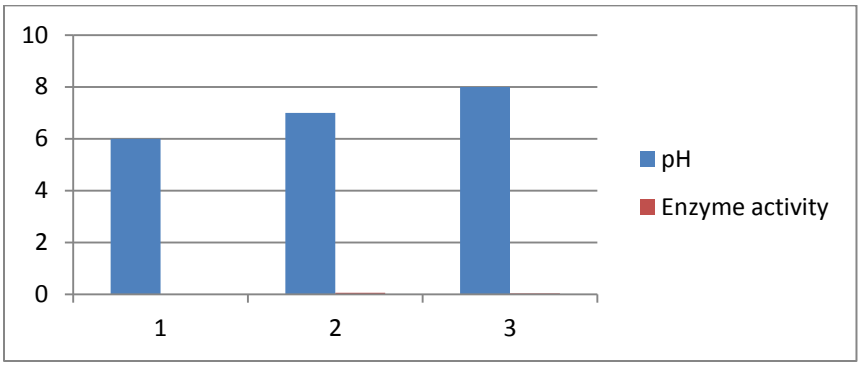
Fig.2. M-9 basal medium plate showing the pinkish color colony

Purification of protein by Dialysis method:

The asparaginase was subjected to partial purification using the ammonium sulphate precipitation method, protein content was precipitated at 80% of salt. Further purification was carried out using the dialysis method.

Effect of pH on M-9 Medium and growth

The response of growth of isolate to pH range of 6,7and 8 was observed. The isolate was able to show growth in the selected pH range. The amino precipitation method is used at 80%.

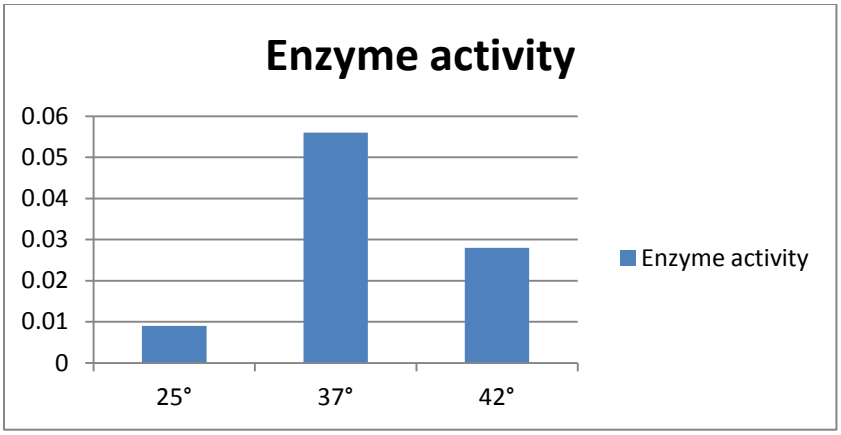


pH enzyme activity graph

The pH enzyme activity was shows at the 7 pH by the spectrophotometer.

Effect of Temperature on M-9 Medium and growth

The response of growth of isolate to temperature from 25°C, 37°C and 42°C.The isolate was able to show growth in the selected temperature. The amino precipitation method is used at 80%.



Temperature activity graph

The enzyme activity of temperature was shows at 37°C by the spectrophotometer.

Conclusion

From the present study it can be concluded that the asparaginase producing organism was screened and isolated. Biochemical characteristics of organism reveal that it may be Bacillus substilis and able to produce the asparaginase enzyme. Isolate shows maximum asparaginase production at pH 7 and 37°C temperature. All this information could be used to design media for large scale production.

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Isolation of Hexavalent Chromium Reducing Bacteria from Industrial Soil Sample

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Abstract

Chromium is an important industrial metal used in diverse products and processes. Effluents from textile, leather, tannery, electroplating, galvanizing, dyes and pigment, metallurgical and paint industries and other metal processing and refining operations at small and large-scale sector contains considerable amounts of toxic metal ions.. Among these heavy metals chromium toxicity can cause serious carcinogenic, genotoxic and immune toxic effects in humans and animals. Bioremediation is a process of contaminant degradation and their subsequent removal. Thus in our study primary screening of the isolates from effluent sample reducing hexavalent chromium were done ,four bacterial isolates were found and selected for further studies. The effect of various parameters like pH, temperature and time interval isolates was determined. Optimum pH for all the three isolate was 7, Optimum temperature for all the isolates was 42^{o}C.

Keywords: Chromium reduction,

Introduction

A pollutant is defined as "a substance that occurs in the environment, at least in part as a result of human activities, and has a deleterious effect on the environment". Pollutants can be divided into two major groups, namely, those that affect the physical environment and those that are directly toxic to organisms, including human beings.

Worldwide about 80 % of the mined chromium is used for metallurgical applications to manufacture ferrous and non- ferrous alloys and most is used in the manufacture of stainless steel. About 15 % used in the chemical industry and the remained is used in the refractory application. Due to improper disposal, leakage and poor storage, chromate has become one of the most frequently detected contaminant at the waste sites (Mishra *et al*., 2010). Not only that chromate is dangerously toxic, it is also difficult to contain and spreads rapidly through aquatic systems and subterranean waterways (DeLeo *et al*., 1994).The ingestion of hexavalent chromium causes death. Occupational exposures to some chromium compounds have been shown to cause bronchial asthma, lung and nasal cancers, nasal and skin ulcers, and allergic reactions in the skin

.The chromate anion is highly soluble and therefore can overcome the cellular permeability barrier. The heavy metals oxyanions interfere with the metabolism of the structurally related non-metals in the living cells (Ezaka, 2011). Thus, chromium has been designated as the priority pollutant.

Materials and Methods

Sample Collection

Soil sample were collected in clean and dry plastic bottles from a Jalgaon MIDC industrial area. Soil samples were taken to the laboratory and stored at room temperature in cool and dry place before use.

Stock Preparation

2.5 LB dissolved in 100ml distilled waterand 0.1gm K_2 Cr_2 O_7 was added in it.

Isolation of Chromium Reducing Isolates:

Materials

Enriched sample was serially diluted upto 10^{-7} using sterile saline. Then 0.1ml of diluted sample from last five dilutions was spreaded on sterile LB / NA agar plate amended with K_2 Cr_2 O_7 . incubated at 37°C for 48hrs. Following incubation, chromium resistance colonies showing growth were selected(Camargo,2003)

Effect of various parameters on Chromium Reduction Kinetics of the Isolates :

Growth of the micro-organism depends upon the various parameters like pH, temperature and chromium concentration. Effect of these factors on the growth kinetics was determined by using appropriate method.

Effect of Chromium Concentration on growth of isolates

Materials : Sterile LB broth of pH 7.5 amended with different chromium concentration.
i.e 200-1400 mg/L Inoculated with isolates and incubated at RT. Turbidity was determined.

Effect of pH Chromium reduction

Effect of pH, was studied by adjusting pH of culture medium amended with 0.1 g K_2 Cr_2 O_7 to 5, 6, 7, 8 and 9 with 1N HCI or 1N NaOH.

Effect of Temperature

For the effect of temperature, culture medium was incubated at room temperature 32°C and47°C respectively. Growth of chromium was determined spectrophotometrically.

Effect of time interval on Growth of Chromium resistant Isolates

For the effect of growth and Cr (VI) reduction / transformation was evaluated with 20mg /l $K_2 Cr_2 O_7$ in shake flask culture containing 100ml LB broth at 37°C, 150 rpm. Sample were withdrawn at 2 hrs intervals. The growth was monitored measuring O.D. at 600nm

Results

The present study was aimed to isolate the chromium resistant isolates and to evaluate the effect of various environmental factors such as pH, temperature and different Cr (VI) concentration on growth kinetics of the microbes.

Isolation of Chromium Resistant microorganisms: Isolates able to reduce chromium in LB medium supplemented with chromium was studied, by observing continuous absorption at 600nm indicating that hexavalent chromium is reduced into its trivalent form. 4 isolates were screen for reduction potential. One isolate showing greater reduction was selected for further studyfour chromium utilizing bacteria CR1, CR2, CR3, and CR4were isolated on solid LB agar plate.

Fig.1 Isolation of Chromium Resistant microorganisms

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Table 2. Screenig for Chromium reduction potential of isolates

Isolate	Initial OD	Chromium Reduced (%)
CR1	0.33	

CR2	0.51	
CR3	0.48	
CR4	0.61	94

Percent chromium removal by microbial isolates: Percent chromium removal by the isolates was determined by using sterile L.B. broth amended with 0.1mg/L of $K_2Cr_2O_7$. was determined spectrophotometrically at 600 nm..

$$\% \text{ Chromium removal} = C_O\text{-}C_1 / C_O \text{ X } 100$$

Where; C_o = Initial chromium concentration in the sample.

C_1 = Final chromium concentration in the sample.

Identification of Isolates

Identification of isolates was done by evaluating their physiological, morphological and biochemical characteristics. On the basis of morphological characteristics on media, staining reaction and biochemical characteristics of isolates, the isolate may be *Bacillus spp.* The results were compared with Bergey's manual of systematic bacteriology.

Effect of Various Parameter on Growth kinetics of The Isolates

Effect of pH

For Effect on growth of isolate, reduction was found to be highest at pH 10 for the isolate. For pH 10 Incubation at 37°C for 24hrs.

Table 3. Effect of pH on Chromium reduction

pH	O.D. at 600 nm	ChromiumReduced (%)
5	0.71	17%
7	1.15	54%
9	0.90	30%
10	0.06	7%

Effect of Temperature

The growth of isolate significantly decreases in activity at 32°C, 42°C and Room temperature. Optimum temperature for chromium reduction was found to be 40°C.

Conclusion

Chromium reducing organism was isolated from industrial soil sample. The isolate was found remarkable in reduction of chromium. Temperature, pH at initial substrate concentration plays key roles in determining the rate of chromium reduction.

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BIOTECHNOLOGY

Biochemical investigation of *Artocarpus heterophyllus* Lam. Latex

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Abstract:

The *Artocarpus heterophyllus* belongs to Mulberry family (Moraceae). It is commonly called as jackfruit. Jackfruit tree latex is a natural, milky substance found in the jackfruit tree. Comprehensive biochemical investigation of *Artocarpus heterophyllus* latex, encompassing various analytical techniques such as solubility tests, phytochemical screening, quantitative estimation of protein content, antimicrobial activity and partial purification of protease enzymes was carried out. Phytochemical screening revealed the presence of primary and secondary metabolites. Antimicrobial activity of latex was checked against *S. aureus*, *Bacillus* sp., *E. coli*, and *P. aeruginosa* by well-diffusion method. Linear increase in inhibitory zones with the increasing concentrations of jackfruit latex was observed. Quantitative estimation of protein content elucidated the nutritive value of the latex, while partial purification of protease enzymes showcased its enzymatic activity.

Key words: *Artocarpus heterophyllus*, jackfruit latex, biochemical investigation

Introduction:

The *Artocarpus heterophyllus* belongs to Mulberry family (Moraceae). It is also known by other names like jackfruit (Eng.), Kathal, Panas (Hindi), Kanthal (Beng.), Palaa (Tamil), Phanas (Guj & Mar) & Chakka (Malayalam) (A.M. Rahaman.et.al 1999). It is a monoecious tree and both male and female inflorescences are found on the same tree. The fertilization is by cross-pollination and the propagation is mostly through seeds (C.E.Elevitch @ H.I.Manner 2006). It is native to Western Ghats of India, Malaysia and also found in central and eastern Africa, south-eastern Asia, the Caribbean, Florida, Brazil, Australia, Puerto Rico and Pacific Islands (A.M.Rahaman.et.al 1999).

Jackfruit tree latex is a natural, milky substance found in the jackfruit tree (Artocarpus heterophyllus). It is often extracted from the tree's bark and contains various compounds including proteins, carbohydrates, and alkaloids (M. Azizur Rahman et.al 1999) Jackfruit latex has gained attention for its potential applications in various industries, including medicine, as it possesses antimicrobial properties and has been studied for its potential wound-healing properties (Shipra Jaha & A K Srivastava 2013). Additionally, it has been explored for its use in

adhesives, coatings, and even in the manufacturing of certain types of rubber. This versatile substance continues to pique interest for its diverse range of potential uses (C.E. Elevitch & H.I. Manner 2006).

Jackfruit latex, a milky exudate from the bark of the Artocarpus heterophyllus tree, has garnered attention for its diverse applications spanning botanical, agricultural, culinary, and medicinal realms. Current research work mainly focuses on phytochemical screening, antimicrobial properties and partial purification of protease enzyme.

Materials and Methods:

Jackfruit tree latex was collected from jackfruit tree located in the Ch. Shivaji Nagar, near railway station road, Jalgaon. Jackfruit latex was successfully collected in a sterile container from the bark of tree and kept in cold storage at 4°C until use.Solubility of latex was checked in various solvents (from non-polar to polar).

Phytochemical screening:

The various qualitative chemical tests were performed in triplicate for establishing a profile of the Jackfruit latex extract for their chemical composition by standard methods given by Plummer D. (1988), Saad R. et al, (2014). Alkaloids were detected by Mayer's test, Wagner's test and Dragendorff's test. Detection of carbohydrate was carried out using Molisch's test, Fehling's test and Benedict's test. Detection of proteins and amino acids was done with the help of Biuret test and Ninhydrin test. Detection of fixed oils and fats was carried out by Spot test. Detection of phenolic content was carried out by Ferric chloride test and Lead acetate test. For detection of flavonoids, Alkaline reagent test was used. Detection of Tannis, steroids and terpenoids also carried out.

Estimation of protein was carried out by Lowry's Method. Caseinolytic activity of a protease was monitored using 1% casein as a substrate with certain modifications in the method given by Singh et al 2018. The reaction mixture

Partial purification of protease enzyme (Balakirev AV & Zamyatin AA,2019):

All purification steps were performed at 4° and centrifugations at 5° at 10 000 g. Repeated freezing and thawing followed by centrifugation removed the gum. The supernatant was passed through 4 layers of cheese cloth and to the clear latex serum. Solid ammonium sulphate was added to bring it to 45% saturation. After standing overnight at 4°, the ppt was recovered by centrifugation, dissolved in 10 mM Tris-STT- EDTA buffer and dialyses against the same buffer.

And protein was estimated by Lowry's method.

Antimicrobial activity of the jackfruit latex was carried out by well diffusion method against *S. aureus*, *Bacillus* sp., *E. coli*, and *P. aeruginosa*.

Results and Discussion:

Solubility test:

Jackfruit latex was found to be soluble in distilled water (Polar). It showed the presence of water-soluble compounds or polymers within the latex.

Sr. No	Solvent	Solubility
1	n-Hexane	Not Soluble
2	Chloroform	Not Soluble
3	Methanol	Not soluble
4	Ethanol	Not soluble
5	Distilled Water	Soluble

Qualitative phytochemical screening:

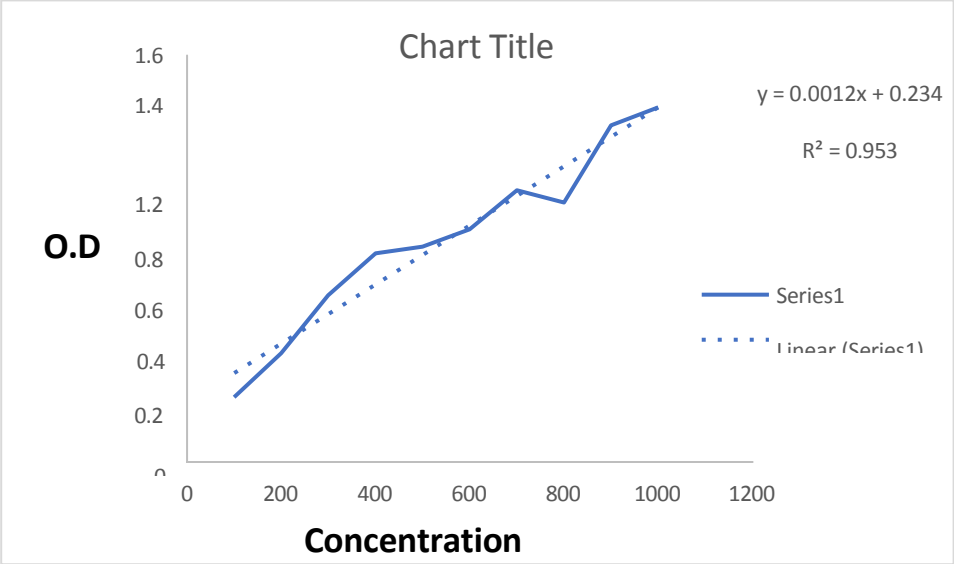
Qualitative phytochemical screening involves identifying various chemical compounds present in jackfruits latex. Common tests include primary metabolites and secondary metabolites. Each test targets specific compounds, aiding in the identification of potential medicinal or pharmacological properties of the jackfruit's latex.

Sr. No	Phytochemical test	Result
1.	Alkaloids Mayer's test, Wagner's test, Dragendorff test	Present
2	Carbohydrate Molisch test, Fehling's test, Benedicts test	Present
3	Saponins Foam test	Present
4	Proteins Biurets test Ninhydrin test	Present
5	Fats Spot test	Present
6	Phenolic content Ferric chloride test lead acetate test	Present
7	Flavonoids Alkaline reagent test	Present
8	Steroids	Absent
9	Terpenoids	Present
10	Tannis	Absent

The results of phytochemical screening typically involve identifying various bioactive compounds present in jackfruit latex, such as alkaloids, flavonoids, tannins, and terpenoids. These compounds often have medicinal properties and can provide insight into the potential health benefits of the jackfruit latex (Shrestha P et al, 2015).

Quantitative estimation of proteins by Lowry's Method: -

Estimation of protein in jackfruit latex was successfully done by Lowry's Method. The quantitative analysis of the protein is evaluated for the plotting the Graph. First reaction Reduction of copper under Alkaline Conditions forms a complex with peptide bonds. The second reaction is the reduction of Folin ciocalteu reagent by the copper peptide bond complex. Which subsequently causes a color change of the solution into blue.



Caseinolytic Activity:

The caseinolytic activity of jackfruit latex was evaluated. The results demonstrated significant caseinolytic activity in the jackfruit latex samples.

Sr.no.	Sample	Optimum density (nm)
1	Sample (before Dialysis)	0.346
2	Sample (after Dialysis)	0.679

The observed caseinolytic activity in jackfruit latex highlights its potential as a source of proteolytic enzymes for various applications, including food processing and biotechnology. Caseinolytic activity are increase after the Dialysis. Further investigation into the specific

enzymes responsible for this activity could provide valuable insights for industrial applications (Balakirev AV & Zamyatin AA 2018).

Partial purification of Protease:

Partial purification of a protease using the ammonium sulfate method involves precipitating proteins from a solution by gradually increasing the concentration of ammonium sulfate. Precipitated protein was further purified by dialysis. At each step of purification caseinolytic activity was found to be increased.

Antimicrobial activity:

The agar plates after performing paper disc assay. the positive control (5 go of Azithromycin) was the paper with a visible zone of inhibition (ranging from 10mm to 12 mm). Plate swabbed with S. aureus, Bacillus sp., E. coli, and P. aeruginosa, respectively were resistant to the extract concentrations.

The antimicrobial capacity of jackfruit latex was made apparent from the agar well diffusion assay in the present study. Antimicrobial activity was carried out against *S. aureus, Bacillus* sp., *E. coli*, and *P. aeruginosa*. This is evident from the linear increase in inhibitory zones with the increasing concentrations of jackfruit Latex. This coincides with the findings obtained by Sundarrajan and Pottail 2021.

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Development and evolution of a formulation for the treatment and prevention of inflammatory Bowel disease (IBD)

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Abstract:

The objective of the study was to develop a formulation from Carrot, Radish, Banana and Bael fruit and to use of the fibre content of these natural sources for the study of inflammatory Bowel disease (IBD). This study was carrying out with the help of dietary fibre available in the natural sources for the fibre absorption in the conditions like Inflammatory Bowel disease (IBD) and Crohn's disease. Several studies have reported that IBD is associated with impairment in short-chain fatty acid (SCFA) production, mainly acetate, propionate, and butyrate. Therefore, it is reasonable to consider therapeutic approaches that increase colonic SCFA production, as it can be achieved by administration of dietary fibre to IBD patients. Unfortunately, there is quite limited documentation of efficacy of dietary fibre in properly designed trials. This innovative study gives an idea about the use of dietary fibre in development of a formulation and its mechanisms of action in the treatment and prevention of IBDs**.**

Keywords: Inflammatory Bowel disease, Zone of inhibition, dietary fibres

INTRODUCTION

Term inflammatory bowel disease (IBD) covers a group of disorders in which the intestines become inflamed (red and swollen), probably because of an immune reaction of the body against its own intestinal tissue. Inflammatory bowel disease (IBD) is an idiopathic disease, The two major types of IBD are ulcerative colitis (UC) and Crohn disease (CD). As the name suggests, ulcerative colitis is limited to the colon. Crohn disease can involve any segment of the gastrointestinal (GI) tract from the mouth to the anus (Talley N, 2018).

The major effects of dietary fibre occur in the colon. Here each type of dietary fibre interacts with the microflora, and the colonic mucosa and muscle to produce several possible effects. The actions of an individual fibre source depend largely on its fermentability. The range of fermentability of different fibre is great and difficult to predict. Dietary fibre, however, can be roughly divided into those which are rapidly fermented, such as oligosaccharides, those which

are more slowly fermented, such as gums, and those which are hardly fermented at all, such as wheat bran.

MATERIALS AND METHODS:

Procedure of making suspension:

1) Raw Materials:

• Banana, Carrot, Radish, Bael fruit, Sorbitol, Glycerine, Na Benzoate, Water

(The fruits and vegetable are bought from local market and shredded in small pieces, and they were dried in hot air oven. After dried completely, they were crushed in mortar and piston. The fine powder was threshed by using strainer.)

2) Production:

• The powder of dried fruits weighed accurately as per calculation.

• The sorbitol, glycerine and water were added aseptically to form the suspension.

• The suspension was placed on the rotary shaker for 24 hours for complete dissolving of the powders.

3) Recovary:

• After the 24 hours the suspension was removed from the Rotary Shaker.

• The suspension was firstly filter by the muslin cloth.

• After completion of first filtration, suspension was filtered by using the Whatman filter paper.

• After that the suspension was put in the glass bottle and mixed thoroughly, as shown in table.

Sr. No.	**Ingredient**	**Quantity in gram (Per each 5 ml)**
1	Carrot	170 mg
2	Banana	150 mg
3	Bael	140 mg
4	Radish	30 mg
5	Sorbitol	1 ml
6	Glycerine	0.12 ml
7	Na benzoate	0.1 % w/v
8	Water	q.s.

EVALUTION OF FORMULATIONS:

Following evaluation parameters were performed to ensure superiority of prepared suspension.

A] Physical evaluation:

Physical parameters such as colour, odour, appearance and texture were checked visually. pH of 1% aqueous solution of the formulation was measured by using a calibrated digital pH meter at constant.

Ostwald Viscometer can be used to measure viscosity. From viscosity measurement, we can obtain much useful behavioural and predictive information for solutions.

Particle size is a parameter, which affect various properties like spread ability grittiness etc, particle size was determined by sieving method by using I.P Standard sieves by mechanical shaking for 10 min.

B] Qualitative phytochemical screening:

The various qualitative chemical tests were performed in triplicate for establishing a profile of the for their chemical composition by standard methods given by Plummer D. (1988), Saad R. et al, (2014).

Antimicrobial assays were performed.

RESULTS & DISCUSSION:

Organoleptic evaluation -

Sr. No	Parameters	Result
1	Nature	Liquid
2	Odour	Pleasant
3	Colours	Yellowish Brown
4	Texture	Sticky & Thick

Physiological Evaluation -

5	pH	7
6	Viscosity	1.22poise
7	Particle size	Uniform

Phytochemical Evaluation -

Tests for identification of	Name of Test	Result
Alkaloids	1) Mayer's Test	Positive
	2) Wagner's Test	Positive
	3) Dragendrorff's Test	Positive
	1) Molisch's Test	Positive

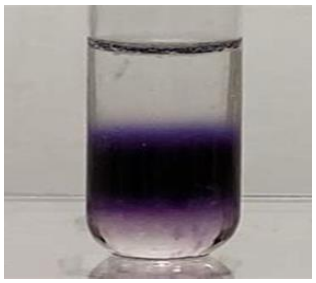
Carbohydrate	2) Fehling's Test	Positive
	3) Benedict's Test	Positive
Terpenoids	1) Salkowski's Test	Positive

1] Alkaloids Detection Test

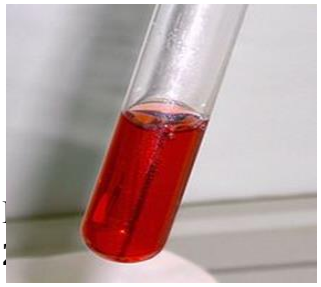
Mayer's Test

Wagner's Test

Dragendrorff's Test



Molisch's Test



Sr. no	Micro-organism	Zone of inhibition (mm)	
		Solution	Standard
1	*Escherichia coli*	10 mm	20 mm
2	*Aspergillus niger*	16 mm	14 mm

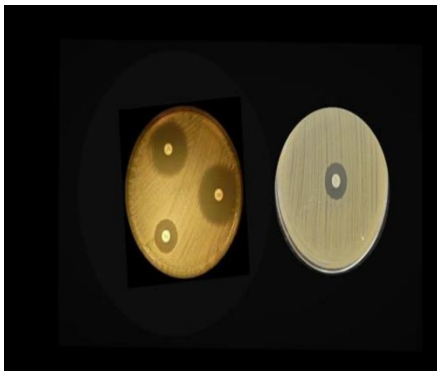


Figure: Zone of inhibition for *Escherichia coli* and *Aspergillus niger*

CONCLUSION

Internationally, the incidence of IBD is approximately 2.2-14.3 cases per 100,000 person- years for ulcerative colitis and 3.1-14.6 cases per 100,000 person-years for Crohn disease. Overall, the combined incidence for IBD is 10 cases per 100,000 annually. The recent conventional therapies for IBD cause serious adverse effects and are used only when potential benefits outweigh the risk. Hence there is a serious need for the development of safer and efficient formulation for treatment of this life-threatening disease. The use of dietary fibres gives a new safer approach for treatment and cure of IBD.

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