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A Compendium of Research Articles by Prospective Researchers 2024-25 Volume VII

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

A Compendium of Research Articles By Prospective Researchers 2024-25 (Volume-VII)

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

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

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

Title of the book: A Compendium of Research Articles by Prospective Researchers volume VII 2024-25

Name of the Editor: Mr. S. N. Patil
Department of Microbiology.

Publisher: Dr. K. B. Mahajan
Principal
Khandesh College Education Society's
Post Graduate College of Science,Arts &
Commerce, Jalgaon, M. J. College Campus, Jalgaon.

Printer: Khandesh College Education Society's
Post Graduate College of Science, Arts &
Commerce, Jalgaon, M. J. College Campus, Jalgaon.

ISBN: **978-81-949083-9-5**

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जा.क्र. KCES/65/2025-26

दिनांक 29/10/2025

FORWORD

I am very happy to know that Khandesh College Education Society's Post Graduate College of Science, Arts and Commerce, Jalgaon is publishing 'A **Compendium of Research Articles by Prospective Researchers' (Volume VII)'** of the academic year 2024-25.

It is a commendable to note that that Principal, Co-ordinator of the PRS scheme and Teachers put their efforts to conduct such research activity for the inculcation of the scientific attitude and temperaments amongst the students. Such activity provides platform for the students for creating innovative ideas and preparing the research projects on the thrust areas of science and technology. The compendium focused on the area like development of methodology of validation by sophisticated instruments, green synthesis and study of microbial activities etc.

I express my best wishes for the PRS activity and compendium of the year 2024-25 (Volume VII).

President,
KCE Society, Jalgaon

KHANDESH COLLEGE EDUCATION SOCIETY ★ JALGAON

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28th October 2025

PREFACE

It is a matter pride for me, that our college is publishing the **compendium of Volume VII** 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 2024-25.

The Research plays an 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 **16** students from **3** departments completed **06 research projects** under the guidance of **05** 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: 20th October 2025

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28th October 2025

FOREWORD

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, and Microbiology have contributed to this volume by undertaking the six research projects, like green synthesis, antibacterial & antifungal activities of microorganisms, synthesis of Schiff's reagent

I take this opportunity to congratulate the principal, co-ordinator of PRS scheme, supervisors and researchers of the project for releasing this magnificent compendium of **Volume VII**. This year the college is publishing compendium of Volume VII 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)

Khandesh College Education Society's
Post Graduate College of Science, Arts & Commerce, Jalgaon
Affiliated To K.B.C. North Maharashtra University, Jalgaon
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From the Desk of the Editor

I am pleased to present to you the seventh edition of *A Compendium of Research Articles by Prospective Researchers*, a publication that highlights the outstanding achievements of our students and faculty involved in the *Prospective Researcher's Scheme* for the academic year 2024-25. This volume showcases a collection of research articles contributed by students and faculty from the science departments of our college.

The *Prospective Researcher's Scheme* is a unique initiative that empowers students to undertake research projects under the guidance of our esteemed faculty. The outcome of these projects is reflected in this compendium, which is published with an ISBN number, serving as a testament to the hard work, creativity, and commitment of both students and faculty.

Throughout the research process, students receive the necessary support and resources to complete their projects. Upon completion, the projects are evaluated by external experts, and the best-performing researchers are recognized and awarded cash prizes for their exceptional work.

The research papers resulting from these projects are compiled into this seventh volume of the *Compendium of Research Articles by Prospective Researchers*, which is now available for distribution. This compendium represents the continued dedication of both our faculty and students, and I am truly proud to present it to you.

I would like to express my sincere gratitude to all the committee members, faculty project guides, and students whose collective efforts have made this edition possible. A special thanks to Principal Dr. K. B. Mahajan, Academic Advisor Dr. V. S. Zope, and our Hon'ble President Shri. N. G. Bendale for their unwavering support and encouragement in bringing this volume to fruition. We also wish to extend our heartfelt thanks to all the contributors for their cooperation and commitment.

I am delighted to hand over this volume to you, confident that it will continue to inspire and motivate future generations of researchers.

Mr. Sandip N. Patil
Editor
28th October, 2025

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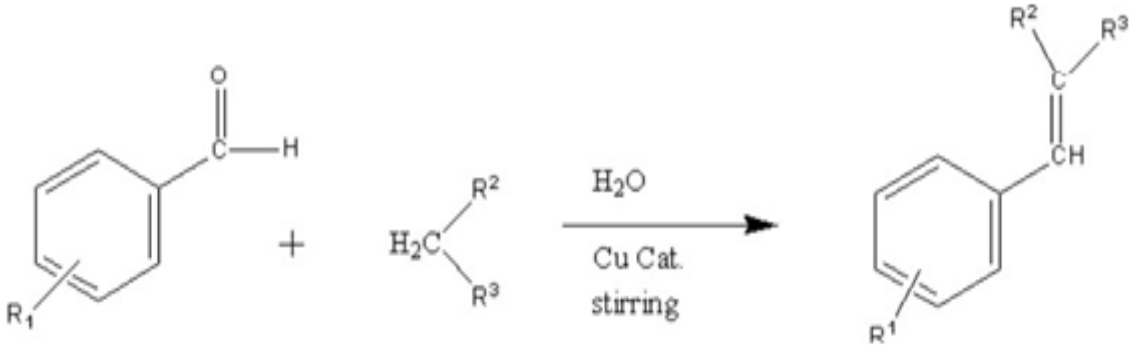
ORGANIC CHEMISTRY



Biosynthesis & characterization of Copper nanoparticles using *Adhatoda Vasica* extract and their application as a heterogeneous catalyst for Knoevenagel reaction

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Abstract

Biological methods for metal nanoparticle synthesis using plant extracts have been suggested as possible eco-friendly alternative to chemical and physical method. In the present study, copper nanoparticle was biologically synthesized using *Adhatoda Vasica* leaf extract as reducing agent. On treatment of aqueous solution of $CuSO_4.5H_2O$ with *Adhatoda Vasica* leaf extract stable copper nanoparticle were formed. Spectroscopic methods were used to monitor the quantitative formation of copper nanoparticle. Also copper nanoparticles were used as efficient catalyst for knoevenagel condensation reaction, the products were characterised by IR spectroscopic methods.

Keywords: Copper nanoparticle, *Adhatoda Vasica* leaf extract, Knoevenagel condensation.

Introduction

Metallic nanoparticles are produced by various methods, the more common ones being chemical and physical methods. The aforesaid methods produce pure and well-defined nanoparticles, but the chemicals used in the synthesis are toxic, energy consuming, expensive, and not suitable for biological applications. The synthesis of metal nanoparticles is covered in the past three decades, but research plant extract based nanosynthesis mushroomed only in the last decade.[1-4.] Biological methods are environmental friendly, economical, simple, and reproducible and require less energy. The nanoparticles exhibit a unique chemical, and biological properties at nanoscale compared to their respective particles at higher scales.[5-7]

Knoevenagel condensation is one of the most widely employed reaction in the industry which is used to produce imperative intermediates for pharmaceuticals, fine chemicals and biologically active materials.[8-9] Benzylidenemalononitrile derivatives are generally synthesized by the knoevenagel condensation reaction of aromatic aldehydes with active methylene compound. In our present work, we are reporting environmentally friendly method for synthesis of benzylidenemalononitrile derivatives of aromatic aldehydes which is clean, efficient and gives excellent yields at room temperature.[10-12] The presence of basic sites within the structure so can promote and accelerate the knoevenagel condensation reaction and is vital for reaction progress.[13-15]

Material and methods

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

Preparation of *Adhatoda Vasica* leaves extract-

Freshly collected *Adhatoda Vasica* leaves were thoroughly washed and grind in mortar pestle. The paste obtained was then re-suspended in 100 mL of distilled water and then filtered with clean muslin cloth at ambient temperature and centrifugation was carried out at 8,000 rpm for 20 minutes in centrifuge to obtain clear solution of *Adhatoda Vasica* extract.

Preparation of Cu nano-particle:

Initially, the $CuSO_4.5H_2O$ solution was prepared by addition of 2.5 g of $CuSO_4.5H_2O$ in 100 mL of deionized water. After, addition of 50 mL of *Adhatoda vasica* extract solution to 100 mL of $CuSO_4.5H_2O$ solution, the pH was kept at 7.0 with NaOH. The solution then underwent to a reflux at a magnetic stirrer. The colour of the solution changed as it was stirring with a from pale-green to a deep- brown while maintaining for 5 h at 70^0C. After centrifuging the solution for 24 hr, it was filtered. The solid precipitate was washed three times with deionised water, followed by 100% ethanol wash for Cu NPs separation, dried and kept at further application.

Result and discussion- Optimized reaction conditions:

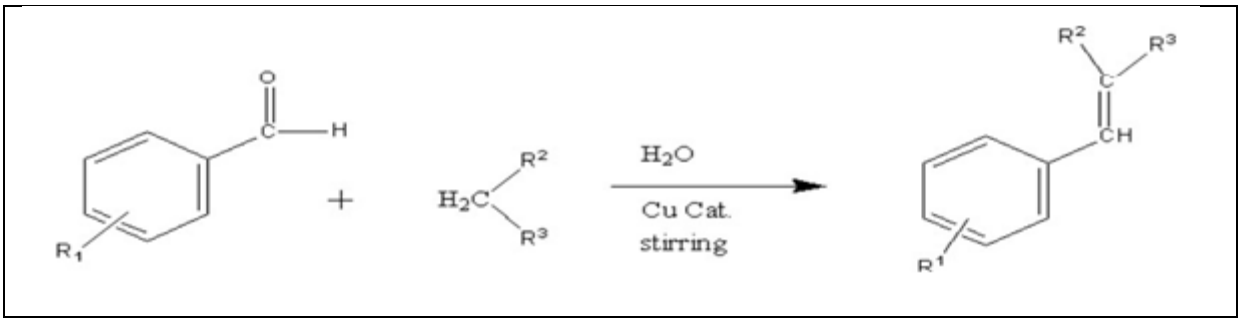
a) Effect of catalyst: Initially we performed reaction without catalyst the yield of product only about 60%. The time required was also more to complete the reaction. To optimize the reaction condition; we performed the model reaction with different amount of copper nanoparticles catalyst loaded as shown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

Table 1: Optimized amount of catalyst loaded

Entry	Catalyst (mg)	Time(Min)	Yield (%)
1.	0	30	70
2.	5	25	84
3.	**10**	**05**	**92**
4.	15	05	90

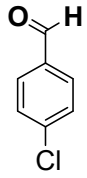
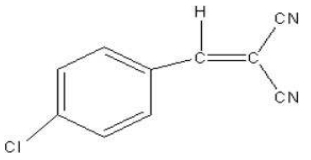
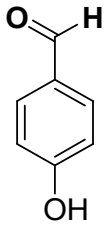
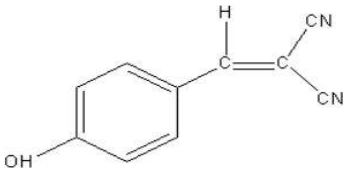
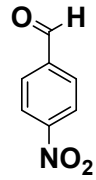
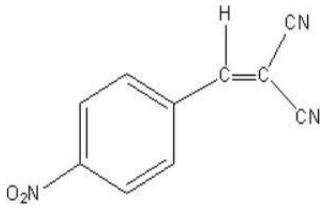
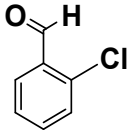
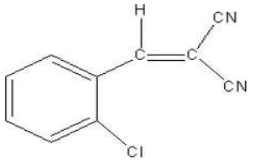
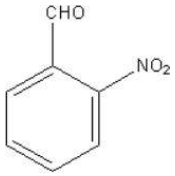
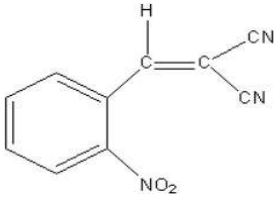
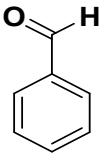
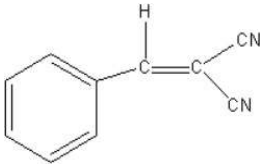
It was found that, the 10 mg catalyst is sufficient to push the reaction forward. Hence the reaction was perform with 10 mg catalyst by optimized the reaction condition.

After the study of optimized reaction condition were explored for the synthesis of series of benzylidenemalononitrile derivatives from various substituted benzaldehyde, malononitrile, using Cu nanoparticles as catalyst as shown in **scheme-1** and the results are in **Table-2**.

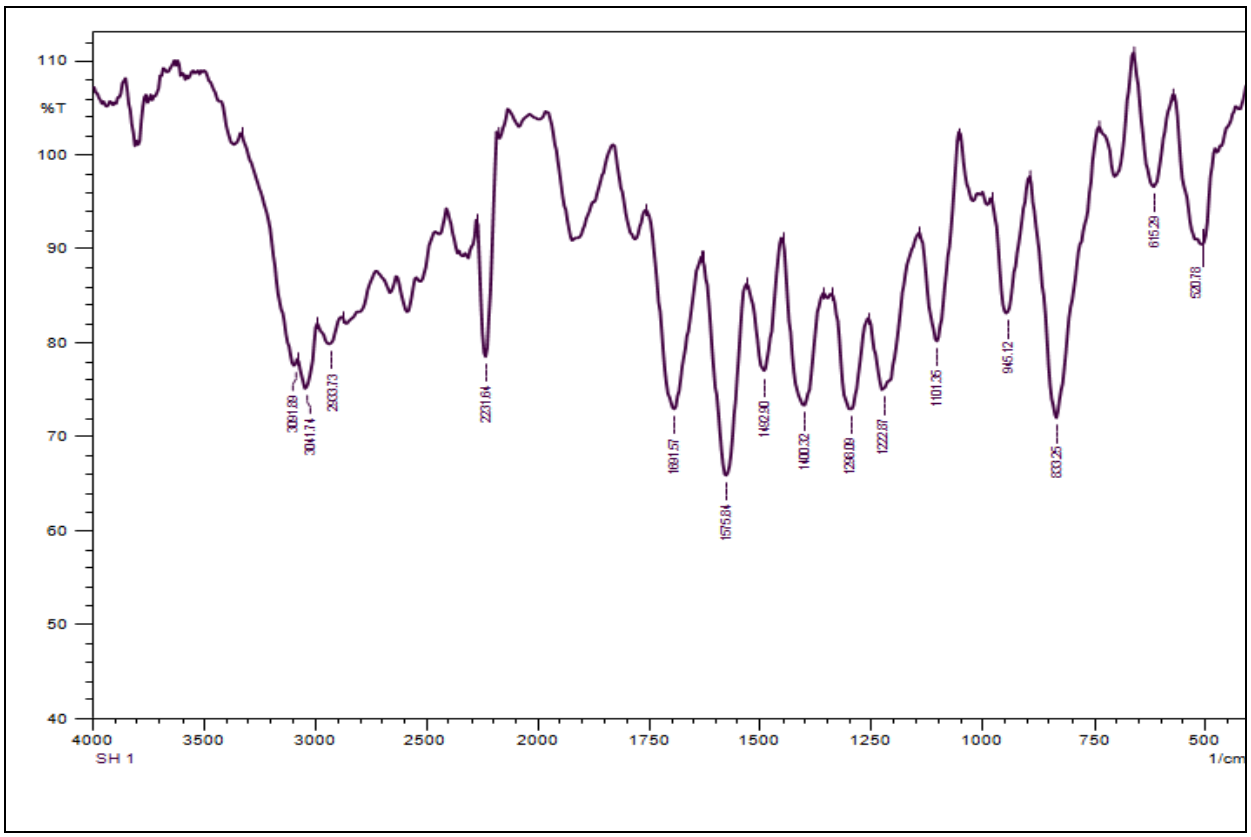


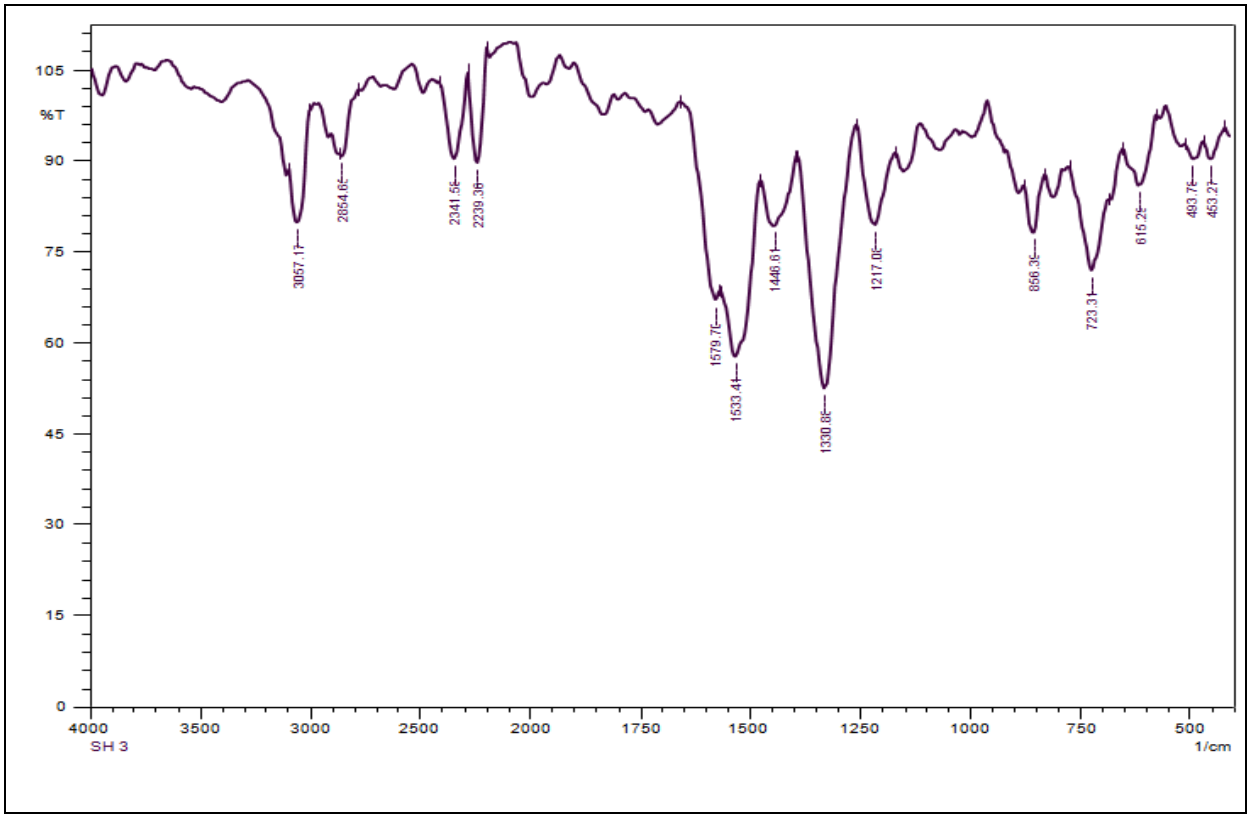
Scheme 1: synthesis of series of benzylidenemalonnonitrile derivatives

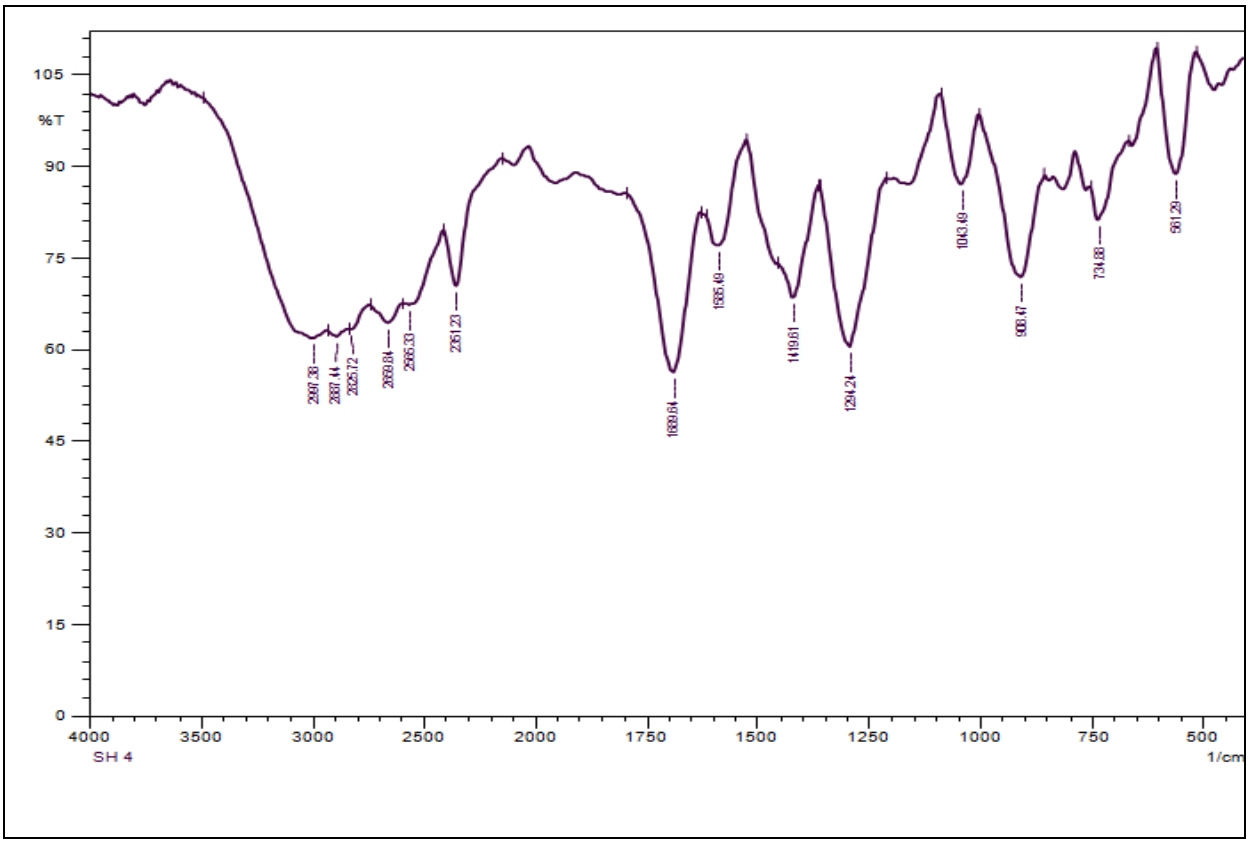
Table-2: Synthesis of of series of benzylidenemalononitrile derivatives

Entry	Starting compounds	Product	Time (min)	Yield (%)	Melting point(^{0}c) Obs. (Reported)
1.			5	88	163
2.			5	88	180
3.			5	96	84
4.			6	90	92
5.			6	91	82
6.			6	85	84

IR Spectra of compounds (1,4 and 5)







IR Spectral data of compounds (1-6)

1. White solid IR (cm^{-1}): 2210.9 (-CN), 1646.5(C=N), 1589.2(C=C), 1330 (C-N), 790.57(C-Cl)
2. Brown solid, IR (cm^{-1}): 2205(-CN), 1631(C=N), 1594(C=C), 1379 (C-N), 3200-3400(C-OH)
3. White solid, IR (cm^{-1}): 2235.99 (-CN), 1603 (C=N), 1598(C=C), 1364 (C-N), 1346.86(NO_2)
4. Brown solid, IR (cm^{-1}): 2211.3(-CN), 1638(C=N), 1589.2(C=C), 1391 (C-N),
5. Pale Yellow solid, IR (cm^{-1}): 2227.(-CN), 1661(C=N), 1589.2(C=C), 1318 (C-N), 1386(NO_2)
6. Yellow solid, IR (cm^{-1}): 2205.99 (-C≡N), 1623.3(C=N), 1578.74(C=C), 1304 (C-N),

Conclusion

We conclude that ***Adhatoda vasica*** can be a good source of reducing agent required for biological synthesis of nanoparticles and it eliminates the use of hazardous chemical that makes synthesis eco-friendly as well as cost effective due to the wide availability of ***Adhatoda vasica*** plants.

We have developed a convenient and practical synthetic protocol of knoevenagel condensation derivatives based on the two component condensation of aromatic aldehyde, malononitrile and ethyl acetoacetate. The use of NPs as a reusable heterogeneous catalyst makes this protocol mild, convenient and environmentally benign. This method offers interesting advantages such as (i) high purity of desired products (ii) short reaction times (iii)

cost effectiveness and (iv) excellent yields. Moreover, avoidance of toxic organic solvents, usage of safe and recyclable Nano-catalyst makes this present synthetic protocol comparatively greener approach.

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Efficient One Pot Synthesis of Schiff's Base by Using Fe-Nanoparticles as a Catalyst

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Abstract:

Nanotechnology has shown a promising future in material science for enormous applications in the field analytical, biological, catalytic, electroanalytical fields and so on. Resent nanoscience has given huge effort to enhance the applicability of nanoparticles through modification or functionalization process. In this modification process nanoparticles are stabilized or factionalized using organic, inorganic metal complexes, and even with Schiff base applicability in catalytic process, antioxidant, and antifungal. Besides, the biological behaviors of the Schiff base functionalized nanoparticle synthesis were prominently noticed due to presence of various functional groups, atoms, metal ions as well as nanomaterial. Even, the imine group (>C=N-) of Schiff base effectively interacts with the cell of microorganisms, and inhibits the growth of cell.

Keywords: Nanoparticles, Nanocatalyst, Schiff's base, antioxidant, antifungal.

Introduction:

Nanotechnology, a science that deals preparation of nano-size particles ranging from 1 to 100 nm. The term 'nanoparticles' was coined from Greek word 'nano' that means 'dwarf' or 'small' and when used as prefix it indicate size 10^{-9} one billionth of meter is equal to 1nm, can be regarded as a nanoparticles based on the SI unit system [1]. These represent the two extremes of what may be regarded as nanoparticles through certainly many in the scientific community do not hold such strict interpretation[2]. The term nanoparticles despite its current acceptance and use are a relatively new term having only come into common use in late 1970 sec and while being used by many others. Green method using leaf extract of *carica papaya* leaves. The papaya plant leaves were collected from premises. The fresh leaves were then washed multiple times with tap water followed by deionized water. The leaves were then dried in oven for an hour and then grinded to form fine powder. The formation of intense black colored solution confirmed the synthesis of iron oxide nanoparticles[3]. The Schiff's bases are also used as versatile component nucleophile addition with organometallic reagent[4] and in cycloaddition reaction[5]. Schiff's bases are known as substituted imine are compound containing azomethane group (-HC=N-) are represented by general formula R^2 –R^3C=NR', they are the condensed product of aldehyde or ketone and were first reported by Hugo Schiff', in 1864[6]. Originally the classical synthetic route for synthesis of Schiff's bases was reported

by Schiff which involves condensation of primary amines with carbonyl compounds[7]. Schiff's bases are condensation products of primary amines and carbonyl compounds. The formation of carbon – nitrogen double bond important role in organic synthesis, this can be achieved by the reaction of aldehydes and amines in acidic medium which leads to synthesis of Schiff's bases (imines). A number of Schiff's bases containing the imino functionally have been shown to have a wide range of biological activities, including antibacterial, antifungal, antidiabetic, antitumor, antiproliferative, anticancer, anticorrosive and anti-imflammatory activites[8-11]. We obtained derivatives of schiff's base by using Fe-nanoparticles as a catalyst and ethanol as a solvent using MCRs give high yield of products. Owing to the biological importance, we developed methodologies for the synthesis of Schiff's bases by using different aldehydes, amine and catalyst continuation of our effort to eco-friendly synthetic approach toward synthesis of bioactive.

Material & Methods:

All reagents used were of laboratory grad. Melting points were determined in open capillaries and are uncorrected. The purity of compounds were checked by TLC.

Preparation of plant extract:

The papaya plant (*carica papaya*) leaves were collected from premises. The fresh leaves were then washed multiple times with tap water followed by deionized water. The leaves were then dried in oven for an hour and then grinded to form fine powder. 20 grams of fine powders was boiled in 1 lit. of deionized water at 80^0C for 30 min and the extract is then filtered using Whatmann no. 42 filter paper. The filtrate was concentrated using rotary evaporator and stored at 40^0C for further use.

Green synthesis of $\propto -Fe_2O_3$ nanoparticles:

Ferric chloride hexahydrate ($FeCl_3.6H_2O$) was use as the precursor for the synthesis of the $\propto -Fe_2O_3$ nanoparticles. 50 ml of papaya leaves extract was added dropwise with 50 ml of 0.1 M $FeCl_3.6H_2O$ solution in 1:1 ratio at room temperature. Following this, 1M NaOH was added till the pH became 11. The resultant mixture was stirred using a magnetic stirrer for 30 min and formation of intense black colored solution confirmed the synthesis of iron oxide nanoparticles. The nanoparticles were separated by centrifugation at 8000 rpm for 20 min and cleaned by subsequent washing with ethanol and water for 2-3 times. The nanoparticles were finally dried in a hot air oven at 80^0C for 3 hr. and store in a seal tight container for further use.

Figure 1.

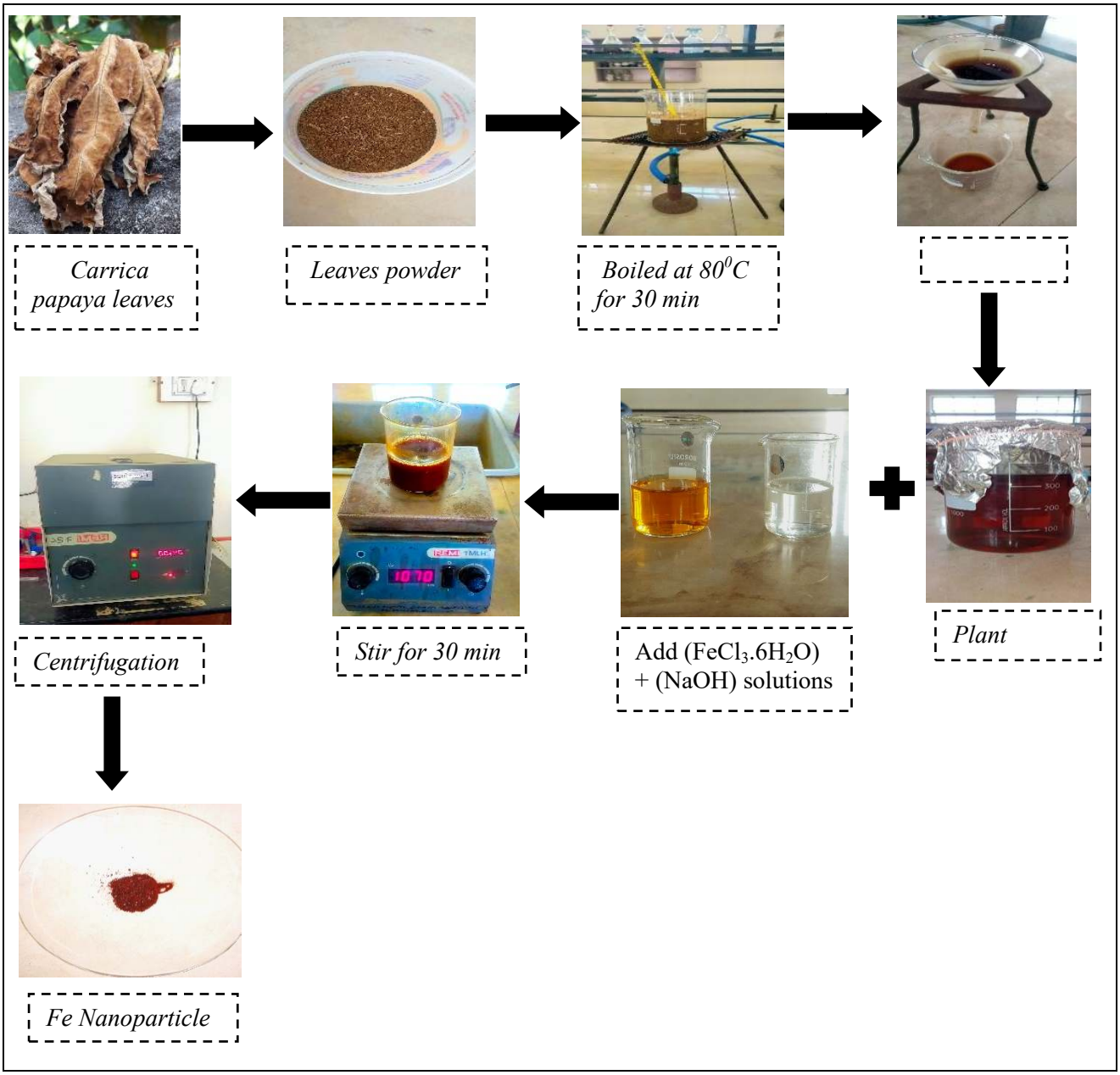


Figure 1-Schematic representation of synthesis of nanoparticles

Result and Discussion:

Optimized reaction conditions:

a) Effect of catalyst:

Initially we performed reaction without solvent the yield of product is only about 50%. The time required was also more (about 30 min) to complete the reaction. But when we used ethanol as a solvent the yield of product increases up to 90% and time also reduced about 10 min. To investigate the role of ethanol reaction was observed that Schiff's base formation was increases in ethanol, while the same reaction occurred slowly in water and another organic

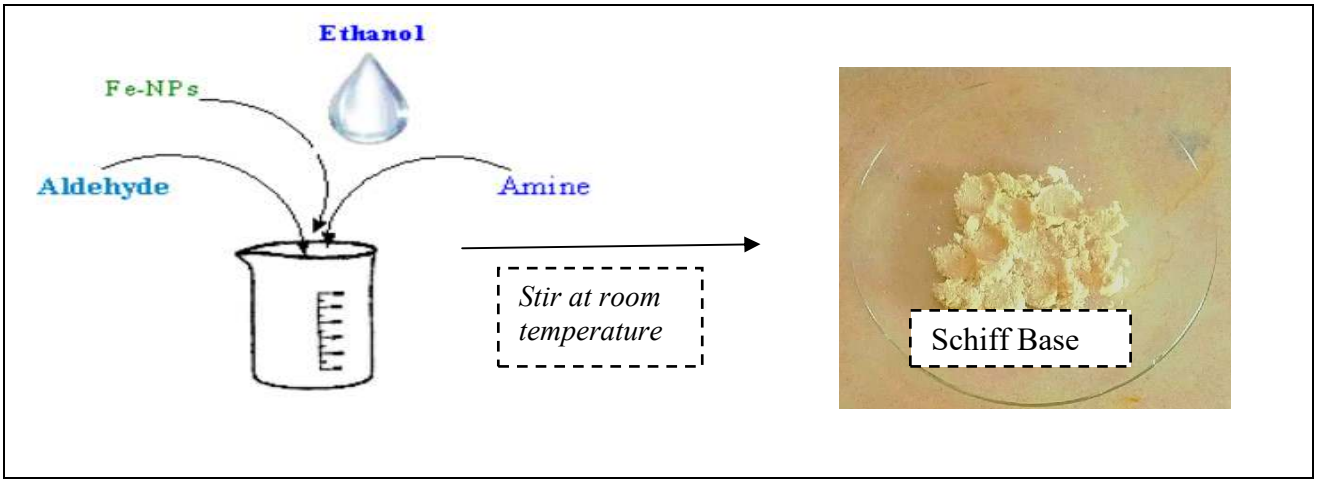
solvent. Hence by using ethanol as solvent we performed the model reaction with different mole % of Fe – NPs to optimized reaction condition as shown in **table-1.**

Table 1: Optimized amount of catalyst loaded

Entry	Catalyst (mole%)	Time (min)	Yield (%)
1	5	5	87
2	10	5	90
3	**15**	**1**	**92**
4	20	1	92

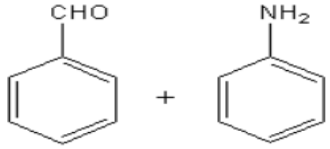
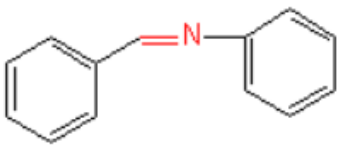
It was found that, the 15 mole % catalyst is sufficient to push the reaction forward. Hence the reaction was perform with 15 mole % catalyst by optimized the reaction condition.

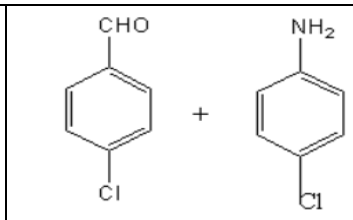
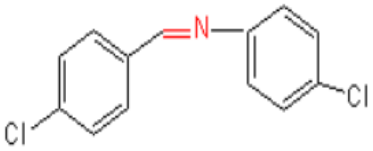
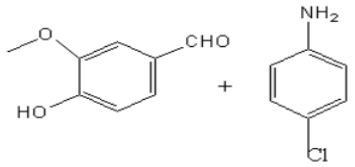
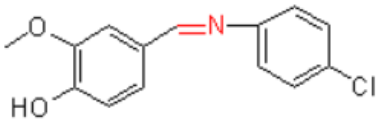
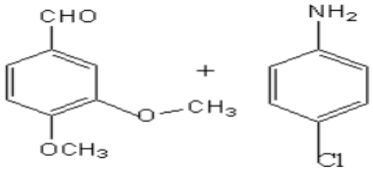
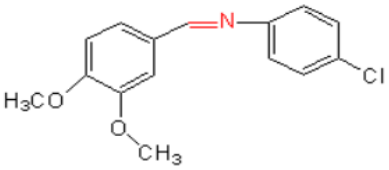
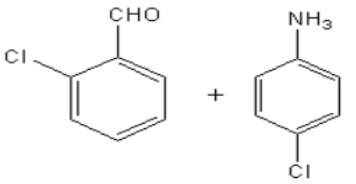
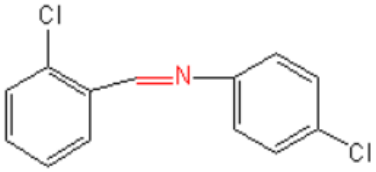
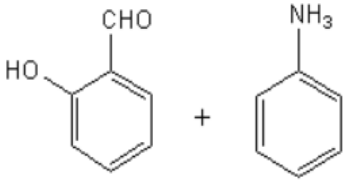
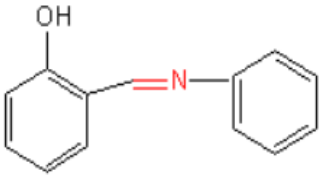
After the study of above optimized reaction condition were explored for the synthesis of series of Schiff's base derivatives from various substituted benzaldehyde and aromatic amines using Fe – NPs as catalyst as shown in **scheme-2** and the results are summarized in **Table-2**.



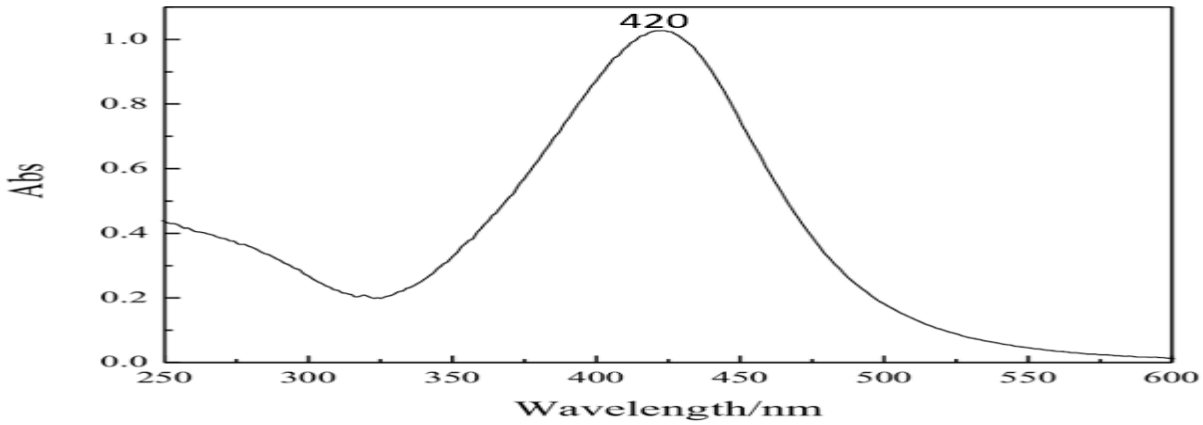
Scheme 2: Synthesis of Schiff's base derivatives by using Fe-Nanoparticle as catalyst

Table-2: Synthesis of Schiff's base derivatives

Sr. No.	Starting compound	Products	Time (Min)	Yield (%)	Melting Point (^{0}C)
1.			7	89	68

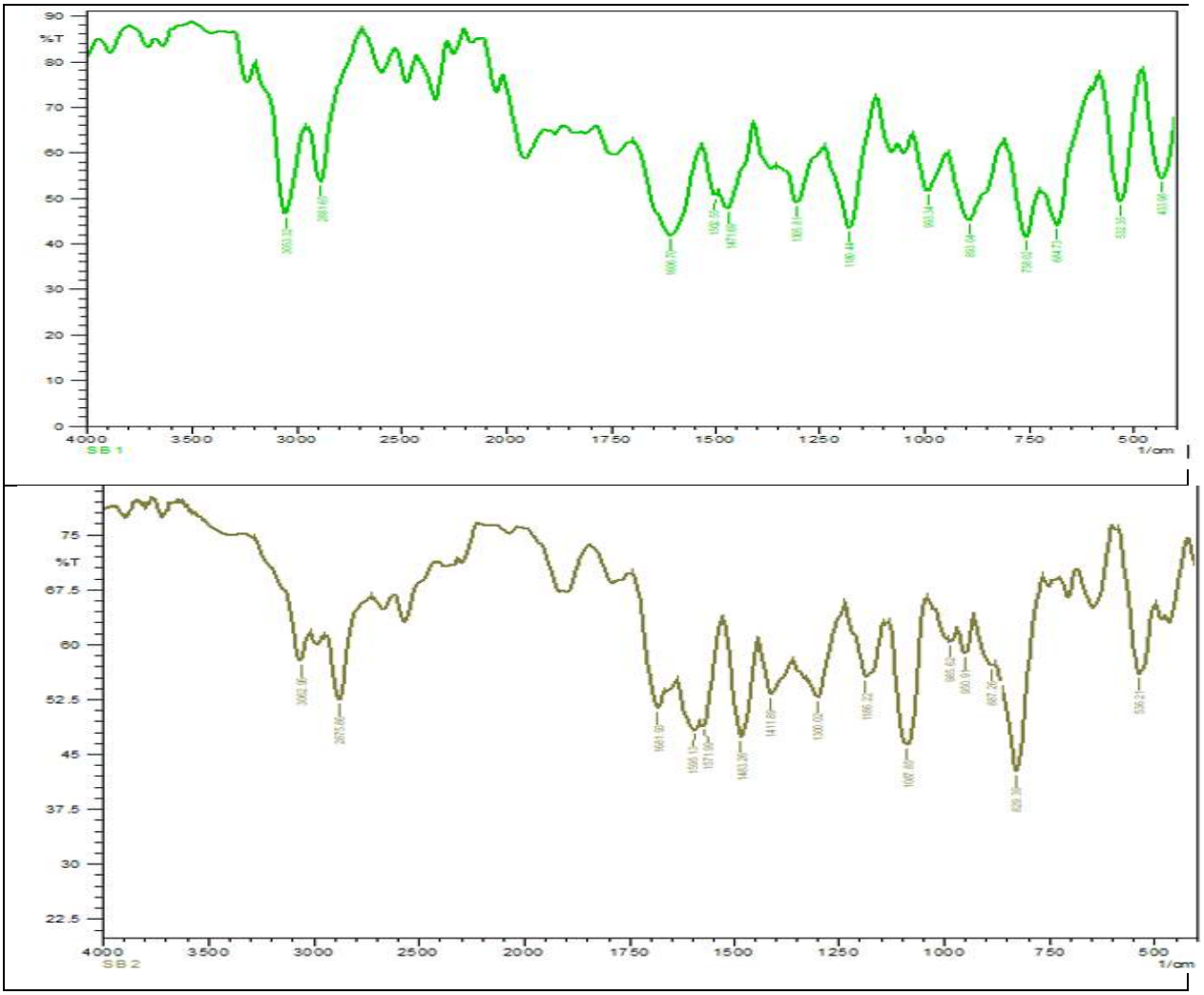
2.			1	90	94 – 96
3.			10	82	118 – 120
4.			3	85	78 – 80
5.			20	92	52
6.			13	85	64

Characterization:-
UV of Fe- Nanoparticles:



***Reference = 422**

IR Spectral of compound 1 &2:



IR Spectral data of compound 1-3:

1. Soft pastel yellow (solid)IR: 1606 cm^{-1}(C=N) 3061.03cm^{-1} (C=C-H) 1305 cm^{-1} (C-N) 2881 cm^{-1} (C-C-H)
2. Off white, (solid) IR: 1681 cm^{-1}(C=N), 3062 cm^{-1} (C=C-H), 829 cm^{-1} (C-Cl), 2875 cm^{-1} (C-C-H)
3. Light brown, (solid)IR: 1598 cm^{-1}(C=N) , 3072 cm^{-1} (C=C-H), 2999 cm^{-1} (C-O), 3034 cm^{-1} (C-H)

Conclusion:

Nanoparticles are prepared by biological method. The green synthesis method must enhance the selectivity, shorten reaction time, and greater yield. Schiff's bases formed by condensation of primary amines with aldehydes. This study focused on green synthetic in order to find the best technique that higher yield in a shorten time in ecofriendly environment.

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ANALYTICAL CHEMISTRY

Study of Oxalate Ions in Sapota and Tomato in different Phases

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Abstract:

The oxalate content of tomato and sapota fruits at different stages of ripening were found out by permagnometric method. Oxalate rich foods are usually restricted to some degree, particularly in patient with high urinary oxalate level. Tomato and sapota fruit have the highest percentage of vitamin C among citrus fruits. It also content oxalate amount, which varies with ripening of fruit. During ripening of tomato and sapota fruit, the oxalate content increases progressively and the fully ripe fruit has the maximum oxalate content.

Keywords:-

Permagnometric method, hyper-oxaluria, hypercalcicuria, vitamin C, oxalate ions, pulp, etc.

Introduction: Oxalate (IUPAC ethandionate) is the dianion with the formula $C_2O_4^{2-}$ written as $(COO)^{2-}$. Either name often used for derivation, such as salts of oxalic acid for example, sodium oxalate $2(Na)^{+1}$-$C2O4^2$ Oxalic acid is the simplest dicarboxylic acid and occurs as the dehydrate C_2H_2O4, $2H2O$. It is a colorless crystalline solid soluble in water. It is a reducing agent and its deprotonated species, oxalate ion ($C_2O_4^{-2}$), is a reducing agent for metal cations. Its main applications include cleaning or bleaching, especially for the removal of rust due to the formation of a stable, water-soluble ferrioxalate ion. Oxalic acid is required in our body for the formation of uracil and orotic acid.

Presence of oxalate is injurious to health. Oxalate rich food is usually restricted to some degree, particularly in patients with high urinary oxalate level. Excessive intake of vitamin C which metabolized to oxalate may lead to hyper calcicuria and an increase in stone formation. Surgical techniques have also been developed to remove kidney stones. Rather than having to undergo treatment, it is best to avoid kidney stone in the first place.

Excessive intake of vitamin C which metabolized to oxalate may lead to hyper calcicuria and increase in stone formation. Pain medication can be prescribed for symptom relief. Surgical techniques have also been developed to remove kidney stone. Rather than having to undergo treatment, it is best to avoid kidney stone in the first place. Avoid calcium rich foods and drink more water. Water helps to flush away that form stone in the kidney.

Methodology:

Materials, Chemicals, and Reagents: Materials used were distilled water, potassium permanganate, oxalic acid and sulphuric acid supplied by S. D. Fine-Chemicals (India) used as received.

Standardization of KMnO4: Potassium permanganate was standardized by using solution of primary standard oxalic acid volumetrically. Slandered 0.059 N solution of potassium permanganate was used for further investigations of free oxalate ions content in samples of tomato and sapota fruits.

Determinations of Free Oxalate Ion in the Fruit (tomato and sapota) Pulps: 50.00 gram of fresh fruit was crushed to fine pulp using pestle and mortar. This pulp was then mixed thoroughly with 50 milliliter of dilute sulphuric acid. The content was transfer into beaker and boiled for about 10 minutes. After cooling the content up to room temperature it was diluted with distilled water in volumetric flask of 100 milliliter capacity. 10 milliliter of diluted solution, 20 milliliter dilute sulphuric acid were poured in to the titration flask, it was heated to about $60^{O}C$ and hot solution was titrated with slandered solution of potassium permanganate. The end pint was recorded at permanent persistence of pale pink color. In this way free oxalate ions were extracted and measured from the fruits. Same procedures were repeated for 50 grams of tomato and sapota fruits of 1, 5, 10, 15, 20 and 25 days old.

Results and discussions: The amount of Free Oxalate Ion in the Fruits (tomato and sapota) Pulps were evaluated by volumetric method using potassium permanganate. The results are as given in table No. 1.

Stages of fruits	**Volume of $KMnO_4$ (ml)**		**Weight of oxalate ion in 50g of fruits (g/L)**	
	Sapota	**Tomato**	**Sapota**	**Tomato**
1day old	49.0	23.1	21.56	9.65
5 days old	50.5	24.7	22.00	10.86
10 days old	52.8	25.2	23.23	11.08
15 days old	57.3	26.6	24.00	11.70
20 days old	63.8	27.4	28.07	12.05
25 days old	69.2	29.8	30.44	13.11

Table 1: The amount of Free Oxalate Ion in the Fruits

The graphical representation of amount of Free Oxalate Ion in the Fruit (tomato and sapota) Pulps is given in Figure No. 1.

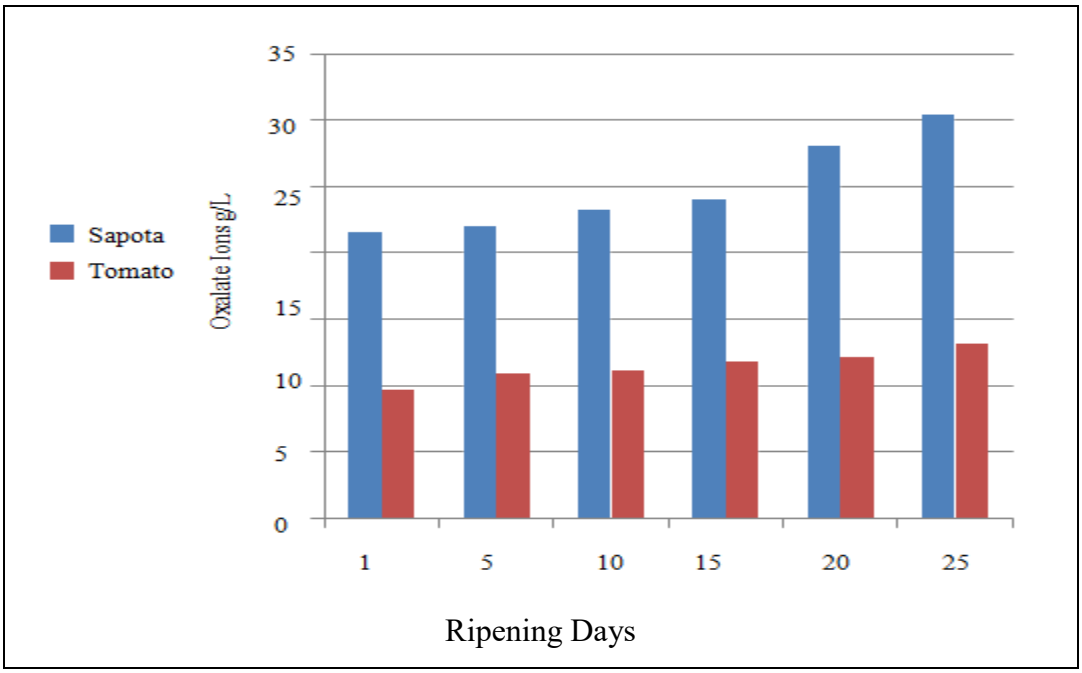

Figure 1: - Amount of oxalate content of Sapota and Tomato fruits during ripening.

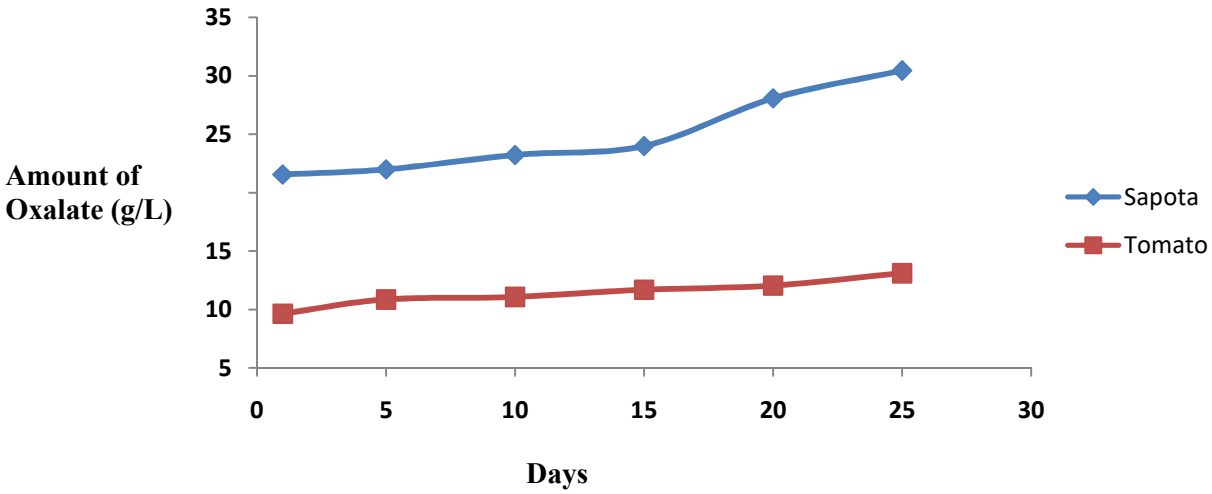

Figure 1: Graph of amount of Oxalate versus days

Figure 1 indicates that the trends of ripening in both the fruits is same and shows gradual increase in value of oxalate content. However, tomato contains significantly more amount (21.56 g/L) of oxalate even at first day (fresh fruit) as compared to sapota (9.65 g/L). It is evident from the data that more oxidation takes place in case of sapota.

Conclusion:-

This project centered upon estimating the amount of oxalate present in the various fruits during ripening. The oxalate content was on the increase in fruits and the days passed on, that is as the ripening proceeded. The oxalate content of both fruits at different time of ripening were found out by permaganometric method. And the days passed on, that is as the ripening proceeded. It should be noted that the increase in oxalate content was mare in sapota than in

tomato.

The amount of oxalate content varies in various ripening fruit at different times. When we cut a fruit for long time, its Oxalate ion quantity will increase. At instant time Sapota and Tomato fruits oxalate ion is low amount. When increase the time period after 5 days, 10 days, 15 days, 20 days and 25 days increasing the oxalate ions in both fruits samples, due to oxidation process.

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UV Spectrophotometric Method Validation Of Azithromycin In Tablet Formulation

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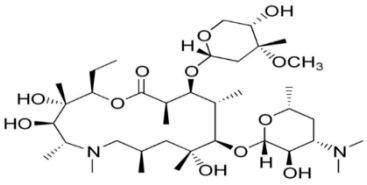
Abstract:

Azithromycin (AZI) is a semi-synthetic macrolide antibiotic drug, effective against a wide variety of bacteria. The present study describes simple, accurate, less time consuming, precise UV Spectrophotometric method for validation of AZI. The absorbance maximum (λmax) for AZI was found to be 275 nm. The method reveals high sensitivity with linearity in the 1 mg/ml to 4 mg/ml range. Curve demonstrated the linear relationship between absorbance and concentration. Result obtained from Spectrophotometric record concluded the validation of drug (AZI) by UV- Spectrophotometric method.

Keywords: Azithromycin, Estimation, UV Spectrophotometer, Validation, Antibiotic, Antimicrobial, ICH guidelines.

Introduction:

Azithromycin is a semi synthetic macrolide antibiotic of the azalide class [1]. It is derived from erythromycin; however, it differs chemically from erythromycin in that a methyl substituted nitrogen atom is incorporated into the lactone ring [2]. It inhibits the translation of mRNA in bacterial cells at the chain elongation step; result in the blockage of trans peptidation. Azithromycin is rapidly absorbed and is widely distributed to tissues and becomes concentrated in cells. Peak plasma concentrations are achieved within 2 to 3 hours. [3] Various methods which are available for AZI are HPLC, Spectrophotometry and microbial assay methods. These methods are either using costly reagents or the method is tedious and time consuming. Therefore, a simple and cheap UV –spectrophotometric method for AZI is used to validate (according to ICH guidelines). [4]



Structure of Azithromycin

Tablet of Azithromycin

Methods and Materials:

Instrument: A double beam UV-visible spectrophotometer Shimadzu, 1900) having two matched quartz cells with 1 cm light path was used for recording of spectra and measuring absorbance. Electronic weighing balance (Shimadzu) and sonicator were used in this study.

Chemicals and reagents: 0.1 M HCl and distilled water was used throughout spectrophotometric method validation. The API use was kindly gifted by kaliberr Bioscience Pvt. Ltd., Dindori, Nashik,(India). Marketed formulation of Azithromycin used for the assay was purchased from local market (EZEE 250 mg tablet).

Preparation of Standard Stock Solution: 500 mg of standard AZI was accurately weighed and taken in a 100 mL clean and dry volumetric flask containing 80 mL of 0.1 M hydrochloric acid and was made up to 100 mL with distilled water. This is considered as the standard stock solution (5 mg/ml). Series of dilutions were prepared over a range of 1 - 4 mg/mL solution**.**

Preparation of Sample Solution: 10 tablets with label claim of 250 mg of AZI were accurately weighed and finely powdered with the help of a mortor and pestle. The powdered drug, equivalent to 500 mg of AZI, was dissolved in 60 mL of 0.1 M HCl. The solution was kept for sonication for about 10 min, followed by filtration (Whatman filter paper no.1). The filter was rinsed 2 times with 0.1 M HCl and then the volume was made up to 100 ml with distilled water.

Weighing

Sonication

Validation by UV Spectrophotometer

VALIDATION OF THE ANALYTICAL METHOD [6]

The analytical performance characteristics which were tested during method validation: linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Linearity:

Standard solutions of AZI over a concentration range of 1 – 4 mg/mL were prepared from a 5 mg/mL stock solution and absorbance was measured. Calibration curve was constructed by plotting the concentration level of drug versus absorbance.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ were estimated from calibration curve used to determine method linearity.

Limit of detection and limit of quantification were calculated on the basis of the Standard Deviation of the y-intercepts of regression lines and the Slope.

The limit of detection (LOD) may be expressed as: LOD = 3.3 x σ/S

The limit of quantitation (LOQ) may be expressed as: LOQ = 10 x σ/S

Where, σ = the standard deviation of the y-intercepts of regression lines

= Standard Error of y-intercept of regression lines / $\sqrt{N}$

N = No. of tests

S = the slope of the calibration curve

The estimation of slope (S) can be done from the data obtained from calibration curve of the analyte. The estimate of σ carried out using standard deviation of the y-intercepts of regression lines.

Analysis of the Marketed Formulation: 2 mg/mL of AZI of the sample solution were applied in 3 replicates and absorbance was taken. The risk and possibility of interference of the excipient in the analysis was studied.

$$\% \text{ Assay} = \frac{Absorbance\ of\ \ sample}{Absorbance\ of\ standard} \times 100$$

Result and Discussion:

1. **Selection of wavelength**

From the literature survey, maximum absorbance λmax of AZI by spectrophotometric method was found to be 275 nm in 0.1 M HCl.

2. **Linearity**

Good linear relationships were obtained over a concentration range of 1 - 4 mg/mL for AZI with respect to absorbance. The linear regression data for AZI is shown in (Table 1 and 2) and graphical representation is shown in (Fig. 1). From the graphical data of AZI, it is obvious that absorbance values of AZI are linear over the concentration range of 1-4 mg/mL with correlation co-efficient 0.9951.

Table 1. Linearity data of AZI

Sr. No.	Concentration (mg/ml)	Absorbance
1	1.0	0.124
2	1.5	0.142
3	2.0	0.154
4	2.5	0.176
5	3.0	0.186
6	3.5	0.208
7	4.0	0.221

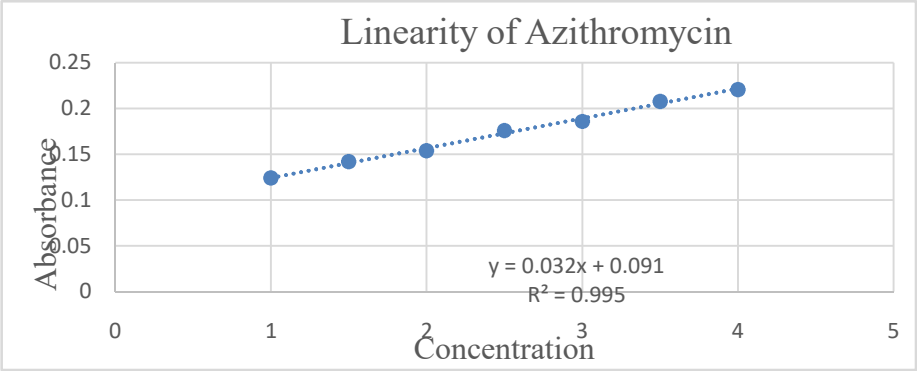


Figure 3: Calibration curve of Azithromycin by simple UV Spectroscopy

Table 2. Linear regression data of AZI

Parameters	Azithromycin
Correlation coefficient r^2	0.9951
Slope	0.0325
Intercept	0.0918
Standard Error of y-intercept	0.0027
Standard Deviation of y-intercept	0.00725

3. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were found to be 0.726 mg/ml and 2.230 mg/ml, respectively.

From data given in table 2, LOD & LOQ can be calculated as

LOD = 3.3 x σ/S

= 3.3 x (0.00725/0.0325) =0.736 mg/mL

LOQ = 10 x σ/S

=10 x (0.00725/0.0325) =2.230 mg/mL

4. Optical Characteristics

Optical characteristics of AZI are tabulated in the (Table 3) given below. The data obtained for the optical characteristics, LOD and LOQ, indicated that the proposed method for AZI is sensitive and small quantities of compounds can be estimated accurately.

Table 3. Optical characteristics of AZI

Parameters	Azithromycin
λmax	275 nm
Linearity (mg/mL)	1- 4 mg/ml
Limit of Detection	0.736 mg/mL
Limit of Quantification	2.230 mg/mL

5. Analysis of Marketed Formulation

Sample solution of 2 mg/mL of AZI was prepared and measured for absorbance (Three trials were carried out). There was no interference from any of the formulation excipients and any other impurities in the sample. The amount of the drug present was found to be 94.8% for AZI.

Table 4: Estimation of azithromycin in marketed formulation by simple UV method

Tablet	Label Claim	Concentration	Mean absorbance	% Assay
EZEE	250 mg	2mg/mL	0.146	94.8%

$$\% \text{ Assay} = \frac{Absorbance\ of\ \ sample}{Absorbance\ of\ standard} \times 100$$

$$= \frac{0.146}{0.154} \times 100 \qquad = 94.8\%$$

Table 5. Summary of validation parameters by simple UV Spectrometric method

Sr. No.	Parameters	Results
1	λmax	275 nm
2	Regression line equation	y = 0.0325x + 0.0918
3	Correlation coefficient (R^2)	0.9951
4	LOD	0.736 mg/mL
5	LOQ	2.230 mg/mL

Conclusion:

The given UV spectrometric method was successfully validated as per ICH guidelines (ICH Q2 R1) and it meets to specific acceptance criteria. It is concluded that this analytical method was simple, fast and economical. Thus, the developed method can be applied for the routine analysis of AZI in tablet formulation.

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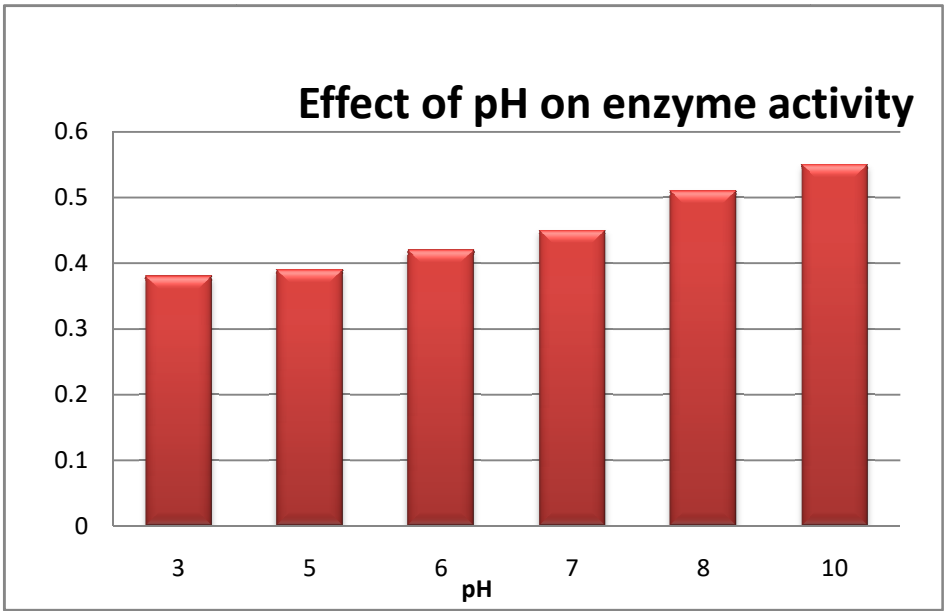

Isolation of Lactic Acid Bacteria from the Nectar of the Flowers

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Abstract

In the present study an attempt was taken for qualitative analysis to detect the presence of LAB Lactobacillus strains from nector part of flowers. The bacterial strain from the flower sample was isolated and tested for biochemical test, salt, pH tolerance and cell adhesion ability, Hemolytic Activity and antibiotic resistivity. Gram staining and colony morphology of each isolated bacteria colony primarily indicate the presence of lactobacillus species which is further confined by different biochemical and selective test for lactobacillus Species. LAB posses' the ability to grow in the range of pH of 4 to 8. The high bile tolerance and cell adhesion test confirm the probiotic potential and application of the lactobacilli strain.

Keywords- LAB, Probiotic etc

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Introduction: Probiotics are living microorganism that when taken by mouth benefit to your health by improving the balance of bacteria in the intestines. Probiotics are useful and friendly microbes. FAO\WHO (2002) defined probiotics as "live microorganisms which when administered in adequate amount confer a health benefit on the host". Probiotics are beneficial bacteria that favorably alter the intestinal microflora balance inhibit the growth of harmful bacteria. The study was aim for investigate lactic acid bacteria that contain an important group of bacterial strain that usually isolated from flowers. These groups of bacteria have been considered as probiotic. In this study bacteria where isolated from plant sources such as Aspilia, Rose, Tuberose, Hibiscus, Curd. The experiments were conducted to explore the diversity of naturally occurring Probiotic lactic acid bacteria (LAB).

Methods and Materials

Collection of sample- The different species of flower were collected from local area in sterile polythene bags. Enrichment and Isolation of lactobacillus strain from nectar part from flower was done by using MRS broth.Nectar part of the flower directly introduce in to the saline on MRS agar media plate and spread with help of sterile glass spreader**.**

Acid Tolerence

MRS broth was adjusted to pH 2.5, 3.5 and 4.5 in different flasks with 1N HCl.Isolates (107 cfu/ml) were then inoculated in the broths of the adjusted pH for about 90 mins at R.T.The determination of

survival was performed by single streaking on MRS agar plates and growth was observed after 48 hrs in microaerophilic condition at R.T.Isolates which were growing on agar were considered to be acid tolerant strains

Bile tolerance

Bile tolerance was carried out by growing the isolated cultures in MRS agar containing 0.1, 0.3, 0.5, 1, 2, 3 , 4, and 5 percent Of bile salt concentration for 24 hours 37 °C under static condition.Control was carried out by growing isolates in MRSagar without bile salts at the same conditions.

Cell adhesion ability

1 ml of hexadecane was added to tube containing 3 ml of Suspension. Hexadecane was chosen as a nonpolar solvent because it reflects cell surface hydro- phobicity and hydrophilicity. The two phase system was throughly mixed by Vortexing for 5min. The aqueous phase was removed after the incubation at Rt and absorbance at 600nm was measured.4.The Influence of the bacterial viability on the the hydrophobicity abilities was analyzed Affinity to hydrocarbons (hydrophobicity) was reported as adhesion percentage according to the formula % adhesion = [A0- A1/A0 x 100] where A0 is absorbance before A1 After extraction with organic solvents, respectively

Haemolytic activity

Material

Analysis of antibiotic resistivity

Antibiotic resistivity was tested by disc diffusion assay.

Results and Discussions

Enrichment and Isolation of Probiotic isolates from flower nectar

Lactic acid bacteria were isolated by pre-enrichment of samples in sterile MRS broth then on sterile MRS agar plates The white creamy colonies were isolated from the Aspillia containing LAB on the MRS agar plate. Isolates were maintained on MRS agar at 4°C and transferred to fresh medium after every 15 days

Acid tolerance

Acid tolerance is an important criteria of probiotic microbes. Only acid tolerant probiotic microbe can survive in the acidic condition of gastrointestinal tract. The isolates shows different time response to different pH treatments. The isolate tolerate pH 5 and 6 upto 30 min but shown 90 min response to pH 8.

Bile tolerance

The colonies was observed on MRS agar plate up to 3% bile salt concentration indicating that lactobacillus probiotic bacteria resistance to bile salt

Hemolytic activity

Hemolytic activities for the LAB were evaluated on blood agar plate. The none of the tested LAB showed alpha haemolytic and beta haemolytic activity when grown on blood agar plate. The tested strain show no haemolytic activity.

Cell adhesion ability

The influence of bacterial viability on the hydrophobicity ability before vortexing and after vortexing were detected under 600nm wavelength. Before the vortexing 0.193nm and after 1hr of vortexing 0.187nm.

% Adhesion =[A0-A1/A0*100]

A0 = Absorbance before vortexing, A1= Absorbing before vortexing .The influence of the bacterial viability on the hydrophobicity abilities was analysed.

Analysis of antibiotic resistivity

Resistance of isolates to antibiotics of different nature, namely bacitracin, streptomycin, amikacin, gentamycin, penicillin G, tetracycline and nalidixic acid was tested by disc diffusion method .After 48hr of incubation no zone around particular antibiotic disc was found and no antibiotic resistivity show the bacteria .

Conclusion

The present study was aimed to isolate and identification of lactic acid bacteria (LAB) bacteria from native source from flower necatr part. An efficient probiotic culture was isolated from feremented food sample. The isolates satisfies all essential characteristics of probiotic organism such as High Acid Tolerance, High Bile Salt Tolerance, High degree of hydrophobicity, Sensitivity to antibiotics, The effective isolate was characterized phenotypically

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Studies on protease enzyme produce by soil bourn microbes of Jalgaon district

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Abstract

This project aims to isolate proteases producing microbes from various soil samples from Jalgaon District. Protease producing bacterial strains was isolated through screening on skim milk and casein agar media. Three strains were selected for enzyme studies. The proteases were partially purified using ammonium sulphate precipitation and dialysis method. The biochemical characterization of these proteases indicated that enzymes showed enhanced activity in the presence of temperature and were found stable various pH. The enzymes found application in the blood stain removal.

Keyword: Proteases, skim milk and blood stain etc.

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Introduction

Protease, a hydrolytic enzyme has attracted much attention because of its wide application in detergent, leather, food, pharmaceutical, agricultural industries and proteinaceous waste bioremediation 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, 2014 and Patil, 2020). Traditionally the proteinases have been regarded as degradative enzymes which are capable of cleaving protein foods. Also they participate in the turnover of cellular protein. (Rani,2012). Proteases constitute >65% of the total enzymes employed in various industrial and commercial purposes (Tiwari, 2014).

Material and method

Isolation of Proteiolytic bacteria

The soil samples were collected from different area of Jalgaon district from 5-10 cm depth.

Screening of Proteiolytic bacteria

Soil sample was serially diluted and spread plated on Skim milk agar and Casein agar and incubates plates at 37^0 C for 24 to 48 hrs. The colonies that had formed a clear zone around the growth were considered as protease positive isolates

Protease production

The positive isolates were further screened quantitatively to assess their protease production potential. Submerged fermentation was carried out for protease production in a protease

production medium.

Protease assay

The protease assay was performed using casein as substrate at a RT temperature. 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 thereaction 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

Fractionation of protein was done by ammonium sulphate precipitation method. The excess of salt was removed by using dialysis bag *(Singh and Sawney1996).*

Effect of pH on enzyme activity

The effect of variable pH on the production of Protease was analysed by growing the selected bacterial isolate in the production medium by varying the pH 4 to 9.

Effect of Temperature on enzyme activity

The effect of variable temperature studied by varying Incubation temperature 30,35,40, 45,50,and 55°C).

Application of protease in de-staining of blood spotted cloth

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

Blood stained cloth dipped in beaker containing only distilled water and containing 2ml enzyme and distilled water and incubated at room temperature for 2-3 hours. After incubation, cloth pieces were taken out, rinsed with water and dried and examine the results.

Results and discussion

Primary screening was performed on skimmed milk agar 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. Further secondary screening of selected isolates was performed by spot inoculating on Casein Agar medium. 13 isolate exhibiting proteiolytic activity were obtained and were name SN1 to SN13 .

Fig 1. Zone of Clearance produces on Casein agar by isolate SN9

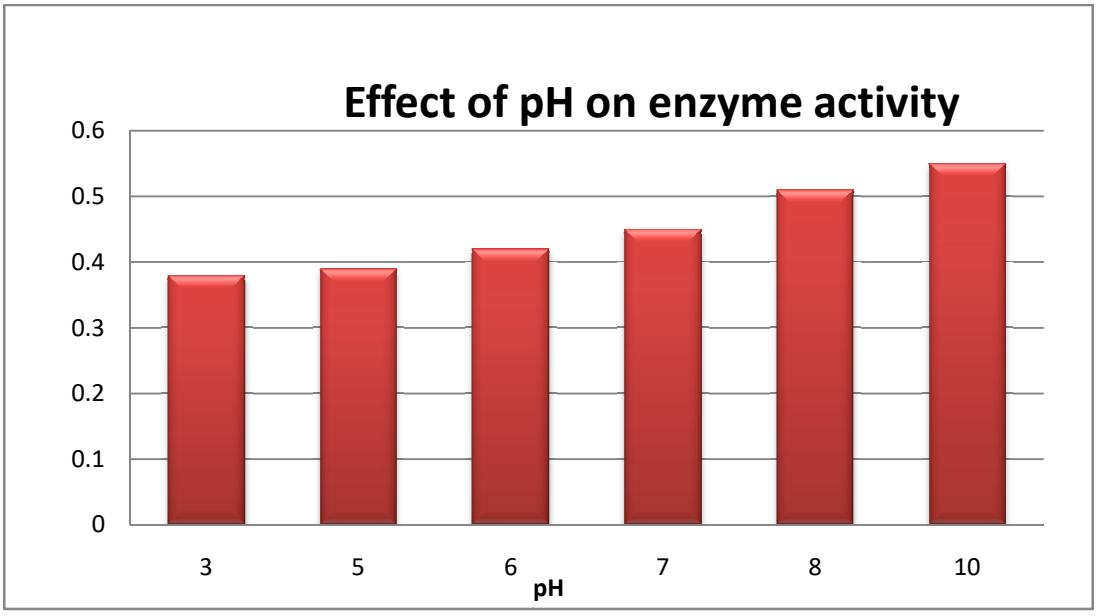
Purification of protease by ammonium sulphate precipitation

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

Effect of pH on enzyme activity

Fig.2. Effect of pH on enzyme activity

Physical parameters play an important role for activity of any enzyme. The positiveisolates were further screened for protease enzyme by quantitative means under submerged condition at 120 rpm at RT. The protease activity was also 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

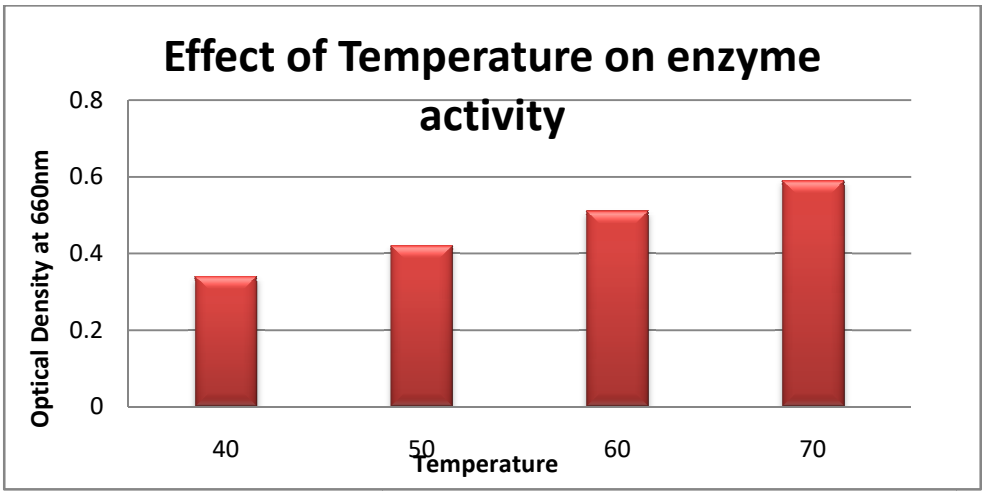


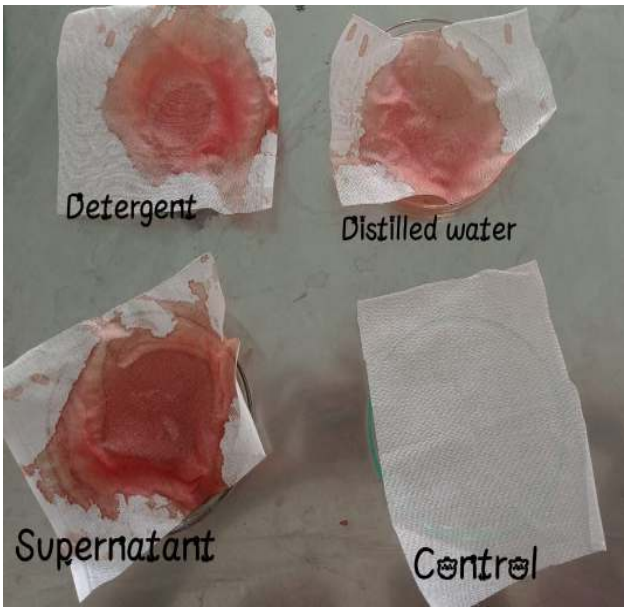
Fig.3. Effect of temperature on enzyme activity

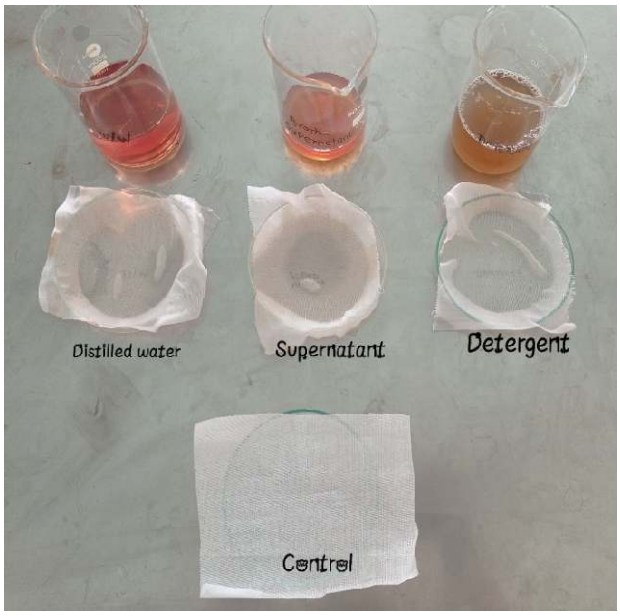
The strain was active over a broad range oftemperature, however, the maximum activity was observed at 60˚C assay temperature.

De-staining of Blood stained cloth

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

Blood stained Clothe Before and after enzyme treatment





Conclusion

- protease producing organism was Screened and isolated.Morphological and biochemical characteristics of organism reveals 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 isolate also exhibit the blood stain removal activity.

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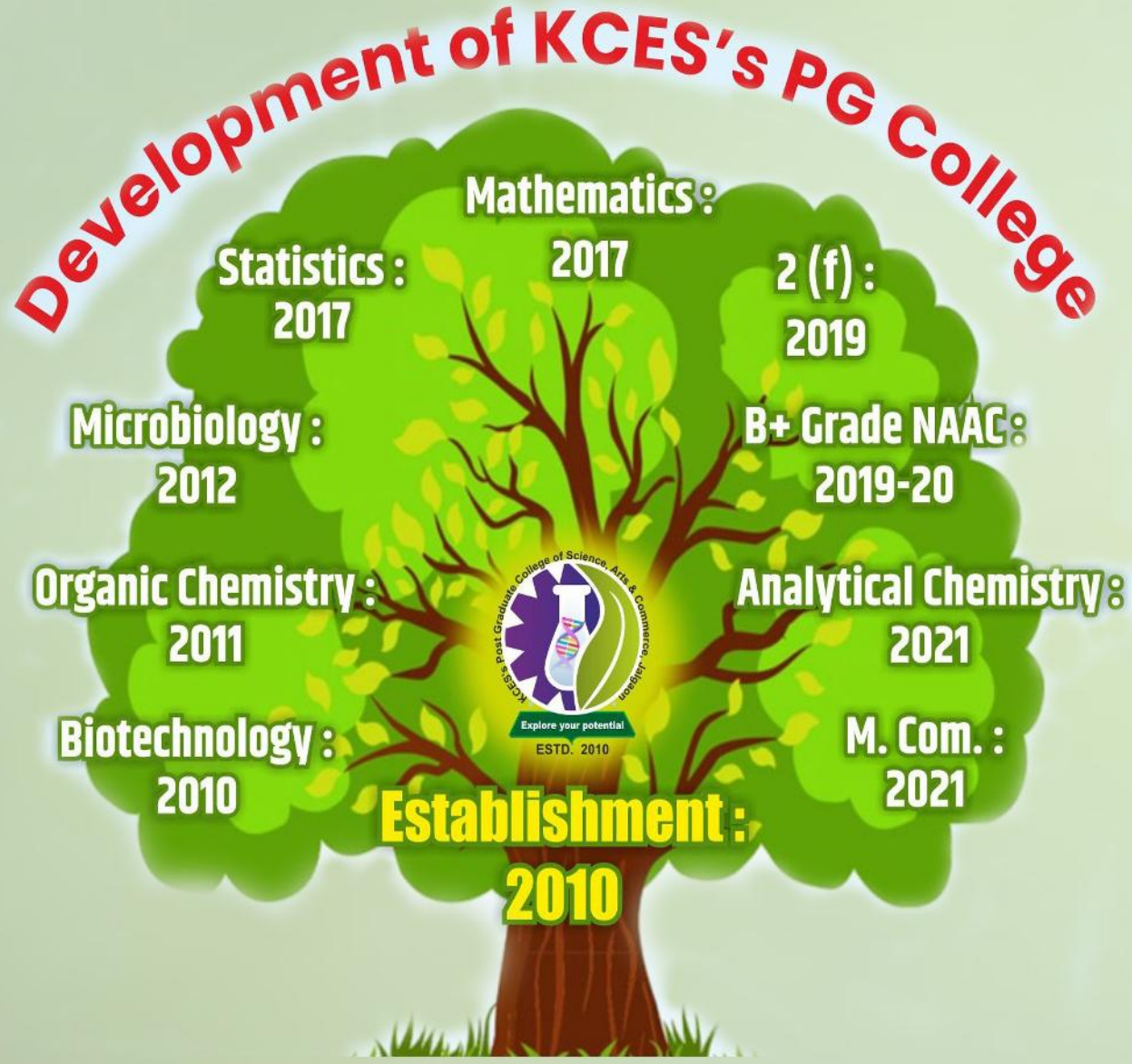

PUBLISHER

Khandesh College Education Society's

Post Graduate College of Science, Arts and Commerce, Jalgaon

Tel. No.: 0257-2236036

Website: http://pgcollege.kces.in

E-mail: pgcollege@kces.in

ISBN 978-81-949083-9-5