Khandesh College Education Society's

Post Graduate College of Science, Technology & Research, Jalgaon

Affiliated to KBC North Maharashtra University, Jalgaon
Conferred 'B' grade with 62 % marks, by academic audit committee of KBC NMU.
Website: www.pgcollege.kces.in


A

Compendium of Research Articles by Prospective Researchers

(2018-19)

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[Under *Prospective Researchers' Scheme* (PRS)]

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Foreword

It gives me great pleasure that KCE society's Post Graduate College of Science, Technology and Research, Jalgaon, is publishing a compendium of research articles prepared by young researcher students under '**Prospective Researchers' Scheme**'. It is a collaborative and concise effort from both, the students and the teachers, hence they deserve compliments. This is an innovative dive to bridge the void since long neglected between formal education and research with a prime objective of improving participation of students in interdisciplinary research activities in both basic as well as applied sciences.

The compendium brings together a spectrum of research articles on various themes belonging to life sciences, chemical sciences and statistics that touch current burning issues of great concern. I am very happy to see the wide spectrum of topics under different disciplines. The prospective researchers have chosen to work on some of the socially relevant topics like statistical analysis of farmer's suicide, eco-friendly synthesis of value added products etc. to name. The topics undertook by the researcher have excellent social and scientific relevance and potential for further knowledge creation, if pursued in right direction. I hope that this activity will establish as a trendsetter and similar innovative activities encouraging researchers in all fields of human needs.

I appreciate the efforts of the students, teachers, co-coordinators of the scheme and other persons involved in bringing out this compendium. I am expecting that the college should continue this activity every year in the large interest of faculty and students.

(Shri. N. G. Bendale)
Hon'ble President, KCE Society, Jalgaon

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Prof. Pramod P. Mahulikar
M.Sc., Ph.D.
Pro-Vice Chancellor

Preface

I am very happy to know that Khandesh College Education Society's Post Graduate College of Science, Technology & Research, Jalgaon is publishing "Compendium of Research Articles by Prospective Researchers" in this year under Prospective Researchers Scheme (PRS). Fifteen articles from various subjects i.e. Organic Chemistry, Biotechnolgoy, Micro-biology & Statistics are describing the research findings of students and teachers of the college as a team work. The enthusiastic endeavors of the college teachers will definitely help to create a vibrant and congenial environment to foster independent thinking and inculcate creativity among the young generation. It will also provide a platform for post graduate students and teachers from all disciplines to fulfill their wishes and realize their dreams.

I highly appreciate the dynamic leadership and guidance of Principal and authorities of college to publish the research work carried out under the Prospective Researchers Scheme (PRS), that will help to inculcate the spirit of research amongst the students right from the entry level of post-graduation. It will also help to boost the creativity and working potency of the teachers and newer researchers.

I take this opportunity to congratulate these future young scientists for their sincere research work as well as Principal and all teachers for their enthusiastic guidance and support.

I wish all the best and bright success to continue this innovative and motivating activity for better achievements. Definitely this activity will help to encourage the young generation towards the research and nation development.

Place: Jalgaon
Date: 01/07/2019

Prof. P. P. Mahulikar
Pro-Vice Chancellor

☎: **(O)**+91- 257 - 2258409, 2257221/222 **(R)** +91- 257 - 2253034
Fax (O) : +91- 257 - 2258403, 2258406 **(Mob.)** : +91 9423185074, +91 9423044000
E-mail : pvc@nmu.ac.in, mahulikarpp@gmail.com **Web** : www.nmu.ac.in

KBCNMU

KHANDESH COLLEGE
EDUCATION SOCIETY, JALGAON
EMAIL : kcesociety@kces.in
WEB : www.kces.in
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TELE/FAX : 0257 - 2239800

Ref. No. _____

Date : _____

Preface

Sciences have been the impetus for technological growth and development. In the drive to provide basic needs and to raise the quality of life of our people, create wealth and to be competitive in an increasingly technologically sophisticated world, harness our natural resources and protect the environment in a sustainable manner. The investments in science today will pay back our technology needs tomorrow. Therefore, the management of KCE society which is always promoting the training of young minds to do science and allied projects through various innovative research activities, is remarkable.

I am pleased to know that KCE society's Post Graduate College of Science, Technology and Research, Jalgaon, has initiated 'Prospective Researcher's Scheme' for the post graduate students. In this scheme, students have carried out research projects under the supervision of the teachers of respective subjects.

This report consists of fifteen research articles distributed in four subjects namely Chemistry, Biotechnology, Microbiology and Statistics. The projects on green synthesis using catalyst and assay of antibacterial and antifungal activities show the awareness amongst the students about environment and a will to use their knowledge for societal concern. I found an article describing statistical assessment of farmer's suicide in Jalgaon district which shows the consciousness of the students regarding social and humanity issues.

This is an innovation and pioneer approach among the college under the KBC North Maharashtra University, Jalgaon. I am sure that this volume will encourage to all teachers and researchers working in the basic as well as allied subjects and provide appropriate platform for exchange of knowledge. I hope the college will continue this activity every year in the large interest of faculty and students.

(Dr. D. G. Hundiwale)
Academic Director, KCE Society, Jalgaon

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Khandesh College Education Society's
Post Graduate College of Science, Technology and Research, Jalgaon
M. J. College Campus, Jalgaon (Maharashtra) INDIA
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Recognised by Govt. of Maharashtra Vide G. R. No. NGC 2010/247/10 & Affiliated to North Maharashtra University, Jalgaon

Date: 12th June 2019

FORWARD

It gives an immense pleasure that KCE society's Post Graduate College of Science, Technology and Research is publishing a compendium of research articles of the research projects undertaken by the students of all the departments of the college. It is due to visionary managements college could start the research a scheme namely **'Prospective Researchers' Scheme'** for the students to inculcate the research attitude among the students and exposure to research methodologies. In the context of present academic curricula, hardly there is an opportunity of real research orientation for the students. It is the glaring lacuna of the present education system. The teaching methodology do not provide sufficient room for subject application and research orientation, hence students find difficult to get proper employment. To eliminate these difficulties initiative of such type scheme plays a great role.

I observed enormous interest of by all the supervisors who guided the project as well as immense curiosity, anxiety and attention among the students about this scheme. All the students who have participated in this project are really the *Prospective Researchers* with bright future. I am sure that they will flourish into the renowned researcher in time to come. I wish that they extend their research activity and pursue advanced research for their doctoral degree. I am also hopeful that industry shall give the impression of this attempt to hunt the young talent. I, hereby, express my firm commitment for such endeavour on sustainable basis for the years ahead.

BEST WISHES

Dr. V. S. Zope
Principal

Editorial!

Dear Students,

I am heartily delighted to present the first edition of ***A Compendium of Research Articles by a Prospective Researchers*** under the activity of '**Prospective Researcher's Scheme**' for the year 2018-19.

PGCSTR has created special attention over the last ten years of establishment, in the educational traditions of Maharashtra, especially Khandesh region, as it is the only post-graduate college in the state, governed by private organization. In the current year, Dr. V. S. Zope has undertaken the charge of college as a Principal and continue the flow of educational journey of college via implementing versatile activities in college including a research scheme, named as 'Prospective Researcher's Scheme' (PRS).

Main objective of PRS is to motivate PG students for excellence by creating a healthy competition between them. Since M. Sc. (II) students have a compulsory research project as a part of their curriculum, PRS has been coordinated with it. Sixty students undertook 15 projects, from four subjects in the scheme, under the supervision of 10 faculties. Research outputs of all the project groups were examined by eminent referees from respective subjects after a power-point presentation and best project work was selected as winners of PRS. On 17/05/2019, all the winners were awarded with cash prizes individually, at the auspicious hands of Dr. Satyendra Mishra, UGC-faculty fellow & renowned researcher from KBC NMU, Jalgaon.

Research articles were prepared by all participants of PRS at the end of their tenure under the guidance of their supervisors and the present compendium has been prepared encompassing all those articles subject-wise. I am pleased to mention that the present compendium is received an ISBN certification. Glimpses of award function of PRS are also appearing on cover pages.

This volume has been completed with worthy support of 'PRS editorial board' & all dear students of PGCSTR for which they all are duly acknowledged. Similarly, on behalf of editorial board, I am heartily grateful to all referees for their valuable time. I am also thankful to college management for providing financial support and all teaching as well as non-teaching staff of college for their co-operation. Last but not the least, I am thankful to KCE Society's ViViDhaTa, a Research & Training Centre for publishing the compendium within time.

Thank you!

Dr. Sarang S. Bari!

INDEX

Chemistry

One Pot Multi-Component Synthesis of Pyranopyrazoles by Grinding Method Using Glycine as a Catalyst

Marathe Gaurav A., Chaudhari Shubham, Patil Kaushal P., Valvi Sunil and Patil R. M.*
Department of Chemistry, Post Graduate College of Science, Technology & Research, Jalgaon

Abstract

We herein report a green and efficient Glycine catalyzed four component condensation of aldehyde, malononitrile, hydrazine hydrate and ethyl acetoacetate to synthesize pyranopyrazoles. This method follows the principle of green chemistry by using environmentally benign synthetic method along with use of cost effective catalyst and green reaction medium.

Keywords: Pyranopyrazoles, Multi-component reaction, Glycine, Green reaction

*Corresponding author: rpatil1734@gmail.com

Introduction

Multicomponent reactions play on importance role in modern organic chemistry, because they generally exhibit higher atom economy and selectivity as well as produce fewer by - product compared to classical multistep synthesis.[1] Furthermore, MCRs are easy to performs inexpensive, quick, consuming less energy and involves simple experimental procedures.[2] The first multicomponent reaction was described in 1850 by strecker,[3] and thereafter many such reactions have been reported in the literature.

Pyrano pyrazole is a fused hetrocyclic compound, which adds provides fruitful area to study the bioactivity. Pyrano pyrazole where first obtained in1973 by the reaction between 3 – methyl 1-phenyl pyrazoline -5-one and tetra cyano ethylene.[4] Pyrano pyrazole has shown bioactivity such as anticoagulant, spasmolytic, hypnotic, diuretic,[5] insecticidal,[6] anti-inflammatory[7], anticancer[8], anti-bacterial and antifungal[9] as well as anti-microbial.[10]

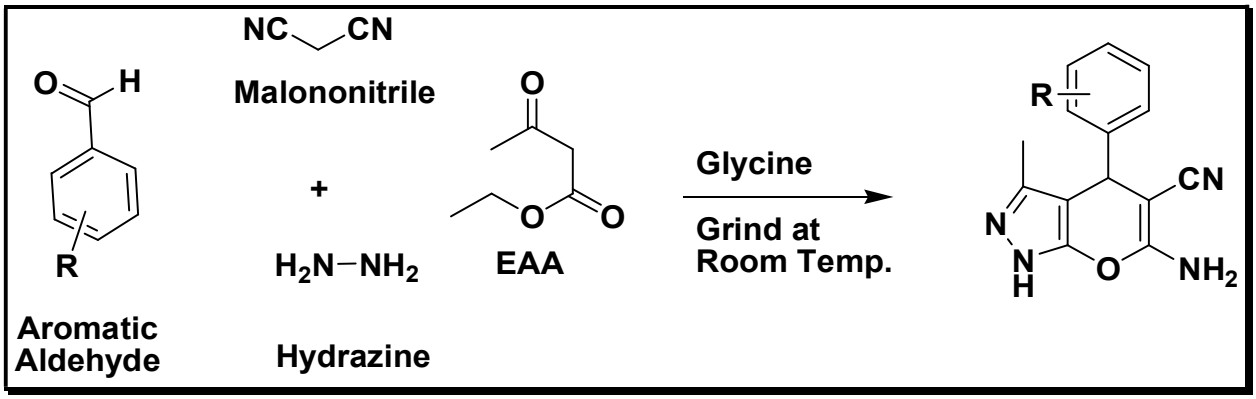
We have use the organo catalyst Glycine an amino acid for the synthesis of pyranopyrazole from aromatic aldehyde, malonitrile, hydrazine hydrate and ethyl aceto acetate at room temperature in solvent free condition with good to excellent yield within 5-10 min as shown in scheme 1.

Materials and methods

All reagents used were of laboratory grade. Melting points were determined in open capillaries and are uncorrected. The purity of compound was checked by TLC, IR spectra were recorded on Shimadzu FT-IR (Affinity Model) using KBr.

General Procedure for Synthesis of Substituted Pyrazole

Aromatic aldehyde (10 mmol), Malononitrile (10 mmol), Ethylacetoacetate (10 mmol), Hydrazinhydrate (10 mmol) and Cat Glycine (20 mol%), were added to a mortar. The mixture was grounded by mortar and pestle, at room temperature for appropriate time given in Table-2. The reaction was monitored by TLC. The solid product was obtained from an intermediate melt and then was laid up at room temperature for 5 to 10 min. The mixture was transferred to cold water and then was filtered off. The crude product was recrystallized by ethanol as per scheme 1.



Scheme 1: Synthesis of pyranopyrazole derivatives using Glycine as a catalyst

Results and Discussion

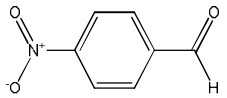
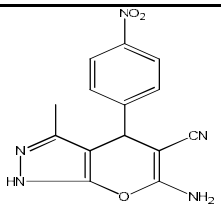
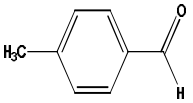
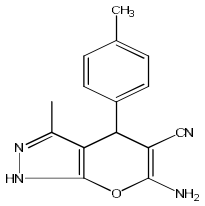
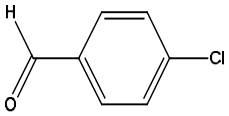
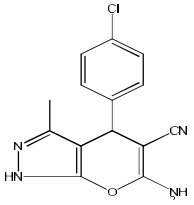
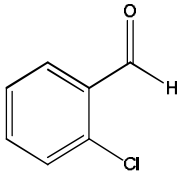
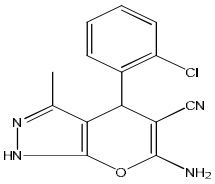
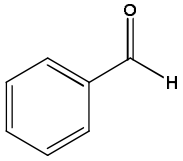
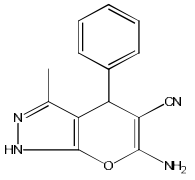
The reaction condition for amount of catalyst was optimized by carrying out the model reaction of benzaldehyde with malanonitrile, ethyl aceto acetate, and hydrazine hydrate, at room temperature with and without catalyst. It was observed that only 60% of the solid product was obtained for one hour reaction time. When the reaction was performed with Glycine as a catalyst, increase in the yield was noticed significantly as summarized in Table 1. Use of 20 mol% Glycine was sufficient to push the reaction forward. Higher amounts of the catalyst of the catalyst did not improve the results to a greater extent. So, Glycine was an efficient catalyst and 20 mol% Glycine was chosen as a quantitative catalyst for reaction, the result summarized in Table 2.

Table 1: Optimized amount of catalyst loaded

Entry	Catalyst (mole %)	Time (min.)	Yield (%)
1.	0	50-60	60
2.	5	15	80
3.	10	12	85

4.	15	10	88
5.	20	5	96
6.	25	5	96

Table 2: Synthesis of pyranopyrazole derivatives

Entry	Aromatic aldehyde	Product	Time (Min)	Yield (%)	M.P (^{0}C)
1			5	95	230-232
2			10	93	196-1998
3			5	92	228-230
4			10	88	240-242
5			10	90	220-222

IR Stretching frequency of the compounds 1 to 5

1) **IR** cm-$^{-1}$: 3266.63 (N-H), 1434.68 (C=N), 2280 (CN), 1604.77 (C=C), 1179.22cm^{-1}(C-O)

2) **IR** cm-$^{-1}$: 3258.07 (N-H), 1480.47 (N=C), 2158.84 (CN), 1614.77 (C=C), 1197.29 cm^{-1} (C-O)

3) **IR** cm^{-1}: 3256.85 (N-H), 1498.33 (C=N), 2176.82 (CN), 1611.71 (C=C), 1123.08 cm^{-1}(C-O)

4) **IR** cm^{-1}: 3348.42 (N-H), 1454.68 (C=N), 2258.64(CN) 1601.92 (C=C) 1151.15 cm^{-1} (C-O)

5) **IR** cm^{-1}: 3388.42 (N-H), 1447.62 (C=N), 2187.28(CN), 1609.85 (C=C), 1121.19 cm^{-1} (C-O)

Conclusion

We have developed a green and simple method for the synthesis of substituted pyranopyrazole from aromatic aldehyde, ethyl acetoacetate, Malononitrile and hydrazine by grinding. These moieties having broad application scope in pharmaceutics. All the products were simply purified by recrystallization from ethanol and hence the method is free from column chromatographic purification. Mild reaction conditions, use of green solvent, high atom economy and the lack of by-products are among the other advantages of this method.

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Efficient one pot synthesis of Schiff's base using amino acid as a catalyst

Patil Suraj G., Suryawanshi Khushal D., Phalak M. N., Borse Sachin M. & Chaudhari Rupali A.*
Department of Chemistry, Post Graduate College of Science, Technology & Research, Jalgaon

Abstract

The reaction of primary aromatic amines with aryl aldehydes is found to be catalysed by glycine as a natural amino acid using ethanol as a solvent to give the corresponding Schiff bases in good yields. This eco-friendly reaction has many advantages like economic, environmental, mild reaction condition & simple work up-with high product yield.

Keywords: Schiff Base, Imine, Glycine, Natural acid, Mild reaction condition.

*Corresponding author: rupalichaudhari887@gmail.com

Introduction

Green chemistry techniques continue to grow in importance. It is an alternative process to help to conserve resources and can reduce costs. The replacement of conventional solvents with green solvent ethanol which is harmless to health and is available in large quantities is on interesting basic approach along this lines[1-3]. MCRs plays an important role in modern organic chemistry, because they generally exhibit higher atom economy and selectivity as well as produce fewer by product compared to classical multistep synthesis[4]. Furthermore, MCRs are easy to perform inexpensive, quick, consuming less energy and involves simple experimental procedures[5]. The recent interest in green chemistry has posed a new challenge for organic synthesis in that new reaction condition needs to be found which reduced the emission of volatile organic solvent and the use of hazardous toxic chemicals[6].

The formation of carbon-nitrogen double bond important role in organic synthesis, this can be achieved by the reaction of aldehyde and amines in acidic medium which leads to synthesis of Schiff's bases (imines). Schiff's bases have attracted considerable attention of organic chemists due to their significant biological activities like anticancer[7], antitumor[8], anti-inflammatory[9] agent and insecticidal[10] activity. The Schiff's bases are also used as versatile component in nucleophile addition with organometallic reagents[11] and in cycloaddition reaction[12]. Schiff's bases are known as substituted imine are compound containing azomethanegroup (-HC=N-) and represented by the general formula $R^3R^2C=NR$, they are the condensed product of aldehyde or ketones and were first reported by Hugo Schiff, in 1864[13]. Originally the classical synthetic route for synthesis of

Schiff's bases was reported by Schiff which involves condensation of primary amines with carbonyl compounds[14].

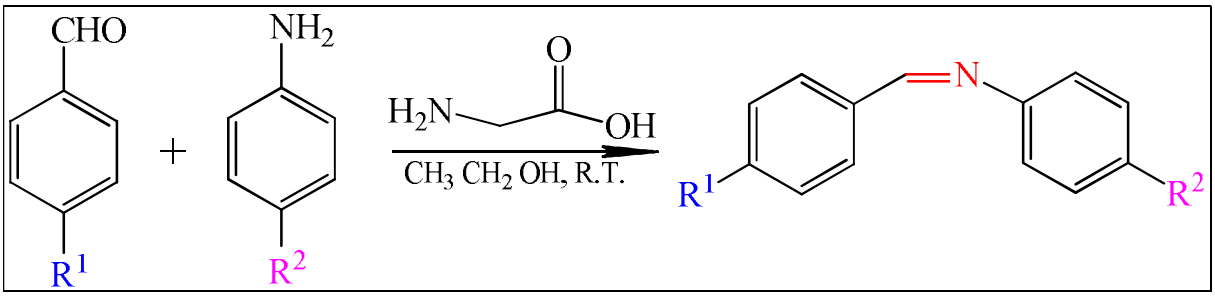
Materials and methods

All reagents used were of laboratory grade. Melting points were determined in open capillaries and are uncorrected. The purity of compound was checked by TLC, IR spectra were recorded on Shimadzu FT-IR (Affinity Model) using KBr.

General procedure for synthesis of Schiff Bases

In 100 ml beaker, a mixture of aromatic aldehyde (10 mmol), substituted aniline (10 mmol) and Glycine (10 mol%) was taken in 2 ml ethanol and stirred vigorously at room temperature for appropriate time (Table-1), the precipitate thus obtained was filtered off. Wash with ethanol & then purified by recrystallization from ethanol to get corresponding Schiff's base in pure and crystalline form.

Scheme 1: Synthesis of Schiff Bases at room temperature



R^1= –H, 2 –NO_2, 2 –Cl, 4 –CH_3 and **R^2**= – H, 4 –CH_3, 4 –Cl, 2 –Cl, 4 –OCH_3, 4 –NO_2.

Result and Discussion

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 carried out in ethanol and water or another organic solvent. It was observed that Schiff base formation was increases in ethanol, while the same reaction occurred slowly in water and another organic solvent.

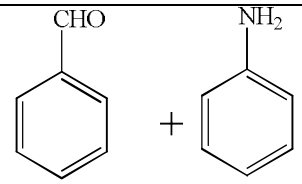
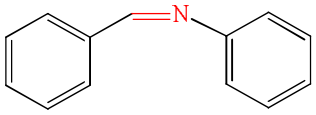
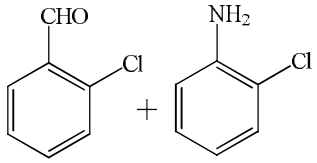
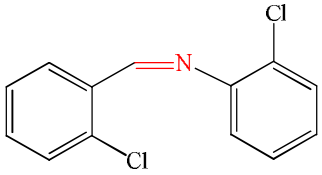
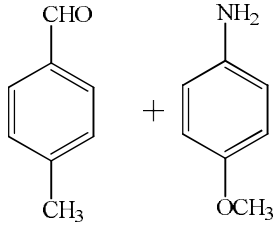
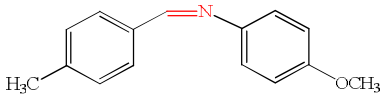
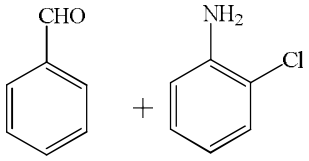
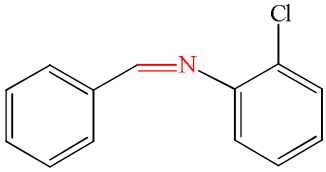
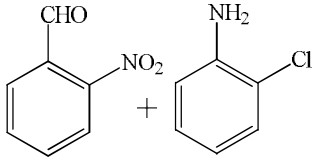
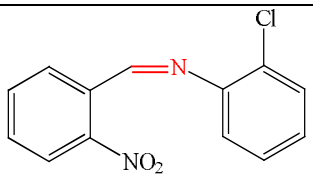
Hence by using ethanol as solvent we performed the model reaction with different mole % of glycine to optimized reaction condition as shown in table.

Table 1: Optimization of amount of catalyst

Entry	Catalyst (mole %)	Time (min.)	Yield (%)
1	5	30	80
2	**10**	**15**	**92**
3	15	15	92

Here we find that the 10 mole % catalyst is sufficient to push the reaction forward. Hence the reaction can perform with 10 mole % as catalyst by optimized the reaction condition.

Table 2: Synthesis of Schiff Bases

Sr. No.	Starting Compounds	Product	Time (min)	Yield (%)	M. P. (^{0}C)
1.			15	92	64
2.			23	88	98
3.			30	80	68
4.			15	74	90
5.			30	90	110

Thus, in the synthesis of biologically active imine compounds we use readily available, inexpensive and environment friendly reagents. The reaction of various substituted aldehyde with amines carried out using glycine (as catalyst) in ethanol (as solvent).

All the products synthesized during the course were characterized by IR.

Spectral data:

N-Benzylideneaniline (SB1): Lemon Yellow solid, m.p.: 64^{o}C, **IR** (cm^{-1}): 1627(C=N), 1305(C–N), 1589 (C=C aromatic), 3061(C=C–H), 2885(C–C–H);

2-chloro-N-(2-chlorobenzylidene)aniline (SB2): Lemon Yellow solid, **IR** (cm^{-1}): 1693(C=N), 1274(C–N), 1460 (C=C aromatic), 3064(C=C–H), 2924(C–C–H);

4-methoxy-N-(4-methylbenzylidene)aniline (SB3): Olive green solid, **IR** (cm^{-1}): 1678(C=N), 1292(C–N), 1242 (C–O), 1504 (C=C aromatic), 3007(C=C–H), 2941(C–C–H);

N-benzylidene-2-chloroaniline (SB4): Yellow solid, **IR** (cm^{-1}): 1691(C=N), 1296(C–N), 1624 (C=C aromatic), 3064(C=C–H), 2870(C–C–H);

2-chloro-N-(2-nitrobenzylidene)aniline (SB5): Lemon Yellow solid, **IR** (cm^{-1}): 1622(C=N), 1352(C–N), 1352 (N–O), 1517 (C=C aromatic), 3080(C=C–H), 2922(C–C–H).

Conclusion

Synthesis of substituted imine derivatives using glycine as catalyst in ethanol which is a green solvent is a highly efficient process for synthesis Moreover, the procedure offers several advantages including high yield, operational simplicity, cleaner reaction, minimum environmental impact and low cost; which make it a useful attractive process for the synthesis of the compound.

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One Pot Synthesis of Pyrazole Derivatives by using $AlCl_3$ Catalyst

Joshi Poornima H., Deore Nitin N., Warude Chetan S., Jagdish V. Padvi, Marathe Ujwala V.*
Department of chemistry, Post Graduate College of Science, Technology & Research, Jalgaon

Abstract

An easy and simple three-component one-pot process for the synthesis of 5-amino-1, 3-diphenyl-1H-pyrazole-4-carbonitrile derivatives using aldehyde, phenylhydrazine and malononitrile has been developed. The method gave 79 to 89% yield in a maximum of 30minutes using $AlCl_3$ as catalyst in aqueous ethanol (1:1)

Keywords: Pyrazole, One- Pot, Multi-component, $AlCl_3$, Lewis acid, Ethanol, Water

*Corresponding author: maratheujwala111@gmail.com

Introduction

Synthetic heterocyclic chemistry using green method has fascinated many researchers in the recent past due to easy operation, environmental friendly and cost-effective approach. Green chemistry technique continues to grow in importance. It is an alternative process to help to conserve resources and can reduce cost. Pyrazole are five members heterocyclic that constitute a class of compound particularly useful in organic synthesis. They are one of the most studied groups of compound among the azole family. The presence of the pyrazole nucleus in different structure leads to diversified application in different areas such as technology, medicine and agriculture. Pyrazole moiety is an important template for many biologically active compound. The derivatives of pyrazole molecule possess wide range of biological activities such as anticancer[1], anti-inflammatory[2], ACE inhibitor[3], MAO inhibitor[4], Cholecystokinin -1 receptor antagonists[5], Estrogen receptor ligand activity[6], antimicrobial[7], anti-fungal[7], antitubecular[8], anti-convulsant[9] etc. One pot multicomponent reaction is the most efficient route for the synthesis of heterocyclic molecules[10-13]. We have developed; $AlCl_3$ catalyzed three component one pot synthesis of highly functionalized pyrazole by using substituted aldehyde, hydrazine hydrate and malononitrile in aqueous ethanol.

Materials and methods

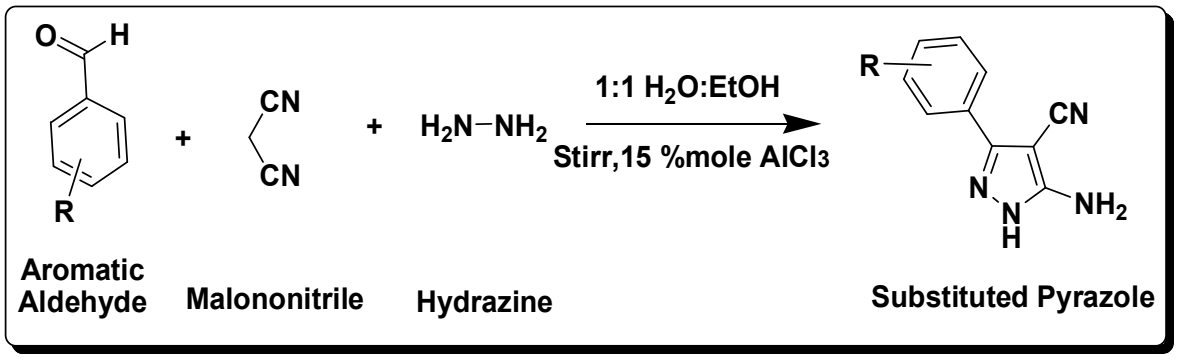
All reagents used were of laboratory grade. Melting points were determined in open capillaries and are uncorrected. The purity of compound was checked by TLC, IR spectra were recorded on Shimadzu FT-IR (Affinity Model) using KBr.

General Procedure for Synthesis of Substituted Pyrazole

In 100 mL round bottom flask, take 4-Methoxybenzaldehyde (10 mmol), Malononitrile (10 mmol), in 15% mol $AlCl_3$ & aq. ethanol (1:1). Reflux the reaction mixture with Stirring. The progress of the reaction was monitored by TLC. After formation of an intermediate, hydrazine hydrate (2 mmol) was added. After the completion of the reaction (monitored by TLC) the reaction mixture was allowed to cool. Then filter the obtained solid product, wash it with water, recrystallized it and record mp.

Results and Discussion

To optimize the reaction condition, we performed the reaction with benzaldehyde, malononitrile and hydrazine hydrate which was considered as model reaction. We optimized the same reaction with water, ethanol and its co-system summarized in table No.1



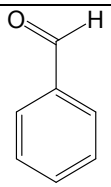
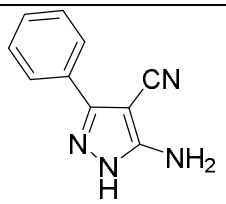
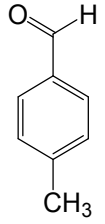
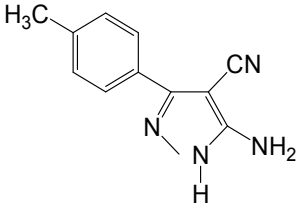
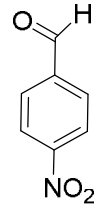
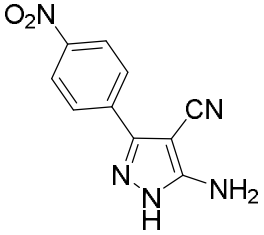
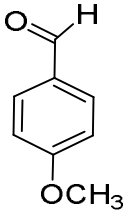
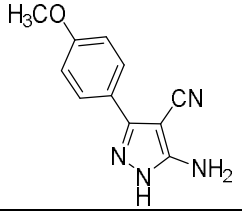
Scheme 1: Synthesis of Pyrazole Derivatives by using $AlCl_3$ Catalyst

To optimize the reaction time, was performed for 1 hour with evaluation at 30 min and 45 min for the completion of reaction and yield. The yield remains constant after 30 min of the reaction. For this we first dissolve benzaldehyde (10 mmol) in ethanol-water (30 ml) and then added 15 % mol $AlCl_3$ followed by malonoitrile (10 mmol). The reaction mixture was stirred till white precipitate was obtained.

Table 1: Optimization of reaction time

Entry	Catalyst (mole %)	Time (min)	Yield (%)
1	15	45	91
2	15	30	89
3	15	15	87

Table 2: Synthesized Substituted Pyrazole Compounds

Sr. No.	Reactant	Products	M.P. (^{o}C) [Lit.]	Time (min)	Yield (%)
1.			161[160][15]	45	91
2.			118[117][16]	30	89
3.			163 [166][14]	45	80
4.			108 [106][15]	30	87

Spectral data: IR Spectra of the compounds

5-amino-3-phenyl-1H-pyrazole-4-carbonitrile (P1): IR (cm^{-1}): –NH- 3282, -CN- 2222, -Ar-C=C-1618, -C=C- 1510, -p-OMe- 1026

5-amino-3-(4-nitrophenyl)-1H-pyrazole-4-carbonitrile (P3): IR (cm^{-1}): 3286(NH), 2225(CN), 1583 (C=C aromatic), 1506(C=C-H), 1330 (p-NO_2);

5-amino-3-(4-methoxyphenyl)-1H-pyrazole-4-carbonitrile (P4): IR (cm^{-1}): 3282 (NH), 2222 (CN), 1618(C=C aromatic), 1510(C=C-H), 1026(p-OMe).

Conclusion

We have adapted a green and simple approach for the synthesis of substituted pyrazole from aromatic aldehyde with Malononitrile and hydrazine in 1:1 EtOH and H_2O with and stirring. These moieties having broad application scope in pharmaceutics. All the products were simply purified by recrystallization from ethanol and hence the method is free from column chromatographic purification. This procedure has simple workup procedure with quantitative yields and high purity of products.

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Solvent Free L-proline Catalysed Synthesis of Pyranopyrazole Derivative

More Lalit S., Patil Tejaswini C., Chaudhari Priyanka B., Rathod Ankush & Patil R. M.*
Department of Chemistry, Post Graduate College of Science, Technology & Research, Jalgaon

Abstract

A facile and convenient protocol is developed for the fast (5–15 min) and high-yielding (88–96%) synthesis of fused pyranopyrazoles from ethyl acetoacetate, phenyl hydrazine, an aldehyde, and malononitrile in the presence of nontoxic, simple, and readily available organocatalyst proline at room temperature. This eco-friendly reaction has many advantages like economic, environmental, mild reaction condition, no solvent use & simple work up.

Keywords: Pyranopyrazole, L-Proline, Natural acid, No solvent use, Mild condition.

*Corresponding author: rpatil1734@gmail.com

Introduction

Green chemistry offers more eco-friendly and green alternatives to conventional chemistry practices such energy efficient energy source, reduction or elimination of the use of toxic and hazardous chemicals in production processes[1]. Pyranopyrazoles are fused heterocyclic compound present in biologically important natural products its one of the privileged significant biological activities such as anti cancer activities[2], antimicrobial[3], insecticidal[4], anti-inflammatory[5], and molluscicidal activities[6]. Multi-component reactions (MCRs) are eco-friendly process as they obey green chemistry principles[7]. MCR has emerged as an efficient green tool for the synthesis of simple and complex building blocks[8]. L-Proline is a readily obtainable naturally occurring amino acid and easy to obtain in high enantiomeric purity it has been reported as an eco-friendly catalyst for synthesis of several heterocyclic[9-10].

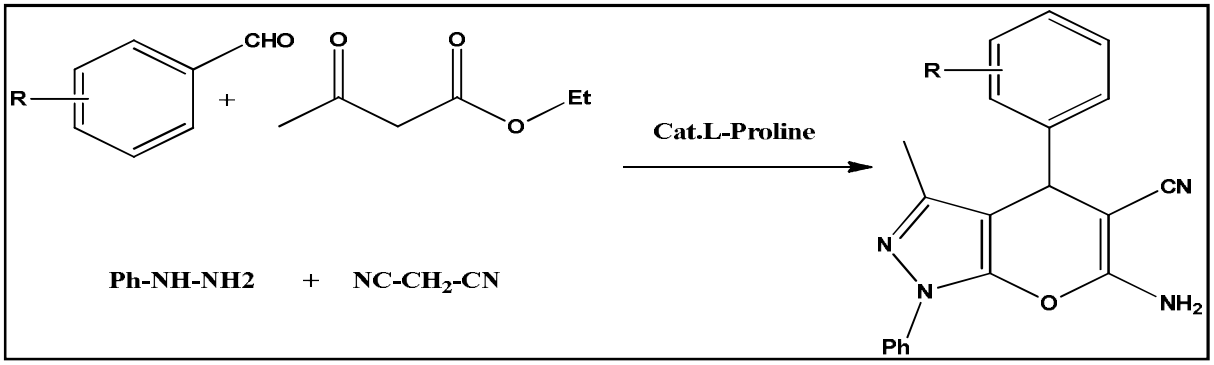
Materials and methods

All reagents used were of laboratory grade. Melting points were determined in open capillaries and are uncorrected. The purity of compound was checked by TLC, IR spectra were recorded on Shimadzu FT-IR (Affinity Model) using KBr.

General procedure for synthesis of pyranopyrazole

Aromatic aldehyde (10mmol), Malononitrile (10mmol), Phenylhydrazine (10mmol) Ethylacetoacetate (10mmol), and catalyst L-Proline (15% of 10mmol), are added to a mortar. The mixture is ground by mortar and pestle at room temperature for a period given in Table 2. The reaction was monitored by TLC. The solid product is obtained from an intermediate melt and then is laid up at room temperature for 5 to 10 min. The mixture is transferred to ice water and then is filtered off. The crude product is purified by recrystallization by ethanol.

Scheme1: Synthesis of pyranopyrazole derivatives



Results and Discussion

In the first instance, 4-chlorobenzaldyde, malononitrile, ethyl aceto acetate were ground in the presence of L-Proline under solvent- free condition. The appropriate reaction conditions are investigated with the results summarized in table 1.

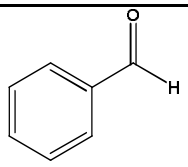
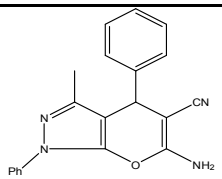
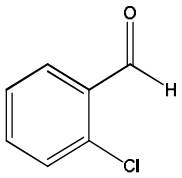
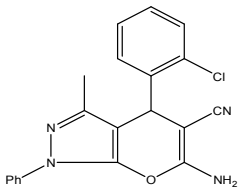
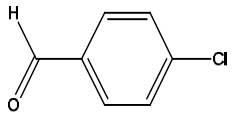
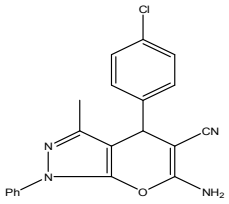
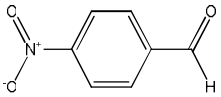
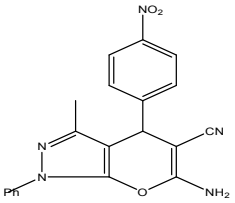
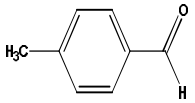
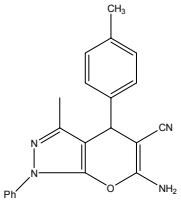
Without catalyst the product was not obtained for 15 min using grinding method. Increasing the amount of the L-proline to 5 mol%, 10 mol%, 15 mol% and 20 mol% result in accelerating the reaction and the yield of product were 75%, 81%, 94% and 94% respectively, and the solidified time was 15 min,10min, 5min and 5min respectively, as shown in Table 1.

Table 1: Effect of amount of catalyst loaded on synthesis of pyranoprazole

Entry	Catalyst (mol %)	Grinding time (min)	Yield (%)
1	0	40	0
2	5	15	75
3	10	10	81
4	**15**	**5**	**94**
5	20	5	94

Use of 15 mol % proline was sufficient to push the reaction forward. Higher amounts of the catalyst of the catalyst did not improve the results to a greater extent. So, L-proline was an efficient catalyst and 15 mol % proline was chosen as a quantitative catalyst for reaction, the result summarized in Table 2.

Table 2: Synthesis of pyranopyrazole derivatives

Entry	Aromatic aldehyde	Product	Time(Min)	Yield (%)	M.P(^{0}C)
1			5	94	164
2			5	92	150
3			5	94	174
4			5	94	144
5			10	90	174

Spectral data of compounds (1-5)

1) IR cm-$^{-1}$: 3288.63 (N-H), 1444.68 (C=N), 2260 (CN), 1604.77 (C=C), 1159.22cm^{-1}(C-O),

2) **IR** cm-$^{-1}$: 3358.07 (N-H), 1450.47 (N=C), 2258.84 (CN), 1604.77 (C=C), 1157.29 cm^{-1} (C-O),

3) IR cm^{-1}: 3336.85 (N-H), 1508.33 (C=N), 2196.82 (CN), 1660.71 (C=C), 1163.08 cm^{-1}(C-O).

4) IR cm^{-1}: 3348.42 (N-H), 1444.68 (C=N), 2258.64(CN) 1600.92 (C=C) 1161.15 cm^{-1} (C-O)

5) IR cm^{-1}: 3378.42 (N-H), 1448.62 (C=N), 2187.28(CN), 1602.85 (C=C), 1181.19 cm^{-1} (C-O).

Conclusion

In conclusion, we have described a highly efficient procedure for the preparation pyranopyrazole derivatives by a multi-component reaction via a one-pot grinding method using L -Proline as catalyst under solvent free conditions. Moreover the procedure offers several advantages including high yields, operational simplicity, cleaner reaction, minimum environmental impact, no chromatographic technique is required for purification and low cost which make it's a useful and attractive process for the synthesis of these compounds.

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Biotechnology

Allelopathic Effect of Some Aphrodisiac Plants on Mung Bean (*Vigna Radiata*) Seeds with respect to Biomarker and Biomass Study

Wagh Vaishali, Somvanshi Tejal, Desale Bhuvaneshwari, Patil Puja, Pratiksha Wankhede*
Department of Biotechnology, Post Graduate College of Science, Technology and Research, Jalgaon

Abstract

The present study was conducted to investigate the alleleopathic effect of selected species of aphrodisiac plants on Mung bean Plant (*Vigna radiata*). Aqueous extract with different concentration i.e. 0.5%, 1.0% and 2.5% were applied on mungbean seeds. As compared with control, as the concentration of extract increases, the biomass in the form of dry weight was also increases. The effect of plant extracts were also checked by extracting chlorophyll contents and results indicates that the Chlorophyll-a and b of test mungbean seeds was highest in *Chlorophytumborivilinum (CB)* at 0.5% than other concentrations. The protein content of test mungbean plant was found to be highest in *Chlorophytumborivilinum (CB)* 18%, moderate 16% in *Curculigoorchoides (CO)* and lowest with *Mucunaprurnies (MP)*. The acid phosphatase used as biomarker to study the effect of different concentration, the order of acid phosphatase found highest in *Chlorophytumborivilinum (CB), Curculigoorchoides (CO)* and less in *Mucunaprurnies (MP)*. Our results justify to say plants under taken are ideal examples of phytobiotics.

Keywords: Aphrodiasic plants, allelopathic, seed germination, chlorophyll and biomarker

*Corresponding author:

Introduction

Agriculture is the science and art of cultivating plants. Traditionally several agricultural as well as medicinal plants were domesticated in India. *Chlorophytum borivilinum* was used for physical illness and to increase general body immunity. Its aphrodisiac properties have proved very much useful for the people suffering from erectile dysfunction and to increase male potency. *Curculigo orchoides* is also popularly used in treatment of menorrhagia, leucorrhoea, gonorrhea, dysuria and menstrual derangements, jaundice, asthma, piles etc. It is also considered as an effective anti-infective and healing agent. *Mucuna prurnies* is used as antidepressant drug. It is useful to decrease Prolactin level, also reduce symptoms of Parkinson's Disease and used for treatment of male infertility. The allelopathic effects of plant on seed germination were studied by Oudhia et

al (1998). The aphrodisiac plants have ability to effects on seed germination. The Allelopathic effect of aphrodisiac plants on seed germination as well as changes in seedling plants were studied by *Blumea lacera L*.on rice on agricultural crops (Ridenour et al., 2001 & Cheema: et al., 2012). Acid phosphatase is reckon as a metabolic biomarker.

Materials and methods

Collection of plant material

The amorphous powder form of root of selected species of aphrodisiac plants *Chlorophytum borivilinum (CB), Curculigo orchoides (CO)* and powder of shoot of *Mucuna prurnies (MP)* were collected in the form local herbal medicine market of Jalgaon district, Maharashtra, India.

Preparation of plant extract

Different plants material collected from local market were subjected to prepare aqueous extract. The collected sample of each plant material was mixed with Distilled water in different concentration (0.5, 1.0 & 2.5%). The aqueous suspensions of different concentrations were incubated for 5 hours at 70 ^{o}C. The extracts were filtered through filter paper and take a clear filtrate as aqueous extract.

Selection of appropriate concentration of aphrodiasiac plants for seedling growth

The effect of aqueous extract on model plants at different concentration was checked by using common Mug bean (*Vigna radiata)*. The seeds of *Vigna radiata* were procured from the local market of Jalgaon district (Maharashtra). Seeds were sterilized in a 0.1% Mercury chloride solution for 10 minutes. Observe the growth at 0, 1, 3 and 5th day after 7 day of growth, the shoot and root lengths were long enough to measure using a ruler. Fresh and dry weights were also measured.

Estimation of Chlorophyll

Chlorophyll A, chlorophyll B and total chlorophyll of all aqueous extract treated plants along with the control plant were tested by the method of Stir ban (1985).

Protein Estimation at different aqueous concentrations

Protein content of all aqueous extract treated plants along with the control plant was measured according to Lowry et al. (1951).

Acid phosphatase activity

Acid phosphatase activity of aqueous extract of ripe and unripe banana peel treated germinated seeds of Vigna radiata was determined according to, (Hussain, 2016), by using the standard para-nitro phenol.

Results and Discussion

Effect of aqueous extract (0.5, 1.0 & 2.5%) of three aphrodisiac plants on growth of aerial and underground tissues of *Vigna raidata*

The treatment of aqueous extract result in changes in the length either shoots or roots. At 2.5% and 1% concentrations the shoot length highest in CB and CO and lowest in MP and root length was found more in CB and MP and less in CO. The mung bean shows the best growth response for shoot and root length and it was observed at concentrations of 0.5% of aqueous extract over control. At this low concentration, more growth of shoot length observed in CB and CO. However, MP treatment could not gave same results in mungbean plants.

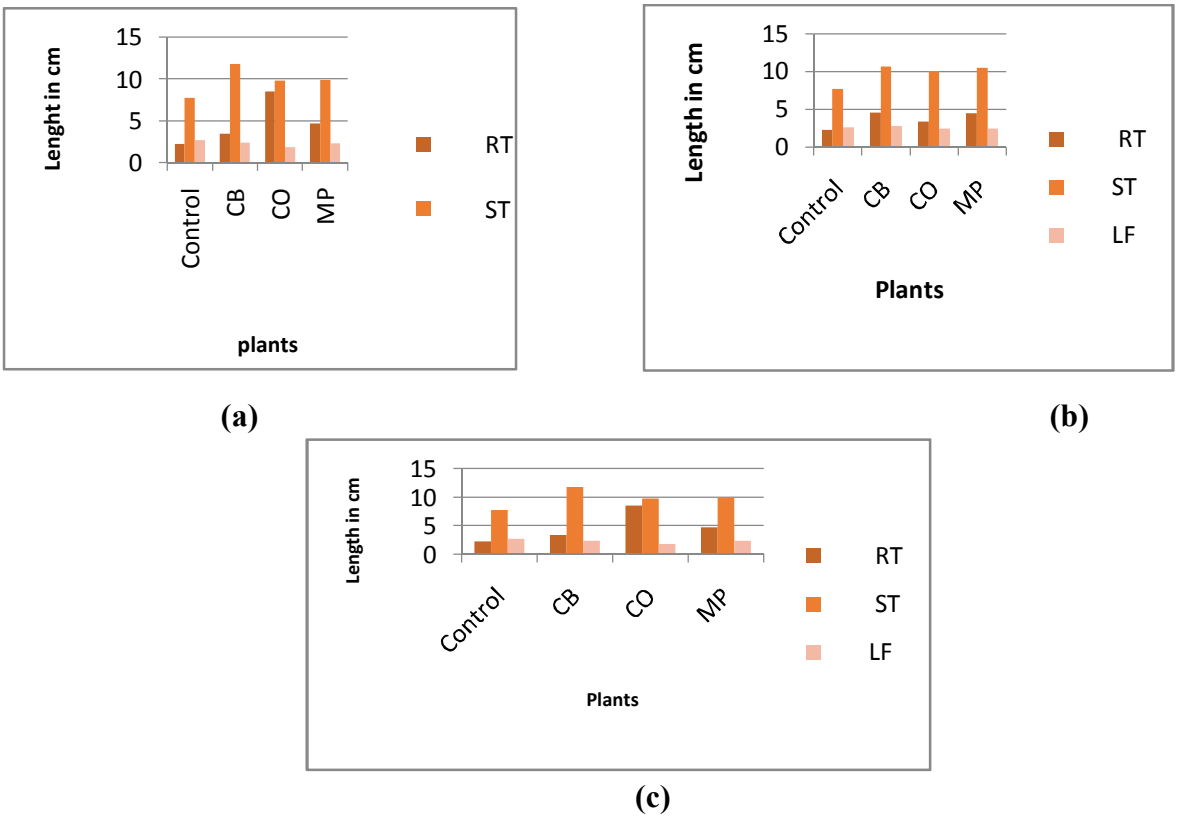

Figure 1: Effect of aqueous extract of three aphrodisiac plants at different concentration (a) 0.5%, (b) 1%, (c) 2.5%

Estimation of total dry weight

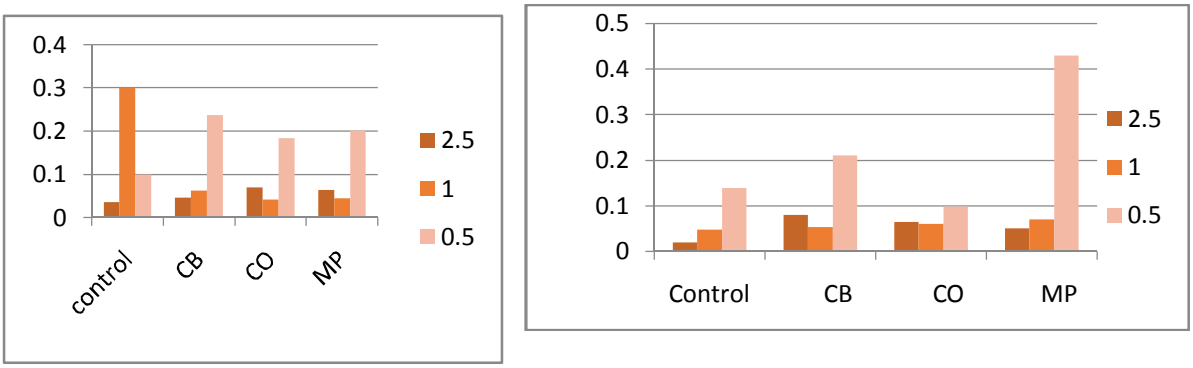

Figure 2: Total dry weight of plantlet *Vigna radiata* treated with aqueous of three aphrodisiac plants

Estimation of Chlorophyll content

Table 1: Chlorophyll content of *Vigna radiata* treated with aqueous extract of different concentration of some aphrodisiac plants

Concentrations (%)	Group	Total chlorophyll (mg/lit)	Chlorophyll A (mg/lit)	Chlorophyll B (mg/lit)
0.5	Control	0.05082	0.0049	0.0031
	CB	0.01690	0.0071	0.0049
	CO	0.01632	0.0070	0.0091
	MP	0.0160	0.0073	0.0085
1.0	Control	0.05082	0.0049	0.0031
	CB	0.01193	0.0077	0.0040
	CO	0.0125	0.0076	0.0046
	MP	0.0133	0.0076	0.0056
2.5	Control	0.05082	0.0049	0.0031
	CB	0.01819	0.0096	0.0083
	CO	0.0152	0.0089	0.0062
	MP	0.01835	0.0095	0.0086

Protein content in leaves of mung bean under different treatment

The protein content of treated plant leaves of mung bean is given in Figure 3. The highest protein content was found in CB and CO and lowest in MP plant.

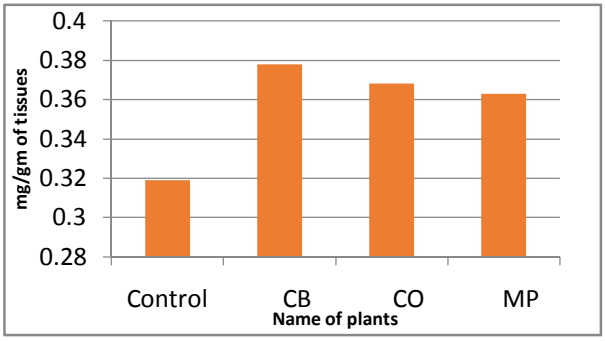

Figure 3: Total protein content of leaves plantlet of *Vigna radiata* treated with aqueous extract of Aphrodisiac plants

Acid phosphatase activity (Biomarker)

Comparative account on (Biomarker) Acid phosphatase activity in germinated *Vigna radiata* seeds on seven day after treatment with three plants aqueous extract

Table 2: Acid phosphatase activity in germinated *Vigna radiata* seeds on seven day after treatment with three plants aqueous extract

Sr. no	Parameter	Total activity (µM/ml)	Enzyme activity (µM/ml/min)	Specific activity (µM/ml/min/mg)
1	Control	4.72	11.8	36.99
2	CB	9.85	24.6	65.07
3	CO	4.40	11	29.89
4	MP	4.15	10.3	28.37

In table 2, it is observed that specific activity of acid phosphatase was highest in CB as 65.07µM/ml/min/mg, moderate specific activity in CO as 29.89µM/ml/min/mg, while less specific activity in MP as 28.37µM/ml/min/mg.

Discussion

In current investigation, on the basis of literature survey, we have selected some aphrodisiac plants to check their stimulation or inhibitory effect on germinating seeds of (*Vigna radiata*) as a

model (Srinive 2007). We found that percentage of germination is more in tested plant treated with (CB, CO, MP) as 100%, followed by CB 90%, MP as 80% respectively, while in CO less growth was observed. Dry weights were found more with shoot & root lengths for Corresponding different concentrations treatment. Control study reveals at a lower concentration of extract less biomass from (root & shoot) over untreated plant. Similar observations are given by Ishii et al (1984) & Bhattacharjee (2008). It is interesting to note that not a single reference occurred in literature indicating Allelopathic effect of aphrodisiac plants in seed germinating and growth however in other plants change in germination however, Allelopathic effect in mung is reported by using *Blumea lacera L*. on rice on agricultural crops (Cheema et al., 2012). The effect of aqueous extract on chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll content) of mung bean (*Vigna radiata*) shows that highest total chlorophyll in 0.5% and 2.5% concentrations of extract evidenced in CB. Also highest protein content was noticeable as increases in CB 18% lowest in MP 13% and moderate protein in CO 16%. But Amira (2001) studied effect of salt stress on plant growth and metabolism of bean plant *Vicia faba*. Biomarker is used to check out the provided treatment is beneficial or harmful to the plants. Acid phosphatase biomarker is used in this *Vigna radiata*. The highest concentration of acid phosphatase observed in CB plants. CO gave less result than MP plants. We found the aqueous extract of three aphrodisiac plant at 0.5% and 1% concentration by standard deviation showed statistically significant stimulation on shoot and root elongation of the tested plant CB,CO and MP (mung bean).

Conclusion

- The result indicates that positive response in growth of CB.
- The length of shoot of seedling is more than control evidence chlorophytum is best.
- During the treatment of CB, more positive response was obtained concomitantly in addition to morphological study.
- We observed positive biochemical changes during the treatment of germinated seed.
- Biochemical parameters like protein & chlorophyll also increased than control seedling.
- The response is best in CB, moderate in CO & good is MP. Present work warrants to conduct further study at various stages of plant growth, in order to implement such treatment for cultivation of plant in the field.

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Utilization of Banana Peel as a Natural Fertilizer and its Impact on Growth of Germinated Seeds of Mung Bean (*Vigna Radiata*) – Waste Management

Dapke Rahul, Patil Sandeep, Patil Umakant, Javed Khan*
Department of Biotechnology, Post Graduate College of Science Technology and Research, Jalgaon

Abstract

Group of farmers in India include Maharashtra, have grown bananas and produced several products from bananas for widely used. Banana peel are become serious environmental problem caused by the bananas production. Studies on utilization of banana peel as a natural fertilizer prepared from the waste banana peel on soil and plant. Prepared ripe and unripe banana peel powder incorporated in the soil, and observed that ripe banana peel powder induce soil fertility. Mung bean (*Vigna radiate*) seeds were used to study as a natural fertilizer of banana peel powder and extract as a natural growth enhancer. After incubation banana peel powder added soil, analyse the soil fertility and after 14 days of application of fertilizer, plant growth was measured. Among the ripe and unripe banana peel powder and extract, ripe banana peel extract was found more suitable for plant growth, soil fertility and also induce the germinated seeds protein content and as well as acid phosphatase activity while unripe banana peel powder and extract doesn't shows any effect on growth of mungbean.

Keywords: Agricultural waste, Banana peel, soil fertility, mungbean, natural fertilizer

*Corresponding author:

Introduction

Agriculture was practiced for thousands of years without using any artificial chemicals. Soil management practices have recently increased the use of chemical fertilizers to help crop yields by improving nutrient supply. Moreover, the organic wastes were increased and become environmental problem caused by banana peels in the processed food products from the bananas. Under this circumstance it is essential to test the soil for knowing the fertility status of field on this basis, fertilizer recommendation can be made for particular crop waste **(**Panwar, 2015**).** In the present study, we have efficiently prepared natural fertilizer by waste banana peel to improve soil fertility and consequently induced more plant growth. This is the first report on natural fertilizer based on waste banana peel utilization, to improve soil fertility and plant growth. Besides this, it involves all the biocompatible precursors to provide simple cost effective route

for preparing high performance eco-friendly natural fertilizer. Acid phosphatase plays the important role of plant phosphorus metabolism, (Stephen Duff, 1994).

Material and methods

Collection of Banana peel sample

Waste banana ripe peel collected from market area and unripe banana peel collected from banana chips manufacturing unit of Jalgaon, Dist - Jalgaon, (MS), India, and it is identified by Dr. Tanveer Khan, Taxonomist Department of Botany, Hajjan Halimabi Jamaloddin Thim College of Arts & Science, Mehrun, Jalgaon.

Preparation of peel powder

The ripe and unripe banana peel waste was collected from different market places of Jalgaon, and dry it under sunlight for three to four days, after drying makes banana peel powder with the help of mixer or blender, and collect the powder separately.

Preparation of aqueous extracts of ripe and unripe banana peel extract

The aqueous extracts of ripe and unripe banana peel powder was prepared along with water on water bath at 70 ^{o}C for 3 hours, then extract were filtered through filter paper and take a clear filtrate as aqueous extract.

Collection of Soil and treatment of banana peel powder and its analysis

Soil samples were collected from Botanical garden and green house of Moolji Jaitha College, Jalgaon (MH) India, during December. Soil samples were taken randomly within garden and mixed into a composite sample representative of that particular site. The samples were collected in polythene bags and transported to laboratory, where stones in the samples were removed and the soils were homogenized through a 2 mm sieve and then placed in composting bags, each bag having 0.5 kg of soil and suitable water. In one bag ripe banana peels powder having weight 100g was added and this tray labeled as bag 1, unripe banana peel powder having weight 100kg was added and this bag labeled as bag 2 and soil without banana peels powder labeled as control bag 3. These bags were kept in greenhouse of Moolji Jaitha College, Jalgaon for 10 days incubation at 30 ^{o}C for composting, after incubation analyze physicochemical properties of soil. The collected soil sample in bag 1, 2 and 3 were analyzed for various physicochemical parameters. The soil content determined according to manual of Soil Testing in India, Department of Agricultural and Ministry of Agricultural Government of India. January, 2011.

Primary soil testing was carried out by using soil test kit at Shree Analytical Testing and Research Laboratory Jalgaon.

Effect of 2.5% and 1% concentration of ripe and unripe banana peel powder and aqueous extract on *Vigna radiata* plantlets

The experimental design was performed with six replications, (S_1 to S_6,). The test species were mung bean (*Vigna radiata*) as a biological material considering its economic importance for agriculture and foods. Seeds of plant material of *Vigna radiata* were obtained from the local market of Jalgaon district (Maharashtra). Healthy and uniform seedlings were allowed to grow in pots and observe the growth at 0, 1, 3, and 5^{th} day and after 14^{th} day of growth, the shoot and root lengths were long enough to measure using a ruler. Number of fibrous roots was also measured.

Chlorophyll content

Chlorophyll A, B and total chlorophyll content of plantlet of mung bean was determined by (Stirban, 1985)

Allelopathic effect of 0.5% aqueous extract on seed germination of *Vigna radiata*

Petri dish experiment

The healthy and uniform seeds of *Vigna radiata* were selected and surface sterilized with 0.1% mercury chloride and thoroughly washed with distilled water to avoid surface contamination .germination experiments were carried out in sterilized petri dishes lined with double layer of Whatman filter number 1. Five sterilized seeds were taken in each petri dishes containing 0.5% concentration of aqueous extract of ripe and unripe banana peel, while tap water was taken as control and incubated at 26 ^{o}C to 30 ^{o}C for germination. The growth parameter like number of fibrous root and root and shoot length was measured after 7 day.

Protein content

The protein content of aqueous extract of ripe and unripe banana peel treated germinated seeds of *Vigna radiata* was determined according to Lowry's method (Lowry et al, 1951), using Bovin Serum Albumin as a standard.

Acid phosphatase assay

Acid phosphatase activity of aqueous extract of ripe and unripe banana peel treated germinated seeds of *Vigna radiata* was determined according to, (Hussain, 2016), by using the standard para-nitro phenol.

Results and Discussion

Primary soil chemical test results

The collected soil sample in bag 1, bag 2 and bag 3 as a control samples were analyzed for various physicochemical parameters such as pH, electrical conductivity, total organic carbon, nitrogen content, available potassium and phosphorous pH values of soil samples treated with unripe and ripe banana peel powder was given in Table 1. The pH value of soil increased after added of unripe banana peel powder 8.32, decreased on added of ripe banana peel powder 7.50 as compared with the control 7.76. The electrical conductivity of soil increased after added of unripe banana peel powder 1.54 ms, decreased on added of ripe banana peel powder as compared with the control 1.25 ms, Organic carbon, Phosphorus, Potassium and Nitrogen content of soil was increased in ripe treated soil while decreased in unripe banana extract as compared with the control.

Table 1: Effect of banana ripe and unripe peel powder on soil fertility

Sr. No	Parameter	Control	Unripe	Ripe
1	pH	7.76	8.32	7.50
2	EC ms	1.25	1.54	0.90
3	Organic carbon %	0.72	0.68	1.10
4	Phosphorus mg/kg	31.1	31.1	39.1
5	Potassium mg/ kg	505	455	995
6	Nitrogen kg/hectare	398.5	334.4	505

Effect of 2.5% banana peel powder on *Vigna radiata* plantlets

In Figure1, it is observed that average length of root was highest for ripe banana peel extract, lowest average length was seen in unripe as compared with the control. From this study, it is clear that average number of fibrous root was highest for ripe banana peel extract, lowest in the unripe banana peel extract as compared with the control. Average length of shoot was highest for ripe was 14.8 cm, while lowest average length of shoot 8.41 cm was observed in unripe, as compare with the control.

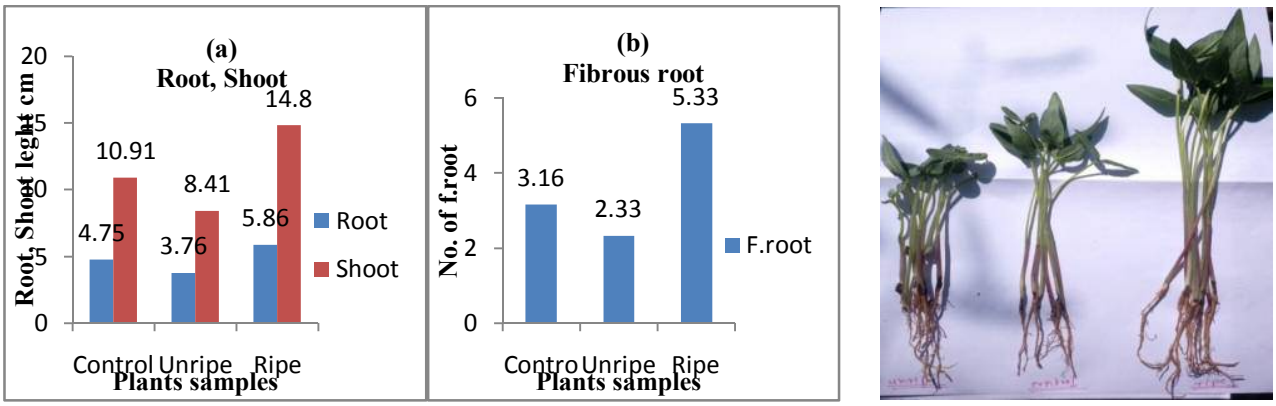


Figure 1: (a) Comparative study of effect of 2.5% powder of ripe and unripe banana peel on growth of root and shoot of *Vigna radiata* plantlets, (b) on growth of fibrous roots of *Vigna radiata* plantlets

Effect of 1% aqueous extracts on *Vigna radiata* plantlets

The average length of root was highest for unripe peel extract, as 13.75 cm, moderate growth was observed in ripe as 11.75 cm, while lowest average length 9.16 cm was seen in control (Figure 1). From this study, it is clear that average number of fibrous root was highest for ripe banana peel extract, while moderate average length was observed in unripe as compared with the control and average length of shoot was highest for ripe, while lowest average length of shoot was observed in control.

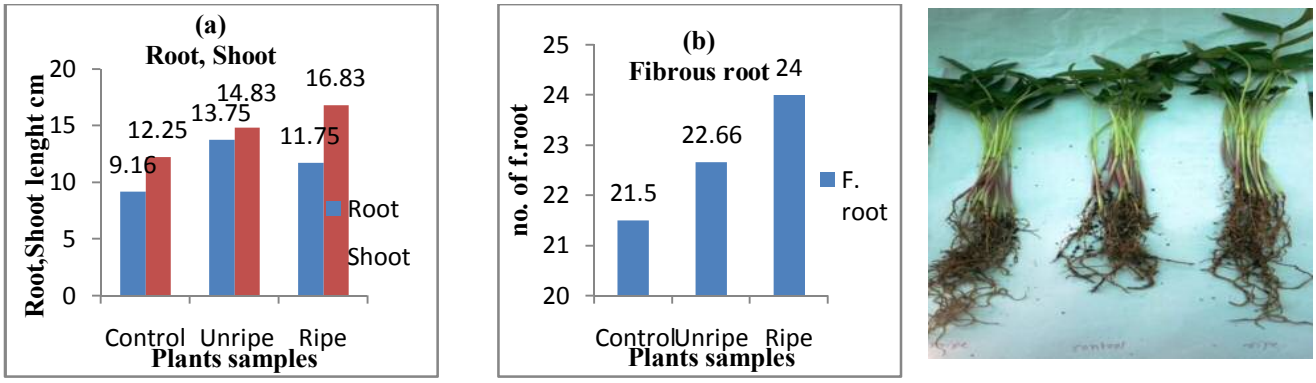


Figure 2: (a) Comparative study of effect of 1% aqueous extract of ripe and unripe banana peel on growth of root and shoot of *Vigna radiata* plantlets, (b) On growth of fibrous roots of *Vigna radiata* plantlets

Chlorophyll Estimation

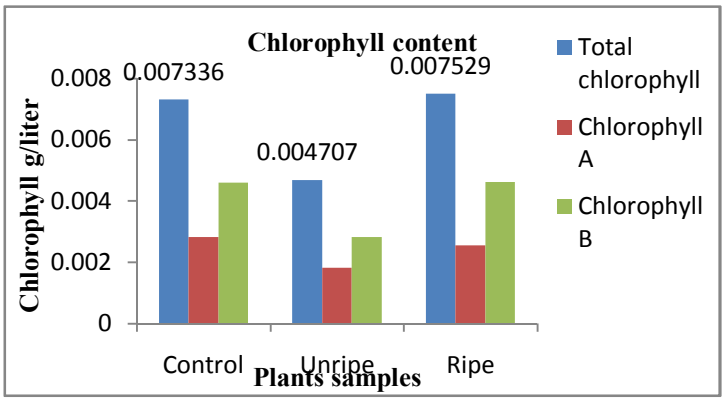


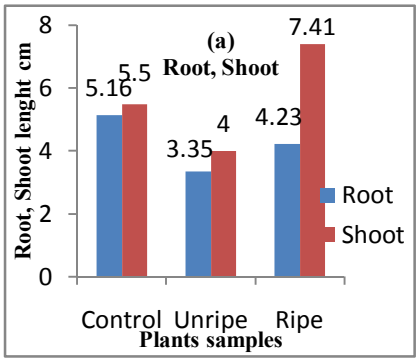
Figure 3: Effect of 1% aqueous extract of ripe and unripe banana peel on chlorophyll content of *Vigna radiata* plantlets

The study of effect of aqueous extract of ripe and unripe peel extract on chlorophyll content (chlorophyll A, B and total chlorophyll) of mung bean (*Vigna radiata*) induce rise in chlorophyll over control as shown in (Figure 3). At a 1 % concentration, total chlorophyll was observed highest in ripe banana peel extract, whereas noticeable changes in chlorophyll A and chlorophyll B, as compared with control and unripe banana peel extract was observed.

Allelopathic effect of 0.5% aqueous extract on seed germination of *Vigna radiata*

Petri dish experiment

The average length of root was lowest for unripe, while moderate average length was seen in ripe as compared with the control. From this study, it is observed that average no. of fibrous root was highest for ripe banana peel extract, while moderate average length was observed in unripe as compared to the control and average length of shoot was highest for ripe was 7.41 cm, while lowest average length of shoot 4 cm was observed in unripe as compared with the control (Fig4).



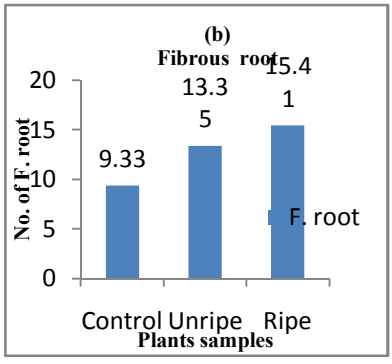


Figure 4: (a) Comparative study of effect of 0.5% aqueous extract of ripe and unripe banana peel on growth of root and shoot of germinated seeds of *Vigna radiata* (b) on growth of fibrous root of germinated seeds of *Vigna radiata*

Protein content at 0.5% aqueous extracts treatment

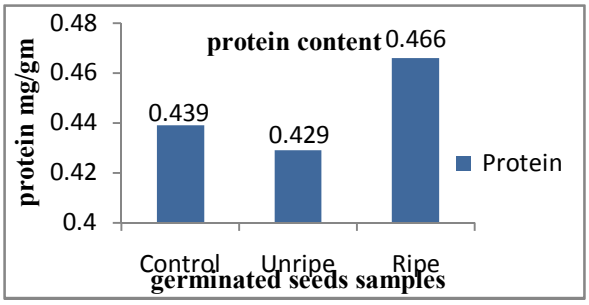


Figure 5: Effect of 0.5% aqueous extract of ripe and unripe banana peel extracts on total protein content of germinated seeds of *Vigna radiata*

The protein content of germinated plant seeds of mung bean is shown in (Figure 5). The highest protein content was observed in ripe banana peel extract and lowest protein content was observed in unripe banana peel extract as compared with the content.

Acid phosphatase activity on germinated seeds of *Vigna radiata*

The specific activity of acid phosphatase enzyme as 53.71 µM/ml/min/mg was highest for ripe banana peel extract. Moderate specific activity was observed in unripe banana peel extract as 40.32 µM/ml/min/mg, which is close to control group 40.31 (Figure 6).

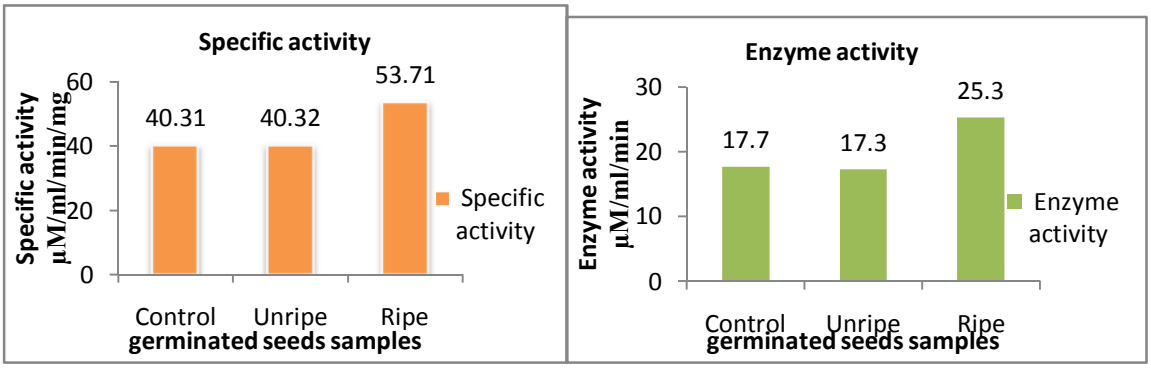


Figure 6: (a) Effect of 0.5% aqueous extract of ripe and unripe banana peel extracts on acid phosphatase specific activity of germinated seeds of *Vigna radiata* (b) on enzyme activity

Discussion

To study the effective dose whose concentration ranges from 0.5 to 2.5 % were used to observe any allelopathic effect on rate of germination and growth of plantlet. Aqueous extract of unripe banana peel extract helps to significantly induce the fibrous root as compared with the content. Unripe banana peel powder doesn't show any effect on growth of plantlets. Until now no scientific literature is available on unripe banana peel use as fertilizer. This is the first observation on unripe banana peel extract as fertilizer. The ripe banana peel powder added soil increase organic carbon percentage and our results was in- consistent with studies on physiochemical characteristics and fertility of soil by addition of banana peel by (Panwar, 2015), reported that studies by after addition of ripe banana peel in soil organic carbon percentage increase the day by day. The observation shows that the aqueous extract of ripe banana peel powder induces the plant growth like shoot length, no. of fibrous root and root length as compare to control and aqueous extract of unripe banana peel powder. Because banana peels is rich in minerals including potassium, phosphorus, and calcium, (Ascher et al., 1994). Our results are very much similar with Patrick is (2017), observation, where he mentioned that phosphorus play a significant role in seed germination and viability. Total chlorophyll, chlorophyll A and chlorophyll B of *Vigna radiata* increased by the treatment of ripe banana peel extract, and greatly decreased on treatment of unripe banana peel extract as compared with the control and these were much identical with the, (Ahemed bakry et al., 2016). Our results are in accordance with the reports of Aisha Wazir (2018). The ripe Banana peels contain the three macronutrients i.e. Nitrogen, Phosphorus and Potassium, as well as many micronutrients. It was observed that protein content of seeds was increased on treatment with an aqueous extract of ripe banana peel was 0.466 mg/gm. However, treatment with aqueous extract of unripe banana peel gave 10% less protein content. These results are very much collated with the Study on Effect of Banana Peel Extract or Tryptophan on Growth Yield and Some Biochemical Aspects of Quinoa Plants under Water Deficit reported by Ahmed Bakry et al., (2016). They reported that the increase in carbohydrate, protein and antioxidant potential. In our study we found that during the germination of seeds, the acid phosphatase activity was induced by treating with ripe banana peel extract, as compared with the unripe banana peel extract and with control. Agoreyo B.O, (2010), reported that acid phosphatase activity increased continuously in the banana fruit and peels, throughout the period of ripening.

Conclusion

- Aqueous extract of ripe banana peel extract helps to significantly induce the fibrous root as compared with the control while unripe banana peel powder doesn't show any effect on growth of plantlets.
- Banana ripe peel powder and extract may be used as organic fertilizer to the agricultural field. However before putting in the field exhaustive experimentation is necessary, to record fruiting data at the maturity state of plant.

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In vitro antimicrobial activity of rhizome of halad (*Curcuma longa l*)

Patil Satish, Shinde Sominath, Satyendra Mishra[a], Bari Sarang*
Department of Biotechnology, Post Graduate College of Science, Technology and Research, Jalgaon
[a]University Institute of Chemical Technology, KBC NMU, Jalgaon

Abstract

Turmeric is used as a dietary spice and coloring agent in foods and a treatment for a wide variety of ailments. Different solvents such as acetone, methanolic and n-hexane extracts of turmeric exhibit wide ranges of antibacterial activity against *Staphalococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*, while *in vitro* antifungal activity was also shown to some extent against *Aspergillus niger* and *Candida albicans.* Antifungal activity was also shown to some extent against *Aspergillus niger* and *Candida albicans* is resistant to three extracts under taken for present study.

Keywords: Antibacterial activity, Antifungal activity, Turmeric extracts, Agar-well diffusion

*Corresponding author: bari.sarang@rediffmail.com

Introduction

The turmeric *(Curcuma longa)* plant, a perennial herb belonging to the ginger family, is cultivated extensively in south and southeast tropical Asia. The rhizome of this plant is also referred to as the "root" and is the most useful part of the plant for culinary and medicinal purposes. The most active component of turmeric is curcumin, which makes up 2 to 5% of the spice. The characteristic yellow color of turmeric is due to the curcuminoids. Curcumin is an orange–yellow crystalline powder practically insoluble in water. The structure of curcumin (C_{21} H_{20} O_6) was first described by Lampe and Milobedeska as it is described by (Bagchi et al. 2012). It is widely used in traditional Indian medicine to cure bleary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis. Turmeric paste in slaked lime is a popular home remedy for the treatment of inflammation and wounds (Bagchi et al. 2012). Extensive investigation over the last five decades has indicated that curcumin reduces blood cholesterol (Aggarwal et al. 2006).

Turmeric (*Curcuma longa* L.) rhizome is the commonly used additive which gives flavor, color and add spices to food preparation in south east Asian countries (Geethanjali *et al.* 2016) ,used

in Ayurvedha, Unani and Siddha medicine for various diseases (Junaid *et al.* 2015). Turmeric is known for its anti-diabetic, antiseptic, antibacterial, anti-asthmatic, antiulcer drug, insect-repellant and wound healing properties (Ammon *et al.* 1991). Indian turmeric is preferred due to its high Curcumin content as compared to other countries.

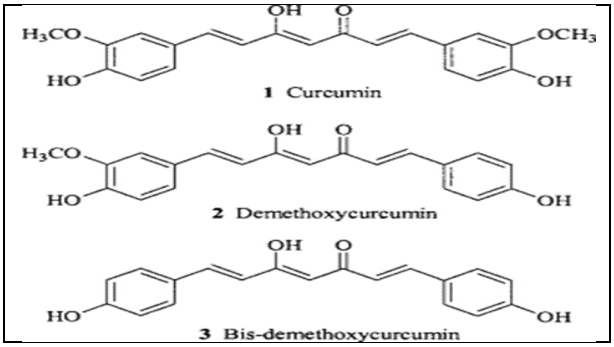

Figure 1: Structure of *Curcumin*

Materials and methods

Plant materials

Turmeric sample are procured from local market of Jalgaon, Maharashtra, INDIA and rhizome of *Curcuma longa* from industry.

Preparation of turmeric extract

25 gm of dry powder was packed in Soxhlet apparatus for extraction of respective soluble bioactive molecules from the rhizome by the use of different solvent (hexane, acetone, methanol and water) base on polarity of volatile solvents, were concentrated on water bath (50 - 70 ^{o}C) under reduce pressure.

Test microorganisms

Four different strains were used for testing antibacterial activity includes *E.Coli, Pseudomonas aeruginosa, Staphalococcus aureus* and *Bacillus subtilis* etc. and two fungal strains for testing antifungal activity includes*, Aspergillus niger* and *Candida albicans* as test organisms were used.

Antibacterial Assay

The effect of various plant extracts on (100mg/2ml) the several bacterial strains were assayed by Agar well diffusion method.

Agar- well diffusion method

Petriplates containing 20 ml Muller Hinton medium were seeded with 24h culture of (0.1 ml) bacterial strains *E. Coli, Pseudomonas aeruginosa, Staphalococcus aureus* and *Bacillus subtilis.*

Wells of approximately 6 mm was bored using a well cutter and 2, 3, 4 and 5 mg/well of the *Curcuma longa* rhizome extracts (Hexane, Methanol and Acetone extracts) were added. The plates were then incubated at 37°C for 24h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Gentamycin, a standard antibacterial agent was used as the positive control (Praveen *et* al. 2014).

Antifungal Assay

The microorganisms; *Candida albicans* and *Aspergillus niger* were used for antifungal assay. The activities of the plant extracts on various fungal strains were assayed by agar well plug method.

The fungicidal effect of the Curcumin extracts can be assessed by the inhibition of mycelial growth of the fungus and is observed as a zone of inhibition near the wells.

Potato Dextrose Agar (PDA) medium was prepared and poured on to the petri plates. Wells of about 6.0 mm diameter were aseptically punched on each agar plate using a sterile cork borer, Fungal strain viz *Aspergillus niger* and *Candida albicans* (0.1 ml) were aseptically inoculated and evenly spread using sterile glass spreader on the surface of potato dextrose agar plate. Add 2, 3, 4 and 5 mg/well of the curcumin extracts (Hexane, Methanol and Acetone extracts) were then inoculated into the wells. The plates were then incubated at 37 °C for 72h. Flucanazole was used as antifungal control. The diameter of zones of inhibition was recorded in mm (Borate *et* al. 2014).

Industrial waste of rhizome of *Curcuma longa*

Industrial waste of rhizome of *Curcuma longa* Collected from Agro industry of Faizpur.

Estimation of curcuminoids: By Spectrophotometric analysis

Preparation of standard

The reference Curcumin purchased from HI-MEDIA Company in India. 1 mg of pure Curcumin was dissolved into methanol and water such that concentration was 60 µg/ml, got reading at 420 nm. They are shown in Figure 1. This graph was referred graph as a standard.

Spectrophotometric analysis

To find out concentration of extracted sample by using spectrophotometer, 1 mg of sample were mixed with methanol and water same as standard solution, OD was taken at 420 nm, because all three components of curcuminoids has λ max at 420 nm (Kulkarni *et* al .2017).

Results and Discussion

Turmeric extract

After drying and extraction through Soxhlet apparatus the % yield were calculated as weight percentage of *Curcuma longa* rhizome obtained and are shown in Table 1.

Table 1: Weight % extract of *Curcuma longa* rhizome

Solvent	Weight % extract
Methanol	15.68
Acetone	22.8
Hexane	6.5

Antimicrobial activity

The result of the antimicrobial activity of the *Curcumin* extracts in methanol, hexane and acetone showed antimicrobial activity against both Gram positive and Gram negative bacteria are as shown in the Table 2 and 3. Table 2 shows antibacterial and Table.3 shows antifungal activity.

Antibacterial activity

The antibacterial activity of Curcumin extract against two Gram-positive (*S. aureus and B. subtilis)* and two Gram-negative (*E. coli and Pseudomonas aeruginosa).* The maximum zone of inhibition was seen in methanolic extract against *S. aureus* (10 mm) at the concentration of 4 mg/well and minimum zone of inhibition was seen in methanolic extract against *Pseudomonas aeruginosa* (3 mm) at the concentration of 2 mg/well. The maximum zone of inhibition was seen in hexane extract against *B. subtilis* (10 mm) at the concentration of 4 mg/well and minimum zone of inhibition was seen in hexane extract against *Pseudomonas aeruginosa* (5 mm) at the concentration of 2 mg/well. The maximum zone of inhibition was seen in acetone extract against *E.coli* (10 mm) at the concentration of 4mg and 5mg/well and minimum zone of inhibition was seen in hexane extract against *Bacillus subtilis* (5 mm) at the concentration of 2 mg/well, and Gentamicin used as a standard positive control (Table 2).

Antifungal activity

The maximum zone of inhibition was seen in hexane extract of rhizome of *Curcuma longa* against *Aspergillus niger* (25 mm) at the concentration of 5 mg/well and minimum zone of inhibition was seen (8 mm) at the concentration of 2 mg/well. No zone of inhibition was observed for *Candida albicans* (Table 3).

Industrial waste of Steam rhizome

Fresh rhizomes are collected from Agro product industry of Faizpur.

Estimation of curcuminoids: By spectrophotometric analysis.

The reference curcumin purchased from HI-MEDIA Company in India.

Standard Curcumin

Concentrations of extracted dried sample were analyzed on spectrophotometer at 420 nm with respect to standard graph (Figure 2) the method described by (Kulkarni et al.2017). The content of curcumin was 2.4 gram per 100 gram of powder.

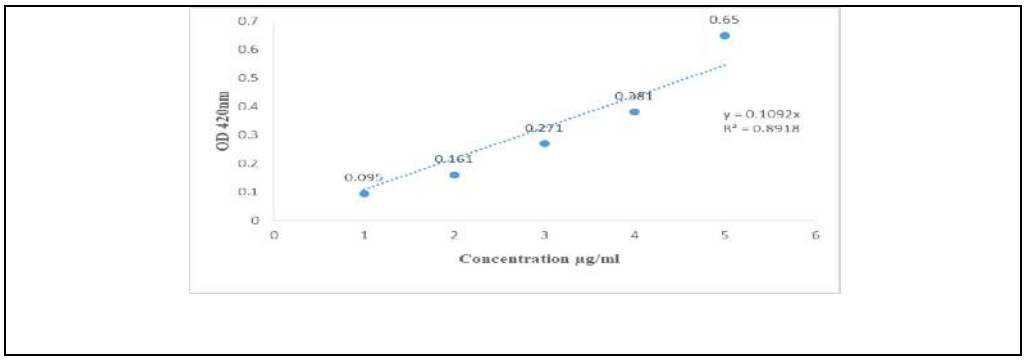

Figure 2: Standard calibration curve of Curcumin

Table 2: Antibacterial activity of *Curcuma longa rhizome* extract against *E. coli, Pseudomonas aeruginosa, Staphalococcus aureus and Bacillus subtilis*

Organisms		Zone of Inhibition (mm)												
		Methanolic extract (mg/ well)				Hexane extract (mg/ well)				Acetone extract (mg/ well)				Control (Gentamicin standard)
		2	3	4	5	2	3	4	5	2	3	4	5	20 µl/well
Gram (-ve)	*E.coli*	5	7	6	8	6	8	8	9	8	9	10	10	17
	Pseudomonas aeruginosa	3	7	4	7	5	6	6	7	7	9	10	9	16
Gram (+ve)	*S. aureus*	5	6	10	8	7	8	7	9	6	8	8	8	17
	B. subtilis	7	7	8	8	6	7	10	9	5	7	7	8	17

Table 3: Antifungal activity of *Curcuma longa rhizome* extract against *Candida albicans* and *Aspergillus niger*

Organisms	Zone of Inhibition (mm)												
	Methanolic extract (mg/ well)				Hexane extract (mg/ well)				Acetone extract (mg/ well)				Control
	2	3	4	5	2	3	4	5	2	3	4	5	20µl/well
A. niger	-	-	-	-	8	13	20	25	-	-	-	-	14
Candida	-	-	-	-	-	-	-	-	-	-	-	-	15

Table 4: Antibacterial activity of industrial waste of steam rhizome treatment (100µl/well) against *E. Coli, Pseudomonas aeruginosa, Staphalococcus aureus and Bacillus subtilis*

	Organisms			
	E. coli	*Pseudomonas aeruginosa*	*Staphalococcus aureus*	*Bacillus subtilis*
Zone of Inhibition (mm)	1	2	1	1

Discussion

In present study, it was observed that, acetone, methanolic and n-hexane extracts of turmeric exhibit wide ranges of antibacterial activity against *Staphylococcus aureus, Escherichia coli, pseudomonas aeruginosa* and *Bacillus subtilis*, while antifungal activity was also shown to some extent against *Aspergillus niger* and *Candida albicans in vitro.*

The methanolic extract was active against *Staphylococcus aurues* showing zone of inhibition ranges between 6 mm and 10 mm at 100 mg/ml and these results were comparable with already existing reports (Gul et al,.2015 with zone of inhibition 7.5 to 12.5 mm) for the antimicrobial activity. They use and found potential use in food industry (Gupta et al, 2015). They antimicrobial activity of *Curcuma longa* rhizome extract against S. aureus. Our study indicates *E. coli* inhibition range in between 5 mm to 8 mm and these results differ with the (Gul et al,. 2015), (Zone of inhibition 22.5 mm) more or less similar result observed by result (Singh et al,. 2012), (20 mm Zone of inhibition). In present study, we found *pseudomonas aeruginosa* zone of inhibition ranges between 8 mm to 10 mm at 100 mg/ml. In *Bacillus subtilis,* inhibition ranges was in between 6 mm to 10 mm at 100mg/ml.

Antifungal activity was also shown to some extent against *Aspergillus niger* and apparently in result may be due to not in *Candida albicans*. The hexane extract were active against *Aspergillus*

niger showing zone of inhibition ranges between 8 mm to 25 mm at 100 mg/ml and these result were comparable with (Singh et al,. 2012), (18 mm zone of inhibition), Zero zone of inhibition was observed for *Candida albicans.* These results are similar with the (Gul et al, 2015).

Conclusion

In this study, the antibacterial activity of turmeric extracts in different solvents, such as n-hexane acetone and methanol were evaluated for their inhibitory effect on bacterial strains of *Staphalococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. The methanolic extract of *C. longa rhizome* is most effective against *S. aureus* and *B. subtilis* and the hexane extract of *C. longa rhizome* is good effective against *B. subtilis* and *E. coli* ,where as acetone extract of *C. longa rhizome* is more effective against *E. coli* and *pseudomonas aeruginosa* as compare to other bacterial species. In our study, the more effective results were noted in the acetone and n-hexane extract of *Curcuma longa* rhizome. The antifungal activity of *Curcuma longa rhizome* extracts was evaluated for their inhibitory effect on standard fungal strains of *Aspergillus niger* and *Candida albicans.* The hexane extract was most effective against *Aspergillus niger* as compared to acetone and methanolic extract whereas no inhibition of organisms observed in all organic solvent extracts against *Candida albicans.* People used halad for wound healing in skin injury as a home remedy. Therefore present results justify upto certain extent as antiseptic. Experimental data encourage us to prove its efficacy thoroughly by using animal model.

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Microbiology

Antibacterial & Antifungal activity of herbal plants against human pathogen

Baviskar Pavan S, Khan Nasir, Rathod Bhavusaheb, Pawra Vinesh and Mali Manisha S.*
Department of Microbiology, Post Graduate College of Science, Technology, Research, Jalgaon

Abstract

This study was carried out with an objective to investigate the antibacterial & antifungal potentials of leaves of herbal plants. In the present study, the antimicrobial activities of herbal extracts were tested against two Gram-Positive & Gram negative human pathogenic bacteria and two fungal strains. Zone of inhibition of extracts were compared with that of different standards like streptomycin for antibacterial activity & nystatin for antifungal activity. The comparison of medicinal plant with gel, the antibacterial property of plant extract promoted to prepare gel as phytobiotic in vitro therefore utility of these plants for skin health and food is advocated

Keywords: Herbal plants, Methyl paraban

***Corresponding author:** malimanishas1589@gmail.com

Introduction

Herbal medicines play a significant role in health care system throughout the world. The focus is mainly on those herbal medicines that are easily available, cheaper, time tested and considered safer than most of the synthetic drugs. The 80% of world population, the usage of antimicrobial agents derived from plants are considered as traditional health remedies (B. Mahesh 2018). Gel is a topical preparation usually for application to skin. There are two types of gel, oil in water (o/w) type creams which are composed of small droplets of oil dispersed in a continuous watery phase and water in oil (w/o) type creams which are composed of small droplets of water dispersed in a continuous oily phase. The main uses includes provision of a barrier to protect the skin, cleansing, emollient effect, and as a vehicle for drug substance such as anti inflammatory, hormones, antibiotics, antifungal or counter-irritants, skin irritation, urinary joint and muscle pain, headache. In human being, skin is the most susceptible part for entering of various pathogens, microorganisms and spreading of diseases. The skin is continuous, with the mucous membranes lining the body's surface. To keep skin healthy, clear and glossy, a balanced nutrition is required. Among various changes, dryness, roughness and pimples are most common. The pathogenesis of this is bacterial over growth and inflammation. Microorganisms such as

Staphylococcus, Bacillus substilis and *Escherichia species* are responsible for the formation of acne.

Materials and methods

Herbal plant collection

Fresh Leaves of Neem (*Azadirachta indica*), tulsi (*Ocimum tenuiflorum*), Aloe Vera, (*Aloe barbadensis*) Green Tea (*Camellia sinensis*) were collected from local area from vavurda, district Jalgaon Maharashtra. Lemon (*Citrus limon*), Turmeric (*Curcum alonga*) and Honey were collected from local market of Jalgaon, Maharashtra. The collected plants were authenticated at the department of Botany Moolji Jetha College, Jalgaon.

Preparation of extract

The leaves of neem (*Azadirachta indica*), Tulsi (*Ocimum tenuiflorum*) were washed thoroughly 2-3 times with running water and once with sterile distilled water. The dried material of plants was ground into powder using mortar and pestle. The impurities of Green Tea (*Camellia sinensis*) and Turmeric (*Curcum alonga*) powder were removed by sieve using 2-3 times. The powder (10 gm) of all plants leaves obtain was successively extracted in methanol and Distilled water (1:1) by using boiling water bath at 70-80 ^{0}C for 8 hr.

Extraction of Aloe Vera

The aloe Vera leaves were collected and slice with a sharp knife, crushed and collected in conical flask.

Extraction of Lemon

The fresh lemon fruits were collected and washed thoroughly. Then crushed fruits and remove the lemon juice collected in flask.

Test microorganism

Bacterial strains *Escherichia coli* (EA), *Staphylococcus aureus* (SA), *Bacillus subtilis* (BS) and Fungal strain *Aspergillus niger* (AN) and *Candida albicans* (CA) were used for Antibacterial activity of plant extract

Antifungal activity of plant extract

The antifungal activity of methanol plant extract on different fungal strains was performed using agar well diffusion method.

Gel formulation

Take 5 ml of distilled water and required quantity of Methyl parabean were dissolved by heating on water bath. Solution was cooled and Sodium Lauryl Sulphate added. Further required quantities of extract were mixed and add this solution into glycerol (Anurag et al 2012).

Table 1: Composition of gel

Sr. no	Name of Ingredients	Quantity
1	Extract of Aazadirachta indica (Neem)	1g
2	Extract of Aloe Vera	2g
3	Extract of Curcuma Longa	2g
4	Extract of Ocimum tenuiflorum (Tulsi)	1g
5	Extract Camellia sinensis (Green Tea)	2g
6	Citrus limon (Lemon juice)	1ml
7	Honey	2ml
8	Methyl paraben	0.2g
9	Glycerol	5ml
10	Sodium lauryl sulphate	5g

Physical evaluation

Appearance of gel was checked visually and was yellowish green in color. The product was applied twice a day for 2 days on hand and was observed for 10min. No skin affect of this product was observed. It was washable (Thombre, 2018). The pH of various gel formulations was determined by using digital pH meter. 1gm of gel was dissolved in 100 ml distilled water. The measurement of pH was interpreted. The corresponding viscosity of the gel was noted (Jamshiya, 2017)

Results and Discussion

The Antimicrobial activity of all the extracts was tested against the test microorganisms and fungus cultures. Methanolic leaves extract of all herbal plants exhibited antimicrobial & antifungal activity against test microorganisms at different concentration prepared gel also exhibit pronounced antibacterial activity. The present results were compared to earlier reports of Shamasu, 2017, Thombre 2018, Ekawat et al 2019 and findings are in accordance to them.

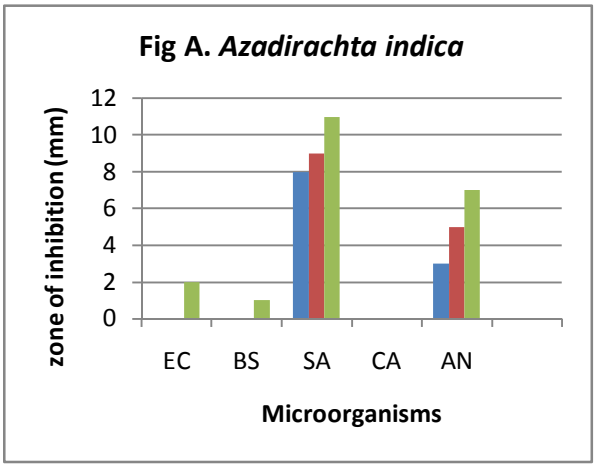


Fig A. *Azadirachta indica*

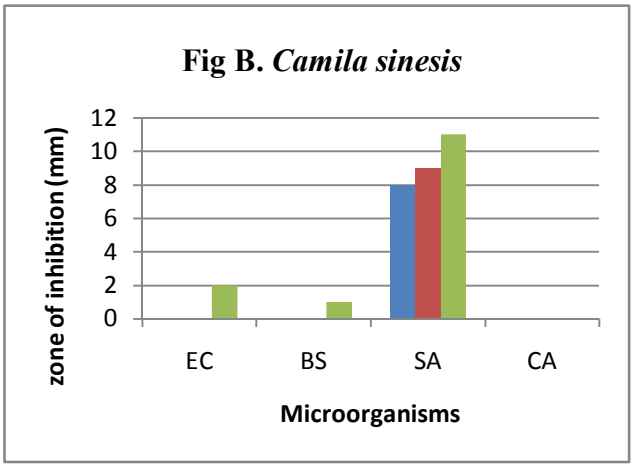


Fig B. *Camila sinesis*

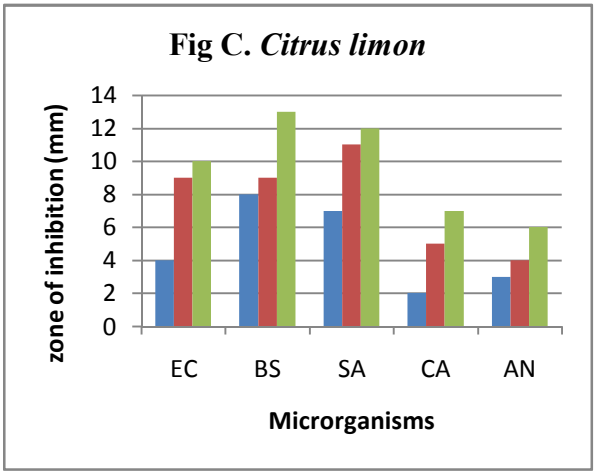


Fig C. *Citrus limon*

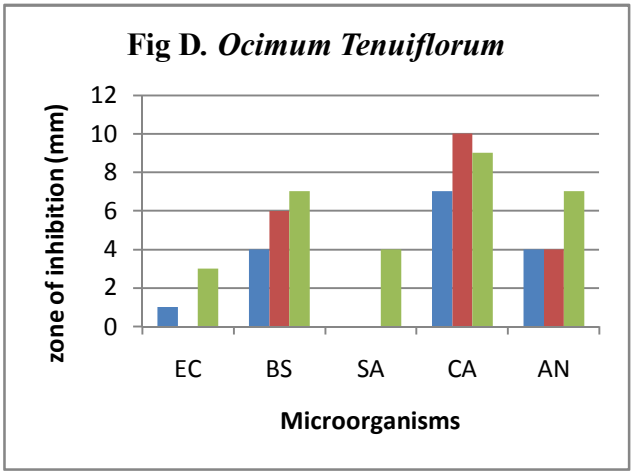


Fig D. *Ocimum Tenuiflorum*

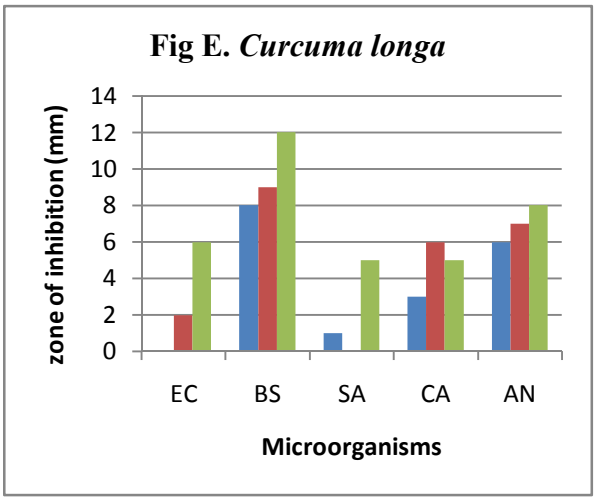


Fig E. *Curcuma longa*

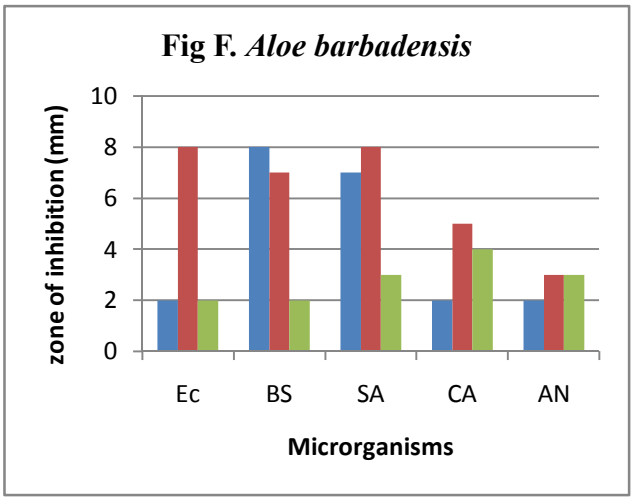
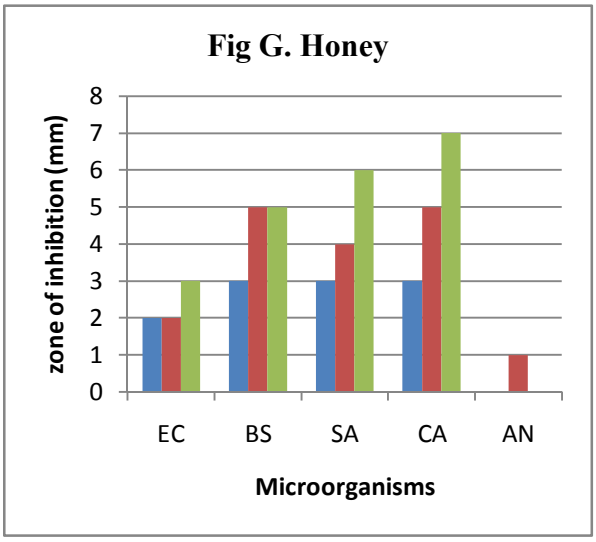
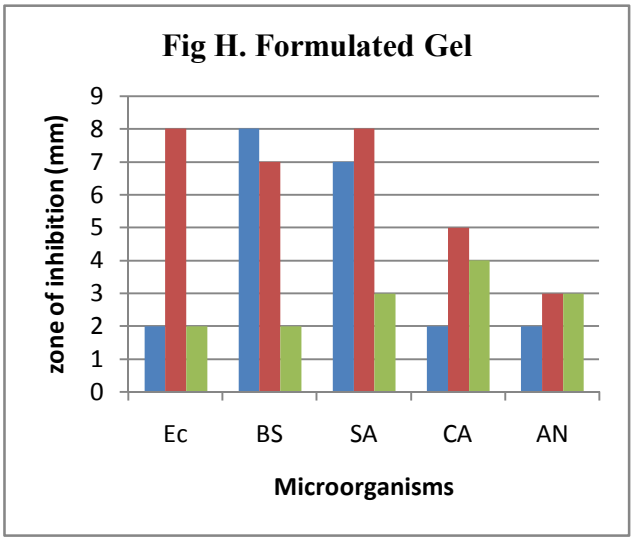


Fig F. *Aloe barbadensis*





500, 750 and 1000 microgram of herbal extract

Figure	Plant Name	Sensitivity		Resistance	
Figure A	*Azardirachta Indica*	SA	AN	BS,EC	CA
Figure B	*Cammelia sinesis*	EC>BS	AN	SA	-
Figure C	*Citrus Limon*	BS>SA>EC	CA>AN	-	-
Figure D	*Ocimum tenuiflorum*	BS	CA	Ec>SA	CA
Figure E	*Curcuma Longa*	SA>BS		EC	AN
Figure F	*Aloe barnadensis*	SA	AN	EC	CA
Figure G	Honey	CA>SA	AN	-	-
Figure H	Formulated Gel	SA>BS>EC	AN	-	-

Conclusion

- The antimicrobial activity of plant extract against gram negative & gram positive microorganism and fungal strains (*Aspergillus niger & Candida albicans*) was established.
- The formulated gel from plant extracts demonstrated excellent in vitro anti acne effects.
- The results are needed to use these plants in cosmetic preparation & wound healing property of gel was also is advocated for further experimentation.

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Reinvestigation of Antibacterial and Antifungal activity of *Curcuma Longa L* in vitro

Pathak Mahesh P, Rathod Jagdish G, Shaikh Tanveer Shaikh Tasleem and Bhat Diksha*
Department of Microbiology, Post Graduate College of Science, Technology & Research, Jalgaon

Abstract

Curcuma longa L. (turmeric) is a medicinal plant that botanically is belongs to 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. Acetone, chloroform, n-hexane, methanolic and water extracts of turmeric were prepared tested against common pathogens In present study Antibacterial and Antifungal activity of *Curcuma longa L* was established against pathogens *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* and *Bacillus subtilis*, while antifungal activity was also shown to some extent against *Aspergillus niger* and *Candida albicans in vitro.* The results directed to use for preparation of antiseptic cream.

Keyword: Antibacterial, Antifungal, Curcuma longa L

*Corresponding author: dikshabhat96@gmail.com

Introduction

It is known that more than 400,000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine. Turmeric has been shown to have anti-bacterial, anti-fungal, antioxidant and anti-inflammatory effects, to which can be added possible anti-ulcer, wound-healing, liver-protective and anti-cancer properties. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. There are several reports on the antimicrobial activity of different herbal extracts indifferent regions of the worlds. Turmeric extract is an oleoresin consisting of a volatile oil (light fraction and a yellow–brown colour (heavy) fraction. It contains a number of curcuminoids, monoterpenoids and sesquiterpenoids. The compounds showing yellow colour are three curcuminoid compounds; curcumin, demethoxycurcumin and bisdemethoxycurcumin. Curcumin, a yellow bioactive pigment, is the major component of turmeric. It has been shown that curcumin have a wide spectrum of biological activities such as antifungal, antidiabetic, antioxidant, anti-inflammatory, anticancer, anti-allergic, anti-protozoal

and antibacterial activities(Gupta et al, 2015). The volatile oil of C. longa reported for anti-inflammatory, antibacterial and antifungal activities.

Chemical Composition of *Curcuma Longa*

Turmeric contains protein (6.3 %), fat (5.1 %), minerals (3.5 %), carbohydrates (69.4 %) and moisture (13.1 %). The essential oil (5.8 %) obtained by steam distillation of rhizomes has a-phellandrene (1 %), sabinene (0.6 %), cineol (1%), borneol (0.5 %), zingiberene (25 %) and sesquiterpines (53 %). Curcumin (diferuloylmethane) (3–4 %) is responsible for the yellow color, and comprises curcumin I (94 %), curcumin II (6 %) and curcumin III (0.3 %). Demethoxy and bisdemethoxy derivatives of curcumin have also been isolated (Figure 1).. It has a melting point at 176–177 °C; forms a reddish-brown salt with alkali and is soluble in ethanol, alkali, ketone, methanol, hexane, acetic acid and chloroform (Geetanjali 2016).

Methods and materials

Plant Materials: Turmeric sample are procured from a field situated at Faizpur, Jalgaon, Maharashtra

Preparation of Turmeric extracts

25 gm of dry powder was packed in Soxhlet apparatus for extraction of respective soluble bioactive molecules from the rhizome by the use of different solvent (hexane, acetone, chloroform, methanol and water) on the basis on polarity of volatile solvents, were concentrated on water bath (50^0-70^0c) under reduce pressure. (Sahne et al 2016). The concentrated extract kept in desiccator till use. Standard sample of curcumin obtain from HI-MEDIA Mumbai

Microorganisms used

The *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Salmonella typhi* strains were employed for the present study, for testing antifungal activity *Aspergillus niger and Candida albicans*as test organisms were used. The fungal cultures were maintained on potato dextrose agar (PDA) and preserved on the same medium at 40 ^{o}C. The cultures were sub-cultured periodically (5-7 days) under stationary condition on the same medium at 28 ±20 ^{o}C.(Najah et al ,2015)

Antibacterial Assay

The effect of various plant extracts on (50mg/ml) the several bacterial strains were assayed by Agar well diffusion method. A sterile borer was used to prepare cups of 6 mm diameter in the Muller Hinton medium spread with the microorganisms. 0.1 ml of inoculums (*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Salmonella typhi)* was

spread on the Muller Hinton medium plate by spread plate technique. Accurately measured (30µl/well) solution of each sample (Hexane, Methanol, Chloroform, Acetone and Water extracts) and standard samples (streptomycin) were added to the cups with micropipette. All the plates were kept in a refrigerator at 2 to 8 ºC for a period of two hours for effective diffusion of test compounds and standards (Geetanjali et al, 2016). Later, they were incubated at 37 ºC for 24 hrs. The presence of definite zones of inhibition around the cup indicated antibacterial activity. The diameter of the zone of inhibition was measured and recorded.

Antifungal Assay

The microorganisms; *Candida albicans* and *Aspergillus niger* were used for antifungal assay. The activity of the plant extracts on various fungal strains was assayed by agar well plug method.

Agar Plug Method

Potato Dextrose Agar medium was prepared and poured on to the petriplates. Wells of about 6.0 mm diameter were aseptically punched on each agar plate using a sterile cork borer, Fungal strain viz *Aspergillus niger* and *Candida albicans* (0.1 ml) were aseptically inoculated and evenly spread using sterile glass spreader on the surface of potatoes dextrose agar plate. Add 30µl of the curcumin extracts (Hexane, Methanol and Acetone extracts) were then inoculated into the wells. The plates were then incubated at 37 °C for 72 hrs Flucanazole was used as antifungal control. The diameter of zones of inhibition was recorded in mm.

Results and Discussion

Antibacterial activity

The antibacterial activity of curcumin extract against two Gram-positive (*S. aureus and B. subtilis)* and three Gram-negative (*E. coli, P aeruginosa and S typhi)* was evaluated. The maximum zone of inhibition was seen in Chloroform extract against *P aeruginosa* at the concentration of 1000µl/well and minimum zone of inhibition was seen in Chloroform extract against *S. typhi* and *E.coli* at the concentration of 1000 µl/well The maximum zone of inhibition was seen in methanolic extract against *P. aeruginosa* at the concentration of 1000 µl/well and minimum zone of inhibition was seen in methanolic extract against *B. subtilis* at the concentration of 1000 µl/well (Table 1). The maximum zone of inhibition was seen in hexane extract against *P. aeruginosa* at the concentration of 1000 µl/well and minimum zone of inhibition was seen in hexane extract against *S typhi* and *E.coli* at the concentration of 1000 µl/well. The maximum zone of inhibition was seen in acetone extract *P. aeruginosa* at the concentration of 1000 µl/well and minimum zone of inhibition was seen in hexane extract against *S. typhi* at the concentration of 1000 µl/well. The maximum zone of inhibition was seen

in water extract against *P. aeruginosa* at the concentration of 1000 µl/well and minimum zone of inhibition was seen in hexane extract against *B. subtilis* at the concentration of 1000 µl/well, and Streptomycin used as a standard positive control. *Pseudomonas* is more sensible to non polar to polar extracts. Polar solvent extract exhibit more activity than non polar extract.

Table 1: Antibacterial activity of *Curcuma longa rhizome* extract

Extracts	***B.subtilis***	***E.coli***	***Psudomonas aeruginosa***	***S. aureus***	***Salmonella typhi***
Hexane	13	15	8	6	5
Chloroform	10	4	8	8	8
Acetone	8	5	9	6	4
Methanol	12	10	10	9	6
Aqueous	13	11	11	---	---
Control Streptomycin std.	20	16	15	15	16

(Zone of inhibition given in mm)

Antifungal activity

The antifungal activity of curcumin extract was obtained against two fungal species *Aspergillus niger* and *Candida albicas.* The Hexane, Chloroform and water extracts were not shown the activity against *A. niger* and Acetone extracts shows activity against *Candida albicans*. While the water extracts shows the highest activity against *Candida albican.* (Figure 1). The present results are in accordance with Gupta et al 2015, Nazha et al 2015 and Singh et al 2011.

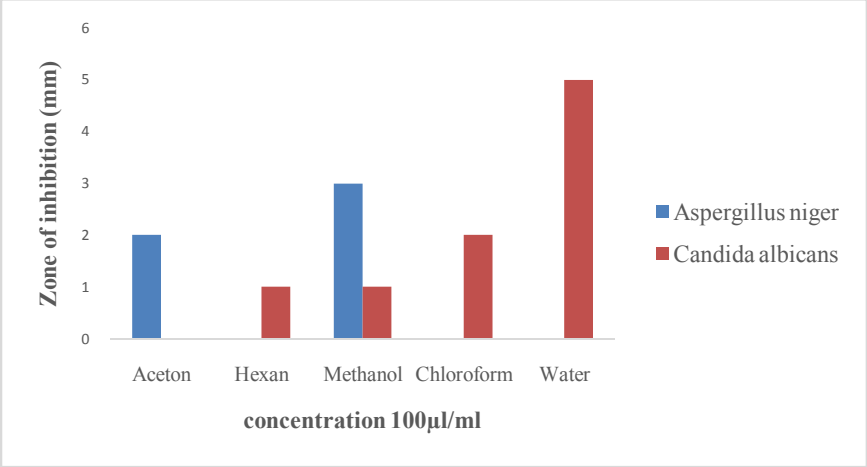

Figure 1: Antifungal activity of *Curcuma longa rhizome* extract

Conclusion

In this study, the antibacterial activity of turmeric extracts in different solvents, such as n-hexane acetone and methanol were evaluated for their inhibitory effect on bacterial strains. The methanolic extract of *C. longa rhizome* is most effective.. In our study the more effective results were shown in the acetone and n-hexane extract of *Curcuma longa* rhizome. The antifungal activity of *curcuma longa rhizome* extracts was evaluated for their inhibitory effect on standard fungal strains of *Aspergillus niger* and *Candida albicans.* The aqueous extract was most effective against *Candida albicans* as compared to acetone, chloroform, hexane and Methanol extract whereas the methanol extract is most effective against *Aspergillus niger* as compare to Acetone, Hexane, Chloroform and Water extract against *Aspergillus niger.* Rhizome of *Curcuma longa* could be best candidate to use it in cosmetic biotechnology to restore skin health and supplement as a skin food. Therefore results of present investigation warrants for preparation of either ointment or cream.

Acknowledgement

We are thankful to farmers of Faizpur village, who gift us *Curcuma longa* rhizome sample.

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Production of extracellular laccase from *Bacillus spp* using agro residue as a potential substrate

Patil Dhanashri, Mahajan Sudharshana, Marathe Jagruti, Sapkale Sushma and Mali Manisha S.*
Department of Microbiology, Post Graduate College of Science, Technology & Research, Jalgaon

Abstract

Laccases are increasingly being used in food industry for the production of cost effective & healthy foods. To sustain this trend widespread availability of laccase & efficient production system has to be developed. Laccase producing bactrerium, *Baciilus subtilis* was subjected to optimization by conventional techniques & was partially purified using ammonium salt precipitation method. The agroresidue substrates used to higher yield of laccase were wheat bran. The enzyme is often associated with lignin peroxidase, Manganese dependant peroxidase, or both because of its importance in bioremediation. Bacterial cultures were screened for laccase positive production by plate test method using the guaiacol indicator.

Keywords: *Bacillus subtilis*, Guaiacol laccase, wheat bran

***Corresponding author:** malimanishas1589@gmail.com

Introduction

Laccases are the members of multi-copper protein family, belonging to the group of blue copper protein. They are widely distributed among plant, fungi, and bacteria. Due to broad substrate, a specificity and ability to oxidised wild range of phenol and poly-phenols. Laccases play vital role in detoxification of textile effluents and bioremediation application. The produced maintain their activity neutral to alkaline condition (Narkhede and R Mahajan, 2013). Ton of laccases from these agro industrial wastes is of great significance owing to its reuse of industrial waste. Most bacterial laccases are highly thermotolerent arising during disposal and reduced can be utilized for various industrial applications. The selection of suitable natural substrate is greatly influence by high lignin contain. So in the present study wheat bran has been selected for the production of laccase. Laccases are widely distributed in nature and have been occured in fungi, plants, and insects and more recently in bacteria and archaea, indicating that the laccase redox process is ubiquitous in nature. Laccase plays an important role in several metabolic steps, including those involved in fungal pigmentation, plant lignin biodegradation humus turnover and cuticles clerotization, where in naturally occurring low molecular weight phenolic compounds and natural fibre polymers are utilized as substrates (Shekhar, 2011).

Materials and methods

Sample Collection:

Soil sample was collected from botanical garden of college campus, farm.

Isolation and characterization

The soil sample was serially diluted (10^{-1}– 10^{-8}) in sterile saline solution. Then, 0.1 ml of appropriate dilution was spread on NA plate.The plates are incubated at 37^0C for 24 hrs.

Selection of natural substrate

Natural substrate such as wheat bran was collected. The substrate where washed 2-3 times with distilled water and boiled for 15 min. The water was then decanted and the substrate where dried in an oven at 60 ^{0}C and powdered .The powder was sieved using a 40 micron mesh and stored. Exactly 2 gram of wheat bran was taken in flask and moistened with 100 ml of Mineral Basal Salt Solution (MBSS). The initial moisture level in the medium was 20%. The flask were sterilised, cooled to room-temperature and inoculated with *Bacillus* followed by incubation for 24 hrs. After incubation, 5 mm glycin- NaOH Buffer under shaking condition and centrifugation at 10,000 rpm for 10 min.

Optimization of media

Effect of incubation period on production

20 ml of MBSS and 0.16 of wheat bran were taken in each test tube. The flask were sterilized Cool to room temperature use and inoculated with Bacillus and incubated at different at different time intervals namely 24, 48, 72, 96, 120 and 144 hrs respectively at room temperature. The contents of the flask were centrifuged at 10,000 rpm for 10 min and the supernatant was used to assay the enzyme activity at 420 nm.

Effect of temperature on production

20ml of MBSS and 0.16 of wheat bran were taken in each tube. The tubes were sterilized, cooled to room temperature and inoculated with *Bacillus* and incubated at different temperature (25, 30, 35, 40, 45 & 50 ^{o}C) for 96 hrs. The content of the tubes were centrifuged at 10,000 rpm for 10 min and the supernatant was used to assay the enzyme activity at 420 nm.

Effect of pH on production

The influence of hydrogen ions on biological activities is related to their hydrogen ion concentration on enzyme activity. The 20 ml of MBSS and 0.16 gm of wheat bran were taken in each tube and pH was adjusted in each of the test tube from 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 respectively by addition 1N HCL and 1NaOH. The flasks were sterilized cooled to room temperature and inoculated at optimized incubation period (96 hrs) and temperature 40^{o}C. The

contents of the flask were centrifuged at 10,000 rpm for 10 min and the supernatant was used to assay the enzyme activity at 420 nm.

Effect of carbon source

Varying concentration (1-5%) of carbon source, namely glucose, maltose, sucrose, starch in tubes containing 20 ml of MBSS medium with 0.16 gm of wheat bran. Flask were sterilized, cooled to room temperature and incubated at optimized incubation period (96 hrs). The contents of the tube were centrifuged at 10,000 rpm for 10 min.

Effect of nitrogen source

The production medium was enriched with varying concentration (1-5%) of inorganic and organic nitrogen sources, namely ammonium sulphate, sodium nitrate, potassium nitrate, peptone, and beef extract in separate tubes containing 20 ml of MBSS medium with 0.16 of wheat bran. The flasks were sterilized cool to room temperature and Bacillus inoculated in each tube. The tubes were incubated at optimized incubation period. Centrifuged at 10,000 rpm for 10 min and the supernatant were used to assay the enzyme activity at 420 nm.

Determination of laccase activity

Laccase activity was measured by monitoring the oxidation of 1 mm guaiacol buffered with 0.2 M sodium phosphate buffer at 420 nm for 1 min. The reaction mixture contains 300 µl of 1 mm guaiacol, culture filtrate, and 0.2 M sodium acetate buffer. One unit of enzyme activity was defined as the amount of enzyme that oxidised 1 µmol of guaiacol per minute. The enzyme activity was expressed in µg/ml.

Partial Purification

Enzyme supernatant was subjected to protein fractionation by 70% ammonium sulphate precipitation.

Immobilization

Purified lipase was immobilized on sodium alginate beads by entrapment method. Two ml of purified was suspended in 10 ml of 3 % (w/v) sodium alginate solution (3 gm dissolved in 100 ml warm distilled water). Alginate drops were solidified upon contact with $CaCl_2$ forming beads. The activity of the immobilized enzyme and activity of free enzyme were calculated.

Results and Discussion

Screening of laccase producing organism

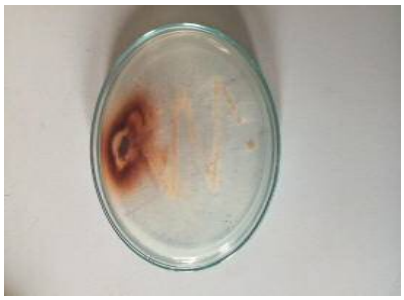

Figure 1: Test for screening of laccase

Laccase producers were isolated using solid media, containing 0.02 % guaiacol as an indicator compound The oxidative polymerization of guiacol to redish brown zones in the medium indicate laccase production.

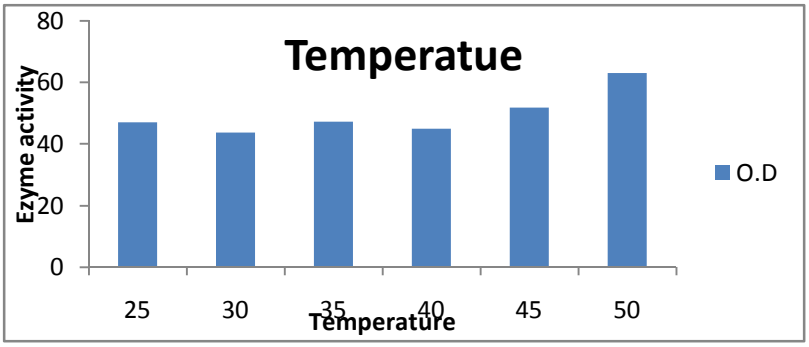

Figure 2: Effect of Temperature of Laccase activity

The isolated bacteria show higher laccase activity of 63.01 µg /ml /min at temperature 50 ^{o}C

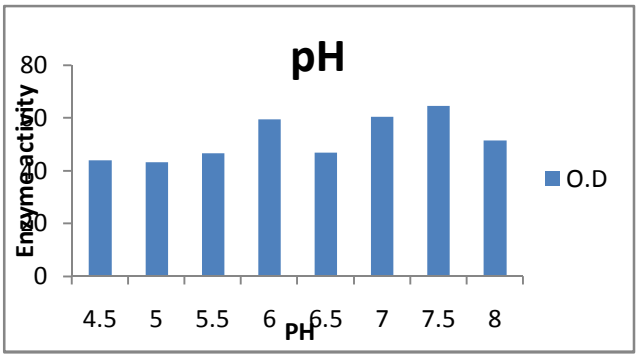

Figure 3: Effect of pH on laccase activity

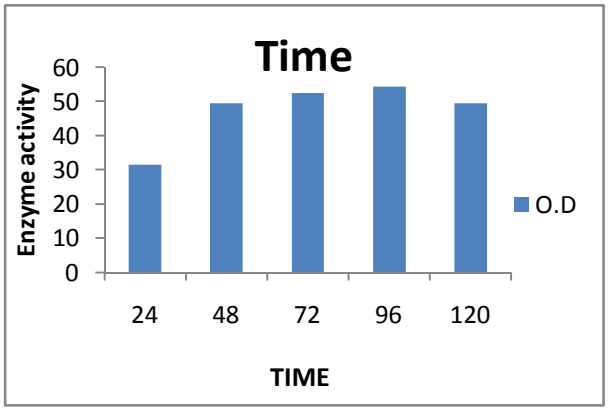

Figure 4: Effect of time on on laccase activity

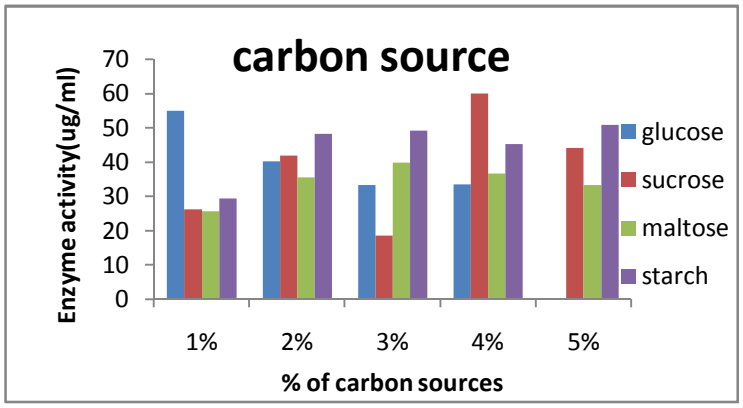

Figure 5: Effect of carbon on laccase activity

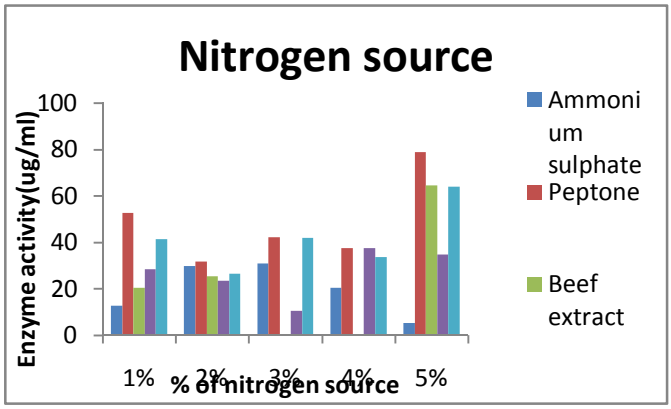

Figure 6: Effect of nitrogen on laccase activity

The Optimization studies suggested that isolated bacteria show higher laccase activity of at p^H 7.5, preferred carbon source sucrose, nitrogen source optimization showed that peptone was the ideal condition for laccase production by selected isolates. Our results are agreed with the reports of Shekhar, 2011, Poonam 2009, and Mahajan et al 2013.

Conclusion

- Laccase producing bacterium *Bacillus subtilis* isolated and subjected to optimization by conventional techniques and precipitation method.
- The agro residue substrate used for higher yield of lassase was wheat bran achieved at temperature 50 ^{0}C.
- The carbon & nitrogen sources resulted in high enzyme yield at 4 % sucrose & 5 % peptone.
- The laccase exhibited optimal activity at 50 ^{o}C.

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Studies on Lipase Producing Organisms from dairy waste

Saindane Meenakshi R, Patil Komal S, Saindane Jagruti S Patil, Rohini G and Patil Sandip N*
Department of Microbiology, Post Graduate College of Science, Technology & Research, Jalgaon

Abstract

Microbial lipases constitute an important group of biotechnologically valuable enzymes, mainly because of the versatility of their applied properties, ease of mass production and low production cost. This study was carried out with an objective to investigate the lipase production potential of bacteria, immobilization of enzyme and optimization of media. Bacterial cultures were isolated from fermented food samples and screening of isolate was done for lipase production using phenol red indicator. Organisms showing clear halo around colony was selected for study. The Isolate was capable of producing enzyme at pH 7.5, temperature 35℃, carbon source as glucose and nitrogen as yeast extract.Immobilization of enzyme in sodium alginate bead exhibited more enzyme activity as compared to free enzyme activity.

Keywords: Lipase, immobilization

*Corresponding author: snpatil012@gmail.com

Introduction

Lipases (EC 3.1.1.3), triacylglycerol hydrolases, are a group of enzymes that catalyse the hydrolysis of acyl glycerides and other fatty acid esters under aqueous conditions and the synthesis of esters in organic solvents (Angkawidjaja,2006). These enzymes also catalyse the exchange of ester bonds (transesterification) when present in nonaqueous media. Lipolytic enzymes are involved in the breakdown and thus in the mobilization of lipids within the cells of individual organisms as well as in the transfer of lipids from one organism to another (Beisson et al., 2000) Some important lipase producing bacterial genera include *Bacillus, Pseudomonas And Burkholderia* (Gupta et al., 2004). Based on three-dimensional structure of various lipases, all have been classified as serine hydrolases. Peoples are using a number of enzymes in the food isolated from microorganisms and lipase is one of them.

Material and methods

Screening and isolation of lipase producing organism

Collection of sample

Samples of dairy waste were procured from local dairy from Jalgaon. The care was taken to avoid gross contamination with environmental material. One gram of sample was dissolved in 1 ml of peptone water (pH 7.0) plating of 0.1 ml aliquots of appropriate dilutions (up to 10−5) was done aseptically on nutrient agar plate and incubated at 37 °C for 24 hrs. The isolated stain was screened for the production of lipase enzyme by streaking the isolated stains on selective MRS media with phenol red indicator and incubated at 37 ^{o}C for 24-48 hrs and observe zone of clearance. The obtained isolate characterized by colony morphology, gram staining and biochemical test (Bergey, 2005).

Optimization of media (Effect of pH, Temperature, Carbon and Nitrogen)

Various pH 7.0, 8.0, 9.0, 10 adjusted with 1N HCL and 1N NaOH solutions per requirements.

0.1 ml of cultures was inoculated in each tube Incubation was made at 37 ℃ temperature for 24 hrs and the by enzyme activity was measured. Effect of temperature was studied by incubating media at various temperatures (Dalmau et al, 2000). After 48 hrs, the enzyme activity was measured. Similarly, effect of different nitrogen and carbon sources on enzyme production was studied by adjusting mineral salt medium with various nitrogen and carbon sources.

Measurement of Enzyme activity by Titrimetric method

The 1m of tris HCl buffer (1ml) + deionized water (2.5 ml) in reaction vessel was taken.

3 ml of olive oil as a substrate and 1 ml of supernatant as enzyme were added to it. After incubation, 3 ml of 95 % ethanol was added to stop the reaction and 3-4 drops of phenolphthalein as an indicator. This reaction mixture was titrated against 50 mm NaOH.(Beisson,2000)

Calculations

$$\frac{Units/ml of Lipase \;=\; (NAOH)\,(Molarity of NaOH)\,(1000)\,(2)\,(df)}{(1)}$$

(NaOH) = volume (in ml) of reagent used for test – volume (in ml) of reagent used in blank

1000 = conversion factor from milli equivalent to micro equivalent

2 = time conversion factor from 30 min to 1 h (unit definition)

Df =dilution factor

1 = volume (in ml) of enzyme used

Production and partial characterisation of Lipase

Mineral Basal salt medium was optimised and inoculated with 4% of inoculums of isolated strain and incubated for 48 h. Broth was centrifuge and supernatant was collected and subjected to protein fractionation by ammonium sulphate precipitation.

Enzyme immobilization

Purified lipase was immobilized on sodium alginate beads by entrapment method. The activity of the immobilized enzyme and activity of free enzyme were calculated.

Results and Discussion

Screening and isolation of lipase producing organism

The isolate showing clear zones of yellow orange halos which indicated the production of the lipase enzyme was isolated. Olive oil used as a substrate for screening of lipase producing strain.

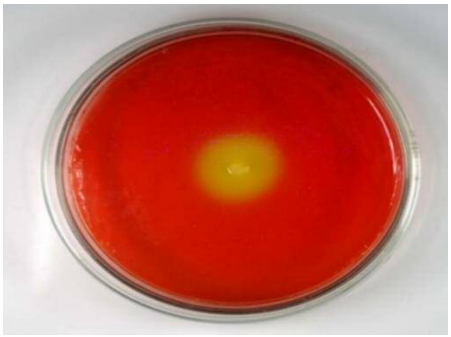

Figure 1: Screening of Lipase producing strain

Identification of isolate

Morphological characteristics on media, staining reactions and biochemical characteristics of isolate, the strain was found to be *Lactobacillus sp*. We refereed specification and parameters for identification of microorganisms (Aneja, 2013)

Optimization of media

Table 1: Optimization of carbon source

Parameter	Carbon source (%)	1	2	3	4	5
Enzyme activity (u/ml)	Glucose	11.65	12.04	13.54	10.82	15.31
	Sucrose	8.32	9.38	8.60	8.10	5.31
	Lactose	8.43	13.85	8.76	9.10	11.04

Media components were optimized by different % of carbon sources. 5% glucose showed higher lipase production.

Table 2: Optimization of nitrogen sources

	Nitrogen source in g%					
Enzyme activity (u/ml)		**1**	**2**	**3**	**4**	**5**
	Yeast extract	**5.38**	**8.94**	**9.30**	**10.02**	**13.28**
	Urea	**4.89**	**5.15**	**5.06**	**4.59**	**4.63**

Media components were optimized by different percentage of nitrogen sources. 5 % yeast extract showed higher lipase production.

Table 3: Optimization of pH

	pH							
pH	**4**	**4.5**	**5.5**	**6.0**	**6.5**	**7.0**	**7.5**	**8.0**
Enzyme activity (u/ml)	**0.17**	**0.22**	**0.27**	**0.96**	**0.62**	**0.64**	**64.64**	**51.58**

Table 3 exhibits enzyme work best at alkaline pH which is not true for pH below than 7.

Table 4: Optimization of temperature

Temperature						
Temp (℃)	**25**	**30**	**35**	**40**	**45**	**50**
Enzyme activity (u/ml)	**47.01**	**43.75**	**63.01**	**45.02**	**51.84**	**47.31**

Bacterial strain showed higher yield of lipase activity at temperature 35 ℃. Less growth was observed above 45 ℃ as high temperature inhibits the growth of isolate.

Partial purification

The salt precipitated sample gave lipase activity after the dialysis. Also the activity of dialyzed enzyme was higher than that of initial activity. In this study primary investigation is base on titrimetric method for assay of lipase from microorganism for further study adaption of spectrophotometric method is warranted.

Table 5: Partial purification of enzyme

Fold purification	**Initial**	**Ammonium sulphate precipitate**	**Dialysed protein (U/ml)**
Enzyme activity (U/ml)	**63**	**120**	**823**

Conclusion

- Lipase producing organism was screened and isolated.
- Morphological and biochemical characteristics of organism reveal that it belongs to genus *Lactobacillus.* The isolate is able to produced optimum lipase at pH 7.5, temperature 35 ℃, carbon source as glucose and nitrogen as yeast extract.

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Applications of medicinal plants for oral health care

Chaudhari Krutika A., Patil Tejaswini D, Patil Reshma G Gosavi Samrudhi S and Bhat Diksha*
Department of Microbiology, Post Graduate College of Science, Technology and Research, Jalgaon

Abstract

In this modern era, the knowledge and experience of usage of herbs are being blend with advanced oral health to develop a safe and effective product. The objective of the present work was to study the antibacterial activity of herbal medicinal plants for oral health care. The total 5 species of oral care medicinal plants, distributed among genera belonging to 5 families were studied for antimicrobial activity. The species were belonging to Asteracece, Euphorbiaceae, Meliaceae, and Theacae. Plant leaves were the dominant part in oral care uses following by stem, bark, root, methanol extracts of *Madhuca longifolia* and *Camellia sinensis*, the *Emilia sonchifolia, Jatropha gossypholia,* and *Azadirachta indica* extracts (juice) is used in the antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Streptococcus* and *Salmonella typhi*. These microorganisms isolated from infected tooth or oral swab. The Muller Hinton agar was used in the antimicrobial activity of organisms. The antimicrobial activity of plants was studied against the isolated organisms. The plants under study showed the antimicrobial activity against these organisms and the highest antimicrobial activity obtained for the *Bacillus subtilis.*

Keywords: medicinal plant, oral health

*Corresponding author: dikshabhat96@gmail.com

Introduction

The use of traditional medicine for treating human diseases remains widespread in many parts of the world. World Health Organization (WHO) estimates that approximately 80% of the population in low-income countries relies mainly on traditional medicines for their primary healthcare (WHO, 2002a). Medicinal plants have many medicinal properties due to the antioxidant materials and used for control of poisoning and medical and pharmaceutical errors by pharmaceutical companies and used for any disorders etc. These plants are used for the dental care like toothache; tooth decay and pyorrhea are the common dental problems of the mouth observing the area (R. T. Mahajan et al 1999). Tooth decay is caused by intra oral factors such as

dental plaque of food and bacteria sticking to teeth, anatomy and position of teeth. On the basis of literature, we selected *Emilia sonchifolia* (L.) DC. (Asteraceae) *Sadhimandhi, Azadirachta indica A. Juss (*Meliaceae) *Neem, Jatropha gossypifolia L*. (Euphorbiaceae) Chandrajyot, *Madhuca longifolia* (Koenig) Macbride (Sapotaceae) Mahuv, *Camellia Sinensis* (Green Tea). Some of the plants have folkloric reputation to heal dental plaque, control bad breathe. The selection of solvent and plant part is based on earlier report.

Methods and material

Collection of plants material

The leaves, bark and stem were collected from the local area of Jalgaon. The *Emilia sonchifolia, Camellia sinensis and Azadirachta* indica leaves were collected and *Jatropha gossypifolia*, *Madhuca longifolia* stem were collected. The plant parts were sun dried and pulverized into course powder samples were sieved and stored in bottle until when required for use.

Preparation of aqueous extracts, from *Azadirachta indica, Emilia sonchifolia, Jatropha gossypifolia*

The plants extracts were prepared using the mortar pestle. Firstly plants leaves are collected and washed it and then the leaves grinded in mortar pestle. Mixture was then filtrated through Whatman No.1 filter paper. Extracts were stored in refrigerator.

Preparation of *Camellia sinensis* and *Madhuca longifolia* plants extract

The dried material of plant was grinded into powder using grinder. The powder obtained and was extracted in methanol in ratio (1:1) by using boiling water bath for 8 hrs. Filtration of extract was done by using simple filter muslin cloth.

Isolation of organisms from mouth sample

Oral swab samples were collected from dental hospitals from Jalgaon, followed by isolation on selective media. The isolated strains were identified by colony morphology, gram staining and biochemical characteristics of isolate. The observations were compared with specification, parameter and characteristics given by Aneja 2016.

Determination of antimicrobial activity

The Muller Hinton plates were prepared and seeded with the test organisms. Wells of 6.0 mm diameter each were made in the plates with a sterile cork borer and filled with 1mg /ml extracts respectively. The inoculated plates were allowed to congeal for 30 min to allow pre diffusion

time and incubated at 37 °C for 24 hrs. The plates were examined for evidence of zones of inhibition which appear as a clear area around the holes. The streptomycin was a positive control for bacteria and used solvent was negative control.

Following plants were used for the oral health care, *Azadirachta indica, A.Juss*. (meliaceae) neem: Toothbrush of neem stem is valued for healthy teeth and gums; paste or juice of stem is applied for swelling or bleeding of gums.

Jatropha gossypifolia (L) (euphorbiaceace) chandrajyot: Small stem is used as toothbrush to cure toothache.

Madhuca longifolia (Koenig) macbride (sapotaceace) mahuv: Toothbrush of small stem is used to cure toothbrush, emerging in mustard oil to cure toothache. (Badgujar, 2008 , Achuta, 2010)

Emilia sonchifolia (L) DC (Asteraceae) sadhimandhi.: Juice of leaves is applied to toothache.

Camellia sinensis (theaceae) green tea: The green tea leaves used against oral microorganism. It is boon to health (Gupta et al, 2013).

Results and Discussion

Antimicrobial screening

Neem stem is commonly used and adopted it for to justify the experimental results *Emilia sonchifolia* , *Madhuca longifo, Jatropha gossypifolia, and Camellia sinensis L though* reported in literature to control the tooth decay and appeared as a text , no experimental evidences were available therefore we assessed in vitro antibacterial potential of them against the organisms isolated from tooth decay. MIC determination and further thoroughly experiments are required to utilize them in toothpaste. Test strain of bacteria were found to be sensitive to streptomycin and methanol was used to the negative control which did not show any zone of inhibition against tested bacteria .result of the agar well diffusion method are shown in table. The extract exhibited antibacterial activity against all the tested bacteria. The 1mg/well extract exhibited significant antimicrobial activity on the *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, and Streptococcus mutan.* On the basis of result four new plants as reported to control the tooth decay would be the good candidate for controlling the tooth decay. Our results are more or less similar to results of earlier authors Joshi et al, 2011, Wishwas, 2002.

Table 1: antimicrobial activity of some medicinal plants

Name of organisms/ diameter of zone (mm)	*Madhuca longifolia*	*Jatropha gosypifolia*	*Emilia sinesis*	*Camelia sinesis*	*Azadirachta indica*
Bacillus subtilis	16	10	4	7	20
Staphylococcus . aureus	12	10	8	14	19
Peudomonas aeruginosa	12	12	10	14	10
Streptococcus sp	15	15	12	10	15
Salmonella typhi	--	16	14	16	14

Zone of inhibition in mm

Conclusion

- The methanolic extracts of *Madhuca longifolia* and *Camellia sinensis*, *Emilia sinensis, Jatropha gossypifolia and Azadirachta indica* were extracted in appropriate solvent.
- Microbes mouth sample were identified as *Staphylococcus spp, Pseudomonas spp, Bacillus spp, Streptococcu spp and the Salmonella spp.*
- The antimicrobial activity of plants extracts against the isolated organisms show an variation in antimicrobial activity against these organisms and the highest antimicrobial activity obtained for the *Bacillus subtilis.*
- Are the above said plants are really act as a phytobiotics or not? Answer to these questions provide new for further experimentations.

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Isolation and characterization of Bacteriocin producing Probiotic organisms

Chavhan Priyanka D., Patil Hema A., Sonar Hiral R., Sonawane Shubhangi M., Patil Sandip*
Department of Microbiology, Post Graduate College of Science, Technology & Research, Jalgaon

Abstract

Probiotics are the beneficial microorganisms that modulate intestinal environment and thus improve host health. Lactic acid bacteria have been extensively used as probiotics because of their fermentative ability as well as health and nutritional benefits. Present research work investigated isolation of a Bacteriocin producing probiotic lactic acid bacterium. Lactic acid bacterial cultures were isolated from fermented food samples and were tested for probiotic characteristics, antibiotic susceptibility, and haemolytic activity and Bacteriocin production. The isolate was evaluated for bile tolerance, acid tolerance, haemolysis, antibiotic resistance, and anti-gastrointestinal pathogenic activity Strain showed excellent tolerance to the highly acidic condition (pH 2), and pepsin, lysozyme of the simulated synthetic gastric juice. They showed ability to resist highest bile salt concentration (1.0 %). The strains under study were found to produce remarkable amount of bacteriocin under optimised conditions.

Keywords: Probiotics, Lactic acid bacteria

*Corresponding author: snpatil012@gmail.com

Introduction

The word ‘probiotic’ comes from Greek language ‘pro bios’ which means ‘for like’ opposed to ‘anitibiotic’ which means against life. Probiotics can be used in the treatment and prevention of enteric infections and chronic inflammatory disorders of the gastro intestinal track (GIT). They are non-pathogenic, acid and bile tolerant, adhere to gut epithelial tissue and produce antimicrobial substances (Bali et al 2011), including organic acids, hydrogen peroxide and bacteriocins. Bacteriocins are ribosomally synthesized antimicrobial peptides produced by one bacterium that are active against other bacteria, either in the same species (narrow spectrum), or across genera (broad spectrum) and, as with host defence peptides, cell signalling mechanisms also be involved’ The bacteriocins are of very high perspective because not only may they be used for food bio preservation but also they have a potential to be utilised as antibiotics(Cleverland et al 2001), exploited in animal health care and marine environmental (Cotter et al. 2013 and Ananou 2007). Bacteriocins are proteinaceous antibacterial compounds

that are bactericidal to many pathogens associated with food spoilage and food borne illnesses (Leroy et al, 2001).

Material and methods

Isolation and screening of Bacteriocin producing microbes

Sample collection

Samples of dairy waste, sweet lime Juice, Honey and curd were collected from a local market in Jalgaon. The care was taken to avoid gross contamination with environmental material. One gram of sample was dissolved in 1 ml of peptone water (pH 7.0). Plating of 0.1 ml aliquots of appropriate dilutions (up to 10−5) was done aseptically on nutrient agar plate and incubated at 37°C for 24 h (Rodgers et al 2002). Screening of bacteriocin producer was done by using the gram positive and gram negative bacteria indicator strains, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas, Salmonella typhi,* and *Proteus* were used. The colonies were selected which shows zone of inhibition against microbial strain.

Identification of isolates

The selected strain was identified by colony morphology, gram staining and biochemical characteristics of isolate (Sharpe, 1996).

Evaluation of Probiotic Properties of isolates

Bile salt tolerance

Bile tolerance of isolate was carried out by growing the isolated cultures in MRS agar containing 0.1, 0.3, 0.5, 1, 2, 3, 4 and 5% of bile salt concentration at 37^0C for 24 hours under static condition.

Acid tolerance

Acid tolerance assayed by sterile nutrient agar medium adjusted to pH 1, 2, 3, 4, and 5 with 0.1 N HCL. 0.1 ml culture suspension of each isolate was spread on the respective plate. These plates were incubated at 37^0 C for 24 hours. Isolates which were growing on agar were considered to be acid tolerant strains.

Gastric Juice Tolerance

To determine the effect of gastric juice 0.9ml artificial gastric juice was transferred to sterile test tube and 0.1ml culture of each isolate was added into it separately and mixes gently. At time intervals of 0, 30, 60, 90, 120, 180, and 240 minutes 0.1ml of culture suspension was drawn and

spread on the sterile MRS agar plates respectively. The plates were incubated at 37^0C for 24 hours. Isolates which were growing on agar were considered to be tolerant strains.

Antibiotic Sensitivity Test

The susceptibility of the isolate to tetracycline, ciprofloxacin, gentamycin, co-trimoxazole, nitrofurantoin, streptomycin, ampicillin and Colistin were determined in Oxoid Muller Hinton (MH) agar plates with octadisc (Himedia).

Haemolytic activity

Hemolytic activity of strains was determined by streaking the isolates on Blood agar plate containing 5 % blood.

Extraction and partial purification of Bacteriocin

The pure culture of isolate were propagated in M.R.S broth and incubated for 48 hours at 37 ^{o}C. Bacterial cells were separated by centrifugation at 10000 rpm for 12 min. The supernatant of each sample was collected and partially purified by ammonium sulphate precipitation at 80 % (w/v) saturation. Then, mixture was centrifuged at 10000 rpm, 4°C for 30min and the pellets obtained containing proteins were resuspended in 2 ml of potassium phosphate buffer and dialyzed against the same buffer for 24 hours at 4°C in the dialysis tubing. The partially purified Bacteriocin were collected in sterile containers and stored at 4°C.

Results and Discussion

Isolation of microorganism from Probiotic food samples

Bacterial isolates were obtained from honey, curd, sweet lime juice by screening on MRS agar medium .Isolates were maintained on MRS agar at 4^oC and transferred to fresh medium after every 15 days.

Identification of organisms

Isolate was identified as *Lactobacillus sp* by Morphological characteristics, biochemical's, sugar fermentation and gram staining as per Aneja's protocol.

Evaluation of probiotics properties of isolates

Acid tolarance of isolate

Acid tolerance is an important criterion of probiotic microbe. Only acid tolerant probiotic microbes can survive in acidic condition of gasrtointestinal tract. The isolate was found to survive at pH 2. No growth was observed at pH 1.2.

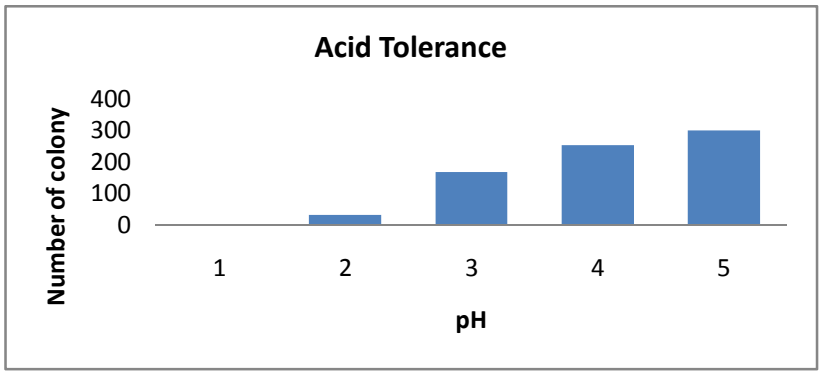

Figure 1: Acid tolerance

Bile salt tolerance of isolate

Isolate survive up to 1% bile salt solution. As concentration of bile salt increase above 1% viability (tolerance) of isolate, ceased drastically

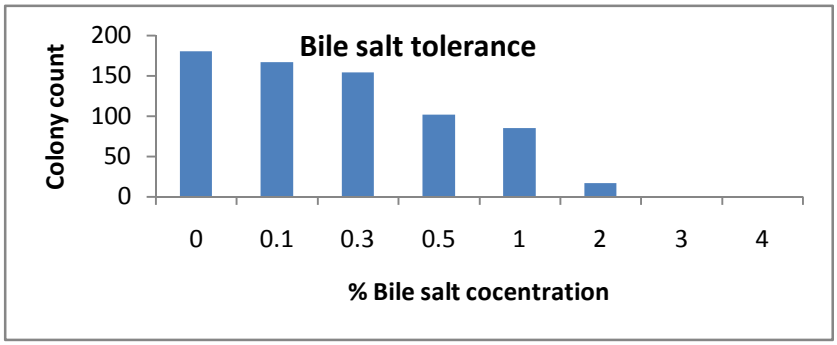

Figure 2: Bile Tolerance

Gastric Juice tolerance

The isolate were able to tolerate gastric environment up to 150 min in per ml of artificial gastric juice after the incubation.

Antibiotic sensitivity test

The isolate exhibit resistance to tetracycline, ciprofloxacin, gentamycin, co-trimoxazole, nitrofurantoin, streptomycin, ampicillin and is sensitive to Colistin.

Hemolytic Activity

The isolate shows no hemolytic activity on human blood agar plate after the 24 hours of incubation at 37 ^{o}C.

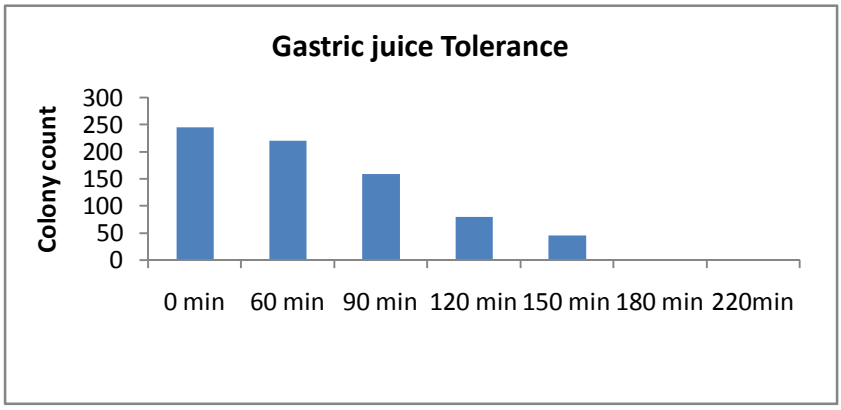

Figure 3: Gastric Juice Tolerance

Conclusions

- An efficient Bacteriocin producing probiotic culture was isolated from food sample.
- The isolate satisfies all essential characteristics of probiotic organism such as High Acid Tolerance, High Bile Salt Tolerance, and Non-hemolytic in nature, Resistant to antibiotics.
- The effective isolate was characterized phenotypically, the isolate was found to be Lactobacillus Sp.
- Characterization of isolated bacteriocin chemically is advisable to justify its commercial value

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Statistics

Statistical Assessment of Farmer Suicide in Jalgaon District for 2012-2018

Thakur Aishwarya, Bagwan Tausif, Wagh Akash and Wagh Yogita*
Department of Statistics, Post Graduate College of Science, Technology & Research, Jalgaon

Abstract

India is an agrarian country with around 70% of its people depending directly or indirectly upon agriculture. Farmer suicide account 11.2% over committed incidents in India. The aim of this study was to identify the socio-economic strata of farmers committing suicides and evaluate the proximate reasons behind these suicides. The pattern and distribution of framers' suicides for the data during year 2012-18 has been studied. Results of present study reveals that 41-45age of farmer is sensitive, higher incidents happened in Parola taluka and land holder's group is 0-5 acre. However, this study of circumstances reveals multiple risk factors – economic downfall, agrarian crisis, social disgrace among others.

Keywords: Farmers suicide, Agriculture, Chi-Square Test for Independence

*Corresponding author: yvj.jalasri@gmail.com

Introduction

Jai Jawan, Jai Kisan - Lal Bahadur Shastri, This slogan of a visionary prime minister had lost its potential over the time. Suicidal behaviour is a major problem across the world. Suicide is a conscious act and concerned person is fully aware about its results.

India consisting of 16% of world's population sustains only on 2.4% of land resource. Agriculture sector is the only livelihood to the two-third of its population which gives employment to the 57% of work force and is a raw material source to large number of industries. Despite of portrayal of farming as a healthy and happy way of life, agriculture sector experiences one of the highest numbers of suicides than any other industry. Farmers' suicide is not only reported in Vidarbha region of Maharashtra, but also from Punjab, Uttar Pradesh, Kerala, and Karnataka. Many enquiry commissions were formed and recommendations were implemented especially in Punjab. The problem of suicide is not only reported in India, but also reported in different parts of the world like England and Wales (Gruere & Sengupta 2011)

The first state where suicides were reported was Maharashtra. Soon newspapers began to report similar occurrences from Andhra Pradesh. In the beginning it was believed that most of the suicides were happening among the cotton growers. More than 1 lakh farmers have taken their lives since 1997. 86.5 percent of farmers who took their own lives were financially indebted. Their average debt was about Rs. 50000. On an average, there has been one farmer's suicide every 32 minutes since 2002. In farming families it is mainly the bread-earning men who have

committed suicide. Mishra et al 2006elaborated their study and analyzed 192 news reports in a Marathi daily news paper.

The issues of agriculture sector of Maharashtra

Seasonal, uneven, uncertain and ill timed rainfall is one of the important issues in Maharashtra. Almost 80 percent of farmers are dependent on rainfall for cultivation. The high deficiency of farm labour in peak season and mass unemployment during lean season leads to migration of landless and marginal farmers rather than only male earners. There is heavy pressure of dependent and increasing fragmentation and division of land. Large number of uneconomic holdings and it leads to confusion of weather doing farm business or not among the farmers community. Cost of production exceeds over the value of production of various crops is burning issue in Maharashtra.

Objectives

- To study the proximate reasons behind these farmers suicides?
- To identify the pattern, distribution of Suicides.
- To study the yearly distribution of suicides

Data collection and description

The data was collected from Municipality of Jalgaon district for year 2012-2018 under the 'Right to Information' Act. There are total 898 observations. There are total 15 talukas in Jalgaon district that are Parola, Amalner, Chalisgaon, Jamner, Pachora, Chopda, Dharangaon, Bhadgaon, Jalgaon, Muktainagar, Erandol, Raver, Yaval, Bodwad, Bhusawal. The data include the following Variables:

Farmers Name: The name of farmer who committed suicide.

Address: This includes the name of the taluka from Jalgaon district in which the farmer committed suicide.

Age: This column shows the age of the farmer when he died.

Incident Date: It is the date on which farmer committed suicide. This gives every specification about the day i.e. exact date, month and year of suicide day.

Way of Suicide: 'How farmer committed suicide?" the various ways accepted by farmers to commit suicides are

Poison	Train Accident
Hang	Drunk
Drowning	Shock
Burn	Wrist Cutting

Land: Total land belongs to farmer which is measured in Acre.

Loan Amount: Total Loan amount taken by farmer either by bank or by any other private contacts.

Nominee: Family members after the death of family's head.

Sub Divisional Opinion: This is the opinion given by sub divisional officer on Farmers suicide after considering all the constraints if the farmer suicide is because of Indebtness or not. If yes then the opinion will be passing otherwise Fail.

Police Opinion: This shows police opinion on farmer suicide

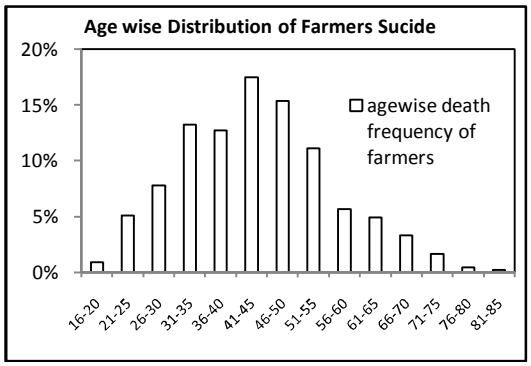
Farmers Society Opinion: This committee either approves or denies the farmers death is because of unpaid debts.

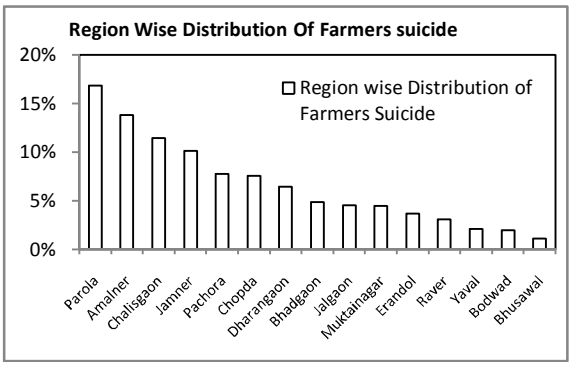
Codes used in Data:

F: Not approved P: Approved W: Pending Opinion.

Materials and Methods

To study the age-group and region-wise distribution of farmers suicide the following graphs plotted.





70% of farmers committed suicide between age 31 to 55 and Majority of the farmers' was from the region Parola, Amalner, Chalisgaon, and Jamner.

The distribution of farmers who committed suicide has Land (in Acre)

Land (Acre)	0-5	5-10	10-15	15-20	20-25	25-30	35-40	125-130	135-140	160-165	165-170	360-365	Grand Total
F	670	162	38	18	3	1	1	1	1	1	1	1	898
%F	75%	18%	4%	2%	0%	0%	0%	0%	0%	0%	0%	0%	100

We observed that the percentage of farmers who committed suicides having the 0 to 5 acre of land was 75%.

Farmers adopted the means for committing Suicide

Type of Suicide	Poison	Hang	Drowning	Burn	Train Accident	Drunk	Shock	Wrist Cutting	Grand Total
Count	467	309	90	16	13	1	1	1	898
Percentage	52%	34%	10%	2%	1%	0%	0%	0%	100

96% farmers committed suicide by poison, hang and drowning.

- **Chi-Square Test for Independence**

a) H_0: Sub-divisional opinion and police opinion on farmer suicide are independent.

H_1: Sub-divisional and police opinion on farmer suicide are not independent.

Rows: Subdivision Opinion Columns: Police

	F	P	ALL
F	325	163	488
P	82	328	410
ALL	407	491	898

Pearson Chi-Square = 195.229, DF = 1,p-value = 0.000

Likelihood Ratio Chi-Square = 204.994, DF = 1, p-value = 0.000

As p-value < 0.05, We reject null hypothesis i.e. Sub-divisional opinion and police opinion are dependent.

b) H_0: Police and Farmer society opinion on farmer suicide are independent.

H_1: Police and Farmer society opinion on farmer suicide are not independent.

	F	P	PENDING	ALL
F	357	47	8	407
	74.64	93.994	0.043	
P	140	340	11	491
	61.871	77.913	0.036	
ALL	492	387	19	898

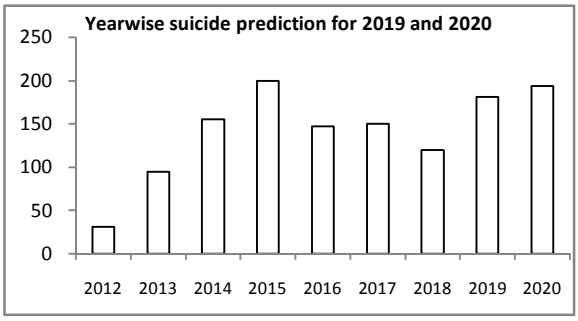
Pearson Chi-Square = 308.497, DF = 2, *p*-value = 0.000

Likelihood Ratio Chi-Square = 337.289, DF = 2, *p*-value = 0.000

p value < 0.05, We reject null hypothesis of police and farmer society opinion are dependent.

- **Time Series: The time series is a statistical tool used for prediction.**

Since, we assume that there is linear relationship between year and number of farmer suicide. If the conditions are not changed then the number of farmer suicide will eventually increase.



- **Correlation between land (acre) and loan amount** = 0.064

The correlation between Loan amount and land (acre) are not strongly related. The farmers who are having land in 0-5 acres have mostly taken loan below one lakh and committed suicide.

Conclusions

The important reason of farmers' suicides can be classified as Indebtedness, Family Disputes and Addiction and Health related Problems. Indebtedness and consequent financial distress causing damage to social status in turn creating difficulties in arranging marriage of daughters or sisters, are overlapping reasons and as such can be classified under a single group of factors indebtedness. Family disputes constitute the second group, while the third group comprise

addiction and health-related problems. Of these three groups, the most dominant one is of course, the indebtedness.

In Jalgaon district mostly 70% of farmers committed suicide between the age group of 31 to 55. Majority of the farmers' was from Parola, Amalner, Chalisgaon, and Jamner talukas. The maximum percentage of farmers who committed suicides have 0 to 5 acre of land. Farmers adopted the means for committing Suicide was poison, hang and drowning.

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The Statistical Analysis of Various Characteristics of Drip-Line Plant

Baviskar Amol B., Patil Sneha A., Bonde Bhagyashri V. and Wagh Yogita S.*
Department of Statistics, Post Graduate College of Science, Technology & Research, Jalgaon

Abstract

The aim of this study was to evaluate drip irrigation as a process, by monitoring the average flow applied by the emitter using tools of statistical quality control. Two kinds of drippers were selected, inline Low-density polyethylene (LDPE) type and online. The selected quality parameters of drip pipes was thickness, Outer Diameter, Weight, RPM and load of machine of drip line round pipe bundle. Our parameters of interest were thickness, Outer Diameter and weight (bundle). For measuring these parameters, instruments used were Digital micrometer and Vernier Calliper. We studied the process and observe that the process is in control for thickness and weight. The performance of process is studied through process capability analysis and conclude that process is highly potential capable for weight.

Keywords: Drip irrigation, Inline and online process, Process capability, Thickness, Hostelling T^2 statistics

*Corresponding author: yvj.jalasri@gmail.com

Introduction

Drip irrigation is a controlled method of irrigation, consisting of tubes with emitters. It allows increasing water use efficiencies by providing precise amounts of water directly to the root zone of individual plants (Burt & Styles 2007). Drip irrigation (also known as trickle irrigation or micro irrigation) is an irrigation method, which minimizes the use of water and fertilizer by allowing water drop by drop slowly to the roots of plants, either onto the soil surface or directly onto the root zone, through a network system of valves, pipes, tubing, and emitters.

Jain irrigation system limited, Jalgaon is a private limited company established in the year 1986 under the hands of its founder Mr. Bhavarlal Jain with a dream to serve mostly represented class of India. The company has many projects situated around the city Jalgaon, Maharashtra viz. Jain™ Plastic Park, Jain™ vally, Jain™ Hills etc. The company produces various products; few of them are Micro Irrigation system (Drip Irrigation), PVC Pipes and Fitting, Polyethylene pipes and Fitting, Plastic Sheet- Foam Sheet, Food Processing, Protected cultivation, Renewable Energy- Solar Products, Sprinkler's irrigation system.

Objective and Scope

- To observe industrial manufacturing process and industrial environment and culture
- To identify source and causes of variation in industrial process.
- To test the mean thickness measured at 4 different places are equal
- To check the capability of the process

Data collection and description:

The field data collection was done for 10 days at Jain Irrigation System Limited, Jalgaon from 24th Jan 2019 to 4th Feb 2019 for Inline Drip extrusion Line and Online Drip extrusion Line. Since, we are interested in observing and learning industrial manufacturing process of Pipe and Fitting, we collected data on 3 machines for online and 3 machines for inline process. The quality parameters of drip pipes are thickness, Outer Diameter and weight (bundle). For measuring these parameters instruments used were Digital micrometer and Vernier Calliper. Data collected on thickness, Outer Diameter, Weight, RPM and Load of Machine of drip line round pipe bundle, after that flow rate data was collected. For each of 400m's pipe bundle which was continuously produced after every 4 or 5 minutes and of 600m's pipe bundle after every 6 or 7 minutes the readings was taken.

Materials and Methods

Statistical quality control is a set of tools that allows solving problems and achieving stability in the process, reducing their variability. It provides information on various parameters of the process and their stability over time, allowing monitoring and increased efficiency. A fundamental objective of statistical quality control is to quickly detect the effect of unassigned causes or changes in the process, so that corrective actions can be taken at the appropriate time (Montgomery, 2009). Among its applications are the applications of pesticides (Silva et al. 2018). However, this set of techniques has been little used in the evaluation and improvement of irrigation quality.

Pareto chart is useful technique to identify the most important defects which occur most frequently due to different causes. Pareto chart of error is plotted for Online Dripper Pipe.

Puncher tube	3
Coiler problem	2
Lance Marking	1
Load Variation	2
Yellow Line Problem	0
Printing Problem	0

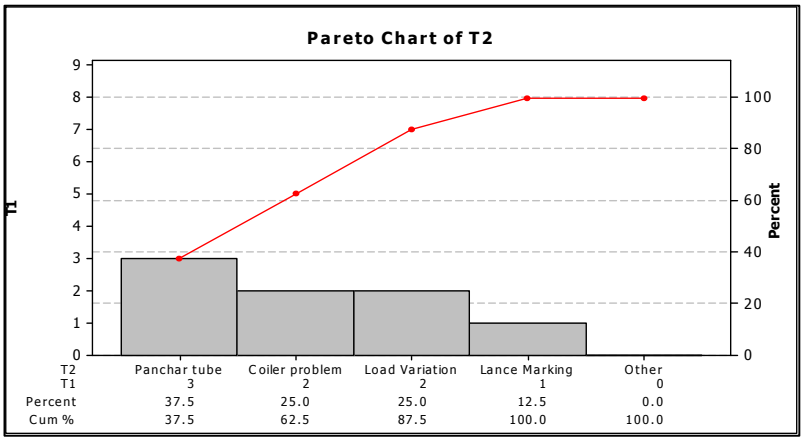


Figure 1: Pareto chart of error for Online Dripper Pipe

The Pareto Chart concludes that 80% defects in dripper round pipe are due to three main causes i.e. Puncher tube (37.5%), coiler problem (25%) and Load variation (17.5%).

Pareto chart of error of Inline Dripper Pipe

Puncher tube	1
Missing Dripper	2
Coiler problem	1
Lance Marking	0
Load Variation	0
Yellow Line Problem	1
Printing Problem	0

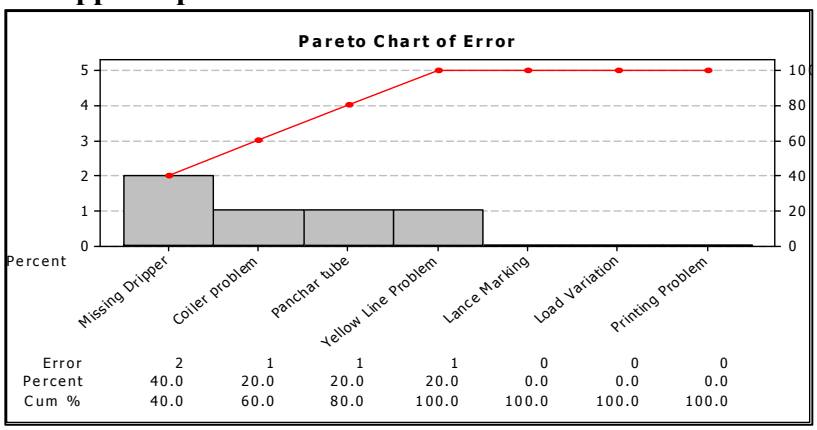


Figure 2: Pareto chart of error for Inline Dripper Pipe

The Pareto Chart concludes that 80% defects in dripper round pipe are due to two main causes i.e. Missing Dripper (40%), Coiler problem (20%) and Puncher tube (20%).

I-MR chart plots the moving range over time to monitor process variation for individual observations. MR chart was used to monitor process variation when it is difficult to group measurements into subgroups. When data are collected as individual observations, the standard deviation for each subgroup cannot be calculated. The moving range is an alternative way to calculate process variation by computing the ranges of two or more consecutive observations.

We use the moving range of two successive observations as the basis of estimating the process variability. The moving range defined as

$$MR_i = |x_i - x_{i-1}|, \qquad i = 2,3, \ldots, n$$

For the control chart for individual measurements, the parameters are,

$$UCL = \bar{x} + 3\frac{\overline{MR}}{d_2},$$

$$LCL = \bar{x} - 3\frac{\overline{MR}}{d_2} \quad \text{and}$$

$$center\ line = \bar{x}.$$

We plotted I -MR chart for quality parameters thickness and weight for 3 different machines where $T1, T2, T3, T4$ be the thickness at 4 different places.

Table 1: Correlation for Thickness and weight

Thickness and weight	Correlation (p)	$p - value$
Thickness P1 and weight	-0.356	0.005
Thickness P2 and weight	0.003	0.983
Thickness P3 and weight	-0.120	0.361
Thickness P4 and weight	0.079	0.550
Thickness mean and weight	0.058	0.662

From above table of correlation, we see that there is significant correlation between thickness of position 1 and weight, and for other points there is not significant correlation. We plotted the I-MR chart for rest of the 5 machines and observed that the process is in control. If few points goes beyond the control those points are deleted from the process. Since, the data is the collected continuously; we prefer the I-MR chart instead of $\bar{X}$-R chart. After deleting those points, the process capability analysis has been done for in control points.

Hotelling T-square Statistics for Thickness

For Dripper round Pipes the thickness measured at four different places and we interested to test whether mean thickness was equal.

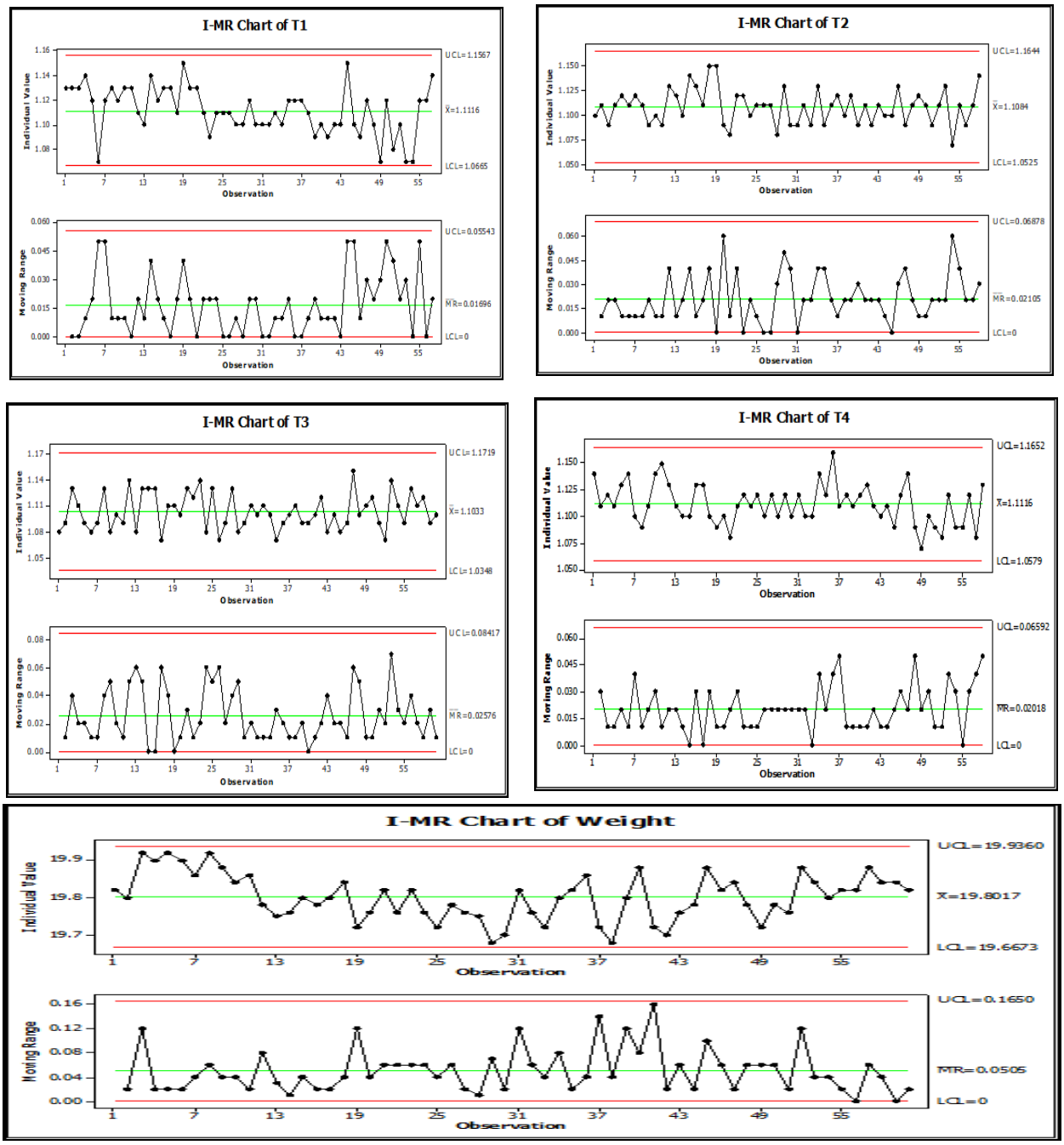


Figure 3: I-MR chart for T1, T2, T3, T4 and Weight

Take $\underline{X} = (X_1, X_2, X_3, X_4)$' where X_i is thickness measured at i^{th} places, $i = 1,2,3,4$.

To test,

$H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4$ Vs

H_1: At least one $\mu_i \neq \mu_j$ for $i \neq j$ Where $\mu_i = E(X_i)$, $i = 1,2,3,4$ for $\alpha = 0.05$.

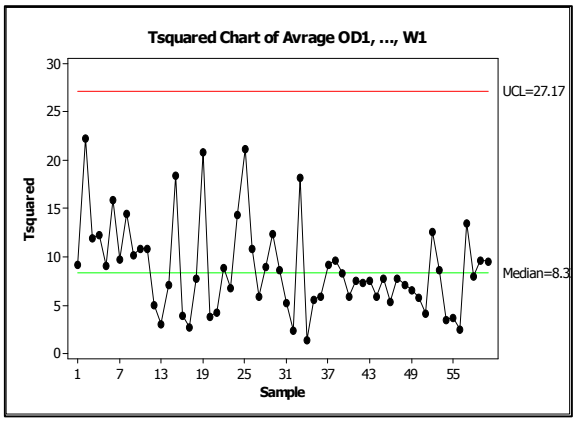
Table 2: Result of Hotelling T-square Statistics for Thickness

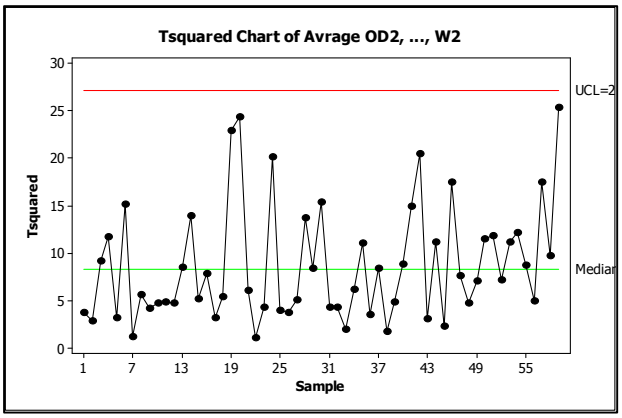
Machine No.	Parameter Size	p-Value	Decision
01	TO-16mm-2class-400mAP	0.68	Accept H_0
27	TO-16mm-2class-400mAP	0.4294	Accept H_0
29	TO-16mm-2class-400mAP	0.0001	Reject H_0
31	L-16mm-4lph-40-1-250m	0.000001	Reject H_0
32	L-16mm-4lph-50-1-400m	0.9801	Accept H_0
33	L-16mm-4lph-50-1-400M	0.000262	RejectH_0

For machine no. 29, 31 and 33 equality tests was rejected i.e. mean at each point is not same. For machine no. 01, 27 and 32 equality of mean test has failed to reject which implies that mean thickness at all 4 different places are same.

T-Square Control Chart for Outer diameter and Weight

We plotted the T-square Control Chart for Outer Diameter, Weight (w) and OD1 = Outer Diameter and W1, W2 and W3.





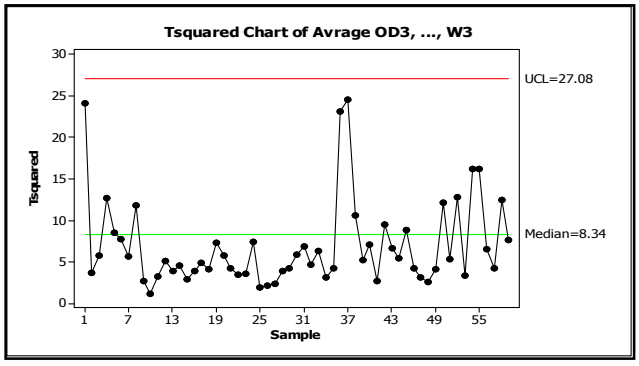
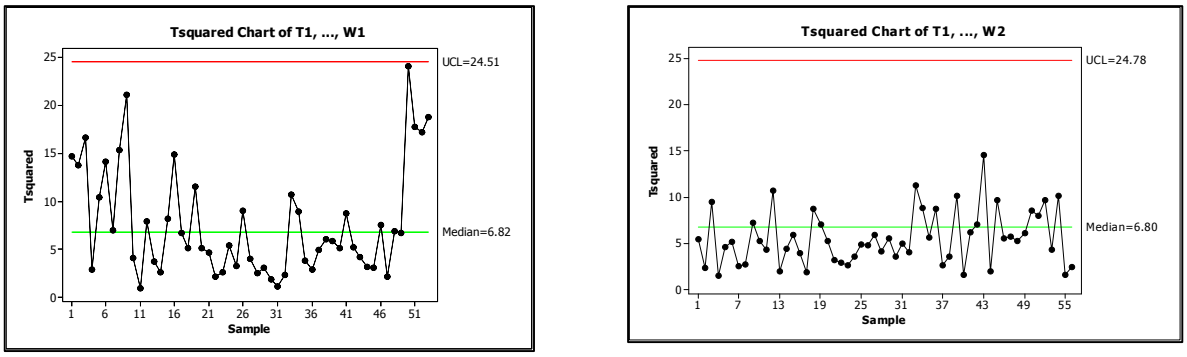


Figure 4: T-square Control Chart for Online Dripper Pipe Outer Diameter, Weight (W1, W2 and W3)



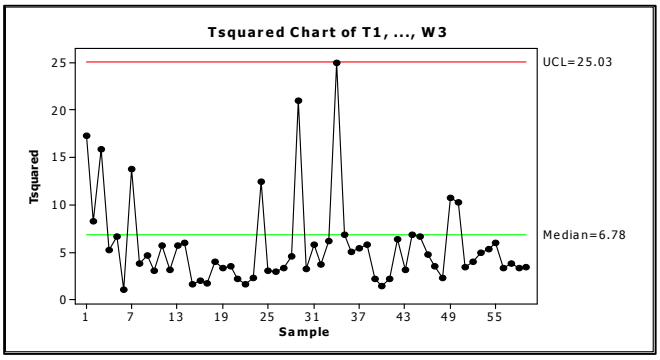


Figure 5: T-square Control Chart for Inline Dripper Pipe Outer Diameter, Weight (W1, W2 and W3)

From above all Figure 4 and 5, we see that by eliminating few of the points which are out of control, we get the stability of the process for all the parameters like thickness, outer diameter and weight which is nothing but multivariate process stability.

Kruskal-Wallis Test for equality of K population medians

We want to test

$\boldsymbol{H_0}$: The median equality of thickness of Dripper Round Pipe is equal Vs

$\boldsymbol{H_1}$: The median equality of thickness of Dripper Round Pipe is not equal

Table 3: Result of Kruskal-Wallis test for median equality for thickness of Dripper round pipe

Machine No.	Parameter Size	p-value	Decision
01	TO-16mm-2class-400mAP	0.472	Accept H0
27	TO-16mm-2class-400mAP	<0.04	Reject H0
29	TO-16mm-2class-400mAP	<0.001	Reject H0
31	L-16mm-4lph-40-1-250m	<0.001	Reject H0
32	L-16mm-4lph-50-1-400m	0.899	Accept H0
33	L-16mm-4lph-50-1-400M	<0.001	Reject H0

For Machine No. 01 and 32 the hypothesis of median equality of thickness of Dripper round pipe was fails to reject and we say that the median thickness is equal. While for other machines it is rejected.

Capability Analysis of quality parameter

Capability Indices are simplified measure to quickly describe the relationship between the variability of the process and the spread of the specification limits. Cp - It is the ratio of specification spread to the process spread.

$$C_p = \frac{USL - LSL}{6\sigma}$$

Where, USL and LSL are upper and lower specification limits respectively. The Cp is measure of the ability of the process to manufacturer products that meet specifications.
Cpk - It is simply the one-sided process capability ratio for the specification limit nearest to the process average.

$$C_{pk} = min\{C_{pu}, C_{pl}\}$$

Table 4: Capability of thickness

Machine No.	St.Dev.	Pp	Ppk	Ppm	Distribution
1	0.02088	1.6	0.62	31728.36	Normal
27	0.01212	2.75	0.31	176674.79	Normal
29	0.008913	3.74	1.22	133.08	Normal
31	0.01703	1.96	1.21	140.44	Normal
32	0.01656	2.01	0.56	47422.09	Normal
33	0.01351	2.47	1.39	15.45	Normal

From above table 4, we observed that machine no. 01, 27 and 32, P_{pk}<1 for thickness, so process is not potentially capable. For machine no. 29, 31 and 33 thickness is P_{pk}>1 means the process is potentially capable.

Discussion and conclusion

From the above study shows that for online process 80% defects in dripper round pipe are due to missing dripper, Puncher tube, coiler problem and load variation. I-MR chart for thickness and Weight shows that the process is under statistical control. We can observe that there is not much correlation between Thickness and Outer Diameter and Weight. This concludes that Thickness and Outer Diameter do not have much impact on Weight of coil and there might be some other machine parameter which affects weight. By using multivariate T-square, we get the stability of the process for all the parameters like thickness, Outer Diameter, and Weight which is nothing but multivariate process stability. It has been observed that $Ppk > 1$ for Length so process is highly potential capable for Weight.

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