



Government of Maharashtra
Ismail Yusuf College
of Arts, Science and Commerce
(Affiliated to University of Mumbai)
Jogeshwari (East), Mumbai -60

Re-accredited 'A' Grade by NAAC (CGPA- 3.14).

PROCEEDINGS

of

INTERNATIONAL e-CONFERENCE

on

Emerging Methodologies in Pharma,
Environmental and Life Sciences.
EMETHPELS 2020

18th and 19th June, 2020

Jointly organized by
Department of Botany, Zoology, and Biotechnology

ISBN: 978-93-83112-09-8



Government of Maharashtra
Ismail Yusuf College
of Arts, Science and Commerce
(Affiliated to University of Mumbai)
Jogeshwari (East), Mumbai -60.

PROCEEDINGS

of

INTERNATIONAL e-CONFERENCE

on

Emerging Methodologies in Pharma,
Environmental and Life Sciences.
EMETHPELS 2020

18th and 19th June, 2020

First edition 2020

Copyright (c) Government of Maharashtra Ismail Yusuf College of Arts, Science and Commerce, Mumbai - 60.
All rights reserved.

Editor

Dr Swati D Wavhal

Editorial Committee Members

Dr. Bhavita Chavan, Smt. Swati Phadke, Dr. Archana Rangari,
Shri. Yuvraj Shinde, Dr. Tulsiram Dadmal, Dr. Ulka Aade,
Smt. Trupti Sapkale, Dr. Pralhad Harinkhede,
Dr. Deepali Sawant, Smt. Sonali Patil

© No portion of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, recording or by any information storage or retrieval system without permission in writing from the publisher.

All information in this book is given in good faith without warranty.

Opinions expressed in this book do not necessarily represent those of the Publishers.

ISBN: 978-93-83112-09-8

Published by:

Colour Publications Pvt. Ltd.

126-A, Dhuruwadi, A.V. Nagwekar Marg,
Prabhadevi, Mumbai-400 025 (India).

Tel.: 022-2430 6319, 9318; E-mail: colorpub@colorpub.in.



ABOUT THE COLLEGE

Ismail Yusuf College, popularly known as I.Y. College, is a prestigious multi-faculty College owned and managed by the Government of Maharashtra. It is the oldest College in North Mumbai and the fourth oldest College in the city of Mumbai. It was established in 1930 with a big treasure-trove of donation from Sir Mohammed Yusuf Ismail, K.T., on the Jogeshwari Hill. The original campus of the College was spread over 120 acres of land which has now shrunk to 54 acres due to encroachments and the construction of the Western Express Highway, cutting the beautiful hillock on the College campus into two parts. The Campus enjoys the immense beauty of nature with innumerable banyan, palm and other trees, rippling brooks and glittering ponds in the rainy season. The foundation of the College stone was laid by Sir Leslie Orme Wilson, the then Governor of Bombay in 1924.

The vision of the founding fathers shaped up a temple of learning in sandstone in the regal Persian style with arches and spacious corridors, surrounded by countless big abundantly bearded banyans and palms stretching heavenward, dotting the horizon. Beginning with a moderate number of a few hundred students, today, the College has about four thousand students receiving instructions at Junior Level, Degree Level, Post-graduate Level and Research Level in all three faculties, viz. Arts, Science and Commerce. Most of our students are first generation learners with poor economic and weak social backgrounds.

The College is humming with research activities with majority of its teachers equipped with Ph.D. degrees, and many of whom are recognised research guides at the research centres of not only the University of Mumbai but many other universities across the state of Maharashtra. The College has been awarded six patents for its contribution to the field of research and development activities. The College takes special care of socially and economically underprivileged students and students with special needs through remedial coaching, bridge courses and mentoring system. The College organises state and national level seminars, workshops, conferences and lecture series on regular basis.

CHIEF PATRONS



Hon. Shri. Uday Samant
Minister, Higher &
Technical Education,
Maharashtra State



Hon. Shri. Prajakt Tanpure
State Minister,
Higher & Technical
Education,
Maharashtra State



Hon. Shri. Saurabh Vijay (IAS)
Secretary,
Higher & Technical
Education,
Maharashtra State

PATRON



Hon. Dr. Dhanraj R. Mane
Director
Higher Education,
Government of Maharashtra.

CONVENER



Dr. Swati Wavhal
Principal

CO-CONVENER



Dr. Vijay Narkhede
Head, Department of Statistics

CO-ORDINATOR



Dr. Amit Saraf
Head, Department of Botany



Dr. Bhavita Chavan
Head, Department of Zoology

ORGANIZING SECRETARY



Dr. Arati Nagarsekar
Co-Ordinator, Science Faculty



Dr. Archana Rangari
Assistant Professor, Botany

ORGANIZING COMMITTEE



Dr. Ulka Aade
Assistant Professor, Zoology



Shri. Nitin Shelke
Assistant Professor, Botany



Dr. Nilambari M. Paulkar
Lecturer, Botany



Dr. Heena Pathan
Assistant Professor,
Biotechnology
(Contractual Staff)



Shri. Yogesh Parsekar
Assistant Professor,
Biotechnology
(Contractual Staff)



Dr. Pooja Shinde
Assistant Professor,
Biotechnology
(Contractual Staff)

ADVISORY COMMITTEE



Dr. Bapu Thorat
Head, Department of Chemistry



Dr. Ravindra Kalesh
Head, Department of Physics



Shri. S. K. Tripathi
Assistant Professor, Physics



Smt. Darakshan Shaikh
Assistant Professor,
Statistics



Shri. Manoj Kaurav
Assistant Professor,
Mathematics



Dr. Dattatraya Bhangare
Assistant Professor,
Chemistry



Dr. Jaywant Deshmukh
Assistant Professor,
Chemistry



Shri. Aashish Bhaina
Assistant Professor,
Physics



Shri. Pravin Rathod
Assistant Professor,
Physics

EDITORIAL COMMITTEE



Dr. Bhavita Chavan
Assistant Professor,
Head, Department of Zoology



Smt. Swati Phadke
Librarian



Dr. Archana Rangari
Assistant Professor, Botany



Shri. Yuvraj Shinde
Assistant Professor,
Head, Department of English



Dr. Tulshiram Dadmal
Assistant Professor,
Chemistry



Dr. Ulka Aade
Assistant Professor,
Zoology



Smt. Trupti Sapkale
Assistant Professor,
English



Dr. Pralhad Harinkhede
Lecturer,
Zoology



Dr. Deepali Sawant
Lecturer,
Botany



Smt. Sonali Patil
Assistant Professor,
Biotechnology (CHB)

TECHNICAL COMMITTEE



Shri. Amol Sonawane
Head, Department of
Mathematics &
Computer Science



Smt. Sybal Dias
Assistant Professor
Computer Science
(Contractual Staff)



Smt. Amita Vakil
Assistant Professor
Computer Science
(Contractual Staff)



Smt. Snehalata Agarwal
Assistant Professor
Computer Science
(Contractual Staff)



Smt. Charul Singh
Assistant Professor
Computer Science
(Contractual Staff)



Dr. Ashwin Mehta
Assistant Professor
Computer Science
(Contractual Staff)

MESSAGE



Hon. Shri. Ravindra Waikar
MLA Jogeshwari Constituency,
Former State Minister Higher Education

It is a pleasure to address the International e-Conference on “**Emerging Methodologies in Pharma, Environmental and Life Sciences**’ (EMETHELS-2020) organized by Ismail Yusuf College, an Institute with 90 years of legendary foundation.

Dear friends, higher education has been going through tremendous changes during last few months. We have realized that we have to use online resources for getting connected to each other during lock down. We have started thinking over changing the traditional pattern of examinations, evaluation, administration related work and such other major responsibilities that have to execute through using online ICT tools.

Research and advancement in methodologies is an important aspect to address the global sustainability. We are facing the impact of pandemics and natural calamity like “Nisarga” rainstorm. The studies are to be spearheaded to resolve the issues. Such e-Conference will surely provide platform for multidisciplinary forum to provide an insight into current scenario. I am sure that there will be sharing of research finding, technological advancements and new ideas in diverse facets of sciences. The COVID-19 pandemic across the globe and natural disasters has impacted every one of us. The e-Conference will be great resource for learning and teaching in the period of social distancing. **We will stay connected and move forward together!**

I hereby give my best wishes to Convener Principal Dr. Swati Wavhal, Organizers, the speakers and participants for the great success of this e-Conference!

Thank you one and all!

MESSAGE FROM THE DESK OF CONVENER



Dr. Swati Wavhal

Principal

Ismail Yusuf College of Arts,
Science and Commerce, Mumbai-60

It gives me immense pleasure and I am proud to welcome all the distinguished speakers and participants to the international e-Conference on “Emerging Methodologies in Pharma, Environmental and Life Sciences” (EMETHPELS-20), organized by the Botany, Biotechnology and Zoology department of Government of Maharashtra's Ismail Yusuf College. The college is celebrating its 90th Foundation Year, and to mark the occasion we have organized this e-Conference.

We at Ismail Yusuf College encourage interdisciplinary research and training for both students and faculty. They are dedicated to undertake research projects to obtain scientific knowledge for which we are well equipped with various sophisticated high-end instruments. Our endeavor is to nurture performance-based hands on training on these instruments to the undergraduate and post graduate students.

The various sessions and talks of eminent researchers and distinguished professionals in their respective field will certainly enrich and inspire young minds to find solutions to many global, environmental and health challenges. I am confident that this scientific congregation will provide valuable data and fresh perspectives on new methodologies and experimentation facilities. The conference will be very informative, productive and beneficial for researchers, academicians and students.

I appreciate and congratulate all my staff members at Ismail Yusuf College for taking this initiative and organizing e-Conference during this phase of social distancing and Covid-19 crisis across the globe. They have worked hard and made this conference possible.

I extend a hearty and warm welcome, and my best wishes to all the honored guests, participating delegates and all the faculty members of the college for the great success of this e-Conference and publications.

Thank you.

MESSAGE



Dr. V. N. Magare
Pro-Vice Chancellor SNDT Women's University, Mumbai

It's indeed a great pleasure to witness an International e-Conference being conducted in an unusual manner on the backdrop of Corona! Conference is an endeavor of coming together of the people to think, discuss, deliberate, decide and resolve on a issues which is of immense importance, common concern and common good to them in a rational manner and perspective. Because of prevalence of Corona though the people are moved by common cause and concern, can not come together physically for safety reasons. However, it does not deter the like minded and motivated people like teachers of 'ISMAIL YUSUF COLLEGE' from coming together! They have found out a new way to it, by establishing a dialogue via e-communication vis a vis e-Conference.

Corona has brought the entire humanity including the world of Science at the crossroads for not knowing as to which way one should go and fight and eradicate Corona, tackling it on fronts, of "prevention" and "cure" both these fronts are essentially methodological in nature and approach and as such demand discipline of high degree of precision and accuracy in managing and developing the means found to be effective and efficacious in curbing this pandemic, whether it be production of vaccine or disciplining the people.

For a scientist and the scientific world, the instruments, equipment's, apparatus, tools and techniques serve to a great extent the need of being precise and accurate in their endeavours in finding out answers to the research problems.

The Ismail Yusuf College strengthened with a band of teacher researchers have identified rightly the necessity of discussing this aspect of "sense of proportion" vis a vis precision and accuracy, that manifests into the principle of finding out "Satyam, Shivam and Sundaram" of Science by organizing an International e-Conference on "Emerging methodologies in Pharma, Environmental and Life Sciences".

Thereby, they have provided a platform to the scholar's world over to listen to the experts and authorities in their fields of work, study and training and also to reflect on, respond to, discuss and deliberate on the subjects under consideration. And also, to put up their own work for consideration and assessment of others.

I appreciate and congratulate the IY College, its teachers and the Principal for having organized this International Conference at an opportune time. I am sure that at the end of the Day every one associated with this conference, in any capacity, would find that there is something to be learnt and taken home, I wish all success to the organizers in their endeavour to make this conference a grand success!

Jai Hind!! Jai Maharashtra!!

KEYNOTE SPEAKER



Dr. Rakesh Kumar

Director, CSIR-NEERI, Nagpur, India
Ph.D (Env. Engg.), M. Tech (Env. Engg.)
IIT Mumbai, ISO 14001, Lead Auditor EARA-UK, RAB-USA

About the Speaker

Dr. Rakesh Kumar is Director of National Environmental Engineering Research Institute (NEERI), part of CSIR (Council and Scientific and Industrial Research). He completed his M.Tech in Environmental Science & Engineering from IIT Bombay in 1987 and later got Ph.D. in Environmental Engineering. He is also qualified ISO 14001 EMS auditor through RAB UK and EARA, USA. He is visiting adjunct Professor at CESE, IIT Bombay besides visiting faculty to Drexel University under Obama-Singh Education Initiative.

His main area of expertise is in development of appropriate technology for environmental quality improvement encompassing the field of air pollution, particularly vehicle pollution, hazardous waste management, waste water treatment and disposal besides Climate Change and Health related subjects. Urban environment management is another area where numerous works related to carrying capacity, urban heat island effect, waste management and water sector analysis have been carried out.

He has been awarded the best scientist award, 1994 for NEERI and nominated for CSIR Young Scientist Award, 1995,1996. He is also the recipient of Commonwealth Commission Award, UK, in 1994. He has been awarded with "Environmental Leadership Award" by US Asia Environmental Partnership and US-AID for the year 2005 for outstanding contribution in improving quality of life for the population of Asia.

In NEERI, he has been awarded with best patent for the year 2005-2006. He has been also awarded distinguished personality by MIDC in August 2007 for his immense help in providing environment friendly direction to MIDC policies. He has been also awarded for Best Patent for Technology Patent by NEERI in 2008-09.

He has been given an award for largest number of technology transfer for low cost waste water treatment-PHYTORID in the year 2012. He has been given VASVIK award for 2012 for his exemplary work for urban environment improvement and sustainable technology "Phytoid" for sewage treatment for better environment.

He has ten patents on pollution control devices, of these two international patents, besides more than 83 papers in national and international Journals, 92 papers in national and international conferences. He has authored three Self Learning Books on various topics of Environmental Science and Engineering, one of them for Commonwealth of Learning, Canada.

He is member of various committees such as member of IAEA, Vienna on the use of radioisotope in surface water pollution studies, Auto Fuel Policy, GOI, Quality Committee of Indian Register of Quality System, IRS, various courts related PIL matter etc. He has worked on various assignments with many international agencies such as WHO, the World Bank, IAEA, UNEP, etc.

SPEAKERS OF THE e-CONFERENCE

Entry of Nanotechnology in Diagnostic arena

Dr. Madhuri Sharon

Director, Walchand Centre of Research in
Nanotechnology & Bio-nanotechnology, India



HPTLC and its applications

Dr. Melanie Broszat

Scientific Business Development Manager,
CAMAG, Muttenz, Switzerland

Enhanced Approaches to Analytical Method Development and
Management in Support of Forthcoming ICH Guidelines

Dr. Stephanie N. Harden

Market Development Manager, Pharmaceutical Development
and Manufacturing EMEA Waters Corporation Switzerland 03



Biopharma with Bioaccord

Dr. Guillaume Bechade

Biopharma Field Marketing Manager Fr St Quentin

Global Challenges in Environmental and Occupational Health

Dr. Arthur Frank

Chair Emeritus & Prof. in Medicine,
Drexel University, Philadelphia, USA



Small Stories Big Impacts: DNA in Life Science Research

Dr. G. D. Khedkar

Director, Paul Herbert Centre for DNA Barcoding &
Biodiversity Studies, Dr. Babasaheb Ambedkar Marathwada
University, Aurangabad, India

CRISPR Genome Editing Technology

Dr. Jacinta Dsouza

Chairperson, School of Biological Science, CBS, Mumbai, India



Advances in Atomic Absorption Spectrophotometry

Dr. Anja Jungnickel

Analytik Jena AG, Germany

CONTENTS

No.	Authors Name	Title	Page No.
01	Chetti S. O., Chutke N. L. and Kulkarni K. V.	Application of Analytical Techniques in Forensic Science: A Review	15
02	Gavhane U. V. and Adsule N. D.	Impact of Covid-19 On the Environment	20
03	Khade Ranjana G.	Isolation of bio plastic producing bacteria for management of congress weed	24
04	Kshirsagar Aboli	Effect of Azospirillum on Quantity of Saponin in Roots of <i>Chlorophytum borivilianum</i> (Safed musli)	29
05	Mole, Megha Nandkishor	Antifungal activity and GC-MS analysis of <i>Chaetomorpha</i> and <i>Padina</i>	32
06	Patil Madhuri, Phulari Megha, Murumkar Chandrashekhar	Extraction and characterization of tannins from <i>Cassia tora</i> Linn leaves	37
07	Uttarwar Monica Laxmanrao	Covid-19 and Its Effects on Economy and Environment	41
08	Saraf Amit and Saraf Aparna	HPTLC fingerprinting: A tool for simplified analysis of terpenoids in medicinal plants	46
09	Raut Mansi and Shinde Pooja	Study of phytochemical, antifungal and antibacterial activity of extracts of aerial roots of <i>Ficus benghalensis</i> L.	51
10	Bisen Dineshwari D. and Harinkhede Pralhad R.	Virtual Labs: An Effective Tool in Education	54
11	Harinkhede Pralhad R.	Index Based Bio-Monitoring Using Macroinvertebrates	59
12	Ulhe P. P.	Seed Coat Study of Some Species of Family Fabaceae	64
13	Hegde V. N., Chavan Bhavita , Dalvie V. V.	Study of Histopathology of some tissues of <i>Macrobrachium rosenbergii</i> (de Man), exposed to pesticides, Chlorpyrifos and Dimethoate, individually and synergistically.	69
14	Ravindra Kalesh, Ravindra N. Kambale, Syed Habibuddin Syed Abed Ali, Vaishali Bambole	Structural and Magnetic Properties of $Mn_{0.8}Co_{0.2}Fe_2O_4$ Ferrite Nanoparticles	77
15.	D. Shanthi, K. Raja and R. Saravanan	Antioxidant potential of aqueous extract of immature fruit and developing shoot of <i>Solanum torvum</i> (Swartz)	81

Application of Analytical Techniques in Forensic Science: A Review

S O Chetti¹, N L Chutke² and K V Kulkarni³

¹Director, Directorate of Forensic Science Laboratories, Mumbai and corresponding Author

²Deputy Director, Mini Forensic Science Laboratory, Solapur

³Assistant Director, Mini Forensic Science Laboratory, Solapur

Email- sandeepchetti@gmail.com

ABSTRACT

Forensic science is an application of all branches of science and helps in crime investigation. It is also known as Science for Justice. Forensic science is application of chemical, physical and biological sciences. Chemical science includes analysis of narcotic drugs, explosive, chemical and toxicology, Physical science includes ballistics, document examination and Biological sciences includes biological fluid detection, DNA analysis...etc.

Analytical techniques like GC, GC-MS, GC-HS, HPTLC- Densitometer, HPLC, LC-MS, EDXRF, UV visible spectrophotometer, FTIR are used in forensic science. Analysis of crime exhibits is again a challenge to forensic scientist as purification and extraction is required and so modern techniques like GC, HPLC, GC-MS help in separation, identification and quantification of organic compounds present in crime exhibits. Similarly, non-destructive techniques like EDXRF, Raman spectroscopy are too useful when trace quantity of crime exhibit is available. DNA finger printing technique helps in paternity, unknown person identification and sexual offences. Scanning electron microscope, velocity analyser are modern tools for ballistics examination. VSC 5000 helps in document examination. In this article, application of analytical techniques used in Forensic science is discussed.

Keywords : Forensic Science, Analytical technique, Chromatography, Spectroscopy, Explosive analysis, Narcotics.

Types of samples analysed

In Forensic Laboratory following samples are received for analysis.

1. Explosive, post explosion residues after bomb blast.
2. Narcotic drugs under NDPS act received for analysis.
3. Biological samples like urine and blood for narcotic drugs in consumption of narcotic drugs cases are received.
4. Petroleum products like diesel, petrol, kerosene under possession and their adulterations under E.C Act.
5. Samples received for detection of inflammables, petroleum hydrocarbon residues in fire & arson cases.
6. Chemical, vegetable oils and fats in crime cases.
7. Cement, mortar and concrete in building/construction/bridge collapse cases.
8. Examination of alcohol content in liquor under Bombay Prohibition Act.
9. Blood sample for estimation of alcohol in blood

under Bombay Prohibition act / Motor Vehicle act in hit and run case.

10. Viscera (stomach, intestine, liver, spleen, kidney), stomach wash, vomit, blood received in toxicology division in homicidal and suicidal cases to analyse poison, alcohol, drugs and toxins
11. Application of DNA in murder, sexual assault, attempt to murder and paternity cases.^[1]

Analytical techniques

Physical, Chromatography, Spectroscopy, combination of chromatography and spectroscopy techniques are used in analysis.

Analysis of Explosive and Narcotic drugs

In case of high explosive and its post explosion residues are analysed by HPTLC, HPTLC-densitometer, HPLC, LC-MS, Gas Chromatography, Gas Chromatography- mass spectroscopy, Gas Chromatography- Thermal energy analyser.

After bomb blast, proper sample should be collected from the scene of crime. RDX, TNT, HMX, PETN, Nitro glycerine and Nitro cellulose are high explosives. High explosives are soluble in acetone, hence acetone is preferred for extraction of high explosives and then analysed.

High performance thin layer chromatography (HPTLC)- In this technique sample extract and standard explosive extract are spotted and Rf values are compared after it was run in solvent system and different spray reagents are also used for visualization. In HPTLC- Scanner/ densitometer, sample extract and standard extract are spotted on HPTLC plate and then run in solvent system and further spots are scanned at different UV range in densitometer. Similarly, sample and standard extract are injected to HPLC and GC and their retention times are compared. In case of the sample extract injected in GC-MS and LC-MS, after separation in GC and LC, the compounds are analysed in Mass spectroscopy and further from mass spectra the compound is identified. In this case is not required standard sample.

Similarly, narcotic drugs and psychotropic substances like heroin, cocaine, Lysergide(LSD), *m e t h y l a m p h e t a m i n e*, methylenedioxymethamphetamine, mephedrone, benzodiazepines are soluble in methanol and ethanol. Hence, methanol/ ethanol extracts of these drugs are further used for analysis. Techniques like HPTLC, HPLC, GC, GC-MS, LC-MS are used for analysis. UV and FTIR spectroscopy are also useful in narcotic drugs analysis, narcotic drugs show typical UV spectra in acidic, basic or neutral medium. Drugs also show IR spectra. Optical isomers are found in few drugs in which only one form falls under NDPS act and are identified by FTIR or polarimeter. Similarly, Raman spectroscopy is also used. Some of the typical analysis of explosive and narcotic drugs are as follows:

HPTLC - In this technique the solvent system and spray reagent are different for different analyte which are available in literature.^[2]

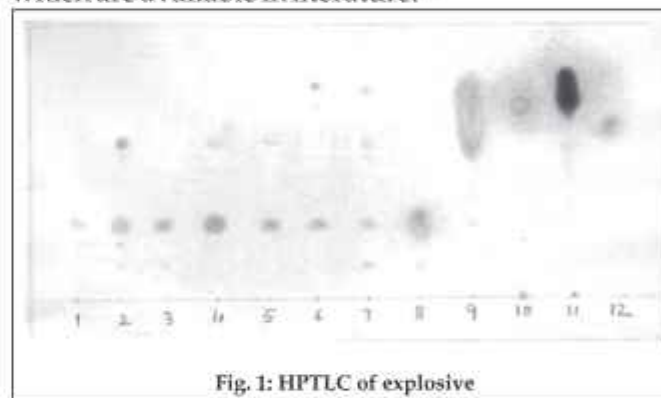


Fig. 1: HPTLC of explosive

(1 to 7 -sample exhibit, 8 to 12- Standard explosive RDX, PETN, NC-NG, TNT, Teteryl.

Solvent system- Trichloroethylene: Acetone

Spray reagent- Alcoholic NaOH, heat for 10 min at 100 °C thereafter Griess reagent (Figure 1).

UV spectroscopy

UV visible spectrophotometer technique is often present used for analysis of drugs. Depending upon the substance present in acidic, basic or neutral medium the typical UV spectra is obtained (Figure 2).

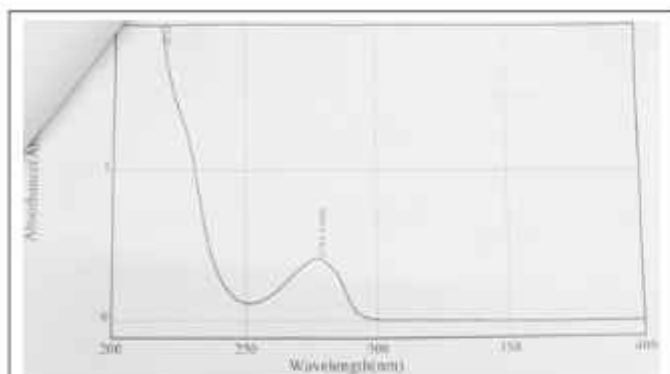


Fig. 2: UV spectra of Heroin. This spectrum of sample (λ_{max} at 278nm) showed presence of heroin.

sample should be thermally stable. The vapors of solutes should pass through the column and depending upon polarity of solutes, solutes get separated in column and then detected in detector.^[2]

Selection of detector is also important. Now a days, Mass spectroscopy is preferred in Forensic science laboratory.^[4]

GC-MS is used in analysis of explosives, narcotic drugs, poisons, unknown chemicals and many compounds (Figure 3). Mass spectroscopy is a

Gas Chromatography

Gas liquid chromatography is the technique used for identification and quantitation of narcotic drugs, explosives, unknown chemicals and many other compounds. In gas chromatography, selection of column and column temperature, detector and flow rate of carrier gas is essential. Depending upon polarity of sample, column should be selected. Obviously there are some intermediate polar material used in column and so can be used for analysis of polar and nonpolar samples (Table 1). Some examples are given below. The injector temperature, detector temperature, oven temperature / column temperature depends upon the compounds melting/ boiling points. The

Sr No	Column	Chemical types	Polarity	Application
1	HP-1	100% Methyl Polysiloxanes	Non polar	Solvents, VOCs and Pet. Products
2	HP-5	5 % Methyl 95% Phenyl Polysiloxane	Non-Polar	Aromatics, Drugs, Perfumes
3	HP-17	50 % Methyl, 50% Phenyl Polysiloxane	Medium Polar	Plasticizers, Esters, Ketones, Drugs
4	HP-10	14 % Cynopropyl 86% Methyl Polysiloxane	Medium Polar	Alcohols, Phenols, Esters, Ketones, Plasticizers.
5	CP-Wax	Polyethylene glycol	Very Polar	Alcohol, Esters, Acids, Amines, solvents

Table 2: Types of detectors

Detector	Sensitivity gs^{-1}	Linear range	Characteristics	Application
Thermal Conductivity Detector TCD	10^{-1}	10^4	Robust, nondestructive	Universal Detector
Flame ionisation detector FID	10^{-12}	10^7	Excellent sensitivity and linear dynamic range, Best detector	Pet hydrocarbon, drugs, explosives, solvents...etc
Nitrogen Phosphorous Detector NPD	10^{-14} (N) 10^{-15} (P)	10^5	Similar to FID, limited linear dynamic range, selective for N and P containing Solutes	Insecticides/ pesticides
Explosives Electron Capture Detector ECD	10^{-12}	10^3	Excellent sensitive for solutes with electronegative elements, Temperature sensitive, limited dynamic range	Organochloro compounds Pesticides/ Insecticides
Thermal Energy Detector	10^{-17}	10^4	Selective for NO and NO_2	Explosives

detector in Gas chromatography. From the mass spectra of individual peaks TIC can identify the organic compound. The sensitivity and linearity of detector is better than other detectors. There are electronic ionization and chemical ionization modes. NIST library provides information for the detection of drugs.

HP- 5 capillary column, 15m X 0.32m film thickness, Injector temperature: 220°C, oven temperature: 180°C-250°C, ramp rate: 10°C per min, final temperature: 250°C, source temperature: 230°C and interface: 180°C. EI mode -70eV

High performance liquid Chromatography-

This technique particularly used for those

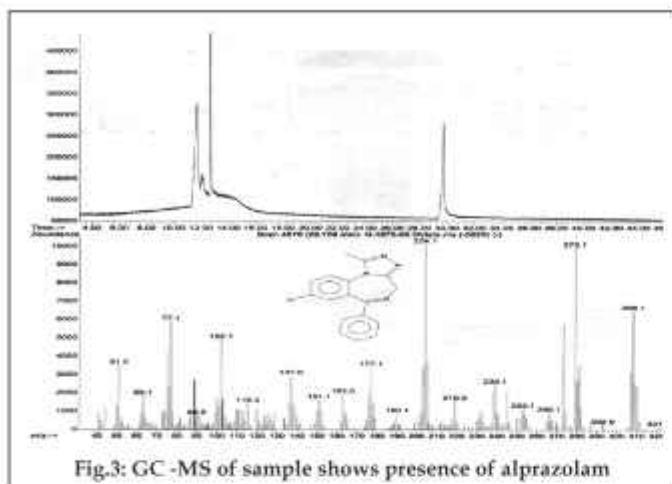


Fig.3: GC -MS of sample shows presence of alprazolam

samples which are thermally unstable, In Forensic science, it is used for analysis of explosives, narcotic drugs, insecticides, herbicides...etc. In HPLC selection of column and detector is as important as in GC. Selection of mobile phase, column and detector depending upon polarity of solute that analyze. C8, C18, ODS are commonly used column in HPLC. Reverse phase column has many applications in drugs analysis. Refractive index, UV, fluorescence, ...etc. detector used in HPLC. LC- MS is an advance technique used in forensic science.^[1] (Figure 4)

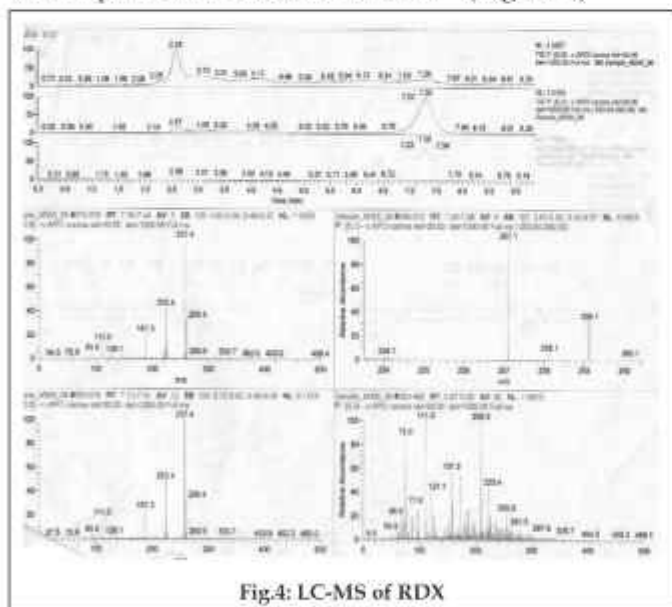


Fig.4: LC-MS of RDX

Petroleum products, fire and arson

In forensic science laboratory petrol, diesel, kerosene, lubricating oil ...etc are received for analysis. The techniques like Distillation analyzer,

flash point, Kinematic viscometer...etc are used for analysis. As per BIS standards the specific gravity/density, distillation parameter, flashpoint, viscosity...etc of sample are analyzed, for e.g. Diesel should distill below 400 degree Celsius.

Gas chromatography with Detailed hydrocarbon analyzer is used in analysis of petrol. Research octane number in petrol is calculated by GC-DHA. Gas chromatography is also used in analysis of petrol, kerosene and diesel. In Fire and arson cases, extraction of partly burnt samples is carried out in solvent like ether and then injected to GC-FID and GC-MS and from peaks obtained in the chromatogram, presence of petroleum hydrocarbon residues are confirmed.^[1]

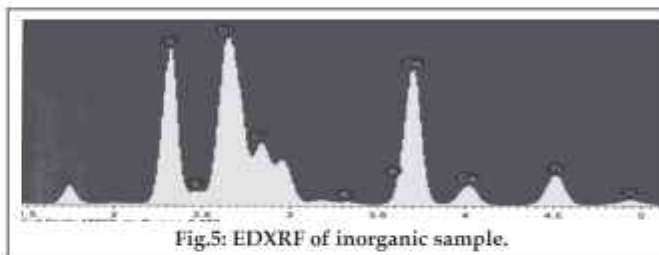


Fig.5: EDXRF of inorganic sample.

Analysis of cement, concrete and mortar

Concrete and Mortar are usually referred to Forensic Science Laboratories in cases of building collapse where the cement : sand ratio is important. In Mortar and Cement : sand : aggregate ratio is significant in concrete analysis. Hence, soluble calcium oxide, silica etc. are determined.

Analysis of Metals

In cheating cases of gold and other metals Inductively coupled plasma atomic emission spectroscopy and atomic absorption spectroscopy, Inductively coupled plasma mass spectroscopy can be used in analysis. Energy Dispersive X-ray Fluorescence (EDXRF) is also useful in trace element analysis in crime exhibit particularly in fire arm cases. EDXRF is non-destructive technique. EDXRF is used in analysis of paints, pigments and inorganic cations.(Figure 5)

Analysis in Toxicology and blood alcohol -

In this division examination of poison in viscera, stomach wash/aspirate, vomit, blood and other biological material is carried out. Organic volatile poisons like ethanol, methanol, formaldehyde can be determined by using Head Space - Gas liquid chromatography (Figure 7). Inorganic poisons like mercury, lead, arsenic...etc. can be determined by using ICP, ICP-MS, Ion exchange chromatography can be used for analysis of inorganic poisons.

Head space method is especially suitable for the very fast separation of volatile components (alcohols, acetone, aldehydes) in complex biological matrices specially blood, in mass liquor and prohibition law related cases. This method has the advantages of avoiding the risk of contamination of non-volatile components, which may be eliminated due to on-line analysis by gas chromatography.

A person who commits any crime under the influence of alcohol, such as Murder, Rape or while driving vehicle and leading to accident is a serious offence and can be charged under IPC section and Bombay Prohibition Act.

The concentration of blood alcohol is given below^[1]

- (1) Excitement 50 - 150 mg %
- (2) In coordination 200 - 500 mg %

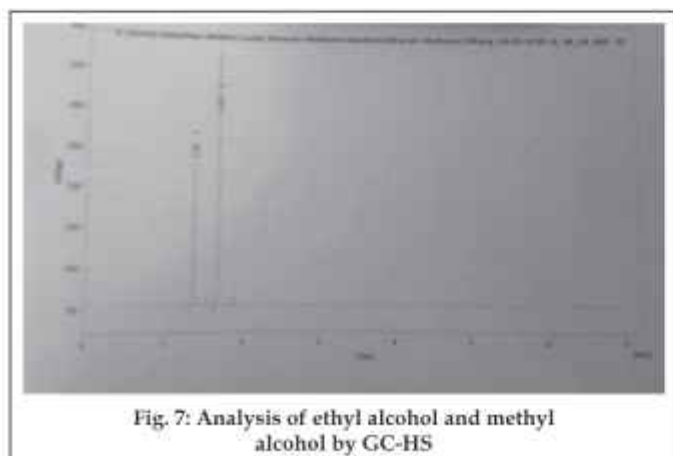


Fig. 7: Analysis of ethyl alcohol and methyl alcohol by GC-HS

- (3) Narcosis above 500 mg %
- (4) Fatal dose 700 to 1000 mg %
- (5) Period 12 to 24 hours.

LD50 = orally in rat 13.7 gms/kg.

Extraction of poisons like insecticide, pesticides, drugs, plants poisons (Figure 8) in biological material is essential, liquid-liquid extraction and solid phase extraction commonly used. HPTLC, HPTLC-densitometer, HPTLC-MS, HPLC, GC, GC-MS and LC-MS techniques can be further used for identification and quantitation of poisons.

Conclusion

With the advent of sophisticated instruments and techniques like GC, GC-MS, LC-MS there is always scope of improvement and as their application is useful in forensic Science. HPTLC-MS is one of the best aspect technique and it is simple and fast. New methods may be developed in every technique. Hence, forensic experts should develop simple, fast and reliable methods.

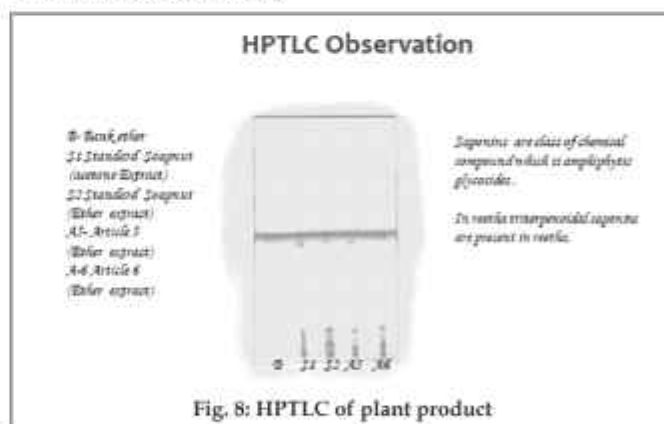


Fig. 8: HPTLC of plant product

References

1. Manual- Directorate of Forensic science laboratory.
2. Symposium "Evolution of gas chromatography in India- Past, present and future".
3. R. Krishnamurthy J Clin Forensic Med Aug-Nov 2006, 13(6-8): 311-5.
4. Sandeep O Chetti Bio Chemical Science, Vol 4, April 2015. pp.73-83

Impact of COVID-19 on the environment

Gavhane U. V.* and Adsule N. D.

PunyaslokAhilyadevi Holkar Solapur University, Solapur
ushag28762@gmail.com

ABSTRACT

After World War II, mankind faced the most hazardous, deadly and crucial pandemic outbreak which threatened mankind. The COVID-19 is considered as the most drastic global health issue. A new infectious disease had emerged in Wuhan, Hubei part of China, was further named by the World Health Organization as COVID-19 (Coronavirus Disease 2019). The causative organism was identified which is able to target the respiratory system causing the respiratory difficulties. This was found to be a new class of Coronavirus, known as SARS-CoV-2, has been found to be responsible for the respiratory disease which has spread globally. According to the WHO, 6080963 are confirmed cases and 431192 deaths over time occurred (WHO as of 1 June, 2020). Though mankind is threatened by this pandemic outbreak, there have been some beneficial impacts on the environment. This research is totally based on the positive and negative impact of COVID-19 on the environment. The study emphasizes the difference in the environment before and after the outbreak of COVID-19. This paper includes the positive impact such as improved air quality, clean water, lakes and beaches, reduced noise pollution as well as negative or secondary aspects like the reduction in recycling, increased medical wastes, etc.

The study also helps to reduce further endangering and activities in the environment and to live simple life without over disturbing the environment.

Keywords: COVID-19, impact, pandemics, global health, environment

Introduction

THE pandemic is in general a serious public health concern which triggers the huge change in socio-economical and political crisis simultaneously causing secondary effects on the environment. Such a destructive kind of virus came across around December 2019, further identified on 7th January, 2020. This was a similar kind of virus previously found causing the SARS outbreak. In 2003, the SARS CoV emerged from China causing the large scale pandemic.^[1] We have evidence that suggests that the transmission of COVID-19 has been occurring human to human by the droplets, tears, fomites and direct contact.^[2] To overcome, the outbreak all the affected countries declared the lockdown in which

there is much restriction to human activities like travelling, gathering, etc. This action of all countries made an observable positive change in the environment of the respective countries which simultaneously changed the global environment including improved air quality.

The studies indicated the positive effects on the environment however, climate experts predict that the greenhouse gas proportions dropped as never before since World War II.^[3] Such a positive change happened only due to the lockdown policy.

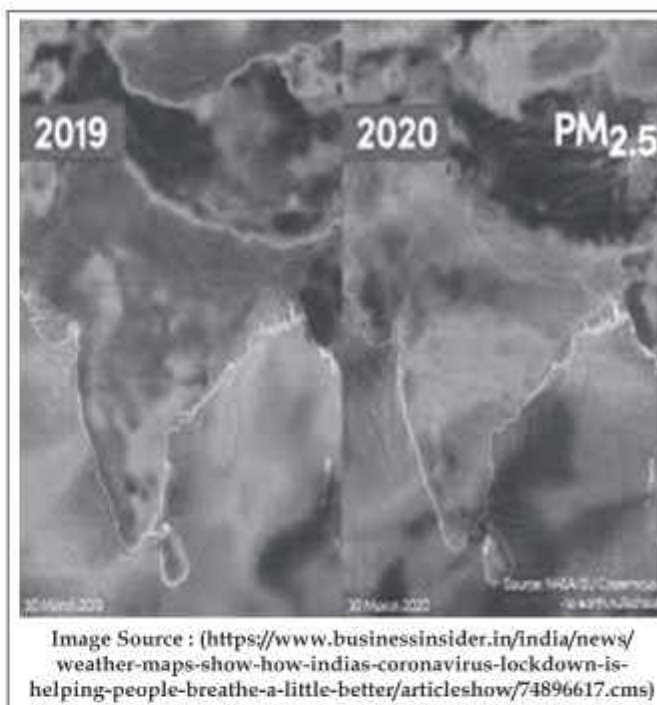
Due to the unusual outbreak of COVID-19, almost every big and small cities and villages in the affected

countries like China, Taiwan, Italy, USA, France, Spain, Turkey, Iran, Germany, S Korea, U.K, India, etc countries are about to remove their lockdown. Various types of industries are not functioning, all types of travels are cancelled. Meanwhile, efforts to restrict transmission of the SARS-CoV-2, by restricting the movement have had an outstanding environmental effect. Due to non functioning of the industries the emissions of the waste from such industries were reduced to the zero level. Likewise there were no vehicles on road, also reduced the emission vehicle gases which together made the positive change and reduced mainly air and noise pollution leading to enrichment in the global environmental health.

Due to lesser demand for power in industries, use of fossil fuels or conventional energy sources have been lowered considerably. Ecosystems are being greatly recovered. In many big cities the inhabitants are experiencing a clear sky for the first time in their lives. The pollution level in tourist spots such as forests, sea beaches, hill areas etc. is also shrinking largely. Ozone layer has been found to have revived to some extent. The pandemic has displayed its contrasting consequence on human civilization, in the sense that, on one hand it has executed worldwide destruction, but created a very positive impact on the world environment on the other hand.^[4]

Positive Impact of COVID-19 on the Environment

1. **Improved Air Quality :** According to the WHO, 91% of the population breathes the poor quality of air.^[5] The consequences of air quality degradation are manifested in a significant percentage of global mortality each year.^[6] The air is the most essential part of all living beings but very few of the human respire in clean air according to the WHO. The lockdown made the environmental air pollution free by the reduction of major air pollutants like NO₂, CO₂, etc due to lesser functioning of the vehicles and the industries.



2. **Reduction in Noise Pollution :** Industries and vehicles are the major sources of noise pollution as the industries have huge machinery and the vehicles have horns and the engines which are the major sources of the noise pollution in the city areas. Noise in the environment is one of the main sources of discomfort for the living beings causing various ill effects on health and also alters the natural conditions of the ecosystems.^[7]
3. **Clean Water Bodies:** Water is an essential part of all living animals and plants but clean water is very important for the assurance of health. Due to complete shut down in many countries, world wide, it directly showed the improvement in the health of the water bodies of corresponding countries. In India, many lakes and rivers have been showing a crystal clear appearance. Beaches are one of the most important natural capital assets found in coastal areas.^[8]
4. **Wild Life:** The most observable change occurs to the wildlife globally. Previously there were very few bird sounds listened to by the people due to huge noise of the vehicles and other machines,

now at the time of lockdown the people witnessed the amazing, attractive, pleasant nature of their surroundings which was totally neglected before. Many ornithologists and bird observers claimed that many birds now come to visit the calmer area where lockdown processes were going on.

Negative Impact of COVID-19 on the Environment

1. Tons of Increase in the Medical Waste: Medical waste is also on the rise. Hospitals in Wuhan produced an average of 240 metric tons of medical waste per day during the outbreak, compared to their previous average of fewer than 50 tons. In other countries such as the USA, there has been an increase in garbage from personal protective equipment such as masks and gloves.^[9]
2. Reduction in the Waste Recycle: During lockdown not only medical wastes but also general day-to-day organic and inorganic wastes were generated which must be disposed or recycled for the better community health. Recycling of waste has always been a major environmental problem of interest for all the countries.^[9]. During the pandemic, some countries like the USA have stopped recycling programs in some of their cities, as authorities have been concerned about the risk of COVID-19 spreading in recycling centres.

Discussion

Coronavirus is a zoonotic disease that has spread from animals to humans and is currently a global concern. Measures taken to eradicate the coronavirus indirectly created a beautiful, attractive and healthy environment in front of the world that could positively purify the global climate. An epidemic caused global warming to become more purified than ever before, as well as significantly improving seawater and river water. Reducing the level of noise pollution also improved human health

and created a stress free environment. In the present world, coronavirus will be eradicated and life and economy will be as smooth as before. But will man learn from the present epidemic and lead a future life?

Waste management has become a major problem in the global epidemic and medical waste is generated but not disposed of to such an extent. Greenhouse gases have remained in the air for decades, causing global warming, but during the period of lockdown, greenhouse gases and vehicle emissions like NO₂ have dropped as never before.

India is a developing country that is using its natural resources to make progress. As India has abundant natural resources, every Indian as well as every country in the world should take a step towards progress by conserving their natural resources. Every human being should respect this environment. If the environment is not respected, then in the future the environment will lead to the destruction of the human race.

References

1. Impact of the COVID-19 lockdown on basic science research in ophthalmology: the experience of a highly specialized research facility in France Marco Nassisi
2. Ong SWX, Tan YK, Chia PY, Lee TH, Ng OT, Wong MSY, et al. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. JAMA. 2020;323:1610-1612. 4. Borrelli E, Sa <https://jamanetwork.com/journals/jama/fullarticle/2762692>
3. Global Carbon Project, 2020. <https://www.globalcarbonproject.org/carbonbudget/index.htm>
4. COVID-19 outbreak: Migration, effects on society, global environment and prevention Indranil Chakraborty, Prasenjit Maity <https://www.ncbi.nlm.nih.gov/pmc/ar>

- articles/PMC7175860/
5. WHO, 2016. https://www.who.int/health-topics/air-pollution#tab=tab_1 Accessed date: 5 April 2020.) COVID-19 outbreak: Migration, effects on society, global environment and prevention (Indranil Chakraborty, Prasenjit Maity)
 6. Zhang, Q., Jiang, X., Tong, D., Davis, S.J., Zhao, H., Geng, G., Ni, R., 2017. Transboundary health impacts of transported global air pollution and international trade. *Nature* 543, 705–709
 7. & 8. Zambrano-Monserrate, M.A., Silva-Zambrano, C.A., Ruano, M.A., 2018. The economic value of natural protected areas in Ecuador: a case of Villamil Beach National Recreation Area. *Ocean & Coastal Management* 157, 193–202
 8. Liu, M., Tan, S., Zhang, M., He, G., Chen, Z., Fu, Z., Luan, C., 2020. Waste paper recycling decision system based on material flow analysis and life cycle assessment: a case study of waste paper recycling from China. *J. Environ. Manag.* 255, 109859
 9. Calma, J., 2020. <https://www.theverge.com/2020/3/26/21194647/the-covid-19-pandemic-is-generating-tons-of-medical-waste>. ■

Isolation of bioplastic producing bacteria for management of Congress weed

Ranjana G. Khade

Department of Microbiology

Seva Sadan's R.K. Talreja College of Arts, Science and Commerce,

Ulhasnagar, Mumbai University, Maharashtra, India.421003.

khaderanjana@gmail.com

ABSTRACT

Congress weed (*Parthenium hysterophorus*) is the most devastating and hazardous weed available in most part of the India. The weed management strategy recommends the use of weed as starting material for production of value added products. Thus, the present study was planned to isolate the bacteria from soil with ability to produce bioplastic (Poly-hydroxybutyrate) using congress weed. Out of 50 isolates, 17 isolates showed bioplastic production ability but only 4 isolates showed bioplastic production in the presence of congress weed hydrolysate. The modified crotonic acid method was used for selection of an efficient bioplastic producing bacteria and production conditions were optimized. The selected *Bacillus* spp. was found to produce bioplastic with 2% concentration of congress weed hydrolysate in presence of sucrose as a synthetic carbon source. FTIR analysis confirmed the extracted PHB as poly-hydroxybutyrate. Thus, the present study concludes the effective way of management of congress weed into eco-friendly bioplastic by bacteria.

Keywords: Poly-hydroxybutyrate, Congress weed, *Bacillus* spp.

Introduction

CONGRESS weed (*Parthenium hysterophorus*), one of the world's most dangerous weed, is responsible for huge losses to the biodiversity, agriculture, economy, and health of livestock and human beings. Industrially it can be used for producing various value added products which can open new avenues for effective management of this violent weed. Spilled oil has various impact on biodiversity (Junfeng D. et al., 2015). Dispersants are generally liquid chemicals which accelerate the dispersion of the oil by reducing the surface tension between the oil and water by reducing the surface tension. These chemicals are expensive and toxic (Ahmad B. et al., 2005). The bioplastic (polybetahydroxybutyrate) is

similar in characteristics as synthetic polypropylene with the added advantage of biodegradability.

Polybetahydroxybutyrate are a form of microbially synthesized biodegradable polyesters accumulated as storage inclusion bodies in the cytoplasm of the cell under the stress condition (Panigrahi S., 2013). Good biodegradable and biocompatible property of the PHB makes them potential alternative of petroleum-based plastics. But major drawback is the high cost of production that pulls down the possible commercialization of PHB. Because refined substrates such as glucose, sucrose are supplemented in production media for polymer production. Much efforts has been devoted



Fig. 1: Qualitative screening of PHB producing isolates using sudan black B.

to reduce the cost of production by using cheap carbon sources which allow high productivity and PHB content (Van D. et al., 2007). It is also important to isolate the microorganism which can utilize the corresponding substrate and produce the relatively good amount of PHB in the substrate media. Moreover, eco-friendly and cost-effective method is required for production of PHB using inexpensive and easily available carbon substrate.

The present study was planned to isolate bioplastic (PHB) producing bacteria from different types of soil sample and formulate media using *Parthenium hysterophorus* (congress weed) as the carbon source for cost effective production of PHB.

MATERIALS AND METHODS
Collection congress weed and soil sample for isolation of bioplastic producers Congress weed was collected from rural area from Thane district, Maharashtra, India and hydrolysate was made (Sabapathy P. et al 2017). Soil sample from different places like garage soil, farm soil, nearby cowdung soil and compost soil were collected for isolation of bioplastic producers.

Isolation and Characterization of PHB producing microorganism from soil sample

The isolation of PHB producing bacteria was carried out using nutrient agar plate with 1% glucose in presence and absence of congress weed

hydrolysate (1%) after enrichment) The production of PHB was analysed by both qualitative and quantitative method by using sudan black B and crotonic acid assay (Parmar S. et al., 2015). The qualitative detection was carried by spreading ethanolic solution of 0.02% Sudan Black B over the Petri plates containing colonies for 30 min. Development of black colonies indicates PHB production (Panigrahi S. et al., 2013). The bacteria with prominent PHB producing ability from congress weed was selected on the basis of production of PHB assessed by crotonic acid assay. Bacterial characterization was done on the basis of morphological, cultural and biochemical tests as per Bergey D., Robert B., Gibbons N., (1974).

Production and Characterization of PHB

The Media was formulated for the production of bioplastic using congress weed as substrate. The effect of different concentration acid treated congress weed hydrolysate (2,4,6 and 8%) on PHB production was studied (Sabapathy P. et al 2017). The production of PHB was also checked in presence of carbon sources like Glucose, Sucrose, Orange peel and Banana peel. (Richard et al., 2014). The PHB production was carried out with optimized concentrations of congress weed hydrolysate and carbon source. The PHB produced was then extracted by solvent extraction method and extracted PHB was analysed by FTIR.

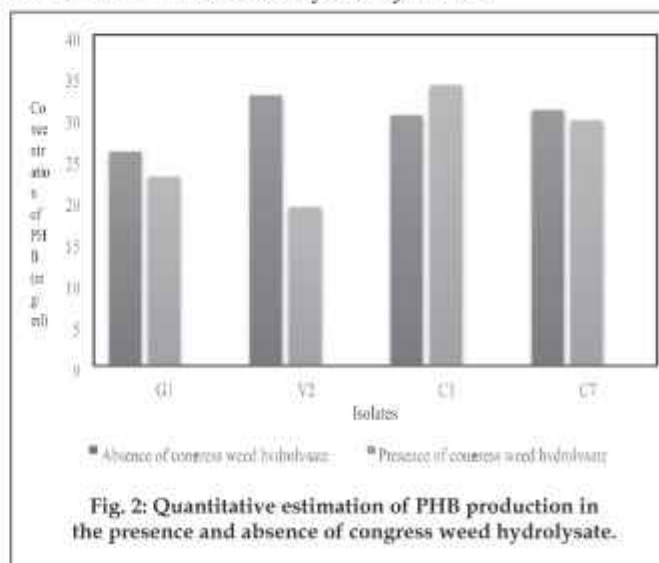


Fig. 2: Quantitative estimation of PHB production in the presence and absence of congress weed hydrolysate.

Results and Discussion

Isolation and characterization of bioplastic producing bacteria

Parthenium hysterophorus an invasive weed throughout India and world. The leaf proteins are reported to be better than cereal and legume proteins. PHB has been reported to be naturally produced by several bacteria such as *Alcaligenes*, *Pseudomonas*, *Bacillus*, *Rhodococcus*, *Staphylococcus* and *Micrococcus* (Singh P. et al., 2011). In the present study, total 6 soil samples were collected from weed, farm, garage and dumping sites. Total 50 isolates were obtained after the isolation of soil samples using Nutrient agar with 1% glucose.

Qualitative screening for PHB producers

The PHB is known to occur as intracellular granules in several genera of microorganisms. The granules are synthesized by prokaryotes using fatty acids, sugars and other carbon sources. PHB molecules are joined by ester bonds between carboxyl and hydroxyl groups of adjacent molecules. PHB accumulated in distinct bodies can be readily stained with Sudan black B and observed under light microscopy and observed as empty holes in the electron microscopy (Swathi and Ranjani, 2015). The lipophilic staining with Sudan Black B has high sensitivity for PHB screening and bacteria containing PHB exhibit dark granules. Out of 50 isolates, 17 isolates showed PHB production ability but only 4 isolates were found to produce PHB on media containing congress weed hydrolysate (Fig. 1).

Quantitative assay for PHB production

All the four isolates which showed PHB production ability qualitatively were checked for quantitative PHB production by modified crotonic acid method. Quantitative PHB production was checked in the presence and absence of 1% parthenium hydrolysate nitrogen deficient media. All isolates showed the PHB production in absence and presence of congress weed but isolate C1 who showed comparatively more PHB production in

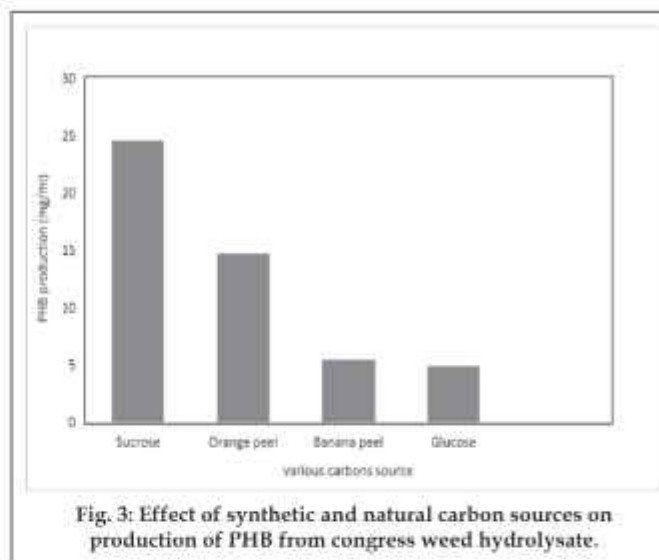


Fig. 3: Effect of synthetic and natural carbon sources on production of PHB from congress weed hydrolysate.

presence of congress weed than without congress weed media (Fig. 2). The PHB production in presence of congress weed hydrolysate of C1 isolate was found to be 33.91mg/ml.

Identification of efficient PHB producing isolate

The isolate showing highest PHB production in presence of congress weed hydrolysate was selected for further study. The selected PHB producing isolate was identified on the basis of morphological, cultural and biochemical test. Gram staining revealed the morphology as gram-positive thick rods. The colonies of the isolate on Nutrient agar plate appeared as big size round shaped, off white colored with entire margin with catalase and oxidase activity. Results of biochemical test of selected gram-positive isolate showed close resemblance with the genus *Bacillus*. Sharma and Bajaj (2014) reported poly-beta-hydroxybutyrate production by newly isolated *Bacillus cereus*.

Cost effective production and characterization of bioplastic

The selected *Bacillus* isolate was studied for growth in presence of different hydrolysate Concentration and 2% concentration of congress weed gave maximum PHB production. Sucrose as synthetic carbon source showed enhanced production of PHB (Fig.3).Sabapathy P et al., (2017)

studied PHB production by *Bacillus* using various congress weed concentration and reported highest PHB accumulation in presence of 6% congress weed media.

Extraction of PHB was performed using chloroform. Film of PHB was observed after evaporation of solvent. Similar method has been followed by Kulkarni S. et. al., (2014) who carried out production of PHA using agro-wastes by *Halomonas campisalis* MCM B-1027. The extracted PHB was analysed by FTIR in order to know the functional group present in the chemical structure of PHB at molecular level. FTIR spectrum of extracted PHB revealed the characteristic peak at 1741.41 cm^{-1} and 1644.98 cm^{-1} which correspond to the C=O stretch (Fig. 4). The characteristic band at 1464.67 cm^{-1} indicates C-H stretch. The peak at 1218.79 cm^{-1} and 1068 cm^{-1} correspond to C-O stretch. Number of additional peaks seen at the region of 3800 cm^{-1} to 3600 cm^{-1} shows the presence of water traces used for sample preparation. The analysis revealed that extracted sample's resemblance with to the member of the family Polyhydroxyalkonates i.e. Polyhydroxybutyrate.



Fig. 4: FTIR analysis of extracted PHB

Conclusion

The isolated *Bacillus* spp. showed potential to utilize congress weed as substrate for PHB production. The PHB was successfully extracted and can be used as an alternative to conventional plastic. Thus, microbial PHB production from the most abundant and hazardous weed, *Parthenium hysterophorus*, helps to reduce the production cost of PHB but also helpful in management of weed along with production of bioplastic.

References

1. Ahmed B., SeyedFoad A., Ahmad M., Reza V.(2005). "Oil spill cleanup from sea Water by sorbent materials" *Chemical Engineering And Technology*. 1525-1528.
2. Bergey D., Robert B., Gibbons N., (1974). "Bergey's manual of determinative bacteriology" Baltimore: Williams and Wilkins. **C a r b o n S o u r c e b y ResponseSurfaceMethodology**" *Journal of Polymers and the Environment* 1-9.
3. Junfeng D., Jing Y., Aizhong D., En X., Shuairan L., Lei Z., (2015). "Evaluation of poly-beta-hydroxybutyrate as a sorbent material for the removal of spilled crude oil from water" *Desalination and Water Treatment* 57. 11233-11241.
4. Kulkarni S., Kanekar P., Jog J., Sarnaik S., Nilegaonkar S., (2014). "Production of copolymer, poly (hydroxybutyrate-co-hydroxyvalerate) by *Halomonas campisalis* MCM B-1027 using agro-wastes" *International Journal of Biological Macromolecules* 784-789.
5. Panigrahi S., Badveli U., (2013). "Screening, Isolation and Quantification of PHB-Producing Soil Bacteria", *IJESI*. 2 (9): 1-6.
6. Parmar S., Malik N., (2015). "Comparitive study for effective extraction method for polybetahydroxybutyrate (PHB) from *Bacillus* spp.", *Life sciences international research journal*, 2: 1-4.
7. Richard H., Bo J., & Florian T., (2014). "Production of Poly(3-hydroxybutyrate) from Waste Potato Starch" *Bioscience, Biotechnology, and Biochemistry* 72 (1): 253-256.
8. Sabapathy P., Sabarinathan D., Preethi K., (2017). "Partheniumhysterophorus: low cost substrate for the production of polyhydroxyalkanoates" *Current Science* 112 (10): 2106-2111.
9. Sharma P., Bajaj B., (2014). "Cost-effective substrates for production of poly-beta-hydroxybutyrate by newly isolated *Bacillus cereus* PS-10" *Journal of Environmental Biology* 36: 1297-1304.

10. Singh G., Mittal A., Kumari A., Goel V., Aggarwal N., Yadav A., (2011). "Optimization of Poly-B-hydroxybutyrate production from Bacillus species" European Journal of Biological Sciences 3 (4): 112-116.
11. Swathi K., Ranjani N., (2015) "Production, isolation, screening and extraction of polyhydroxybutyrate (PHB) from Bacillus spp. using treated sewage sample" Int J Pharma Bio Sci 5 (2): 58-64.
12. Van D., Quillaguama J., Mamo G., and Mattiasson B., (2007). "Utilization of agricultural residues for poly(3-hydroxybutyrate) production by Halomonas boliviensis LC1" Journal of Applied Microbiology 420-428. ■

Effect of Azospirillum on Quantity of Saponin in Roots of *Chlorophytum borivillianum* (Safed musli)

Aboli Kshirsagar

Department of Botany

Arts and Science College, Pulgaon, Wardha, Maharashtra.

Corresponding Email- kshirsagaraboli@gmail.com

ABSTRACT

Saponin are naturally occurring glycoside widely distributed in plants. Saponin consists of a sapogenin as the aglycone moiety and a sugar. The sapogenin may be a steroid or a triterpene and the sugar may be glucose, galactose, a pentose, or a methylpentose. It exhibit antimicrobial properties, guarding your body against fungi, bacteria and viruses. at the same time, they improve immune function by stimulating the production of T-cells. Additionally, they act as antioxidants and scavenge oxidative stress. That's why sapogenin used in some vaccines. These chemical compound contain the 27 carbon atoms forming the core structures; spirostan and furostan. In present work author try to enlighten the influence of symbiotic association of Azospirillum (the nitrogen fixating bacteria) with root nodules of *C. borivillianum* on amount of saponin content in *C. borivillianum*. To increase the percentage (amount) of saponin the treatment of nitrogen fixating bacteria was given the symbiotic association of nitrogen fixating bacteria with root nodules of plants help to increase the amount of biologically active constituent of plant also help to increase the fertility of soil.

Keywords: Saponin, Azospirillum, Nitrogen fixation, *Chlorophytum borivillianum* (Safed musli), medicinal plants.

Introduction

From ancient time the human being used various plants for curing the various diseases . Now a days also these plants are used as an important part of supplement medicine . Safed musli (*Chlorophytum borivillianum*) is a commercially important medicinal plant in India belong to family Asparagaceae . It is one of the very popular herbal drug used in variety of diseases and ailments. It also have an exhaled position as a Rasayana drug of Ayurvedic System of Medicine. The drug containing saponin is considered a valuable medicine because of its wide applications as therapeutic and recreational

purpose saponin containing plant also have good defense activity against pathogen and animals.

Recently the drug has been investigated for various pharmacological activities and chemical constituents. *Chlorophytum borivillianum* has therapeutic application in Ayurvedic system of medicine. This have a very good application to increase immune system. Its aphrodisiac properties make it useful for the people suffering from Erectile Dysfunction and to increase male potency. Its spermatogenic property is helpful in curing impotency as they are rich in important constituent glycosides It is known to cure many physical illness

and weaknesses. Among all the species of Chlorophytum present in India *C. borivilianum* produces the highest yield of saponin. The detergent properties of saponin have led to their use in shampoos, facial cleansers and cosmetic creams.

**Classification of this plant is
Chlorophytum borivilianum (Safed musli)**

Kingdom : Plantae
Division : Angiosperms
Class : Monocots
Order : Asparagales
Family : Asparagaceae
Subfamily : Agavoideae
Genus : Chlorophytum
Species : borivilianum



Material and method

- 1] **Preparation of Azospirillum culture** - The chemical component require for the preparation of culture media is taken with extra purity .The agar solution is used for preparation of culture media.
- 2] **Preparation of land plots for cultivation of plant** - After pre sampling of soil and studying the effect of various fertilizer on biochemical constituent of *Chlorophytum borivilianum* (safed musli), cultivation of seedling tuber which are collected from Chikhaldara, district Amravati, state - Maharashtra from India .
- 3] **Harvesting** - After the yellowing of aerial part of *Chlorophytum borivilianum* the harvesting was done. Washing of tuber under running water for removing adhering soil is important.

- 4] **Peeling** - There after washing peeling off skin from root of *Chlorophytum borivilianum* carried out and roots were dried in shade.
- 5] **Chemical Analysis** - Simlot method a specific method with some modifications used for chemical analysis of saponin content from *Chlorophytum borivilianum*

Observation:- The observed saponin content is given in the following table:

These samples are used after specific interval of day and after specific treatment.		
Sr. No	control condition	Saponin (mg/100 gm)
1	sample1	0.221
2	sample 2	0.119
3	sample 3	0.219
4	sample 4	0.220
5	sample 5	0.219
6	sample 6	0.217
7	sample 7	0.218
8	sample 8	0.220
9	sample 9	0.218
10	sample 10	0.221
11	sample 11	0.216
12	sample 12	0.219
13	sample 13	0.220
14	sample 14	0.218
15	sample 15	0.220
16	sample 16	0.221
17	sample 17	0.221
18	sample 18	0.222
19	sample 19	0.221
20	sample 20	0.221
21	sample 21	0.219
22	sample 22	0.219
23	sample23	0.221
24	sample 24	0.222
25	sample 25	0.219
	Average quantity (mg/100 gm)	0.219

Result and Discussion - From the observations the final conclusion was made that the nitrogen fixing bacteria i.e Azospirillum affect the biochemical content Saponin of *Chlorophytum borivilianum*. The content was increases quantitatively in treated tuber it is 0.219mg/ 100 gm.

Acknowledgement

Author is thankful to UGC (University Grant Commission, Delhi, India) for providing all necessary facilities for completion of this research project. Author is also thankful to Arts and Science College, Pulgaon, Maharashtra, India for their valuable support.

References :

1. Anna petruczynnik,(2012), Central European Journal of Chemistry, 802-835
2. G Manjunatha, S K Tyagi, K Shrinivasan, (2004) Agrobios Jodhpur, India. pp. 12-14.
3. Kothari SK and Singh K., Production technique for the cultivation of safedmusli (*Chlorophytum borivilianum*), J Horticulture Sci Biotech. 2003; 78:2:261-264.
4. Oudhia P (2001). Problems perceived by Safed Musli *Chlorophytum borivilianum* growers of Chhattisgarh (India) region: A Study. J. Med. Arom. Plant Sci. 22/4A & 23/1A, 396-399.
5. P.C. Bordia, A.joshi, A.Simlot, (1995), Medicinal and Aromatic plant advanced in Horticulture,429-451
6. Payal Sharma (2017) and Kaushal K Chandrul. *Chlorophytum borivilianum* (Safed musli): A Vital Herbal Drug, International Journal of Pharmaceutical and Medicinal Research, 5(1):401-411
7. Qiu SX, Li XC, Xiong Y, Dong Y, Chai H, Fransworth NR, Pezzuto JM, Fong HH (2000). *Planta Med.* 66(6):587-590.
8. Rajpal V (2002). Standardization of Botanicals Testing and Extraction method of medicinal herbs. Vol.1, Eastern Publishers. pp. 223-228
9. S K Kothari, K. J. Singh, 2001, Spices Arom. Crops, 40:147-149.
10. S X Qiu , Li XC, Xiong Y, Dong Y, Chai H, Fransworth N R, Pezzuto JM, Fong HH (2000).*Planta Med.* 66(6):587-590.
11. S.G. Wankhade, R.B. Sarode, S.V. Gholap and S.B. Nandanwar,(2008) Saponin content in safedmusli (*Chlorophytum borivilianum*) as influenced by peeling of roots. The Asian Journal of Experimental Chemistry, Vol 3,79-81
12. Shah Taj-Uddin MS., Qureshi NA., Chasti AR., Wani AH. and Ramboo AR., Performance of *Chlorophytum borivilianum* Linn. in Kashmir., National Seminar on Organic Products and their Future Prospects, SKUAST 2003;(K), Srinagar, 98.
13. V K Kumar, Vadiraj BA, Sivadasam CR and Potty SN (2000). Challenges and opportunities in the new century. Contributing papers, Centennial Conference on Spice and Aromatic Plant, Calicut Kerela, India. 20-30,281-283.
14. Y Mimaki, T Kanmato, Y Sashida, A Nishimo , Y Setomi and H Nishino (1996), Drying characteristics of Safed Musli (*Chlorophytum borivilianum*) and its effect on colour and saponin content. Photochemistry 41:1405-1410.
15. Yun DJ., Metabolic engineering of medicinal plants: transgenic *Atropa belladonna* with an improved alkaloid composition, Proc. Natl. Acad. Sci. U. S. A. 1992;89: 11799- 11803.

Antifungal activity and GC-MS analysis of *Chaetomorpha* and *Padina*

Megha Nandkishor Mole

Department of Botany, Mahavir College, Kolhapur
m_pranju@rediffmail.com

ABSTRACT

The antifungal activity and chemical constituents of *Chaetomorpha media* and *Padina tetrastromatica* Collected from Kunkeshwar Sindhudurg district of Maharashtra. Food poisoning method was used to determine the antifungal activity of extractable matter against a *Fusarium oxysporum* and *Rhizopus artocarp*. The methanolic extract of *Padina* inhibited the mycelial growth of *F.oxysporum*(12.39mm) and *R.artocarp* (15.63mm). Gc-ms analysis of *C.media* and *P. tetrastromatica* indicate the existence of different constituents revealing ecological impact. In the methanolic extract of *Chaetomorpha media* and *Padina tetrastromatica* 8 and 5 compounds were identified. The compound with the highest concentration was n-hexadecanoic acid Followed by phytol. Most of the identify compound reported were responsible for antifungal potential of reported in the present study

Keywords: Antifungal, *Chaetomorpha media*, *Padina tetrastromatica*, *Fusarium oxysporum*, *Rhizopus artocarp*, GC-MS analysis.

Introduction

There was a growing demand and need for new bioactive drugs to control many bacterial and fungal diseases of plants and animals. New sources of drugs were tapped to overcome the problem of microbial resistance to antibiotics. Marine organisms caught the attention of researchers, being rich source of structurally new and biologically active metabolites (Ely et al., 2004). Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of primary and secondary metabolites which can be used in pharmaceutical industry (Attaway and Zaborsky, 1993). These bioactive molecules represent a broad range of biological activities such as antibiotic, antimicrobial, antiviral, antitumor and antioxidant (Scheuer, 1990; Tuneyet al., 2006; Patra et al., 2008). Pathogenic fungi are responsible for a considerable loss of plant yield (Sexton and Howlett, 2006). Secondary metabolites extracted from seaweeds are

known to possess antifungal properties (Cordeiro et al., 2006; Khanzada et al., 2007).

Several studies have been reported on the antibacterial activity of marine algae however, available information on their antifungal activity is limited. The present investigation aimed to evaluate the antifungal activity of *Chaetomorpha media* and *Padina tetrastromatica*, collected from Kunakeshwar, Sindhudurg district of Maharashtra against *Fusarium oxysporium* and *Rhizopus artocarp* in order to discover potential antifungal metabolites. Qualitative identification of most potential antifungal extract of these two seaweeds was performed using retention time and mass spectra in the GC-MS analysis.

Material and Methods

Collection and extraction: Fresh and mature thalli of *Chaetomorpha media* (C. Agardh) Kutzand *Padina*

tetrastromatica Hauck was collected during low tide from the submerged marine rocks at Kunkeshwar (164°0.120'N latitude and 7328°0.120'E longitude) in Sindhudurg district along the west coast of Maharashtra (India). The algal sample was cleaned with fresh seawater and then in distilled water to remove epiphytes, suspended matter and sand particles. The material was air dried in shade and after complete drying it was ground to form powder. Ten grams of dry algal powder was extracted in 100ml of methanol solvent for 24 hours on a rotary shaker and the extract was filtered through a Buchner funnel using Whatman no.1 filter paper (Yuvraj et al., 2011). The filtrate was condensed to half of the original volume (50ml) and stored in glass vials until used.

Antifungal Activity

Sensitivity of fungal strains to different algal extracts was analyzed using food poisoning method described by Dekker and Gleink (1979).

Czapek Dox Agar medium plates were prepared by mixing one ml algal extract with autoclaved Czapek Dox Agar in a 30ml marked beaker to make final volume of 30ml. The contents were mixed well and poured into a sterile petriplate. Discs of 8mm of actively growing margins in the plates of eight days old fungal culture were placed inverted on the agar surface of plates at the center. The control was maintained without algal extract. Plates were incubated at 25±20C in an incubator and linear growth was measured after 72hours. Percent inhibition was calculated by using formula-

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

Where C = Diameter of fungus colony in control (mm)

T = diameter of fungus colony in algal extract (mm).

GCMS analysis of seaweeds

Seaweed extracts were analyzed by gas chromatography and mass spectrometry for the quantitative determination of phytochemicals. GC-MS analysis was carried out using Shimadzu QP-2010 with non polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 400C and held for 3 min and the final temperature of the oven was 4800C with rate at 100C [min,sup.-1]. A two µL sample was injected with splitless mode. Mass spectrum was recorded over 35-650 amu range with electron impact ionization energy 70 eV. The total running time for a sample was 45 min.

Identification of components

Identification of mass spectrum was done using data base of NIST (National Institute of Standards and Technology). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The retention time, molecular weight, molecular formula and percent amount of individual compounds were recorded.

Result and Discussion

Antifungal activity observed in methanolic extracts of *C. media* and *P.tetrastromatica* is presented in Table 1. The percent inhibition observed for *Chaetomorpha* and *Padina* extracts were compared with that of standard Streptomycin (Fig. 1).

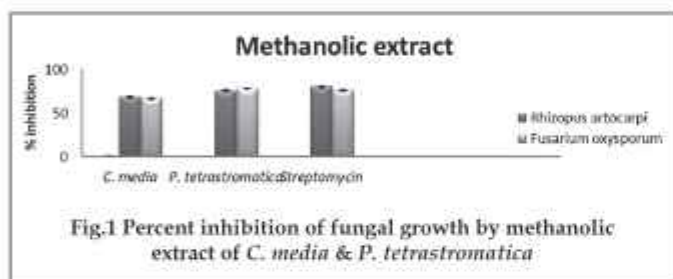
P. tetrastromatica effectively inhibited the growth of *R. artocarpi* (15.63mm) and *F. oxysporum* (12.39mm) as compared to *C. media*. Methanol extract of *P. tetrastromatica* gave better percent inhibition of fungal growth ranging from 76.06 - 78.44%.

C. media was less effective to control *F. oxysporum* and *R. artocarpi* Methanol extract of *C. media* produced more than 60% inhibition in all the fungal strains in the present study

Table 1: Effect of methanolic extract of *Chaetomorpha media* & *Padina tetrastromatica* on fungal growth

Seaweeds	Growth zone (Diameter in mm)	
	<i>Rhizopus artocarp</i>	<i>Fusarium oxysporum</i>
<i>Chaetomorpha media</i>	20.36 ±0.35	18.83 ±0.30
<i>Padina tetrastromatica</i>	15.63 ± 0.70	12.39 ± 0.25
Control	65.30 ±0.26	57.53 ±0.50
Streptomycin	12.66± 0.76	13.50± 0.50

Values are mean of three replicates. ± Values represent SD.



Several workers have reported antifungal activity of ethanol, methanol, hexane and ethyl acetate extracts of *Ulva fasciata* and *Chaetomorpha antennina* against a variety of pathogenic fungi (Febles et al., 1995; Ali et al., 2000). Tuney et al. (2006) found that ethanolic extract of *Padina pavonica* was active against *Candida albicans* but methanolic and acetone extracts of the same alga were inactive against *C. albicans*. Khaled et al. (2012) reported a significant antifungal activity against *Candida glabrata* and *C. krusei* in ethyl acetate fraction of *Padina pavonica*. Saidaniet al. (2012) also observed antifungal effect of *Padina pavonica* against *C. albicans*.

In our study methanolic extract of *P. tetrastromatica* were active against *R. artocarp* and *F. oxysporum*.

GC-MS analysis

In methanolic extract of *C. media* eight compounds were identified using GCMS. The compound with the highest concentration was n-hexadecanoic acid (53.39%) followed by phytol (20.36%) and then methyl ester of hexadecanoic acid (11.74%).

. n- hexadecanoic acid (46.17%) and phytol

((19.16%)) was the most abundant compounds in *P. tetrastromatica*.

Table 2: Chemical composition of methanolic extract of *Chaetomorpha media*

R.T (min.)	Name of Compound	Molecular formula	Molecular weight	Percent composition
16.094	2-Pentadecanone, 6-10, 14- trimethyl	C ₁₈ H ₃₆ O	268	1.02
16.148	1,4- Eicosadiene	C ₂₀ H ₃₈	278	5.32
16.387	7- Octadecyne, 2- methyl	C ₁₉ H ₃₆	264	2.27
16.579	3,7,11,15- Tetramethyl - 2- hexadecen- 1-01	C ₂₀ H ₄₀ O	296	3.16
16.889	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	11.74
17.245	- Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	53.39
18.596	10- Octadecanoic acid	C ₁₉ H ₃₆ O ₂	296	2.75
18.741	Phytol	C ₂₀ H ₄₀ O	296	20.36

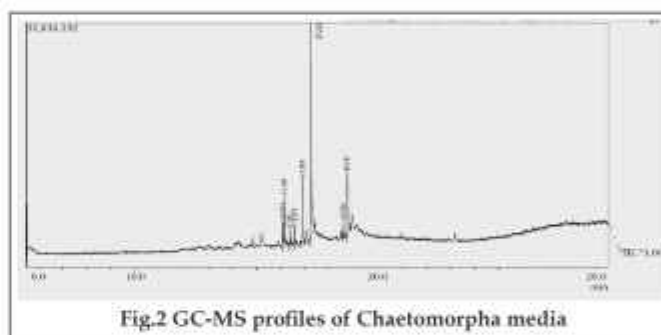
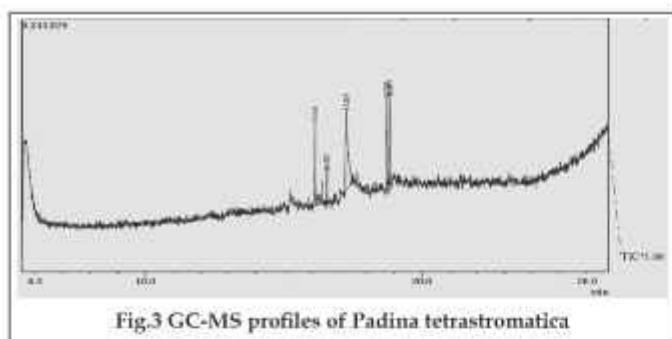


Table 3: Chemical composition of methanolic extract of *Padina tetrastromatica*

R.T (min.)	Name of Compound	Molecular formula	Molecular weight	Percent composition
16.141	9- Eicosyne	C ₂₀ H ₃₈	278	15.19
16.573	E- 11- Tetradecen- 1- 01 acetate	C ₁₈ H ₃₄ O ₂	254	4.91
17.273	- hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	46.17
18.753	Phytol	C ₂₀ H ₄₀ O	296	19.16
18.847	Dicyclomine	C ₁₅ H ₁₉ NO ₂	309	14.56

An investigation on the fatty acid content of brown algae from Indian coast has shown, palmitic acid (hexadecanoic acid) as the major constituent (Dhamotharan, 2002). Palmitic acid (hexadecanoic



acid) has been detected as the major fatty acid in *P. gymnospora* (Parekh et al., 1984), *P. pavonica* (Qasim, 1986). In *Padina pavonica* oleic acid was found to be the main fatty acid and palmitic acid appeared in low concentration (Kanias et al., 1992).

References

1. Ali, M. S., Mazhar, F., Ahmad, V. U. and Usmanghani, K. (2000). Preliminary biological screening of some marine algae collected from Karachi coasts of Arabian sea. *J. Chem. Soc. Pak.* 22(1): 36-41.
2. Attaway D. H. and Zaborsky O. R. (1993). *Marine Biotechnology Volume I. Pharmaceutical and bioactive natural products*, Plenum Press, New York, 504 pp.
3. Cordeiro, R. A., Gomes, V. M., Carvalho, A. F. U. and Melo, V. M. M. (2006). Effect of proteins from the red seaweed *Hypneamuscififormis* (wulfen) Lamouroux on the growth of human pathogen yeasts. *Braz. Arch. Biol. Technol.* 49: 915-921.
4. Dekker, J. and Gleink, A. J. (1979). Acquired resistance to pimaricin in *Cladosporium cucumarinum* and *Fusarium oxysporum* sp. narcissi associated with decreased virulence. *Neth. Plant. Pathol.* 85: 67-73.
5. Dhamotharan, R. (2002). An investigation on the bioactive principles of *Padina tetrastromatica* Hauck and *Stoechospermum marginatum* (C. Ag.) Kuetz. with respect to antimicrobial and biofertilizer properties. Ph. D. Thesis, University of Madras.
6. Ely, R., Supriya, T. and Naik, C. G. (2004). Antimicrobial activity of marine organisms collected off the coast of South East India. *J. Exp. Biol. Ecol.* 309: 121-127.
7. Febles, C. I., Arias, A., Hardisson, A. A. and Lopez, A. S. (1995). Antimicrobial activity of extracts from canary species of Phaeophyta and Chlorophyta. *Phytother. Res.* 9: 385-387.
8. Kanias, G. D., Skaltsa, H., Tsitsa, E., Loukis, A. and Bitis, J. (1992). Study of the correlation between trace elements, sterols and fatty acids in brown algae from the Saronikos Gulf of Greece. *Fresenius' J. Anal. Chem.* 344: 334-339.
9. Khaled, N., Mawlawi, H. and Chbani, A. (2012). Antioxidant and antifungal activities of *Padina pavonica* and *Sargassum vulgare* from the Lebanese Mediterranean Coast. *Adv. Env. Biol.* 6(1): 42-48.
10. Khanzada, A. K., Shaikh, W., Kazi, T. G., Kabir, S. and Soofia, S. (2007). Antifungal activity, elemental analysis and determination of total protein of seaweed, *Solieriarobusta* (Greville) *Kylin* from the coast of Karachi. *Pak. J. Bot.* 39(3): 931-937.
11. Parekh, K. S., Parekh, H. H. and Rao, P. S. (1984). Antibacterial activity of Indian sea weeds. *J. Phycol. Soc.* 23: 216-221.
12. Patra, J. K., Rath, S. K., Jena, K., Rathod, V. K. and Thatoi, H. N. (2008). Evaluation of antioxidant and antimicrobial activity of seaweed (*Sargassum* sp.) extract: A study on inhibition of Glutathione-S-transferase activity. *Turk. J. Biol.* 32: 119-125.
13. Qasim, R. (1986). Studies on fatty acids composition of eighteen species of seaweeds from the Karachi coast. *J. Chem. Soc. Pak.* 8: 223-230.
14. Saidani, K., Bedjou, F., Benabdesselam, F. and Touati, N. (2012). Antifungal activity of methanolic extracts of four Algerian marine algae species. *African J. Biotechnol.* 11(39): 9496-9500.
15. Scheuer, P. J. (1990). Some marine ecological phenomena: chemical basis and biomedical potential. *Science.* 248: 173-177.
16. Sexton, A. C. and Howlett, B. J. (2006). Parallels in fungal pathogenesis on plant and animal hosts. *Eukaryotic Cell.* 5: 1941-1949.
- Tuney, I., Cardira, D. U. and Sukatar, A. (2006). Antimicrobial activities of the extracts of marine

algae from the coast of Urla [Zmir, Turkey].
Turk J. Biol.30:171-175.

17. Yuvraj, N., Kanmani, P., Satishkumar, R., Paari, K. A., Pattulumar, V. and Arul, V. (2011). Extraction, purification and partial

characterization of *Cladophora* glomerata against multiresistant human pathogen *Acinetobacter baumannii* and fish pathogens. World J. Fish Mar. Sci.3(1):51-57. ■

Extraction and characterization of tannins from *Cassia tora* Linn leaves

Madhuri Patil*, Megha Phulari and Chandrashekhar Murumkar

Post Graduate Research Centre, Department of Botany,

Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati (Autonomous)

Corresponding author : patilmadhuri352@gmail.com

ABSTRACT

Cassia tora Linn is a medicinal plant used as laxative for the treatment of leprosy and various skin disorders, belongs to family *Caesalpin : aceae* used in Ayurved and Siddha system. It is common weed of rainy season and even blooms in hot arid environment of drought prone area of Baramati. It is rich in tannins and other biomolecules which may help in its medicinal potential. The objective of this study is to extract tannins from normal and oven dried method and to characterize the extracted tannins using UV and FT-IR methods. *Cassia tora* Linn grow as weed in waste places and thus tannin extraction and its characterization may further be utilized for its bioprospecting potential.

Introduction

Cassia tora Linn. is annual foetid herb which grow in low lying coastal areas, river banks, abundant in waste places and other uncultivated fields. It is most commonly known as 'Sickle pod' due to Sickle shape of pods. Kirtikar and Basu (1975) reported that the leaves of *C. tora* are reported to have antirheumatic activity in folklore practice and decoction of the leaves is used as laxative. The extract of *C. tora* leaves has been found to possess significant hepatoprotective activity and anti-inflammatory activity (Maitya,1998)

Das et al., (2011) proposed that various phytochemicals present in this plant such as anthraquinone glycosides, naphthopyrone glycosides, phenolic compounds, flavonoids, sennosides, rubrofusarinetriglucoside, etc. present in the plant significantly contribute to the diverse biological functions such as antioxidant, antibacterial, antifertility, antitumor, antiinflammatory, antifungal activities. *Cassia tora* leaves are used as antifungal agent due to presence

of chrysophanic acid -9-anthrone (Acharya et al.,1975). Leaves and seeds are used in the treatment of ringworm and itch (Hooker,1979). Sennosides, which are well known for their medicinal importance, have been detected in the leaves of the plants(Lohar,1975).

Besides of its biological evaluation tannin extraction and its characterization from leaves of *Cassia tora* Linn is worth to study for its industrial exploitation. In the present investigation, two

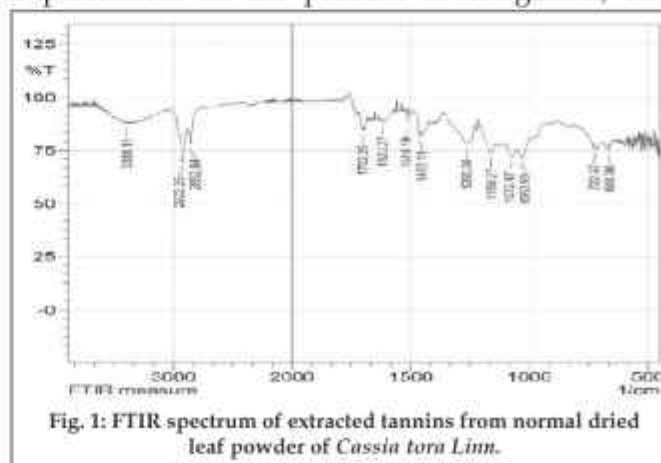


Fig. 1: FTIR spectrum of extracted tannins from normal dried leaf powder of *Cassia tora* Linn.

methods were used to dry the leaves and comparative study on characterization of extracted tannins was carried out.

Materials and Methods

The fresh plant of *Cassia tora* Linn. was collected from Baramati area during the month of July-August 2019 and identified by the taxonomist of Post Graduate Research Centre, Department of Botany, Tuljaram Chaturchand College, Baramati using floras. The leaves were cleaned and dried by natural sun drying and oven dried method. It was grinded to make fine powder filtered through muslin cloth and stored in a vacuum desiccator for further studies.

Extraction of Tannins

For extraction of tannins *Cassia tora* L. normal and oven dried leaf powder of 10gms was macerated in 100 ml of acetone for 24 hours; The supernatant was separated from the residue by filtration using Whatman no.1 filter paper, the fraction concentrated, dried to a constant weight in a vacuum oven at 45°C and the residues obtained stored in refrigerator. The extract obtained was used for further analysis of UV and FT-IR spectroscopy.

UV analysis

Normal and oven dried powder of *Cassia tora* L. leaves were scanned by UV-visible spectrophotometer at the wavelength of 200 - 800 nm on Perkin - Elmer Lambda 25 spectrophotometer. It is basically done for monitoring the extract as UV - VIS spectroscopy is used for the characterization of colloidal particles. Nobel metal particles possess strong surface plasmon resonance (SPR) absorption in the visible region and are highly sensitive to the surface modification. For UV-VIS spectrophotometer analysis, the acetone extract was centrifuged at 3000 rpm for 10 mins and filtered through Whatman No. 1 filter paper.

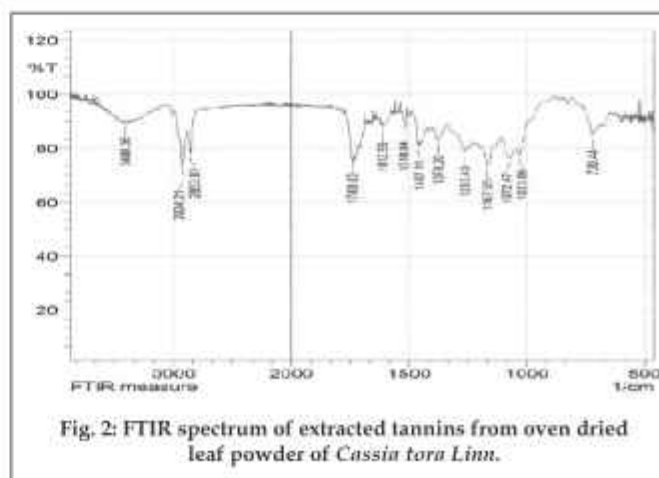
FT-IR Spectroscopy: Fourier transform infrared (FTIR) measurement of normal and oven dried powder of *Cassia tora* L. leaves was performed on the

instrument (Shimatzu). A few mg of sample was placed in the sample holder and scanned the sample between range 4000-500 cm⁻¹ by ATR technique was used to identify the characteristic functional groups in the extract. It provides information about the structure of molecule and obtained from its absorption spectrum.

Results and Discussion

Cassia tora Linn is rich in tannins. Mainly there are three types of tannin present in plants hydrolysable, condensed tannins and phlorotannins plays an important role in defense mechanism against microbial infection. Their molecular weight ranges between 500-3000 Da. Three solvents are commonly used to extract tannin from plant samples: boiling aqueous methanol, aqueous acetone, or acidic methanol. Boiling aqueous methanol is thought to be the most effective solvent for condensed tannin (Bate-Smith, 1975). Aqueous acetone is routinely used to extract hydrolyzable tannins (Foo and Porter, 1980) thus in present investigation 80 % acetone is used for extraction of tannins from leaves of *Cassia tora* Linn. A great interest has been emerged in the protective role of tannins against free radicals and ROS generation helps in anticancer studies (Singh and Kumar, 2019)

Hagerman (1988) concluded that aqueous acetone appears to be the best solvent for extracting tannin from leaf tissue. She also observed that the amount of tannin extracted from fresh leaves of Burr oak,



Quercus macrocarpa; sugar maple, *Acer saccharum*; shagbark hickory, *Carya ovata* collected late in the season was greater than from fresh leaves collected early in the season. The extraction of tannin from the dried leaves was quite efficient for some samples and less efficient for other samples.

Acetone is an effective solvent because it inhibits interaction between tannin and proteins thus prevents tannin from binding to leaf proteins during homogenization (Hagerman and Robbins, 1987). Acetone does not interfere with most chemical assays for tannin (Price et al., 1978).

UV analysis: The qualitative UV-Vis profile of extracted tannins from normal and oven dried leaf powder of *Cassia tora* Linn. was taken at 200 to 800 nm. The peaks are at 210.5nm with max absorption of 4.00 in oven dried sample and in normal dried sample max absorption at 3.68 and 3.42 with max absorption at 214 and 210.5nm. It is very near to standard tannic acid as similar peaks were shown at 214, 271, 235, 323 nm in dried leaf powder of *C. tora* Linn.

Comparative UV analysis indicate that normal drying method for extraction of tannins is quite suitable for extraction of tannins as having same chromophores or functional groups as that of standard tannic acid but oven dried sample is with only one peak.

FT-IR analysis: FTIR measurement of normal and oven dried powder of *Cassia tora* L. leaves is depicted in figure 1 and 2. It is clear from figures that strong absorption around 3388.00 and 3408 cm⁻¹ assigned to NH stretching, 2923.25 and 2924.21 cm⁻¹ due to C-H stretching and 2852.84 and 2853.81 cm⁻¹ assigned for O-H stretching in both samples whereas 1702.25 cm⁻¹ and

1740.83 cm⁻¹ from Normal and 1740.83 cm⁻¹ from oven dried leaf sample is due to C=O stretching. C=C stretching was observed at 1620.27 and 1612.56 cm⁻¹ while 1514.19 and 1518.04 cm⁻¹ peaks assigned for N-O stretch in normal and oven dried leaf sample.

Interestingly in oven dried leaf powder additional peak at 1462.00 cm⁻¹ for C-H bending, 1318.20 cm⁻¹ for OH bending, 1263.43 for C-O stretch whereas the peaks at 1167.95, 1072.47, 1033.89 cm⁻¹ assigned to C-N stretch which may be amines and C=C bond stretching at 720.44 cm⁻¹. It showed that both the samples have same vibrational frequencies.

Conclusion

Global market for tannins is increasing day by day. As compared to synthetic chemicals natural source of tannins can be an alternative green material in new emerging industries for sustainable environment. *Cassia tora* Linn. grows as weed in many parts of India and abroad unexploited for its utilization as source of tannin. Present studies will be helpful for higher yield of tannins from *Cassia tora* Linn. leaves using normal drying method with accuracy with which a chemical component in a biological matrix remain altered in its biological activity and structure. Further microbial studies will throw more light on understanding medicinal potential of tannins.

References

1. Acharya, T.K., Chatterjee, I.B., Lloydia, 38(3), 218-220 (1975)
2. Ann E. Hagerman, (1988) Extraction of tannin from fresh and Preserved leaves Journal of Chemical ecology, vol. 14, no. 2, 1988.
3. Bate-smith, E.C. (1975). Phytochemistry of proanthocyanidins. Phytochemistry 14:1107-1113
4. Das C., Dash S., Sahoo D. C., Mohanty A., and Rout D. (2011). *Cassia tora*: A Phyto-Pharmacological Overview. Int. J. Res. Ayurv. Pharm.; 2(4): 1162-1174
5. Foo, L.Y., and Porter, L.J. (1980). The phytochemistry of proanthocyanidin polymers.
6. Hagerman, and Robbins. 1987. Radial diffusion method for determining tannin in plant extracts. J. Chem. Ecol. 13:437-449.

7. Hooker, J.D., The Flora of British India,II, L.Reeve and Co., England, (1879), p.26.
8. Kirtikar, K.R. and Basu, B.D., (1975)Indian Medicinal Plants, Vol II, Periodical Experts D-42, Vivek Vihar Delhi, 1975, pg.877.
9. Lohar D.L., Chawan D.D. and Garg S.P., Phytochemical studies on *Cassia* species of Indian Arid Zone" Current Science, (1975), 44(2),67.
10. Maitya T.K., Mandal S.C., Saha B.P. and Pal M., Evaluation of hepatoprotective potential of *Cassia tora* leaf extract, Prod. Sci, (1998), 4(4), 226.
11. Price, m. L. Vanscoyoc, S., and butler, I.g. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in *sorghum* grain. J. Agric. Food Chem. 26:1214-1218.
12. Singh Akhlesh P. and Kumar Sunil, (2019), Applications of tannins in Industry BK Tannins - Structural Properties, Biological Properties and Current Knowledge Publisher: Intech Open, London, United Kingdom, DOI: 10.5772/intechopen.85984. ■

COVID-19 and its effects on economy and environment

Monica Laxmanrao Uttarwar

Victoria Melbourne, Australia-3000

Email Address: monicauttarwar14@gmail.com

ABSTRACT

The Corona Virus which started in December 2019, also called as COVID-19 which stands for 'CO' as corona, 'VI' for virus and 'D' for Disease, '19' denotes the year. It is denoted as new virus which was linked to the same family of viruses which is Severe Acute Respiratory Syndrome (SARS). The outbreak started from Wuhan, Hubei province and it was spread in China. Since people were not aware of the severity of the illness and no travel restrictions virus started spreading all over the world. Soon people in China realized the severity of the virus and by early January interventions were introduced like testing and isolation of suspected cases and introduced travel restrictions to other cities across Hubei Province in China. In just around 2 more months epidemic spread across the world. On March 11, 2020 the World Health Organization (WHO) declared the spread of COVID 19 as a pandemic. Till March end 303000 confirmed cases were measured across the world and approximately over 10,000 deaths in 150 countries were counted. It was affecting the older adults and people with serious health conditions. China started the lockdown and it affected the consumption and production and it also affected the supply chain and affected the companies across the globe. Many people lost their jobs due to lockdown; schools were shutting down. Even increase in the cases across the world end other countries started to go into the lockdown including closing borders and travel restrictions, closing schools and shopping malls and marriage halls and closing non-essential entertainment like cinemas, restaurants and bars to maintain social distancing. When lockdowns were introduced the panic buying of goods which in return created shortage in the markets for goods. This action of the government caused economic crisis, affected the share prices in the world and cause of recession.

Keywords: COVID, epidemic, SARS, pandemic, World Health Organisation, Recession.

Introduction

This paper focuses on the impact of COVID on the world economy and the prevention measures which are studied for disease. After declaration by WHO COVID as pandemic Public Health Emergency of International Concern and rapid increase in confirmed cases made the prevention and control of COVID-19 extremely serious^[1]. There are also arguments as on 1st March 2020 around 79,986 cases of COVID-19 were confirmed in China. The reports of cases were restricted to Hubei until 23 January

and there are reports which are studied with travel history and Wuhan has been major source of early cases, this movement increased before Lunar New Year. Travel bans at that time would have restricted pandemic to spread across the world^[2]. COVID has affected 100 countries in weeks, it reduced new cases in China by more than 90%. However, Italy has been affected, number of patients infected since Feb 21 follows an exponential trend, if trend follows for weeks then there will be 30000 infected patients^[3]. In USA influenza is widespread 3499 cases with 6

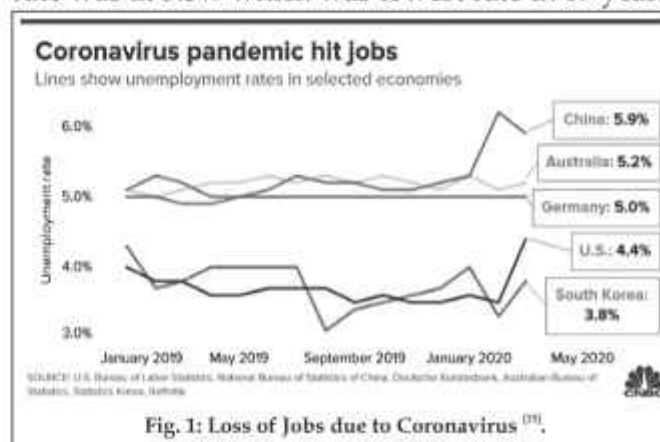
deaths, with most cases in New York (20.1%), Washington (18.4%) and California (12.2%), they also marked the COVID cases increase with older age^[4]. COVID-19 worldwide exploded to 784,794 cases and caused 37788 deaths by 30 Mar 2020. In India first case was reported on 30 Jan. By 30 Mar, India had reported 1251 cases and 32 deaths. As per the prediction more than 364 million cases and 1.56 million deaths with peak in mid-July as per the studied model in the research publication and predicted growth rate for pandemic in India was 1.15^[5].

Related work

Ever since WHO declared COVID 19 as pandemic, there are various health authorities focused on rapid diagnosis and isolation of patients and shown Chloroquine inhibited replication of some corona viruses. Chloroquine is form of quinine, which was synthesized in Germany by Bayer in 1934, is considered drug against malaria and possess antiviral activity against various viruses^[6]. WHO announced major study to compare trial design and also many doctors around world to join, there are 12 treatments being tested which includes drugs in use for HIV, malaria and antibody-rich plasma from people who recovered from COVID-19^[7]. Other studies show the antiviral and supportive treatments are important, however there is debate for other treatment like anti-inflammatory medicine like corticosteroid, debate aspects like for which patient the anti-inflammation therapy should be used? When should the treatment start? Which medication is the best choice? As taking this medicine may delay elimination of virus and increase the risk of secondary infection^[8]. Even though there is no proven therapy for virus, according to this study most promising therapy is remdesivir, as it is not US Food and Drug Administration approved and is currently being tested [9]. Immune-boosting effect of Homoeopathic medicine and its role in preventing viral infection. The Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha, and Homoeopathy (AYUSH) have suggested the use of Arsenicum album - 30 for its

possible role in preventing COVID-19 infection [10]. COVID 19 has led to distress in general public like symptoms of anxiety and depression. Effect of Ayurveda promoted by Indian government as "immune booster" described by Moerman influence positively on effects of symptoms of depression or anxiety and resulting in moderating the risk of infection^[11].

There have been many effects on the economy of the countries, in China we have seen the lockdown which reduced the consumption and production. Even the global supply chains are disrupted, millions of people losing jobs and companies shutting down. Consumers changed consumption pattern, which is resulting in shortages in supermarkets^[12]. When we study the primary sector industries like Agriculture, the global crash in demand of hotels and restaurants has seen prices of agriculture commodities drop by 20%. Secondary sector like chemical industry is predicted to reduce its global production by 1.2% and tertiary industries like Education has been affected from preschool to tertiary education. There was complete closure in Germany and Italy to targeted closure in United Kingdom, additionally over 100 countries imposed nationwide closure of educational facilities and UNESCO estimated close to 900 million learners have been affected by closure^[13]. There are three indicators like stock market volatility, newspaper-based economic uncertainty and subjective uncertainty in business expectation surveys. For example, in USA in February 2020, unemployment rate was at 3.5% which was lowest rate in 67 years,



nearly ten million Americans filled unemployment benefits^[14]. Economist warned the lockdown measures around the world will lose jobs; it is shown in the Fig.1. below^[15].

Warned COVID-19 hit the economic activity, it is expected the global economy to shrink by 3% this year. Only some economies like China are expected to grow as shown in Fig. 2. ^[15].

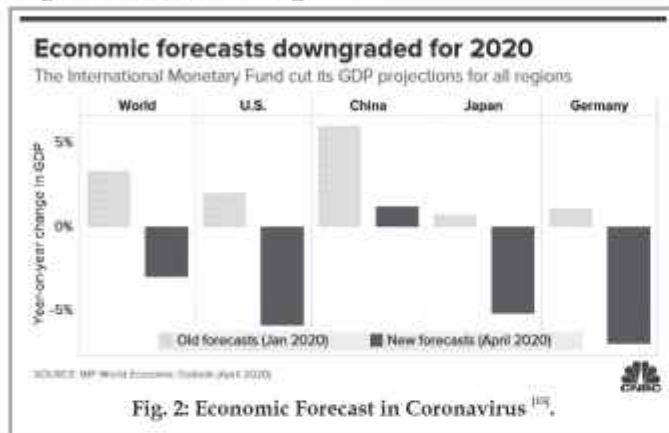


Fig. 2: Economic Forecast in Coronavirus ^[15].

Proposed Solution

Various aspects of COVID-19 and how it all started and affected the humankind. With safety measures which are taken by the government and society to introduce lockdown has led to mixed effects. With all family members staying at home either working from home or staying at home increased the domestic violence which includes physical, emotional and sexual abuse, increased home video-gaming. Self-isolation and loss of job have increased depression among people and decrease the power of spending. COVID has also thought us that you can have nice time with family staying at home. In this time, it is very important to be motivated and have positive mindset irrespective of listening all the depressing and unfavorable news. Along with positive mindset the focus should always be to exercise regularly, practice yoga and meditation, have healthy diet and not to forget self-hygiene, like regularly washing hands for 20 seconds, covering your nose with tissue or your elbow while sneezing, regular use of sanitizers and avoid touching the surfaces.

Discussion

With the progress in the research and developments going on presently across various countries we have to just wait and watch for the results which will be promising for humankind. In the present time it is very important to think outside the box and increase our immunity as we do not know what future has for us. We should all start from the basics to boost immunity, take healthy diet and avoid eating outside food. It is also very crucial for each country to focus on the improvement of their healthcare and increase the availability of the testing amenities as it should be available for all the people in the region.

Conclusion

As there are fears of new recession and financial collapse, the time calls for strong leadership in healthcare, business and government. Relief measures should be implemented for those who are suffering, long term planning should be done to re-energize the economy and new opportunities should be given for the businesses to flourish again. So far, no therapies have shown effective results to date. There is still needed to conduct control randomized trials to confirm the findings to introduce the vaccine for COVID-19. There have been many times in the past that viruses and crisis have impacted the humanity and we are the ones who know how to find the solutions, stand strong and overcome the crisis.

Acknowledgements

I am grateful for completing Masters of Engineering, Department of Electrical and Electronic Engineering, Auckland University of Technology, New Zealand to my guide Dr. Peter Chong. I wish to express my deep sense of gratitude to Dr. Amit Saraf, Head, Department of Botany Ismail Yusuf College of Arts, Science and Commerce for giving me an opportunity to publish my research paper and providing valuable guidance and support during the preparation of the research paper.

References

1. Ying-Ying Zheng, Yi-Tong Ma, Jin-Ying Zhang, and Xiang Xie, " COVID-19 and the cardiovascular system", *Nature Reviews Cardiology*, Volume 17 May 2020
2. Moritz U. G. Kraemer, Chia-Hung Yang, Bernardo Gutierrez, Chieh-Hsi Wu, Brennan Klein, David M. Pigott, Open COVID-19 Data Working Group, Louis du Plessis, Nuno R. Faria, Ruoran Li, William P. Hanage, John S. Brownstein, Maylis Layan, Alessandro Vespignani, Huaiyu Tian, Christopher Dye, Oliver G. Pybus, Samuel V. Scarpino, "The effect of human mobility and control measures on the COVID-19 epidemic in China" Kraemer et al., *Science* 368, 493–497 (2020) 1 May 2020.
3. Andrea Remuzzi, Giuseppe Remuzzi, "COVID-19 and Italy: what next?", *Lancet* 2020; 395: 1225–28 Published Online March 12, 2020 [https://doi.org/10.1016/S0140-6736\(20\)30627-9](https://doi.org/10.1016/S0140-6736(20)30627-9), Vol 395 April 11, 2020.
4. Gerardo Chowell, Kenji Mizumoto, " The COVID-19 pandemic in the USA: what might we expect?", Department of Population Health Sciences, School of Public Health, Georgia State University, Atlanta, GA 30033, USA www.thelancet.com Vol 395 April 4, 2020.
5. Kaustuv Chatterjee, Kaushik Chatterjee, Arun Kumar, Subramanian Shankar, "Healthcare impact of COVID-19 epidemic in India: A stochastic mathematical model", *Medical Journal Armed Forces India* 76, 2020 page 147-155.
6. Christian A. Devauxa, Jean-Marc Rolaina, Philippe Colsona, Didier Raoult, "New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19?", *International Journal of Antimicrobial Agents*, 2020.
7. Kai Kupferschmidt and Jon Cohen, "Race to find COVID-19 treatments accelerates", Published by AAAS, 27 MARCH 2020 VOL 367 ISSUE 6485.
8. Wen Zhanga, Yan Zhaoa, FengchunZhanga, Qian Wanga, Taisheng Lib, ZhengyinLiuc, JinglanWangd, Yan Qine, Xuan Zhanga, XiaoweiYanf, XiaofengZenga, Shuyang Zhang,"The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China" 2020 Elsevier Inc. 25 March 2020.
9. James M. Sanders, PhD, PharmD; Marguerite L. Monogue, PharmD; Tomasz Z. Jodlowski, PharmD; James B. Cutrell, MD, " Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19) A Review", *JAMA* May 12, 2020 Volume 323, Number 18.
10. Dr. Niraj Parikh* (MD Homoeopathy) and Dr. Devang Parikh (MD Pharmacology), " ROLE OF HOMOEOPATHY IN COVID-19 MANAGEMENT - A CLINICAL EXPERIENCE", *World Journal of Pharmaceutical Research*, Volume 9, Issue 5, 2459-2466, ISSN 2277-7105.
11. Ravi Philip Rajkumar, " Brain, Behavior, and Immunity Ayurveda and COVID-19: Where psychoneuroimmunology and the meaning response meet", Department of Psychiatry, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Dhanvantari Nagar Post, Pondicherry 605 006, India journal homepage : www.elsevier.com/locate/ybrbi, Contents lists available at ScienceDirect.
12. Nuno Fernandes, "Economic effects of coronavirus outbreak (COVID-19) on the world economy", *IESE Business School Spain*, April 13, 2020 Version 2.0.
13. Maria Nicola, Zaid Alsafi, CatrinSohrabi, Ahmed Kerwand, Ahmed Al-Jabir, Christos Iosifidis, Maliha Agha, Riaz Agha," The socio-economic implications of the coronavirus pandemic (COVID-19): A review", 12 April 2020, Available online 17 April 2020, 2020 IJS Publishing Group Ltd. Published by Elsevier Ltd, *International Journal of Surgery* 78 (2020) 185–193.
14. Scott R. Baker Nicholas Bloom Steven J. Davis Stephen J. Terry, " COVID-INDUCED ECONOMIC UNCERTAINTY", NBER

WORKING PAPER SERIES, NATIONAL BUREAU OF ECONOMIC RESEARCH, Cambridge, MA 02138 April 2020, JEL No. D80, E17, E32, E66, L50.

15. YEN NEE LEE."7 CHARTS SHOW HOW THE CORONAVIRUS PANDEMIC HAS HIT THE

GLOBAL ECONOMY", WORLD ECONOMY, CNBC LLC., 24 APR 2020, [HTTPS://WWW.CNBC.COM/2020/04/24/CORONAVIRUS-PANDEMICS-IMPACT-ON-THE-GLOBAL-ECONOMY-IN-7-CHARTS.HTMLS.](https://www.cnbc.com/2020/04/24/coronavirus-pandemics-impact-on-the-global-economy-in-7-charts.html) ■

HPTLC fingerprinting: A tool for simplified analysis of terpenoids in medicinal plants

Amit Saraf*¹ and Aparna Saraf²

¹ Ismail Yusuf College, (Affiliated to Univeristy of Mumbai) Mumbai, India

² Dr. Homi Bhabha State University, The Institute of Science, Mumbai - 400032, India

aysaraf@gmail.com

ABSTRACT

Terpenoids constitutes important class of plant secondary metabolites with significant bioactivity. The identification and profiling of such secondary metabolites validates therapeutic activities exhibited by the medicinal plants. HPTLC is an accepted analytical method for the analysis of medicinal plants by WHO and pharmacopoeia across the globe, including the United State Pharmacopoeia. The methanolic extracts of *Abrus precatorius L.*, *Sapindus trifoliatus L.* and *Embelia ribes* Burm. F. were developed on the HPTLC system to study the diversity of terpenoids like compounds like steroids and sterols under different development conditions. Steroids were separated using n-butanol: methanol: water (3:1:1 v/v/v) and Anisaldehyde Sulfuric acid as spray reagent. Sterols were separated on chromatogram by using Chloroform: Ethyl acetate (4: 6 v/v) as solvent system and 10% methanolic Sulphuric Acid Reagent was used for derivatization. Profile of 09 polyvalent phytoconstituents were separated during the analysis of sterols and steroids individually. The HPTLC analysis successfully demonstrated that phenolic secondary metabolites can be effectively separated using the same extract under different development conditions.

Keywords: HPTLC, steroids, sterols, *Abrus precatorius L.*, *Sapindus trifoliatus L.* and *Embelia ribes* Burm. F.

Introduction

The advancement in the sensitivity and precision of analytical techniques has added a new dimension in the evaluation of natural products. These analytical methodologies are often subjected to critical evaluation by various international organizations like FDA, WHO and various pharmacopoeia across the world, before being accepted as a valid tool for phytochemical investigations. High Performance Thin Layer Chromatography (HPTLC) is one such sensitive analytical tool which is being accepted across the globe for herbal evaluation (Frommenwiler et al, 2019). It is being well utilized to study various classes of secondary metabolites on

the basis of their selective solubility in the specially formulated solvent systems (Saraf et al, 2016, Tilak et al, 2019). The present study was performed to utilize HPTLC methodology for separation of terpenoids fraction of few medicinal plants. Terpenoids constitutes a diverse group of secondary metabolites and is classified on the basis of multiple of 5 carbon isoprene unit. Terpenoids exhibits plethora of pharmacological and therapeutic properties (Proshkina E et al, 2020). Steroids and Sterols are two important classes of compounds in the Terpenes family. HPTLC methodology is successfully utilized to profile various classes of compounds (Reich E and Schibli A., 2007).

In the present study three medicinal plants viz *Abrus precatorius L.*, *Sapindus trifoliatus L.* and *Embelia ribes Burm. F.* were subjected to HPTLC profiling to study the diversity of terpenoids like compounds like steroids and sterols under different development conditions. These plants exhibit antifertility activity which is also attributed to the presence of terpenoids compounds targeted in the present study (Gupta and Sharma, 2006).

Material and Methods

Preparation of Extracts:

The methanolic extract of *Abrus precatorius L.* (seeds), *Sapindus trifoliatus L.* (dried pericarp) and *Embelia ribes Burm. F.* (dried fruit) was sonicated for 15 minutes.

HPTLC studies:

Precoated Silica gelTLC plates 60 F254, Merk, Germany was used for the HPTLC analysis. The extracts of various concentration (1 μ l, 2 μ l and 5 μ l) were loaded on 20 X 10 cm plate with CAMAG Linomat V sample applicator. The plates were developed in CAMAG Automatic Development Chamber 2 (ADC2) using n-butanol: methanol: water (3:1:1 v/v/v) and anisaldehyde sulfuric acid as spray reagent for profiling of steroids. Sterols were separated by using the solvent system of Chloroform: Ethyl acetate (4: 6 v/v) and 10% methanolics sulphuric acid reagent was used for

derivatization. The plates were observed in CAMAG Visualizer 2 and were later scanned at the wavelength of 254 and 366 nm using CAMAG TLC Scanner 4. CAMAG vision CATS software was used for the current study (Reich E and Schibli A, 2007).

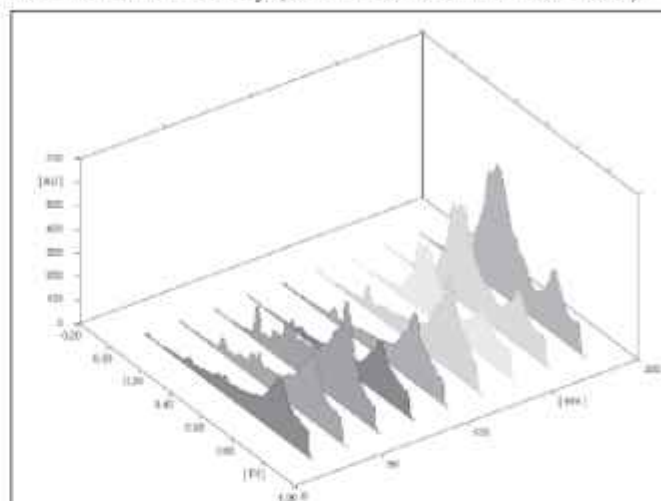
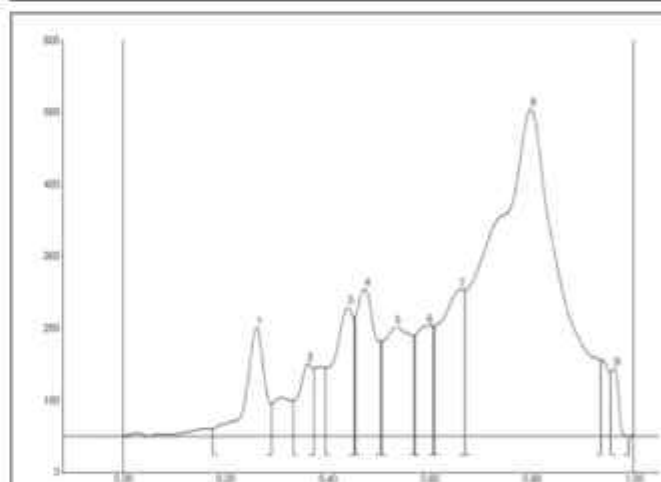


Fig. 1: HPTLC densitogram of steroids in *A. precatorius*, *S. trifoliatus* and *E. ribes*



Densitogram of 5 μ L extract of *A. precatorius* for steroids

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.19	10.4	0.26	151.3	8.90	0.31	45.6	4315.7	5.18
2	0.36	49.6	0.39	101.1	5.95	0.40	93.5	2198.5	2.64
3	0.43	95.3	0.48	180.0	10.59	0.49	164.3	5372.2	6.45
4	0.49	165.1	0.51	205.5	12.10	0.55	132.1	6036.0	7.24
5	0.55	132.3	0.58	152.3	8.96	0.62	140.1	6319.2	7.58
6	0.62	140.3	0.64	154.5	9.09	0.66	153.0	3758.0	4.51
7	0.66	153.2	0.71	204.2	12.02	0.72	203.6	7394.5	8.87
8	0.72	203.7	0.86	454.8	26.76	1.01	105.1	46838.9	56.20
9	1.03	89.3	1.04	95.5	5.62	1.07	0.1	1105.5	1.33

Rf values of 5 μ L extract of *A. precatorius* for steroids

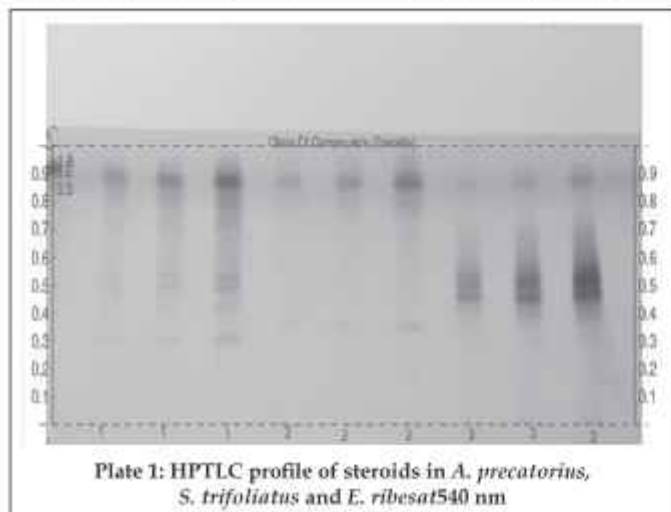
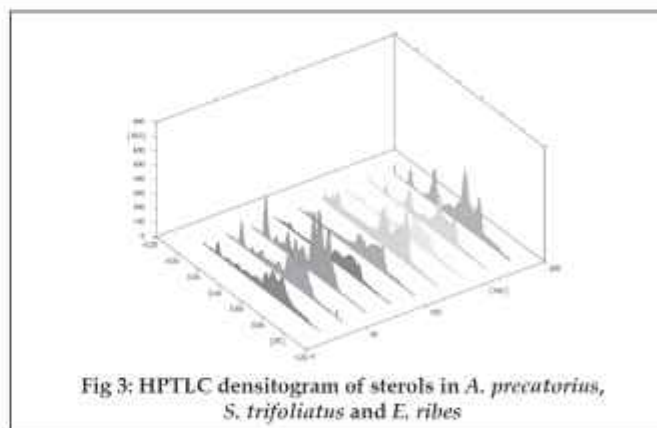
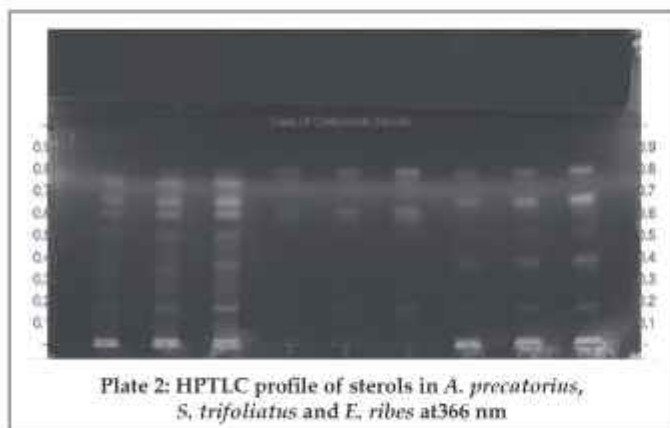
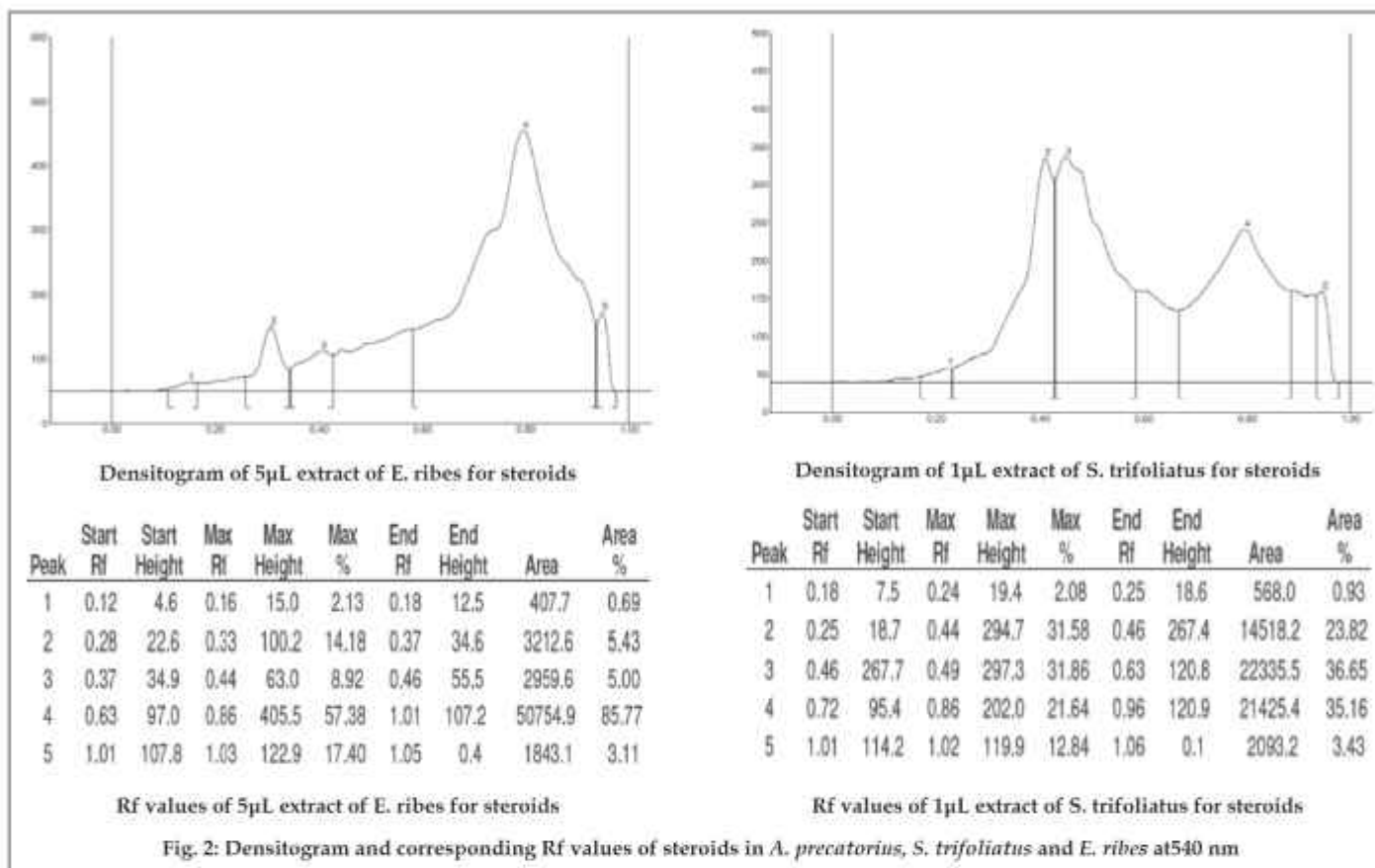


Plate 1: HPTLC profile of steroids in *A. precatorius*, *S. trifoliatus* and *E. ribes* at 540 nm

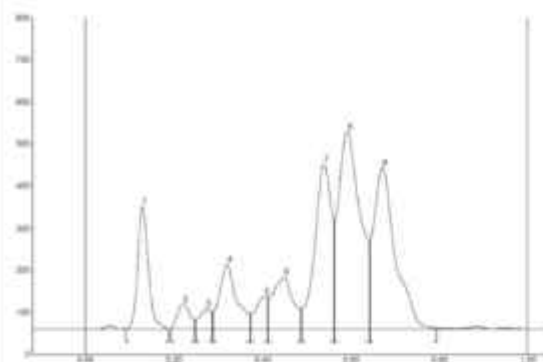


Result and Discussion

The results from HPTLC finger print for steroids shows best results when scanned at wavelength 540 nm. 5µL extract of *A. precatorius* for steroids reveal the occurrence of 9 polyvalent phytoconstituents while *E. ribes* and *S. trifoliatum* profile shows the presence of 5 polyvalent phytoconstituents for 5µL and 1µL extracts respectively. Wide range of

medicinal and nutritional importance of steroids is cited in the literature (Khatun A et al, 2019 and Di Gioia et al, 2019).

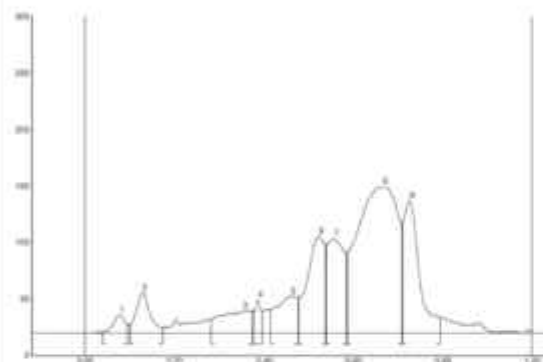
The HPTLC finger print for sterol shows best results when scanned at wavelength 366 nm. 4µL extract of *A. precatorius* and 1µL extract *E. ribes* for sterols exhibits the separation of 9 polyvalents, Whereas 2µL extract *S. trifoliatum* reveal the



Densitogram of 4µL extract of *A. precatorius* for sterols

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.10	0.5	0.14	293.7	14.64	0.20	0.2	5506.2	8.76
2	0.21	0.3	0.24	60.3	3.01	0.27	21.2	1338.9	2.13
3	0.27	21.7	0.30	49.4	2.46	0.31	41.1	986.0	1.57
4	0.31	41.7	0.35	156.1	7.78	0.40	36.7	4720.4	7.51
5	0.40	36.9	0.44	76.7	3.82	0.45	75.2	1605.2	2.55
6	0.45	75.3	0.49	124.7	6.22	0.53	48.8	4464.9	7.10
7	0.53	49.4	0.58	391.6	19.53	0.61	261.5	11577.2	18.41
8	0.61	261.8	0.64	469.6	23.42	0.69	211.5	17912.4	28.49
9	0.70	212.4	0.73	383.6	19.12	0.86	2.3	14767.7	23.49

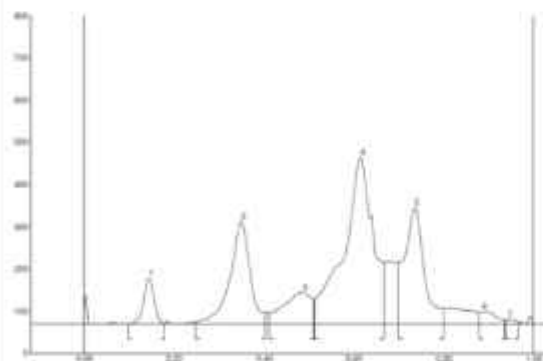
Rf values of 4µL extract of *A. precatorius* for sterols



Densitogram of 1µL extract of *E. ribes* for sterols

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.04	0.7	0.08	15.9	2.90	0.10	7.3	315.5	1.50
2	0.11	7.7	0.14	35.5	6.46	0.19	4.9	861.0	4.09
3	0.30	11.9	0.38	19.8	3.61	0.40	19.2	1042.4	4.96
4	0.41	19.4	0.42	29.4	5.36	0.43	19.7	302.6	1.44
5	0.45	20.8	0.50	32.4	5.89	0.51	31.4	1162.2	5.53
6	0.52	31.5	0.57	85.8	15.61	0.58	78.4	2603.5	12.38
7	0.58	78.5	0.60	83.8	15.24	0.63	71.1	2435.1	11.58
8	0.63	71.1	0.72	129.7	23.59	0.77	97.8	9093.9	43.24
9	0.77	98.3	0.79	117.4	21.35	0.86	13.3	3215.1	15.29

Rf values of 1µL extract of *E. ribes* for sterols



Densitogram of 4µL extract of *S. trifoliatius* for sterols

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.10	0.2	0.15	109.1	9.57	0.19	1.6	1967.8	4.43
2	0.27	5.2	0.38	242.6	21.28	0.44	27.1	8420.5	18.95
3	0.45	27.0	0.53	75.2	6.60	0.55	59.7	3673.6	8.27
4	0.55	59.8	0.66	396.2	34.75	0.72	146.6	19954.0	44.91
5	0.76	146.0	0.80	276.4	24.25	0.86	37.4	9359.7	21.06
6	0.95	28.7	0.96	29.3	2.57	1.01	10.7	873.0	1.96
7	1.01	10.8	1.02	11.1	0.98	1.05	5.2	184.5	0.42

Rf values of 4µL extract of *S. trifoliatius* for sterols

Fig. 4: Densitogram and corresponding Rf values of sterols in *A. precatorius*, *S. trifoliatius* and *E. ribes* at 540 nm

occurrence of 7 polyvalent phytoconstituents. The role of Sterols in human nutrition and its therapeutic potential is highlighted in various studies (Corrêa et al, 2017, Kumar et al, 2012 and Choudhary and Tran, 2011).

Conclusion

Herbal extracts consist of composite mixture of various class of metabolites. Separation and evaluation of such diverse group of compounds

pose a serious challenge. HPTLC stands out as a potential tool for profiling of such diverse group of secondary metabolites. These profiles can be subjected to further evaluation for their nutritional and pharmaceutical potential.

References

1. Corr ea RC, Peralta RM, Bracht A and Ferreira IC. The emerging use of mycosterols in food industry along with the current trend of extended use of bioactive phytosterols. *Trends in Food Science and Technology*. 2017 Sep 1;67:19-35.
2. Di Gioia F and Petropoulos SA. Phytoestrogens, phytosteroids and saponins in vegetables: Biosynthesis, functions, health effects and practical applications. In *Advances in food and nutrition research* (2019) Jan 1 (Vol. 90, pp. 351-421). Academic Press.
3. Frommenwiler DA, Booker A, Vila R, Heinrich M, Reich E and Ca nigueral S. Comprehensive HPTLC fingerprinting as a tool for a simplified analysis of purity of ginkgo products. *Journal of ethnopharmacology*. (2019) Oct 28, 243:112084.
4. Gupta RS and Sharma R. A review on medicinal plants exhibiting antifertility activity in males. *Natural Product Radiance*. (2006); 5(5):389-410
5. Khatun A, Rahman M, Rahman MS, Hossain MK and Rashid MA. Terpenoids and phytosteroids isolated from *Commelina benghalensis* Linn. with antioxidant activity. *Journal of Basic and Clinical Physiology and Pharmacology*. 2019 Nov 26;31(1).
6. Kumar D, Kumar A and Prakash O. Potential antifertility agents from plants: A comprehensive review. *Journal of Ethnopharmacology*. 2012 Mar 6;140(1):1-32.
7. P Choudhary S, S Tran L. Phytosterols: perspectives in human nutrition and clinical therapy. *Current medicinal chemistry*. 2011 Oct 1;18(29):4557-67.
8. Proshkina E, Plyusnin S, Babak T, Lashmanova E, Maganova F, Koval L, Platonova E, Shaposhnikov M and Moskalev A. Terpenoids as Potential Geroprotectors. *Antioxidants*. 2020 Jun;9(6):529. DOI: 10.3390/antiox9060529
9. Reich E and Schibli A. High-performance thin-layer chromatography for the analysis of medicinal plants. Thieme; (2007).
10. Saraf A, Srinivas KS and Chaturvedi A. Validation of HPTLC Method for Quantification of Embelin from *Embelia ribes* Burm. F. F. *Int J Pharmacog Phytochem Res*. 2016;8(9):1492-6.
11. Tilak A, Sharma R, Gangwar SS and Sutar RC. High performance thin layer chromatography fingerprint profile of flavonoids of leaves extract of *Ailanthus excelsa* (Roxb). *Journal of Pharmacognosy and Phytochemistry*. 2019;8(4):1174-7. ■

Study of phytochemical, antifungal and antibacterial activity of extracts of aerial roots of *Ficus benghalensis* L.

Pooja Shinde* and Abhilash Tripathi

Department of Biotechnology, Ismail Yusuf College, (Affiliated to University of Mumbai), Jogeshwari (E)
E mail id - poojas24488@gmail.com

ABSTRACT

Natural products constitute the foundation of traditional herbal medicine. They are still at the core of the primary health care system in majority parts of world. The therapeutic potential of native flora against plethora of diseases have been demonstrated by traditional herbal medicine practitioners. Plant derived compound have always played an important role in the development of several clinically useful antimicrobial agents. *Ficus benghalensis* L. is an important medicinal Plant of the Moraceae family which has been used to treat a wide assortment of diseases. This plant is also widely used in preparation of herbal hair oil which promotes hair growth and reduces hair fall.

Present study is carried out to investigate the phytochemical analysis, Antibacterial and antifungal activity of various extracts such as Methanol extract, Acetone extract of aerial roots of *Ficus benghalensis* L. Phytochemical screening revealed presence of Alkaloids, Phenols, Flavonoids, Sterols, Tannins and Saponins. Bacterial and fungal strains which can cause skin infection were selected for screening of antimicrobial and antifungal activity. The antimicrobial activity was performed using Agar well diffusion method. Ethanol extract has shown good activity against all selected strains as compare to acetone extract.

Keywords: Antifungal, Antibacterial, phytochemical

Introduction

Ficus benghalensis is a tropical, deciduous, evergreen tree with more than 800 species and about 40 genera. *F. benghalensis* is commonly known as Banyan tree and is cultivated as a Garden tree or Spiritual tree. The aerial root is styptic, useful in syphilis, biliousness, dysentery, inflammation of liver etc (Govilet al, 1993). The bark of plant is used in Ayurvedic medicine for the treatment of diabetes. Extract of *F. benghalensis* bark has antibacterial activity (Shansaviet al, 2010). Aqueous extract of aerial root proved to have immuno-stimulant

activity. (Tabassum et al, 2008). The milky juice is aphrodisiac, tonic, and is also useful in piles, diseases of the nose, gonorrhoea (Patel and Gautam, 2014). The chloroform extract of the fruit of *Ficus benghalensis* has shown to have anti-tumor property. (Gopukumar & Praseetha, 2015). Leaf powder of *F. benghalensis* is mixed with coconut oil and applied topically on the affected places for wound healing. Various parts of this plant are known to have many therapeutic uses but still very less work is done on aerial roots. These aerial roots are generally used in preparation of herbal oil, which is used to stop hairfall also induces hair growth.

So, the study was conducted to screen the presence of secondary metabolites present in crude extracts of aerial roots and antibacterial potential of crude extracts against *E. coli*, *S. epidermidis* and *C. albicans* i.e. Skin infection and dandruff causing bacteria were used for study (Sunder et al 2012; Sakeen et al, 2016; Saxena et al 2018)

Material and Methods

Aerial roots were collected in the month of October 2019 from Ismail Yusuf College campus. It was then washed and shade dried. Roots were powdered using mechanical grinder. Powdered roots were extracted in Methanol (Itratkhahet al 2019) and acetone using cold extraction technique. 1 gm of powdered material mixed with 50 ml of each solvent separately then kept on shaker. After 24 hrs again 50ml solvent was added to respective conical flasks. After 24 hrs both the extracts were filtered and used for further analysis.

For Phytochemical screening standard methods were used (Harborne, 1970).

Agar well diffusion method was utilized for the evaluation of antibacterial and antifungal bioactivity of the plant under study. Activity was screened against *Escherichia coli* (MTCC 443), *S. epidermidis* (ATCC12228), *Candida albicans* (SC5314). These bacterial and fungal strains are known to cause skin infections and dandruff (Govinda swami et al 2013). Amoxycillin was used as a standard antibiotic. Filtered extracts were evaporated and reconstituted at concentration of 1 mg/ml in DMSO.

Result and Discussion

Phytochemical screening showed that methanol showed presence of maximum number of secondary metabolites as compared to acetone extract. So, we can say that methanol is better solvent as compared to acetone to get maximum extraction by using cold extraction technique.

Antibacterial and antifungal studies showed that methanolic extract was showing good inhibition against selected strains. Also antibacterial as well as antifungal activity was also reported in acetone extract. Good inhibition by methanolic extract can be attributed to different secondary metabolites that are present in it.

	E.coli	S. epidermidis	C. albicans
Control	19mm	20mm	16mm
Methanol	15mm	16mm	12mm
Acetone	9mm	8mm	2mm




		
<i>S. epidermidis</i>	<i>E. coli</i>	<i>C. albicans</i>
+ve- Amoxycillin, -ve- DMSO, M- methanolic extract, D- acetone extract		

Table 2: Antibacterial screening of aerial root extract of *Ficus beghalensis* L.

Conclusion

Aerial roots of plants are found to be rich in various secondary metabolites. So, it may be concluded that it has many pharmacological activities. Also, aerial roots have known to reduce hairfall and stimulate the growth but the present study also proves that oil from aerial roots may be used to cure various skin infections. Oil rich in aerial root extracts can also be used to control dandruff caused due to bacteria.

The mechanism by which phytochemicals elicit their actions are unclear, it is suggested that some of the secondary metabolites interfere with the transcription and translational process of bacterial cellular membrane (Andreadi, et al 2006). Flavonoids inhibit both cytoplasmic membrane function and DNA synthesis (Zhang, et al 2008; Plaper, et al 2003). Alkaloids kill the microbes by intercalating with DNA (Pandey and Kumar, 2013).

Further studies may provide other novel or innovative ways developing antimicrobial agents from aerial roots of this plant. Use of new and novel bioactive products from plant origin is still to be explored for development of new drugs to improve the healthcare in medical fields. standardization methods of extraction and in vitrotesting will facilitate interpretation of results

References

- Gopukumar, S. T., and Praseetha, P. K. (2015). *Ficus benghalensis* Linn—the sacred Indian medicinal tree with potent pharmacological remedies. *Int. J. Pharm. Sci. Rev. Res.*, 32(37), 223-227.
- Kashem, S. W., and Kaplan, D. H. (2016). Skin immunity to *Candida albicans*. *Trends in immunology*, 37(7), 440-450.
- Ramesh Patel and Piyush Gautam. (2014) *International Journal of Medicinal Chemistry & Analysis. Medicinal Potency of Ficus benghalensis: A REVIEW*, 4(1): 53-58
- Tabassum, K., Pratima, T., Gabhe, S.Y. (2008). Immunological Studies on the Aerial Roots of the Indian Banyan. *Ind. J. Pharm. Sci.*, 70(3): 287–291
- Shandavi, C.B., Vikas, V.P. and Vijay, R.P. (2010). Antibacterial Activity of *Ficus benghalensis* barks on *Actinomyces viscosus*. *Int. J. Pharm. Sci.*, 2(1): 39-43.
- Govil, J.N., Singh and V.K., Shameema, H. (1993). *Glimpses in Plant Research. Medicinal Plants: New Vistas of Research (part 1)*. New Delhi, India, Today and Tomorrow's Printers and Publishers, 10: 69.
- Sunder, S., Haguenoer, E., Bouvet, D., Lissandre, S., Bree, A., Perrotin, D., and Guillon, A. (2012). Life-threatening *Escherichia coli* cellulitis in patients with haematological malignancies. *Journal of Medical Microbiology*, 61(9), 1324-1327.
- Saxena, R., Mittal, P., Clavaud, C., Dhakan, D. B., Hegde, P., Veeranagaiah, M. M. and Misra, N. (2018). Comparison of healthy and dandruff scalp microbiome reveals the role of commensals in scalp health. *Frontiers in cellular and infection microbiology*, 8, 346.
- Kotze, M., Eloff, J. N. and Houghton, P. J. (2002). Extraction of antibacterial compounds from *Combretum microphyllum* (Combretaceae). *South African Journal of Botany*, 68(1), 62-67.
- Etratkhah, Z., Ebrahimi, S. E. S., Dehaghi, N. K., and Seifalizadeh, Y. (2019). Antioxidant activity and phytochemical screening of *Ficus benghalensis* aerial roots fractions. *Journal of Reports in Pharmaceutical Sciences*, 8(1), 24.
- Zhang, C. J., & Cheng, C. G. (2008). Identification between *Stephaniatetrandra* S. Moore and *Stephaniacepharantha* Hayata by CWT-FTIR-RBFNN. *Spectroscopy*, 22(5), 371-386.
- Plaper, A., Golob, M., Hafner, I., Oblak, M., Šolmajer, T., & Jerala, R. (2003). Characterization of quercetin binding site on DNA gyrase. *Biochemical and biophysical research communications*, 306(2), 530-536.
- Harborne, J. B. (1998). *Phytochemical methods*. 3 Ed., Chapman and Hall, London, New York.
- Pandey, A. K., & Kumar, S. (2013). Perspective on plant products as antimicrobial agents: a review. *Pharmacologia*, 4(7), 469-480.
- Andreadi, C. K., Howells, L. M., Atherfold, P. A., & Manson, M. M. (2006). Involvement of Nrf2, p38, B-Raf, and nuclear factor- κ B, but not phosphatidylinositol 3-kinase, in induction of heme oxygenase-1 by dietary polyphenols. *Molecular pharmacology*, 69(3), 1033-1040.
- Tripathi R., Kumar A., Kumar S. and Prakash S. (2015). *Ficus Benghalensis* Linn.: a tribal medicine with vast commercial potential. *Indian Journal of agriculture and allied sciences*

Virtual labs : an effective tool in education

¹Dineshwari D. Bisen and ²Pralhad R. Harinkhede

¹Western College of Commerce and Business Management Sanpada, Navi Mumbai

²Department of Zoology, Ismail Yusuf College, (Affiliated to University of Mumbai), Jogeshwari (E), Mumbai-60

E-mail: dineshwaribisen@gmail.com

ABSTRACT

The present paper deals with various aspects of application of virtual labs. These labs integrate the ICT tools in the subjects of science, technology and engineering. As a new technological approach, they provide a good platform for online distance learning in various disciplines of Science and Engineering. During the situation of COVID-19 pandemic lockdown, when learner from the virtual-education community cannot use his skills factually in the labs, the virtual labs stand one of the best emerging tools that can overcome some of the potential difficulties in the education sector. These labs exercise to provide effective skill acquisition and hands-on experience. They are widely multidisciplinary in character useful for students of all grades, teacher and research scholar. These labs may be built either by enabling the real lab for remote access or replicating as a fully software-based virtual lab. The latter concept offers advantages over remotely controlled real labs. Providing audio and video streaming of an actual lab experiment and equipment to the students make virtual labs more effective and realistic. Students can also remotely trigger an experiment in an actual lab and obtain result of the experiment through the computer interface. Modeling and carrying out simulations provide a version of the 'real-world' experiment.

Keywords : Virtual labs, online learning, virtual environments, modeling, skills

Introduction

Learning experience and development of skills are needed in education. The laboratory experiments make the students confident to deal with real life issues and equipped for the needs of employment market. But these practices in physical labs create more pressure on students by investing time, cost and resources and on universities, institutions and schools due to investment and maintenance of cost, man power and resources (Lynch and Ghergulescu, 2019).

Therefore, equipping the learners with the scientific skills and knowledge to make them trained is utmost important. In this context, the Virtual Labs; also called as Remote labs, are effectively used and integrated into the curriculum. They have been

found to be the emerging solution to increase students' knowledge, skills and performance in examinations and to reduce the burden of universities and institutions (Tuysuz, 2010). They are offered on electronic devices and are increasingly realistic and rapidly increasing in use. Virtual Labs strengthen the learning ecosystem and provide an opportunity to both students and teachers to augment the learning skills and experience. Modern developments augmented reality into virtual labs, giving new dimension to user's experience (Kumar et al., 2018). Hence this paper is an Application of ICT in Indian Education.

Methodology

Most of the virtual labs are already in use as mere additions to traditional lab set-ups. In India, the

virtual labs are still in infancy and a comprehensive knowledge is yet to be built in this field. Hence this paper is a part of research on 'Application of ICT in Indian Education' is a small effort towards grabbing the attention on underlining role of virtual labs in Indian education. For this study, we adapted survey method and a survey of 21 online papers on the application, benefits and challenges of virtual labs was done during the lockdown period of COVID-19 pandemic. On the basis of survey following observations were made.

Observations

Virtual lab vs Physical lab

The sense of touch, hearing, olfaction and vision are the features of the learning process that are important in bridging the gap between virtual labs and real labs. Unlike traditional labs, virtual labs provide an enhanced learning experience equally among the students including differently able students as they remove all physical limitations of a traditional lab (Lynch and Ghergulescu, 2019). Virtual labs are available all year and therefore the students can do experiments without limitations of space or time while physical labs are limited to a specific place and time. Unlike the school labs, the virtual labs make the students acquire better computer skills for lifelong learning and bring together different subjects. The simulations offered a good replacement for physical labs especially where hands-on experimentation (eg. manipulation of equipment) is not a key part of the lab (Ramadhan and Irwanto, 2017). Virtual labs are easy to set up, use and maintain with less cost and time while physical labs are difficult to set up and very time-consuming and costly for the institutions to manage and require a lot of technical expertise to run (Rajendran et al., 2010). Unlike physical labs, virtual labs can be potentially used for dangerous or impossible real life experiments, e.g. practicing surgeries with no risk to the virtual patient or testing the functions of a nuclear reactor where students can learn from mistakes without causing any real damage to themselves or others (NMEICT, 2020).

Lab environment: Virtual lab environment can be divided into following categories;

- (i) Simulations: These are model based processes of OS on computers. They are cheaper, faster, less risky and more affordable than the real processes.
- (ii) Network applets: These are small sized; easily transportable experimental devices which can be used regardless of the OS type.
- (iii) Virtual labs: Simulate a virtual OS, the computer screen, science laboratories that exploit modern media technology. They interact the direct and plausible manipulation of objects and parameters technically.
- (iv) Virtual Reality Laboratories (VRL): These are computer based workshops enjoying artificial 3D optical environment.
- (v) Remote Labs: The virtual labs can be controlled remotely (Panagiota, 2015).

Characteristics of virtual labs

1. Flexible access: Students as well as teachers can access at their convenience. They may be used during regular class or at the best suitable time.
2. Instant feedback: Students can redo experiments on the spot, record the results and make communication with teachers and other students more efficiently.
3. Top-notch equipment: Virtual labs offer cutting-edge technology to the users. They make users compete to stay ahead with the progression of technology.
4. Cost and time saving: Virtual labs provide better learning experience to students with less cost and time as they are easy to set up, use and maintain by quick and fast experimentation, compatible activities, easy and simple observation and safe measurement (Robin et al., 2018).

Benefits of virtual labs in education system: Virtual labs,

- Relate the users directly to the real world by interaction and sharing virtual objects.

- Share the material remotely between institution and students (NMEICT, 2020).
- Students can develop the greater depth, understanding, reasoning, critical thinking, innovative and creative skills within less time, resources and space.
- Solves ethically questionable practices while teaching life science. For example, dissection labs becoming increasingly rare.
- Virtual experiments give students multiple attempts.
- Provide seamless assessment.
- Allow for many outcomes and solutions instead of only one result (Aljuhani et al., 2018).

Challenges faced by virtual labs

Though the virtual labs have revolutionized the teaching and learning process, the gaps in its recognized potential and the actual applications still exist. They lack their real-life feel and unable to teach about health and safety to students if not designed

and implemented correctly. This problem can be resolved with augmented reality, multi-sensorial devices, live videos, interactive videos and serious games, this problem can be resolved to some extent (Wolf, 2010 and Kumar et al., 2018). The data generated by virtual labs relies on the underlying assumptions and lack natural variation and therefore students do not become familiar with uncharacteristic or poor data, nor will they learn how to deal with these data (Lewis, 2014). The ability of the students to handle real equipment can be decreased and can feel as if they are losing out on some stages of practical training available in traditional labs. (De Jong et al., 2014). The incorporation of virtual labs requires adjustment or extension of existing resources available within the labs. Also understanding the creation and implementation of underlying technology behind virtual labs is quite difficult for the teacher. It requires highly skilled programmers and graphic designers, who, in turn, need to cooperate with

Table 1: Some world leading virtual labs, e-learning resources for disciplines of life Sciences (Source: Ray et al., 2012)

So	Virtual labs	Location	Subjects	URL
1	Virtual Biology Labs	Rutgers University, New Jersey	Cell Biology, Plant Biology, Genetics	https://bio.rutgers.edu
2	Virtual Labs at SUMMIT- Stanford	Stanford University Medical Media and Information Technologies	Physiology, Biology, Immunology, Neuroscience, Health Education	https://virtuallabs.stanford.edu/
3	McGraw Hill Online Learning Center	McGraw Hill publishers	Basic Biology, Reproduction, Genetics, Ecology, Virology, Plant and Animal Biology	https://highered.mcgraw-hill.com/sites/0073031208/student_view0/virtual_labs.html
4	Learn.Genetics	University of Utah	Basic Genetics, Stem Cells, Gene therapy, Cloning, Transgenics, Epigenetics, Genetic Technology	https://learn.genetics.utah.edu/
5	MIT Open Course ware	Massachusetts Institute of Technology	Biochemistry, Health Science, Molecular Biology, Cytology, Genetics, Proteomics, Developmental Biology, Neurology, Stem Cells, System Biology, Immunology, Bioinformatics	https://ocw.mit.edu/index.htm
6	Serendip	Bryn Mawr College - Philadelphia	Evolution, General Biology, Neurobiology	https://serendip.brynmawr.edu/serendip/
7	HHMI Biomedical Interactive Virtual Labs	Harvard Hughes School of Medicines	Genetics, Cardiology, Neurophysiology, Microbiology, Immunology, Stem Cells, Genomics, Cancer, ELISA	https://www.hhmi.org/biointeractive/vlabs
8	Virtual Laboratory - Colorado	Laboratory.net inc	Fundamentals of Biology, Genetics	https://virtuallaboratory.colorado.edu

Table 1. Continues....

9	Biotechnology Virtual Labs	International Center for Agricultural Research in Dry Areas - Syria	Farming related and Scientific Research Tutorials	https://www.icarda.org/Training_elearning.htm https://learning.cgiar.org/moodle
10	National Program for Technology Enhanced Learning	Joint Collaboration of several Indian Universities	Biochemistry, Cell Biology, Molecular Biology, Fermentation Technology, Immunology, Proteomics, Microbiology, Biomathematics	https://nptel.iitm.ac.in/courses.php?disciplineId=102
11	"Sakshat" Virtual Biotechnology Engineering Labs	Joint Collaboration of several Indian Universities	Neurophysiology, Biochemistry, Ecology, Immunology, Proteomics, Microbiology, Cell Biology Molecular Biology, Instrumentation, Fermentation Technology,	https://www.vlab.co.in/ba_labs.php?id=6
12	Department of Biology - Virtual Lab	Johns Hopkins University	Basic Lab Techniques, Instrumentation, Gel Electrophoresis, Microscopy	https://bio.jhu.edu/Undergrad/VirtualLabDemos.aspx
13	Cairo University, Faculty of Science E-learning facility	Cairo University Egypt	Biophysics, Genetics, Botany, Zoology, Molecular Biology	https://elearning.cu.edu.eg/moodle/
14	Annenberg Learner	Annenberg Foundation	Proteomics Theory and 2DE, Mass Spectrometry, Protein Interactions and Microarrays	https://www.learner.org/courses/biology/textbook/teprote/index.html
15	Center for Cardiovascular Research - Molecular biology	John A Burns School of Medicine, University of Hawaii	SDS-PAGE, Western Blotting, ELISA, Protein microarrays	https://ccrhawaii.org/index.php/protein-techniques

experts on respective subjects to realistically model virtual objects with their properties (Lynch and Ghergulescu, 2019).

Examples of virtual labs: A variety of virtual labs have been developed by different organizations under following broad areas. (Source: NMEICT, 2020).

1. Electronics and Communications
2. Computer Science and Engineering
3. Electrical Engineering
4. Mechanical Engineering
5. Chemical Engineering
6. Biotechnology and Biomedical Engineering
7. Civil Engineering
8. Physical Sciences
9. Chemical Sciences

Virtual available in India

1. Fading Channels and Mobile Communication Lab (IIT Kharagpur)
2. RF and Microwave Characterization lab (IIT Kanpur)
3. Advanced Network Technologies Virtual Lab (IIT Kharagpur)
4. Digital Electronic Circuits Lab (IIT Kharagpur)
5. Analogue Signals Network and Measurement Lab (IIT Kharagpur)
6. Hybrid Electronics Lab (COE Pune)
7. Digital Electronics Lab (New) (IIT Rooraki)
8. Basic Electronics Lab (IIT Kharagpur)
9. Digital Logic Design Lab (IIT Hyderabad)
10. Signals and Systems Lab (IIT Guvahati)
11. Queuing Networks Modelling Lab (IIT Delhi)
12. Electric Circuits Lab (Amrita Vishwa Vidyapeetham)
13. Digital Signal Processing Lab (IIT Kharagpur)

Labs at IIT Mumbai

1. Simulation of Control of Magnetic Levitation System
2. Urban Transportation Systems Planning Lab
3. Virtual Proteomics Laboratory
4. Single Board Heater system
5. Electronic Devices and Circuits
6. Traffic Engineering Laboratory

Conclusion

The present study permits to conclude that the virtual labs provide remote-access to Labs in various disciplines of Science and Engineering. They are useful for students in problem solving, critical thinking, creativity, conceptual understanding and skills. The teachers can also use virtual labs to improve their teaching quality. Virtual Labs are very useful for students and institutions that lack proper access to good lab-facilities and help them in learning basic and advanced concepts through remote experimentation. Finally the virtual labs have the potential to provide all students with practical experience in their subject of interest. The Indian education scenario is also transforming rapidly to make India a knowledge superpower.

References

1. Aljuhani, K., M. Sonbul, M. Alhabiti and M. Meccawy (2018): Creating a virtual science lab (VSL): the adoption of virtual labs in Saudi schools. *Smart Learning Environments*. 5(16): 1-13.
2. De Jong, T., S. Sotiriou and D. Gillet (2014): Innovations in STEM education: The Go-Lab federation of online labs. *Smart Learning Environments*. 1(3). DOI: 10.1186/s40561-014-0003-6.
3. Kumar D., R. Radhamani, N. Nizar, K. Achuthan, B. Nair and S. Diwakar (2018): Virtual and remote laboratories augment self learning and interactions: development, deployment and assessments with direct and online feedback. *PeerJ Preprints*. Pp. 1-42.
4. Lewis, D. I. (2014): The pedagogical benefits and pitfalls of virtual tools for teaching and learning laboratory practices in the Biological Sciences. *The Higher Education Academy*. www.heacademy.ac.uk/system/files/resources/the_pedagogical_benefits_and_pitfalls_of_virtual_tools_for_teaching_and_learning_laboratory_practices_in_the_biological_sciences.pdf
5. Lynch, T. and I. Ghergulescu (2019): Review of virtual labs as the emerging technologies for teaching stem subjects. A project funded by European Union's Horizon 2020 Research and Innovation programme under Grant Agreement no. 688503. Pp. 1-10.
6. NMEICT (2020): 'Abhasi' a bi-annual newsletter published by virtual labs, (National Mission on Education through ICT) an initiative of MHRD. 4th edition, January 2020. Pp. 2-5.
7. Panagiota, A. (2015): Virtual laboratories in teaching and learning science. *Scientix blog*. www.blog.scientix.eu/about
8. Rajendran, L., R. Veilumuthu and J. Divya (2010): A study on the effectiveness of virtual lab in E-learning. *International Journal on Computer Science and Engineering*. 2: 2173-2175.
9. Ramadhan, M. F. and Irwanto (2017): Using virtual labs to enhance students' thinking abilities, skills and scientific attitudes. *ICERI*. Pp. 494-499.
10. Ray S, Koshy NR, Diwakar S, Nair B, Srivastava S (2012) Sakshat Labs: India's Virtual Proteomics Initiative. *PLoS Biol*. 10(7): e1001353. <https://doi.org/10.1371/journal.pbio.1001353>.
11. Robin Kay, Helene Goulding, Jia Li (2018): Assessing the impact of a virtual lab in an allied health program. *Spring*, 47(1):45-50.
12. Tuysuz, C. (2010): The effect of the virtual laboratory on students' achievement and attitude in chemistry. *International Online Journal of Educational Sciences*. 2: 37-53.
13. Wolf, T. (2010): Assessing Student Learning in a Virtual Laboratory Environment. *IEEE Transactions on Education*. 53(2): 216-222. ■

Index based Bio-Monitoring using Macroinvertebrates

Pralhad R. Harinkhede

Department of Zoology, Ismail Yusuf College, (Affiliated to University of Mumbai),

Jogeshwari (E), Mumbai-60

E-mail: prahari_72@rediffmail.com

ABSTRACT

The present study is an attempt to generate information on comprehensive water quality assessment and macroinvertebrate biodiversity of the River Vainganga in Gondia district using trophic dynamics and the sensitivity of macroinvertebrates to the pollution – an aspect of bio-monitoring. Different levels of sensitivity of the macroinvertebrate species to different abiotic factors determined their assemblages and diversity. A perusal of spatiotemporal results suggested by FFGs, %EPT, BMWP and ASPT scores indicated very good river water quality, acceptable for all purposes. These indices proved to be the emerging and significant tools, providing an important insight into the health of the river and append the knowledge and understanding of the management strategies. The higher values of %EPT and BMWP and ASPT scores at upstream sites than downstream sites and dominance of sensitive species indicated very good water quality. The percent composition of functional feeding groups indicated dominant predators followed by collectors, shredders and scrappers revealed clean and good conditions of the river. The number of macroinvertebrate families encountered was normally higher during winter followed by summer and monsoon. This study offers an essential step to address the consequences of present and future threats of contamination and provide a basis for future action at all levels. These indexes intended for an ecological integrity assessment of biodiversity of macroinvertebrate fauna which in turn provided useful bio-indicators of bio-monitoring approach in order to provide a complete spectrum of information for appropriate water management of freshwater bodies.

Keywords: Biotic indexes, trophic dynamics, biodiversity, water quality, River Vainganga

Introduction

Water is life and rivers are lifelines. They are directly linked to human welfare. These dynamic, renewable, natural water bodies are unfortunately under severe environmental stress and threatened due to developmental activities (CPCB, 2002). Aquatic ecosystems support plethora of aquatic and terrestrial populations and play a significant role in agriculture, fisheries and industries and provide various products used by local people. River is one of the richest sources of biological diversity harboring a plethora of dynamic interdependent

biotic and abiotic factors in its unidirectional flow which produces many effects that determine the diversity of flora and fauna therein (Clegg John, 1974).

Measurement of water quality index determines the extent of pollution. Water quality index has been studied by several workers. It provides a single number that expresses overall water quality at a certain location (Armitage et al, 1983). Water quality index turns the complex water quality data into information that is understandable and usable by

the public (Anbalagan et al., 2004). It also incorporates data from multiple water quality parameters into a mathematical equation that rates the aquatic health with number (Mandaville, 2002).

The macro-invertebrates are extremely diverse, omnipresent, show varying sensitivity levels, easily sampled with life spans ranging from weeks to several years and can be spatio-temporally assessed. Their ecological structure and function as well as quantity and quality significantly influence proper health of a water body. Unlike physicochemical method, bio-monitoring gives both past and present conditions. Hence aquatic macro-invertebrates are used as bio-indicators for assessing water quality (Muralidharan et al, 2010). The changes in the trophic status of a water body are reflected in its biotic community structure. The abundance of benthic fauna mainly depends on physical and chemical properties of their habitat as they respond more quickly if any changes occur in water quality (Kaushik and Saksena, 1995).

Methodology

The river Vainganga is the largest sub-basin of the river Godavari. It is endowed with diverse flora and fauna. Along its course of 120 kms in Gondia district of Maharashtra state, it receives wastes from its tributaries, streams and runoffs which can cause contamination and affect its biodiversity. In this view, a study was undertaken to assess its water quality at 5 sites in Gondia district. Water samples were collected as per APHA (1998) guidelines once a month at each site from February 2011 to January 2012. Macroinvertebrates were collected from slow moving waters, riffles, pools with 1ft. deep area of 100 m², using D-frame pond net and a quadrat kick-net having 1 x 1 m area and 500 µm mesh size for 3 minutes. They were filtered, sorted out, counted and identified through the keys of Tonapi (1980), Subba Rao (1989), APHA (1998) and Naidu (2005). Most of them were set free unharmed and alive in the river water to protect and conserve the biodiversity. A few of them were fixed in 4% formalin and then preserved in 70% alcohol.

Observations

A total of 62024 individuals from 133 species of invertebrates belonging to 71 families of 15 orders comprising 3 phyla were recorded during the study period. Among these phyla, Arthropoda emerged as a dominating group followed by Mollusca and the least was Annelida. Various indices were used during the present study are as follows.

BMWP (Biological Monitoring Working Party): It is the sum of all scores given to each taxon (1-10). The highest score was given to pollution sensitive macroinvertebrates and lowest score was given to pollution tolerant macroinvertebrates. This index is easy, time saving and need limited taxonomic precision. The standard score given by various macroinvertebrates are given in Table-1 (Hellowell 1986 and Armitage et. al., 1983). The BMWP score was calculated highest during the winter, followed by summer and monsoon. While the average BMWP score was observed 130.73 which is in Good water quality range.

ASPT (Average Score Per Taxon): ASPT equals the average of the tolerance scores of all macroinvertebrate families found. It ranges from 0-10. Unlike BMWP, the ASPT score does not depend on the family richness. The overall ASPT scores were calculated highest during summer followed by winter and monsoon with annual average of 7.12 indicating very good water quality range. Annual spatial trend was observed as site-I > site-II > site-III > site-IV and V (Table-2).

EPT: The EPT index uses three orders of aquatic insects namely Ephemeroptera, Plecoptera and Tricoptera. These insects are easily sorted and identified and are commonly used as an indicator of water quality. This index provides rapid resource assessment. It is based on the fact that high quality streams usually have the greatest species richness (Hilsenhoff, 1981). As per the Table 3, %EPT score was found highest during summer followed by winter and monsoon. While the average %EPT value was observed 47.31 which is in very good water

quality range. The spatial trend was observed as site-I> site-II> site-III > site-IV > site-V.

FFG (Functional Feeding Groups): This approach provides the information on the feeding strategies in the benthic assemblage (Uwadiae, 2010). The knowledge of FFGs has been widely studied throughout the world and is central to the River Continuum Concept (Vannote et al., 1980) and is used in water quality assessment, energy transfer dynamics and food chain modelling (Tomanova et al., 2006). The results showed that the predators dominated followed by collectors, shredders and scrappers during summer and winter while during monsoon the trend was predators> collectors> scrappers> shredders (Table-4).

BMWP Score	Water Quality Class	Study Range			
		Winter	Summer	Monsoon	Annual
>150	A. Very good	155.25	145.65	91.3	130.73
101-150	B. Good				
51-100	C. Fair				
16-50	D. Poor				
0-15	E. Very Poor				

ASPT Score	Water Quality	Sites	Study Range			
			Summer	Monsoon	Winter	Annual
>7	Very Good (Natural)	Site-I	8.18	7.64	7.84	7.89
6.0-6.9	Good	Site-II	7.95	7.18	7.65	7.59
5.0-5.9	Fair	Site-III	7.84	7.08	7.45	7.46
4.0-4.9	Poor	Site-IV	6.86	5.65	6.51	6.34
3.0 or less	Very Poor	Site-V	6.87	5.63	6.51	6.34
Average			7.54	6.64	7.19	7.12

EPT	Rating	Sites	Summer	Monsoon	Winter	Annual
>27	Excellent	Site-I	62.31	50.74	59.09	58.58
21-27	Good	Site-II	57.82	42.28	54.32	52.81
14-20	Good-Fair	Site-III	57.1	42.81	50.54	50.77
7-13	Fair	Site-IV	43.09	25.75	40.27	37.75
0-6	Poor	Site-V	43.22	25.37	38.19	36.62
Average			52.71	37.39	48.48	47.31

Season	Predators	Collectors	Shredders	Scrappers
Summer	39.36	33.28	16.94	10.42
Monsoon	39.15	34.42	10.55	15.87
Winter	40.54	31.56	16.16	11.73
Average	40.14	32.69	14.99	12.19

Discussion

The present study revealed that the macroinvertebrate fauna dominated during winter and lowest density was observed during the monsoon, due to influx of more water and high water velocity that might have changed the substratum with higher load of suspended materials and higher turbidity (Joshi et al., 2007). The higher abundance of sensitive species were recorded, indicating good water quality (Merritt and Cummins, 1996) as well as acceptable conditions for biotic communities however, upstream sites showed better water quality than downstream sites.

There were little spatial differences observed in the index values. The high species diversity has been correlated with longer food chains and complex food webs of the ecosystems with stable community (Negi and Malik, 2008). The indices were slightly higher in the upstream than in the downstream. This may be due to unimpacted or unpolluted conditions, while lower species diversity downstream often signified environmental stress due to human activities, urban and agricultural runoffs and unregulated discharges from small industries, nalas and tributaries which have strong influence on the aquatic fauna causing variations in their community structure (Merritt and Cummins, 1996). The sensitive species live consistently in the clean water of upstream. This indicated the natural habitats of the biotic species that truly reflected the assemblages and

distribution of the benthic macroinvertebrates (Akkaraboyina and Raju, 2012). The BMWP and ASPT scores at the upstream and downstream stations indicated conditions, from the clean upstream to the slightly polluted downstream. The BMWP-ASPT biotic index is particularly important in assessing organic pollution (Zamora-Munoz et al., 1995).

According to River Continuum Concept, the predators normally have a similar proportion throughout the stream length and their dominance depends on the availability of prey (Vannote et al., 1980) which was observed during this study. Abundance of collectors, shredders and scrappers indicated optimal detritus accumulation due to canopy on the banks. This represented autochthonous organic matter indicating pristine nature of the river (Suriyawong et al., 2018).

Conclusion

The river stretch under study revealed a cradle for macroinvertebrates harboring more diverse habitats favorable for dense growth of the macroinvertebrates that showed rich diversity. The higher %EPT, BMWP, ASPT and %FFG scores indicated dominance of sensitive taxa indicating good water quality harboring a diverse, healthy and stable macroinvertebrate population. The distribution of the macroinvertebrates in the Vainganga River could remain stable unless the river is disturbed by natural and anthropogenic activities. As the river stretch under study is facing threats, regular monitoring and conservation strategies are needed.

References

1. Akkaraboyina, M. and B. Raju (2012): A comparative study of water quality indices of River Godavari. *Int. J. Eng. Res. Dev.* 2(3):29-34.
2. Anbalagan S., B. Kaleeswaran and C. Balasubramanian (2004): Diversity and Trophic categorization of aquatic insects of Courtallam hills of Western Ghats. *Entomon.* 29(3): 1 - 6.
3. APHA (American Public Health Association) (1998): Standard methods for the examination of water and wastewater, 20th Edition, APHA AWWA WPCF (eds), Washington DC, USA.
4. Armitage P.D., D. Moss, J.F. Wright and M.T. Furse (1983): The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running water sites. *Water Research.* 17: 322-347.
5. Clegg J. (1974): *Freshwater Life*. Frederick Warne: London and New York. Pp. 1 - 261.
6. CPCB (2002): Annual Report 2001-2002: Air and Water Quality Monitoring Network.
7. Hellawell J.M. (1986): Biological indicators of freshwater pollution and environmental management. Elsevier Appl. Sci. Publ., London. Pp. 525 - 546.
8. Hilsenhoff, W.L. (1981): Use of arthropods to evaluate water quality of streams. Tech. bulletin No. 100. Department of Natural Resources. Madison, WI.
9. Joshi P.C., R.K. Negi, T. Negi (2007): Seasonal variation in benthic macro-invertebrates and their correlation with the environmental variables in a freshwater stream in Garhwal region (India). *Life Science Journal.* 4(4): 85-89.
10. Kaushik S. and D.N. Saksena (1995): Trophic status and rotifer fauna of certain water bodies in central India. *J. Environ Biol.*, 16(4): 283-291.
11. Mandaville S.M. (2002): Benthic macroinvertebrates in freshwaters: Taxa tolerance values, metrics and protocols. Canada. (Project H-1), Soil and Water Conservation Society of Metro Halifax. Pp. 1-42.
12. Merritt R.W. and K.W. Cummins (1996): An introduction to the aquatic insects of North America. 3rd ed. Kendall/Hunt, Publishing Company, New York. Pp. 87 - 862.
13. Muralidharan M., C. Selvakumar, S. Sundar and M. Raja (2010): Macroinvertebrates as potential indicators of environmental quality. Review article, *IJBT*, 1(Special Issue): 23-28.
14. Naidu K.V. (2005): The fauna of India and the adjacent countries. ZSI, Kolkata. Pp. 1-294.

15. Negi K.S. and D.S. Malik (2008): Macrobenthic invertebrate population of Chhirapani, a hill stream in Kumaon Himalaya, India. *I.J.E.E.S.* 34(4):329-334.
16. Subba Rao N.V. (1989): Handbook of freshwater Mollusca of India, ZSI, Calcutta. Pp. 225-289.
17. Suriyawong, P., D. Thabanya, E.A. Berghy and P. Chantaramongkol (2018): Aquatic insect functional feeding groups in a mountain stream with a series of check dams in Northern Thailand. *Sains Malaysiana*, 47(7):1379-1386.
18. Tomanova, S., E. Goitia and J. Helesic (2006): Trophic levels and functional feeding groups of macroinvertebrates in neotropical streams. *Hydrobiologia*. 556(1): 251-264.
19. Tonapi G.T. (1980): Freshwater animals of India: an ecological approach. Oxford and IBH Publishing Co., New Delhi, Bombay, Calcutta. Pp. 1-341.
20. Uwadiae, R.E. (2010): Macro-invertebrates functional feeding groups as indices of biological assessment in a tropical aquatic ecosystem: implications for ecosystem functions. *New York Science Journal*. 3(8): 6-15.
21. Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell and C.E. Cushing (1980): The river continuum concept. *Canadian Journal of fisheries and Aquatic Sciences*. 37(1):130-137.
22. Zamora-Munoz, C., C.E. Sainz-Cantero, A. Sanchez-Ortega and J. Alba-Tercedor (1995): Are biological indices BMPW' and ASPT' and their significance regarding water quality seasonally dependent? Factors explaining their variations. *Water Resources*, 29(1): 285-290. ■

Seed coat study of some species of family Fabaceae

Ulhe P.P.

Department of Botany, Narayanrao Kale Smruti Model College of Art, Commerce and Science, Karanja (gh.) Dist:-Wardha .

Email:- pallavi_ulhe27@rediffmail.com

ABSTRACT

For seed morphological investigation scanning electron microscopy is important for seed study. Seed is a complex structure. The seed surface study shows various parameters like size, shape, colour, weight, symmetry, surface, medicinal value etc .For scanning electron microscopy study 4 species of family Fabaceae/Leguminosae were well studied. The species like *Albizia lebbeck* (Linn.) Willd., *Phaseolus vulgaris* L., *Prosopis spicigera*, *Crotalaria Juncea* L. were studied. The spermoderm shows specific pattern. The SEM study shows mostly smooth, plain surface with granulated deposition. Some seeds shows cellular globular mounded ridges. So variations found in seed surface. It help in seed identification processes. Various chemical constituents present in seed coat which gives high medicinal value. It is important for preparation of various drugs. The study is important for micromorphological variations of seed coat, taxonomic study, identification and also for theruptic efficacy.

Keywords: Seed morphology, Scanning electron microscopy (SEM), Fabaceae

Introduction

The Fabaceae or Leguminosae commonly known as legume, pea or bean family are a large and economically important family of flowering plants. The family shows trees ,shrubs and perennial or annual herbaceous plants. The plant is easily recognized due to legume fruit, compound stipulate leaves. Family shows characteristic flowers and fruits in many legumes. (<https://en.wikipedia.org/wiki/Fabaceae>). *Fabaceae* is the third largest family of flowering plants. It shows more than 18,000 described species. It is present in all over the world especially the tropical rain forest. Seed is a complex structure.

The seed shows cells from three generations,a parent sporophyte a female gametophyte and the embryo of the next sporophyte generation.

Fertilized female gamete called a seed. Seed is a fertilized or mature ovule. (Green, et al. 1984). For the identification of seed there are various parameters which are helpful in distinguishing the taxa at suprageneric level. These parameters include morphology, anatomy, information of various types of seeds life size, shape, colour, surface, symmetry, medicinal value of seeds, value of seed in trade and marketing systems. The surface study is essential for observing the different structural, ornamentations of seeds . Various seeds show different type of seed coat structure which are helpful for solving the taxonomic problems. Seed surface sculpturing is useful traits for species identification. There are innumerable variation in the seed size, shape, colour and surface.The surface smooth, wrinkled, striate, ribbed, furrowed, reticulate, tuberculate, alveolate, hairy, pulpy or having pattern like finger prints. (Bhojwani and Bhatnagar,2000).

Scanning Electron Microscopy (SEM) - The shape, size, symmetry, hilum position and shape are also the important parameters that characterize the identity of seeds. The structural form depends on all these three factors. Seed weight factor help for the identification of healthy seeds. The SEM investigation i.e. Scanning electron microscopy play a very important role in differentiating and identification of micromorphological characters of seeds. Number of scientist worked on the seed morphological characters, but SEM investigation is unique one which gives special identity for surface ornamentation pattern. Scanning electron microscopy with higher range of magnification, determines seed surface characters.

The Scanning electron microscopic studies on the seed coat surface (spermoderm) is a recent field. The study of seed coat pattern provide important characteristics. It also provides relationships among closely related taxa. The systematic application of seed surface features observed under SEM clearly depicts that seed characters are influenced by environmental conditions. The structural diversity is found on the seed surface. Their high structural diversity provides most valuable criteria for classification at species and family levels (Barthlott, 1984; Manilal and Pandey 1996; Pandey et al. 1996).

Materials and methods

Sample collection: Seeds of family Fabaceae like *Albizia lebbeck* (Linn.) Willd., *Phaseolus vulgaris* L., *Prosopis spicigera* and *Crotolaria iuncea* L. were collected from local area. For seed coat study, all the seeds parameters were studied using dissecting and binocular microscope. Digital weighing balance was used for weighing the seeds in mg. The morphological observations of seeds were done followed by their photography, using 1 cm. scale.

Seed coat morphology (SEM):- To study the seed coat morphology scanning electron microscopy is most important. For this purpose, the individual seeds were dipped in alcohol for 5-10 min. to remove the dust from them. The seed mounted on pin type

stubs using double sided adhesive tape or conductive silver paint to prevent charging of the surface during scanning and then coated with a very thin layer of gold in a polaron sputter coating unit. For spermoderm study of seed photomicrograph were taken in the scanning electron microscope (SEM) (LEO 430) at Birbal Sahani Institute of Paleobotany, Lucknow.

Observations:- In *Albizia lebbeck* (Linn.) Willd. local name is siras. Externally seed 1.06 cm. - 0.58 cm, oblong ovate, brown (dark), 139.91mg, bilateral, hilum apical, circular or linear, seed surface smooth, flagella like projection present on lateral side, it is present near hilar region. (Fig-01).

In *Phaseolus vulgaris* L. local name is rajma .Externally Seed 1.44 cm. - 0.69 cm, ellipsoidal, reddish ,391.8 mg, bilateral, hilum median, elliptical, surface smooth, hilar region is whitish in colour, seed surface is reddish colour but some brownish patches present on surface, slightly shaded. (Fig-02)

In *Prosopis spicigera* local name is shami, externally Seed 0.51 cm. - 0.28 cm ,oblong, brownish, 101.98 mg, bilateral hilum apical, circular, seed surface smooth (Fig-03).

In *Crotolaria iuncea* L. local name is Ranboru/Khulkhula. Externally Seed 0.46 cm. - 0.34 cm, trigonous, slightly heart shaped, greyish, 27.38 mg, bilateral, hilum apical, circular ,present in notch and whitish, seed surface is smooth i.e.at the apical portion one lobe is broader than other. (Fig-04).

In Scanning electron microscopy (SEM) investigation *Albizia lebbeck* (Linn.) Willd. seed surface shows regular plain surface ,not much variation found in them but some granulated deposition with branched lining on cellular surface. (Fig- 05). In *Phaseolus vulgaris* L. seed surface shows dense irregular thread like ,granulated deposition part in hilar region with plain seed surface. (Fig-06)

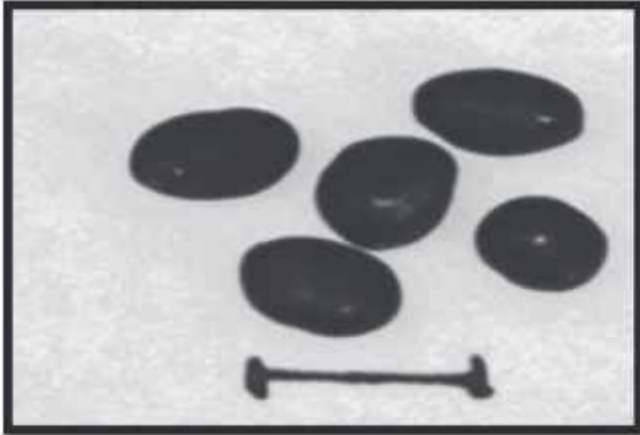


Fig. 1: *Albizia lebeck* (Linn.) Willd

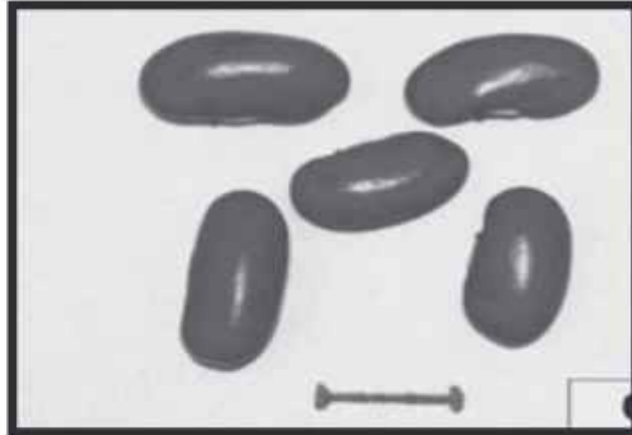


Fig. 2: *Phaseolus vulgaris* L.

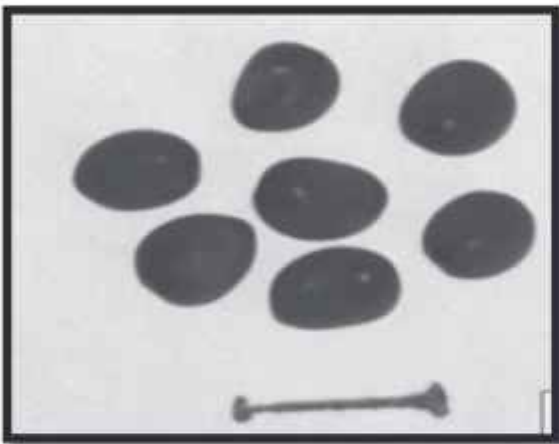


Fig. 3: *Prosopis spicigera* L.

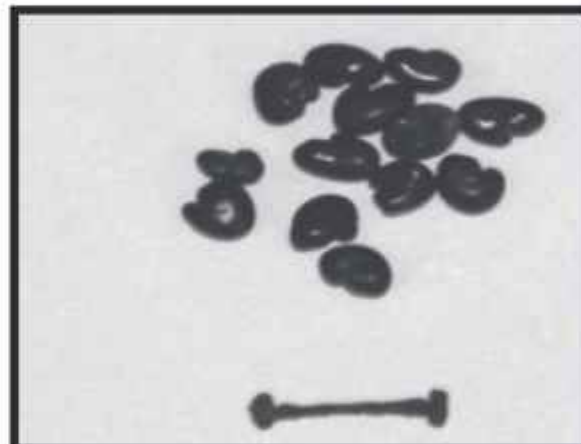


Fig. 4: *Croton juncea* L.

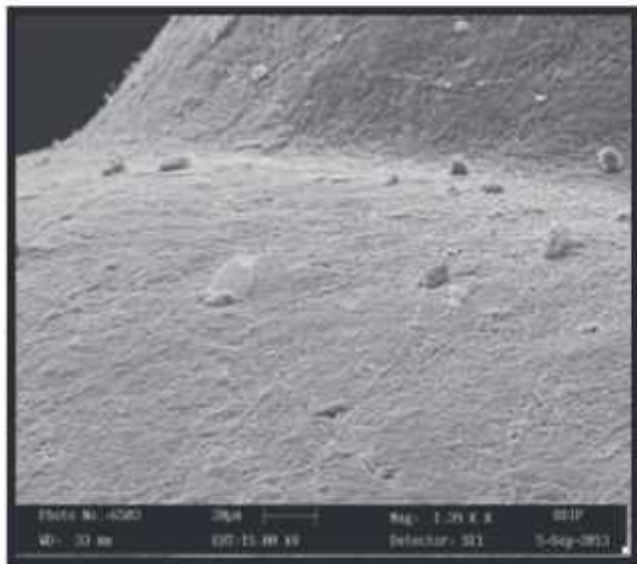


Fig. 5: 1.39 KX- *Albizia lebeck* (Linn.) Willd

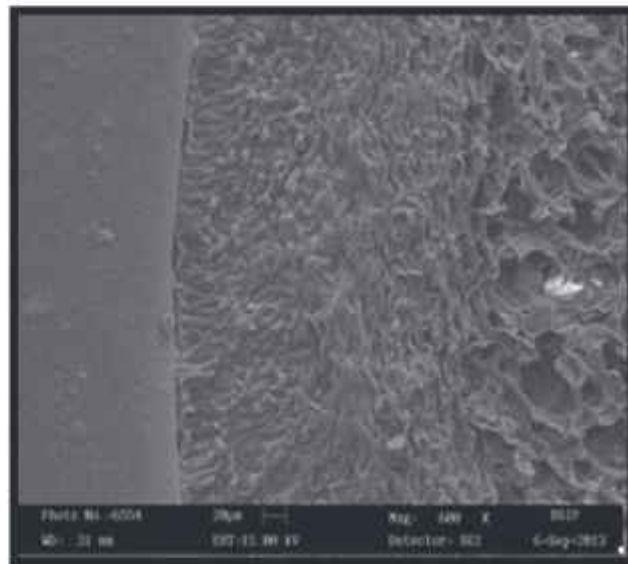


Fig. 6: 600X- *Phaseolus vulgaris* L.

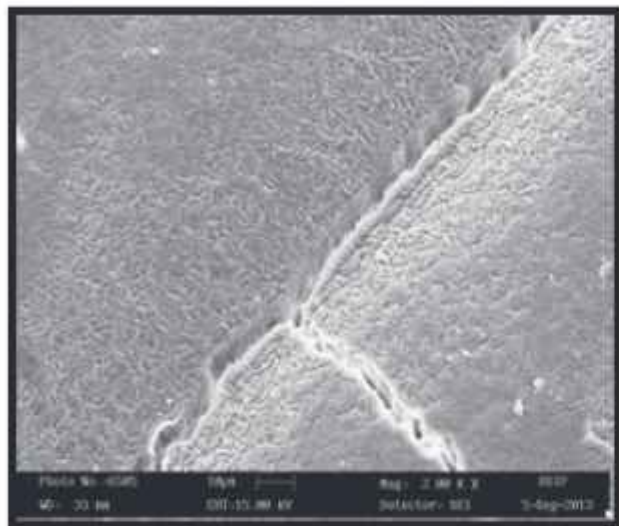


Fig. 7: 2.00 KX -*Prosopis spicigera L.*

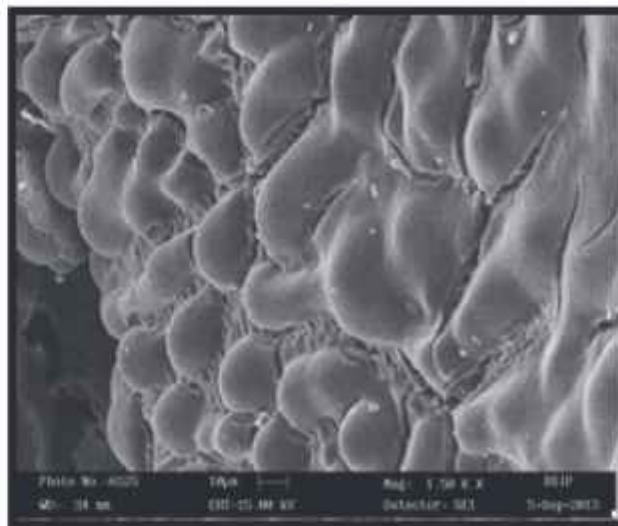


Fig. 8: 1.50 KX- *Crotalaria juncea L.*

In *Prosopis spicigera L.* scanning electron microscopy (SEM) seed surface shows not showing specific variation, cellular surface with elongated raphe region.(Fig-07).In *Crotalaria juncea L.* scanning electron microscopy (SEM) shows cellular variation in which highly globular mounded ridges, below the ridges threads like fibrous attachment present, the ridges and furrows present on surface. (Fig-08)

Medicinal uses:- In *Albizia lebbek (Linn.) Willd.* Seeds astringent, aphrodisiac, restorative and given in piles, dysentery and diarrhoea and used in diseases, gonorrhoea and tuberculous glands. In *Phaseolus vulgaris L.* Cooking, astringent, expectorant and anthelmintic. In *Prosopis spicigera L.* seeds medicinally important. The seed contains relatively large proportion of unsaturated fatty acids, with linoleic and oleic acids (Girase M.V. et al.2016). In *Crotalaria juncea L.* Seeds given to purify blood, in impetigo, psoriasis, as an emmenagogue, toxic to cattles. (Sharma,2003).

Discussion:- Scanning electron microscopy is one of the recent field for furnishing information on seed coat morphology. The morphological variations were well studied during this investigation .Seed surface characters is also

important character for morphological identification. Seed size were taken using average length and breadth of 15 seeds which gave individual size of seed. In *Albizia lebbek (Linn.) Willd.* Seed medicinally important. Morphologically variation on surface region. Seed medicinally very important restoring health and strength of body. In *Prosopis spicigera L.* Seed shows minor variations on surface but anthelmintic activity shows destruction of intestinal worms. In *Crotalaria juncea L.* the hilly region present in notch which represent the position of hilum as a significant characters of this genus. Medicinally useful on skin infection. On the basis of this surface study the seeds can be differentiated on micromorphological characters of seed. These structure helps for the protection of cellular surface from external agents. According to Chuang and Heckard (1972) the seed coat pattern is diversified among species and furnish an important feature for classification. Systematic and scientific investigations of traditional medicinal plants provide important drugs for various therapeutic uses.

Different plant organs are being used for curing various types of diseases, but seed is one of the important organs which is mostly used for preparation of drugs. The seed which we studied for

morphological investigations having medicinal uses. Medicinally whole seed or seed coat used for preparation of various drugs. Various chemical compositions present inside the seed are effective for drug preparation. Phytochemicals from medicinal plants are receiving greater attention in scientific literature, in medicine and in the world economy in general (Bruni 2003). Seeds are astringent, aphrodisiac in *Albizia lebbek*. Seed contains various types of alkaloids which are used for preparation of drugs or medicine. The seeds or seed coat are known to have capacity to store various alkaloids or chemicals. The present investigation helps to determine micromorphological characters of seeds, their identification, detection of various chemical compounds in them. Detection of various compound and surface characters helps to solve taxonomic problems, therapeutic efficacy etc.

Acknowledgement

The authors are thankful to the Director, Birbal Sahni Institute of Paleobotany, Lucknow for extending SEM facilities.

References

1. Barthlott, W.1984.Microstructural features of seed surface.Pp.85-106 in. Heywood V.H. and Moore,M.(eds.),Current concepts in plant taxonomy. Academic press, London.95-105.
2. Bhojwani S.S. and Bhatnagar, S.P. 2000. Seed. The embryology of angiosperms. Vikas pub. House pvt.ltd.15.
3. Bruni, A. 2003. Foreword. Plant derived antimycotics. (Current trends and future prospects) food product press.
4. Chance, G.D., J.D. Bacon,(1984) Systematic implications of seed coat morphology in *Nama* (Hydrophyllaceae) Am. J.Bot. 71: 829-842.
5. Chuang, T.I. and Heckard, L.R. 1972. Seed coat morphology in *Cordylanthus* (Scrophulariaceae) and its taxonomic significance. Amer. J.Bot. 258-265.
6. Girase M.V. Jadhav M.L. Jain A.S. 2016 *Prosopis spicigera*: A Nature's Gift.International Journal of Pharmaceutical Chemistry and analysis.3(1):49-52.
7. Green, N.P.O., Stout, G.W., Taylor, D.J. ed.R.Soper,1984. Evolution of seeds. Biological science (organism, energy and environment) variety of life-fungi and plants. Cambridge University Press. 3: 68.
<https://en.wikipedia.org/wiki/Fabaceae>
8. Manilal, K.S. and Pandey ,A.K.1996.Taxonomy and plant conservation CBS pub. and dis. Matthews, J.F., P.A. Levine,1986.The Systematic significance of seed morphology in *Portulaca* (Portulacaceae) under scanning electron microscopy. Syst Bot. 11 : 302-308.
9. Pandey ,A.K., Verma, S.K., Kumar,A. 1996.Systematic significance of achene micromorphology in Cyperaceae. Taxonomy and plant conservation.CBS pub. and dis.13:91-99
10. Sharma,R. 2003.Introduction.Medicinal plants of India- An encyclopaedia.Daya publishing house xxx.
11. Shetler,S.G.,N.R.Morin,1986.Seed morphology in North American Campanulaceae. Ann.Missouri. Bot. Gard. 73: 653-688.

Study of Histopathology of some tissues of *Macrobrachium rosenbergii* (de Man), exposed to pesticides, Chlorpyrifos and Dimethoate, individually and synergistically.

V.N.Hedge¹, B Chavan² and V. V. Dalvie³

¹Department of Zoology, S.V.K.M.'s Mithibai College (Autonomous), (Affiliated to University of Mumbai), Vile Parle (W), Mumbai 400056
drvnhegde@rediffmail.com

²Department of Zoology, Ismail Yusuf College, (Affiliated to University of Mumbai), Jogeshwari (E), Mumbai 400060.
prbchavan@gmail.com

³Department of Zoology, S.V.K.M.'s Mithibai College (Autonomous), (Affiliated to University of Mumbai), Vile Parle (W), Mumbai 400056.
dalvie@gmail.com

ABSTRACT

Macrobrachium rosenbergii (de Man), the giant freshwater prawn, occupies an important place as a culture aquatic organism only next to the Indian Major Carps(IMCs), especially in the riverine regions of coastal India. The prawns grow to a length of 12 inches or more, the males being longer and identified by their long blue chelae. They live in almost all freshwater bodies and descend into brackishwater regions to breed. The Post Larvae (PL) and juveniles ascend the rivers again, to reach freshwater regions. On their upward journey they enter streams, rivulets, fields, mangroves, ponds and other freshwater bodies. It is in this region that many fishermen collect them for the purpose of selling them as seed to farmers for their culture. The Vaitarna River is one such place which is the habitat of the giant freshwater prawn *Macrobrachium rosenbergii*. The river has its origin in the Trimbak Hills in Nasik district in North West Maharashtra and it empties into the Arabian Sea. On their ascend up the river the juveniles enter banana plantations and rose nurseries on the banks of the River Vaitarna. These plantations and nurseries use the organo-phosphorous pesticides Dursban (Chlorpyrifos) and Rogur (Dimethoate), to control pests on the farms at various intervals. The pesticides affect the growth, physiology and body metabolism of the juveniles and adult which are actually non-target organisms and also enter the human food chain to cause disturbances in body metabolism, since they are harvested after the rains and sold in the local markets. Therefore, the present study is an attempt to assess the damage to the gills, hepatopancreas and nerve fibres of the prawn exposed individually and in combination (synergistically) to the pesticides Chlorpyrifos (C) and Dimethoate (D), C:D (1:1), C:D (1L3) and C:D (3:1) respectively. The histopathology study revealed significant damage in the tissues caused due to the pesticides. The damage was more pronounced in the prawns exposed to the mixture of the pesticides than individual.

Keywords: *Macrobrachium rosenbergii* (de Man), Chlorpyrifos, Dimethoate, individual, synergism, histopathology, gills, hepatopancreas, nerve fibres.

Introduction

Histopathology is the study of the pathological changes in the microanatomy of the tissues. Histopathological studies help in understanding the stress due to pollution; the animal is exposed to, much before any external manifestation of the same. Histopathological studies are now gaining wider importance in the field of aquatic toxicology. However the greatest significance of histological observations as a part of toxicological investigations probably lies in the fact that even extremely low levels of pollutants which may fail to produce any obvious external morphological or physiological changes in animal may still produce suitable damage in its metabolically sensitive tissues.

The gills being the respiratory tissues are considered to represent an area of prime histological interest in aquatic organisms. The gill tissue not only absorbs oxygen but also inorganic salts from the surrounding medium. The crustacean hepatopancreas is an organ of vital importance, being involved in diverse metabolic activities like secretion of digestive enzymes, absorption and storage of nutrient materials, excretion, moulting cycle, storage of inorganic reserve, lipid and carbohydrate metabolism. A major part of neurological activity is controlled by *supra esophageal* ganglia. A lot of work on effect of pesticides on the histopathology of fish gills and liver is available while reports on crustacean species are meagre. (Yamuna, et al., (2009); Satpornavanit, (2006); Thosar, et al., (2001); Reddy, (1986); Ghate and Mulherkar (1979) and Kinne (1971)). Hence, the present work deals with the effects of two pesticides Chlorpyrifos and Dimethoate on the histological structure of gills, hepatopancreas and nerve fibres of the prawn *Macrobrachium rosenbergii*.

Materials and Method

Live prawns were collected from the River Vaitarna and acclimatized under laboratory conditions for 7 days in glass aquaria. They were fed

with commercial feed ad libitum twice a day. The water in the aquaria was well aerated and replaced daily with dechlorinated aged tapwater. After acclimatization healthy prawns were selected and placed in six groups, each group of 10 prawns maintained in glass aquaria containing well aerated, dechlorinated aged tapwater. The prawns were exposed to the pesticides Chlorpyrifos (C) 0.0002 ppm and Dimethoate (D) 0.02 ppm individually and in combination in the proportion of 1:1, 1:3 and 3:1 respectively and control group without the pesticide was maintained. At the end of the exposure period of 28 days, the prawns were sacrificed and tissues gills, hepatopancreas and nerve fibres were fixed in Bouin's fixative and dehydrated using alcohol grades. After clearing with xylene, the tissues were embedded in paraffin wax. Sections were cut at 5 microns and stained with Ehrlich's haematoxylin-eosine (Pedro, 2009). Sections were cleared in xylene, mounted in DPX and observed under binocular research microscope with attached Mitotic Research Microscope@.

Results and Discussion

Gills

The prawn gills are almost crescent-shaped with their size increasing antero-posteriorly and are described as phyllobranch. Each gill consists of two rows of leaf-like rhomboidal gill plates arranged parallel to one another. The two rows of gill plates are separated by median longitudinal groove which extends length-wise in gill axis. The gill plates are longer in the middle region and tapers in the end.

The section of normal gill of prawn from control group showed an excess of base which is almost triangular and consists of a central core of a connective tissue enclosed in a layer of epidermis or hypodermis. Hypodermis externally shows a thin cuticle. Gill plate shows a single layer of cells with cuticle on both sides. These cells are of two types - pigmented and transparent, alternating with each other.

Gills of prawns exposed to the organophosphorus (OP) pesticides viz. Chlorpyrifos (C) and Dimethoate (D), individually and synergistically, showed drastic histological changes. The cuticle being a hard substance showed no significant changes in prawns exposed to individual pesticide.

The lateral longitudinal channels were obliterated or filled with a fluid matrix, fewer pigmented cells and a pattern of pigmentation alternating with cells no longer visible, mucous formation and sticky gill lamellae observed in Chlorpyrifos while in prawns exposed to Dimethoate the gills showed alterations in the structure with a three fold elongation in the gill lamellae with abnormal bulging of gill lamellae tips. Severe damage was observed in the gills of the prawns exposed to the combination of pesticides Chlorpyrifos and Dimethoate (C+D) in the ratio 1:1, 1:3 and 3:1 respectively (Fig.1.1-1.5). Gill plates shrunk in size as compared to normal gills, cuticular disruption accompanied with sloughing of gill lamellae, mucous formation and tissue debris on the whole clearly showed the damage caused by the pesticides, (Bhargava and Bhide, 1987; Prasad et al., 2000). Thus damage to the gill structure affects the respiratory process causing stress to the prawn. The loss in the basic structure of the gills (Banerjee et al., 1987; Khare et al., 2002) may also affect the process of exchange of gases, leading to the disruption of supply of oxygen to vital organs like heart, brain, and the hepatopancreas which in turn may affect body metabolism. Similar findings were reported by Bhavan and Geraldine (2000) in gills of the prawn *Macrobrachium malcolmsonii* exposed to endosulfan.

Hepatopancreas

The hepatopancreas in crustaceans is responsible for digestion as well as other metabolic processes including secretion of enzymes. It plays a very important role in detoxification of substances which enter the prawn body. The effect of pesticides on hepatopancreas forms an important aspect of histopathology.

In the control prawn, the hepatopancreas showed numerous glandular tubules which are branched in a flowering pattern and held together by connective tissue, to form a compact mass. The walls of the tubules are formed of a single layer of columnar epithelium which in turn consists of four types of cells namely granular cells, ferment cells, hepatic cells and basal or replacing cells. All epithelial cells rest on a basement membrane. The hepatopancreas of prawns exposed to Dimethoate (Rodrigues and Fanta, 1998), Chlorpyrifos (Maharajan et al., 2015), and a mixture of both the organophosphorus pesticides, showed that most of the granular cell, hepatic cells and basal cells were ruptured. The lumen appeared larger than in control indicating that the cells have undergone lysis. In Chlorpyrifos exposed prawns the hepatopancreas showed degeneration of tubule, karyomegaly and vacuoles.

This is in agreement with findings reported by Jaiswal, K. and Sarojini, R. (1990). In prawns exposed to the mixture of both the pesticides, Chlorpyrifos and Dimethoate in the proportion of 1C:3D and 3C:1D the damage was more severe with abnormal lumen tubule, degeneration and lysis of the epithelial cells more prominent than in the prawns exposed to 1:1 pesticide mixture. (Fig:2.1-2.5)

Nerve fibres

Major part of neurological activity is controlled by the brain, supra oesophageal ganglion. In the prawn it is a bilobed structure which lies at the base of the rostrum, anterior to the oesophagus. From this region five nerves arise, namely antennary, optic, ophthalmic, antennary and integumentary (supplying to the labrum). Two more short nerves arise from the posterior part of the brain and run downwards and backwards around the oesophagus. These ganglia form a mass in the anterior region called ventral thoracic ganglionic mass. This ganglionic mass is formed by fusion of eleven pairs of segmental ganglia of the cephalothorax. It gives off eleven pairs of nerves on its lateral sides of which the first three pairs are cephalic and the last eight pairs are thoracic nerves

and the five pairs to the walking legs. The ventral thoracic ganglionic mass extends posteriorly to form a thick abdominal nerve cord. It has an abdominal ganglia in each of the abdominal segments. The prawn peripheral nervous system arises from the most posterior part of the brain to the visceral oesophageal ganglia. Since the *supra oesophageal* ganglia and ventral thoracic ganglia are important from neurological point of view, sections of these ganglia were taken for the present study.

In the control both the ganglia appear normal with the neurosecretory cells and the membranes. In the sections taken from prawns exposed to the pesticides, the cells appeared to be elongated with their nuclei being prominent. In the section of the supra-oesophageal ganglionic region, the cells appear as a mass which cannot be distinguished from one another. The cells appeared very hazy and the nucleolus distorted. The neurosecretory cells and the granular cytoplasm reduced as compared to the nerve fibre in control prawn. This indicates that the neurosecretory function responsible for the proper functioning of X and Y organs would be adversely affected, thus thereby affecting the physiological manifestations.

Nerve fibres in Chlorpyrifos treated prawns showed more damage than Dimethoate, whereas prawns exposed to both the pesticides in the proportion of 1:1, 1:3 and 3:1, the damage was severe with degeneration of tissue. The damage showed loss of cytoplasmic inclusions and distortion in the nerve fiber structure (Fig.3.1- 3.5).

The study of the histopathology of the tissues namely the gills, hepatopancreas and nerve fibers showed that Chlorpyrifos is more toxic pesticide as compared to Dimethoate. Further, the combination of pesticides in the proportion of 1:1, 1:3 and 3:1 prove to be more detrimental to the basic structure of the tissues gill, liver and nerve fibres. This is in agreement with Maharajan et al. (2015). The action of Chlorpyrifos is enhanced three fold by Dimethoate indicative of positive synergism, that is Dimethoate increases the toxic

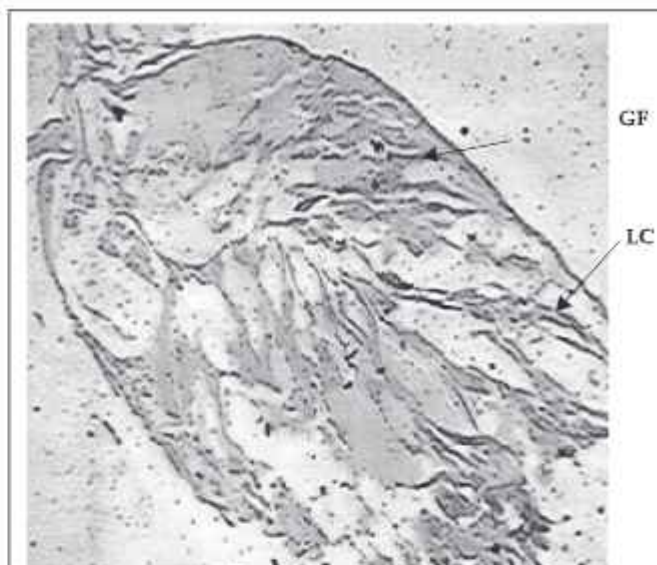


Fig.1: LS of prawn gill of control
GF: Gill Filament, LC: Lymph Channel 100x

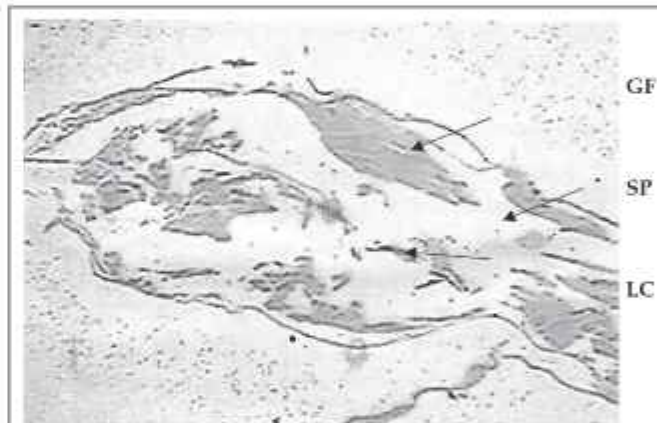


Fig.1.1: LS of prawn gill exposed to Chlorpyrifos (C)
GF: Gill Filament, LV: Lymph Channel, SP: Space 100x

effects of Chlorpyrifos manifold. The combination of 3C:1D is so very toxic that it showed complete destruction of the basic histological integrity of the tissues. According to K Burnett and L Burnett (2015) in decapods aggregates of activated hemocytes and bacteria can occlude hemolymph channels in the gills, impairing respiration and affecting the metabolism of the animal. The toxicity of pesticides to aquatic animals have been studied by many workers (Bakthavatsalam et al.,1984; Jauhar and Kulshreshta,1985; Nagabhushanam et al. 1987; Bhatnagar and Bana 1990; Singh and Sahai 1990; Singh 1993; Karuppswamy,1999; Pawar et al. (2000), Sakthivel and Richardson (1995), SelvaSundari and Perumal (2006).

Conclusion

It may be concluded that pesticides affect the aquatic animals moreover the mixture of pesticides augment and manifest histological damage often leading to mortality.

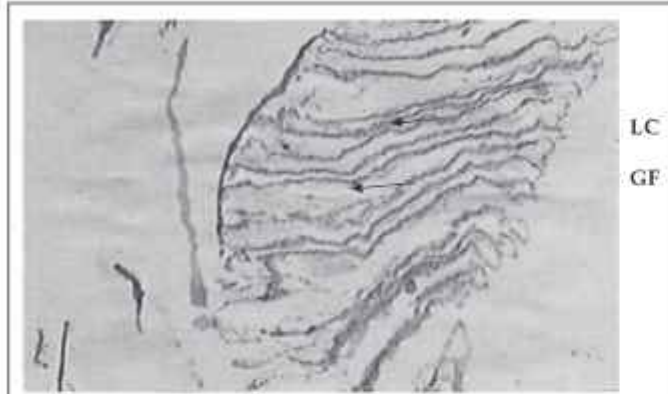


Fig.1.2: L.S of prawn gill exposed to Dimethoate (D) GF: Gill Filament, BV: Lymph Channel 100x

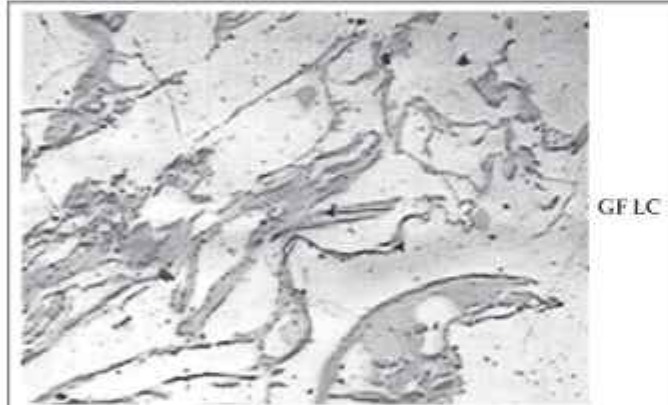


Fig.1.3: L.S of prawn gill exposed to 1C:1D GF: Gill Filament, LC: Lymph Channel 100x



Fig.1.4: L.S of prawn gill exposed to 1C:3D GF: Gill Filament, LC: Lymph Channel 100x

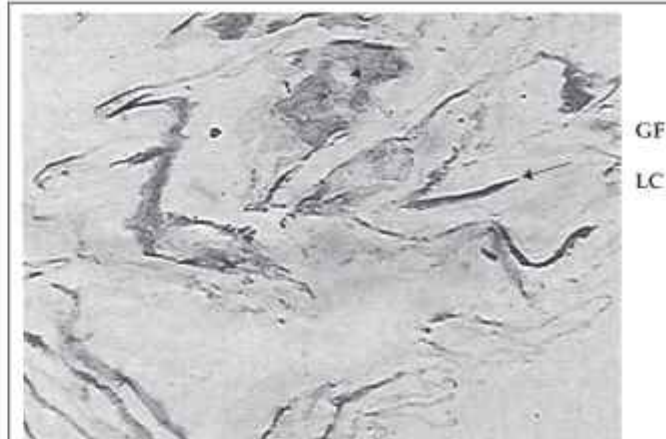


Fig.1.5: L.S of prawn gill exposed to 3C:1D GF: Gill Filament, LC: Lymph Channel 100x



Fig.2: LS of prawn hepatopancreas of control CT: Connective Tissue, VO: Vacuoles, LT: Lumen of Tubule, HPM: Hepatopancreatic Membrane 210x

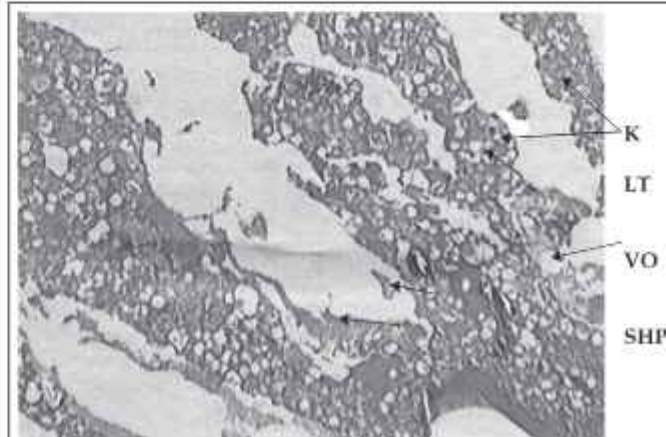


Fig.2.1: LS of prawn hepatopancreas exposed to Chlorpyrifos (C) SHP: Sloughing of HP, VO: Vacuoles, LT: Degeneration of Lumen of Tubule, K: Karyomegaly 210x

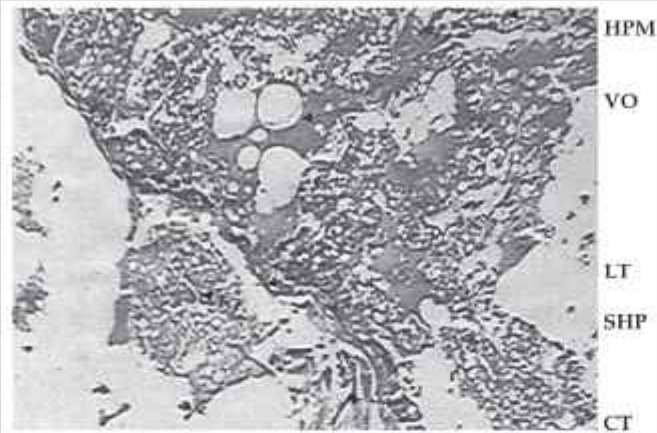


Fig.2.2: LS of prawn hepatopancreas exposed to Dimethoate (D)
CT: Connective Tissue, VO: Vacuole, LT: Lumen of Tubule,
HPM: Hepatopancreatic Membrane 210x

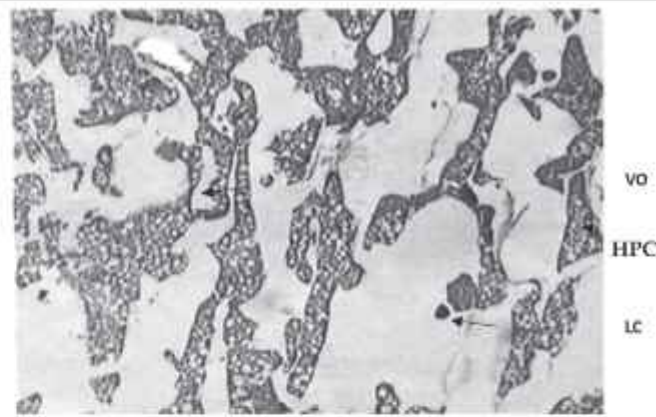


Fig.2.5: LS of prawn hepatopancreas exposed to 3C:1D
VO: Vacuole, LC: Disintegrated Lymph Channel,
HPC: Hepatopancreatic Cell 210x



Fig.2.3: LS of prawn hepatopancreas exposed to 1C:1D
VO: Vacuole, LT: Lumen of Tubule, HPM: Hepatopancreatic
Membrane, HPC: Hepatopancreatic Cell 210x



Fig.3: LS of prawn nerve fibre of control
DT: Dendrite, N: Nucleus, AX: Axon 210x



Fig.2.4: LS of prawn hepatopancreas exposed to 1C:3D
VO: Vacuole, LC: Lymph Channel, HPM: Hepatopancreatic
Membrane, HPC: Hepatopancreatic Cell 210x

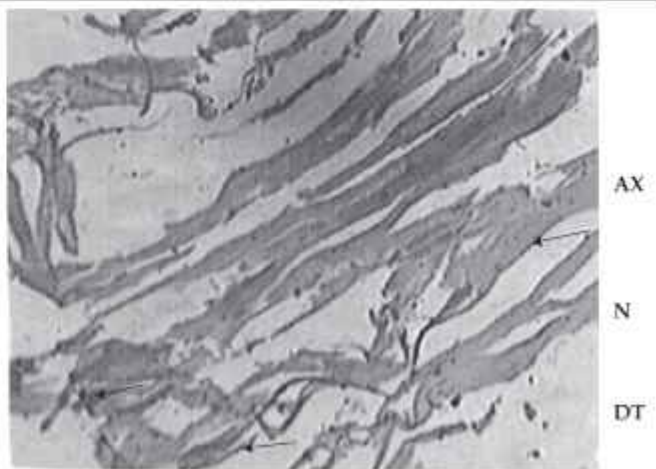


Fig.3.1: LS of prawn nerve fibre exposed to Chlorpyrifos (C)
DT: Disintegrated Dendrite, N: Disintegrated Nucleus,
AX: Disintegrated Axon 210x

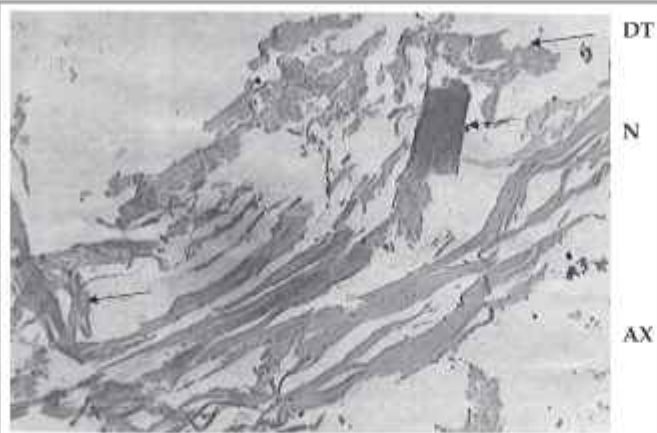


Fig.3.2: LS of prawn nerve fibre exposed to Dimethoate (D)
DT: Disintegrated Dendrite, N: Disintegrated Nucleus,
AX: Disintegrated Axon 210x

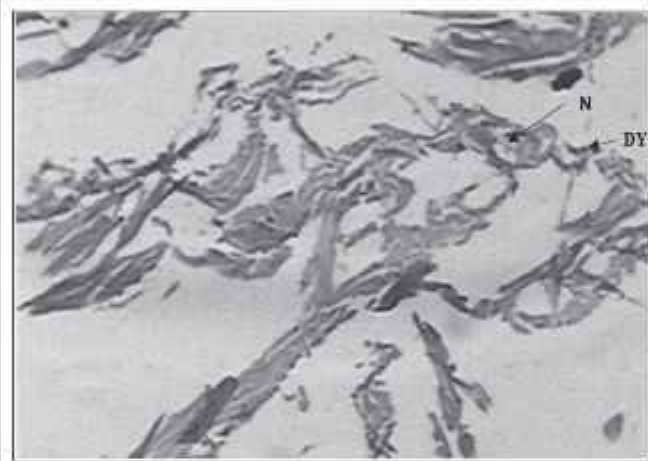


Fig.3.3: LS of prawn nerve fibre exposed to 1C:1D
DT: Disintegrated Dendrite, N: Disintegrated Nucleus 210x

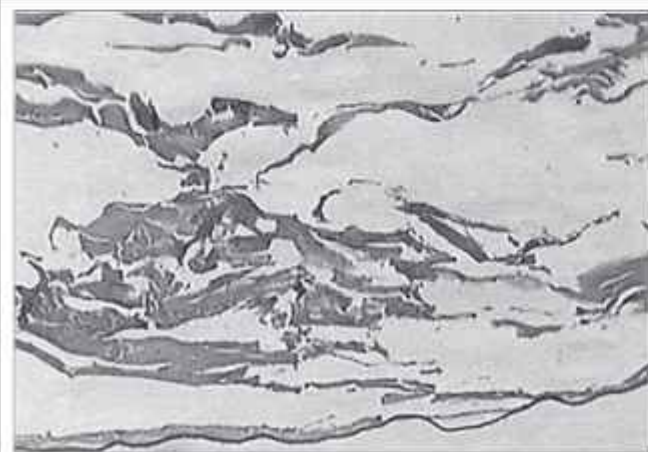


Fig.3.4: LS of prawn nerve fibre exposed to 1C:3D
Sloughing of nerve fibre 210x

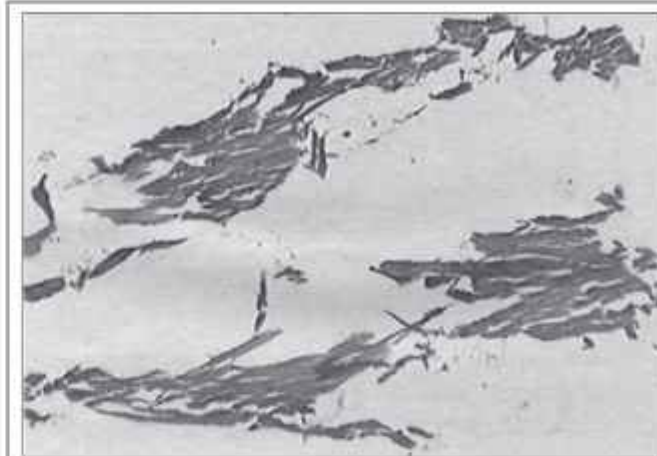


Fig.3.5: LS of prawn nerve fibre exposed to 3C:1D
Disintegration of nerve fibre 210x

References

1. Bakthavasalam, R., Ramalingam, R. and Ramaswamy, A. (1984), Histopathology of liver, kidney and intestine of *Anabas testudineus* exposed to Furadon. Environ. Ecol. 2(4):243-277.
2. Banerjee, G., Rajendranath, T. and Dayakar, P. (1987), Acute and chronic toxicity of Dichlorvos (DDV), on the gills of *Clariasbatrachus* Linn. Abst.Proc.8thAEB, Sess.andSymp. Jammu, 80.
3. Bhargava, S. and Bhide, M. (1987), Histopathological studies in the gills of *Rasbora daniconius* exposed to DDT. Abst.Proc. 8th AEB Sess. Symp. Jammu, 52.
4. Bhatnagar, T. M. C. and Bana, A. K. (1990), Pesticide induced histophysiological alterations in liver of *Channa gachina*. Abstracts, Proc.XI Ann. Ses. Acad. Env. Biol..Aurangabad, Maharashtra:77.
5. Bhavan, P.S. and Geraldine,P. (2000), Aquatic Toxicology 50 (2000), 331-339 Histopathology of the hepatopancreas and gills of the prawn *Macrobrachium malcolmsonii* exposed to endosulfan. Aquatic Toxicology 50, 331-339
6. Burnett, K. and Burnett, L. (2015), Respiratory and Metabolic Impacts of Crustacean Immunity: Are these implications for the insects?, Integrative and Comparative Biology, 55(5):856-868.
7. Jauhar, L. and Kulshreshtha, S. K. (1985),

- Histopathological effects induced by sublethal doses of Sevin and Thiodon on the gills of *Channa striatus* (Bloch). *Acta Hydrochem. Hydrobiol.* 13(3):395-400.
8. Karuppswamy, R. (1999), The effect of phenyl mercuric acetate (PMA) on the physiology, biochemistry and histology of selected organs in a freshwater fish *Channa punctatus* (Bloch), Ph.D. Thesis, Annamalai University, India.
 9. Khare, S., Singh, S. and Mehrotra, A. (2002), Histopathological changes in the gills of *Nandus nandus* induced by endosulfan and carbaryl. *Nat. Environ. Poll. Tech.* 1(1):124.
 10. Maharajan, A. M., Narayanaswamy, Y., Ganapriya, V. and Shanmugavel, K. (2015), Histological alterations of a combination of Chlorpyrifos and Cypermethrin (Nurocombi) insecticide to the freshwater crab *Paratelphusa jacquemontii* (Rathbun). *J. Bas. Appl. Zoo.* 2015, 72:104-112
 11. Nagabhushanam, R, Sambasiva, Rao, K. and Sarojini R. (1987), Histological changes in the gills and hepatopancreas in the marine crab *Scylla serrata* induced by an organophosphate Dimecron. *J. Adv. Zool.* 8(1):46-51.
 12. Pawar, S., Rangnekar, P. V. and Tope, J. (2000), Studies on neurosecretory cells in the lobster *Panulirus polyphagus* (Herbst). International Conference on Probing in Biological Systems, Institute of Science, Mumbai.
 13. Prasad, B. B., Singh, K.M. and Singh, R. B. (2000), Effects of carbamate (Furadan-3G) on the histopathological changes in the gills of *Channa marulius* (Ham.). *J. Env. Poll.* 7 (4):279-281
 14. Richardson, R. J. (1995), Assessment of the neurotoxic potential of Chlorpyrifos relative to other organophosphorus compounds, a critical review of the literature. *J. Toxicol. Environ. Health.* Feb. 1995, 44 (2) : 135 – 165.
 15. Rodrigues, E. L. and Fanta, E. (1998), Liver histopathology of fish *Brachydanio rerio* after acute exposure to sublethal levels of the organophosphate Dimethoate. *Rev Bras. Zool.* 15:441-450.
 16. Sakthivel, V. and Gaikwad, S. A. (2002), Tissue histopathology of *Gambusia affinis* (Baird and Girard) under Dimecron toxicity. *Ecol. Env. Con.* 8(1):27-31.
 17. Satpornavanit, K. (2006), Feeding behavior of prawn *M. rosenbergii* as an indicator species of pesticide contamination in tropical freshwater. Ph.D Thesis, Institute of Aquaculture, University of Sterling, U.K.:254.
 18. Selvasundari, C. J. and Perumal, N. V. (2006), Studies on toxicological effects of Monocrotophos in the tissues of freshwater fish, *Cirrhinus mrigala*. *J. Aqua. Biol.* Vol.21 (1):176-178
 19. Singh, R. K. (1993), Pesticidal effect on the gills, liver and kidney of a freshwater teleost *Cyprinus carpio* Ph. D. Thesis, Barkatullah University, Bhopal, M.P.
 20. Singh, S. and Sahai, S. (1990), Changes in the gills structure induced by BHC, Lindane and Endosulfan in the freshwater teleost *Puntius ticto* (Ham.) *Folio-Morphol Praha*, Vol. 38,ISS 4:423-432.
 21. Thosar, M. R. and Huilgo, I.N.V. and Lonkar, A. N (2001), Observation on the changes in the structure of hepatopancreas in the freshwater gastropod snail, *Vivipara bengalensis*, exposed to sublethal concentrations of insecticide Metasystox. *J. Aqua. Biol.* 16(2): 42-44. ■

Structural and Magnetic Properties of $Mn_{0.8}Co_{0.2}Fe_2O_4$ Ferrite Nanoparticles

Ravindra Kalesh¹, Ravindra N. Kambale², Syed Habibuddin Syed Abed Ali² Vaishali Bambole^{2*}

¹Department of Physics, Ismail Yusuf College,
(Affiliated to University of Mumbai) Mumbai - 400 060, India

²Department of Physics, University of Mumbai,
Vidyanagari Campus Kalina Santacruz Mumbai 400098, India

*Corresponding author: vabphy@gmail.com.

ABSTRACT

Cobalt doped Manganese ($Mn_{0.8}Co_{0.2}Fe_2O_4$) spinel ferrite nanoparticles have been synthesized by green sol-gel auto combustion technique with lemon juice. The prepared sample was sintered at 550°C for 8 hrs. The structure and morphology of prepared sample were investigated by X-ray diffraction (XRD) and Field Emission Gun Scanning electron microscopy (FEG-SEM) technique. The X-ray diffraction pattern confirm the single phase formation and the crystallite size of synthesized $Mn_{0.8}Co_{0.2}Fe_2O_4$ ferrite nanoparticles were found to be within the range of 8 -12nm. The magnetic properties were studied by using vibrating sample magnetometer (VSM). The saturation magnetization, coercivity, remanence, Bohr magneton and anisotropy constant (K) were calculated from the M-H hysteresis loop.

Keywords: Nanoparticles, Saturation magnetization, anisotropy constant and Bohr magneton.

Introduction

Nano structured spinel ferrite have been largely counted during the last decades, many scientists have devoted their effort for studying ferrite nano materials especially for many technological and industrial applications like sensors, data storage devices, medical applications, catalysis for water splitting and microwave absorption etc.. This is because ferrites exhibits excellent chemical stability, moderate saturation magnetization and mechanical hardness [1-4]. Thus much attention has been paid to synthesize and characterization of nanoparticles of spinel ferrites. It is necessary to fabricate new materials of more predictable properties than what are currently available. In addition, the doping of metallic ion like Co in manganese ferrite may increase the nano magnetism

phenomenon. Spinel ferrite in the nano form with large surface to volume ratio and super paramagnetic nature is accountable for improved Nano magnetism in spinel ferrite [5-6]. Co doped manganese ferrite, unpaid to strong ferromagnetism and high Curie temperature, is used in electronic appliances than Mn-Co magnet since it stays magnetized even when the applied magnetic field is removed and it has superior heat resistance, temperature characteristics, and corrosion resistance also, Co doped manganese ferrite is an interesting material because of its good chemical stability and moderate saturation magnetization. [9-12].

Structure and morphology also depends on preparation method of nanomaterials. Spinel ferrites nanoparticles were synthesis by several

methods, such as co-precipitation, spray pyrolysis, solid state reaction and sol-gel auto combustion methods. Among these methods sol-gel auto combustion method with lemon juice is an excellent to prepare nanoparticles with maximum purity. By using different amount of lemon juice we control the size of nanoparticles. Also this method has several advantages over the other synthesis methods due to its low processing temperature, high chemical homogeneity, thermal stability of controlling the size and morphology of particles etc.^[13-17]

The present study includes the synthesis and characterization by different tools like X-ray diffraction and field emission gun electron microscopy for the investigation of structural, microstructural properties and magnetic properties of the sample were studied by vibrating sample magnetometer.

Materials and Method

Cobalt doped Manganese ($Mn_{0.8}Co_{0.2}Fe_2O_4$) spinel ferrite nanoparticles were synthesized using sol-gel auto combustion technique with lemon juice as a chelating agent. AR grade chemicals such cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$), manganese nitrate ($Mn(NO_3)_2 \cdot 6H_2O$) and ferric nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) were used. All nitrate were dissolved in 100ml de-ionized water separately and mixed one another. Certain amount of lemon juice is added in the mixture and stirred continuously for 2hrs. To make solution neutral, ammonium hydroxide was added and obtained solution was heated at $90^\circ C$. After 5hours the solution becomes gel and the gel was turn into loose powder. Finally, $Mn_{0.8}Co_{0.2}Fe_2O_4$ crystalline powder was obtained after calcining the loose precursors at $550^\circ C$ for 8 hours. This powder was used for further investigations of structural properties and magnetic properties.

Results and Discussion

The X-ray diffraction pattern of the $Mn_{0.8}Co_{0.2}Fe_2O_4$ spinel ferrite nanoparticles is shown in Fig. 1. The XRD pattern shows the single phase cubic spinel

structure the sample and there was no any impurity peaks observed in XRD pattern. The sharp and high intensity peaks indicates that the prepared nanomaterial is in high crystallite. The lattice parameter was found to be 8.360. The intensity of (311) plane is more as compared to other planes like (220), (222), (400), (422) and (511) and is chosen for the determination of crystallite size. The average crystallite size of the sample was calculated using Scherer's formula,

$$D = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

k is the grain shape factor (0.9) and λ , θ , and β are the X-ray wavelength, Bragg diffraction angle, and full-width at half-maximum of the diffraction peak respectively.

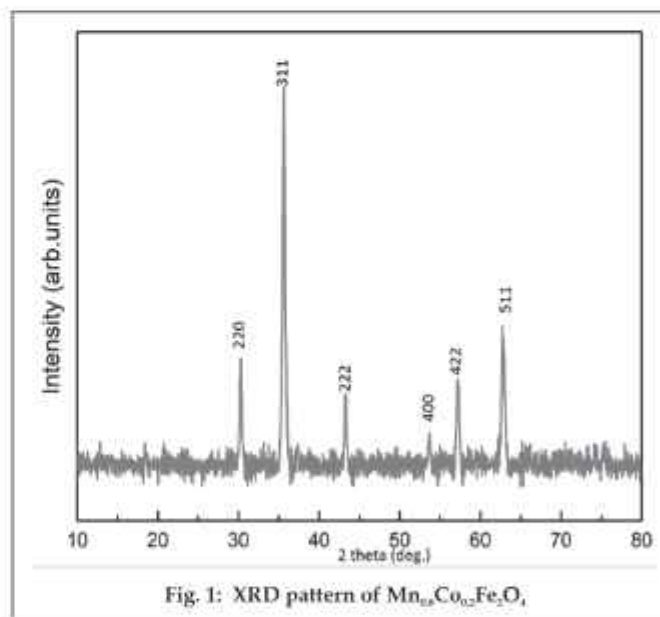


Fig. 1: XRD pattern of $Mn_{0.8}Co_{0.2}Fe_2O_4$

The X-ray density was calculated using the following equation

$$D_x = \frac{8M}{N_A a^3} \quad (2)$$

The lattice constant, X-ray density, and average particle size were calculated using XRD data Table 1.

Sample	Lattice constant (a) (Å)	X-ray density (gcm ⁻³)	Average particle size (nm)
Mn _{0.8} Co _{0.2} Fe ₂ O ₄	8.360	5.262	12

Fig.2 shows morphological pattern of the prepared cobalt doped manganese ferrite nanoparticles taken by Field emission gun scanning electron microscope (FEG-SEM). Evidently, from FEG-SEM image it was seen that the morphology of the particles were almost cubical in shape, but agglomerated to some extent due to the interaction between magnetic nanoparticles. The formation of nano size crystallites was confirmed through FEG-SEM image.



Fig. 2: FEG- SEM image of Mn_{0.8}Co_{0.2}Fe₂O₄ spinel ferrite nanoparticles

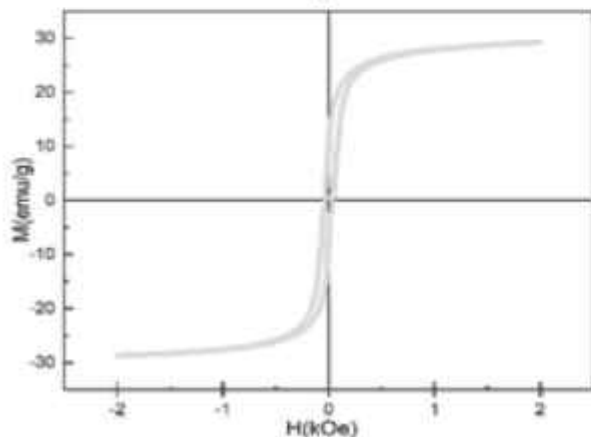


Fig. 3: Magnetic hysteresis loop of Mn_{0.8}Co_{0.2}Fe₂O₄ spinel ferrite nanoparticles

The magnetic properties of the Mn_{0.8}Co_{0.2}Fe₂O₄ spinelferrite nanoparticles were analysed at room temperature by using a vibrating sample magnetometer (VSM) with an applied field -2 kOe ≤ H ≤ 2 kOe [17, 18]. Fig. 3 shows the magnetization (M) versus the applied magnetic field (H) for Mn_{0.8}Co_{0.2}Fe₂O₄ spinel ferrite nanoparticles. The value of saturation magnetization (Ms), remnant magnetization (Mr) and coercivity (Hc) for Mn_{0.8}Co_{0.2}Fe₂O₄ spinel ferrite nanoparticles were calculated (Fig. 3 and Table 2). The following equations were used for calculating the anisotropy constant and Bohr's magneton,

$$\text{Anisotropy constant (K)} = \frac{H_c \times M_s}{0.96} \quad (3)$$

$$\text{Bohr magneton} = \frac{M \times M_s}{5585 \times D_s} \quad (4)$$

Sample	M _s (emu /g)	M _r (emu /g)	H _c (kOe)	Mr/Ms	Anisotropy constant (erg/cm ³)	Bohr magneton
Mn _{0.8} Co _{0.2} Fe ₂ O ₄	29.235	11.177	0.0375	0.382	1.142	0.2302

Conclusion

Cobalt doped Manganese (Mn_{0.8}Co_{0.2}Fe₂O₄) spinel ferrite nanoparticles successfully synthesized by sol-gel auto combustion technique with lemon. From X-ray diffraction, single phase nanosize crystallites were confirmed and particle size of the sample was obtained by field emission gun scanning electron microscopy with the help of Ima-J software and average particle size of the sample is 12nm. The value of saturation magnetization (Ms) from M- H loop was obtained to be 29.235 emu/g and Anisotropy constant 1.142 erg/cm³. It may be concluded that cobalt doped manganese spinel ferrite nanoparticle is a novel material for or microwave absorption in high frequency band.

References

1. S. G .Algude, S.M.Patange, S.E.Shirsath, D.R.Mane, K.M.Jadhav, J. Magn. Magn. Mater. 350, 39-41 (2014).
2. Pradeep Chavan, L R Naik, Vacuum 15. 47-49 (2018)
3. J.F.Hochepped, M.P.Pilani, J. Appl. Phys. 87, 2472 (2000).
4. Jian Ming Gao, Zhi Kai Yan, Jing Liu, Mei Zhang, Min Guo, Material Letters.141, 122-124 (2015)
Zhu Yan, Juhua Luo, J. alloys. Compd. 695, 1185 -1195 (2017).
5. Jian-Ming Gao, Zhi-kai Yan, Jing Liu, Mei Zhang, Min Guo, Materials Letters. 141, 122-124 (2015)
6. M.H.Khedr, A.A.Omar, M.I.Nasr, E.K.Sedeek, J. Anal. Appl. Pyroly. 76, 203 (2006).
7. E Ateria, M A Ahmed, R M Ghouniem, Solid State Sci. 3, 99-106 (2014)
8. Lijun Zhao Hua, Yang Lianxiang, YuYuming, Cui Xueping, ZhaoShouhua Feng, 242, 2, 686-691 (2007).
9. H. Duan, F. Zhou, X. Cheng, G. Chen, Q. Li, J. Magn. Magn. Mater. 424, 467-471 (2017).
10. Ravindra N Kambale, Akhilesh Patel, K G Suresh, Vaishali A Bambole, Int. J. Eng. Tech. Sci. Research. 5, 2, 147-154 (2018).
11. SateeshPrathapani, M. Vinitha, T. V. Jayaraman, and D. Das, J. Appl. Phys. 115, 17A502-1-3 (2014).
12. R. S. Alam, M. Moradi, M. Rostami, H. Nikmanesh, R. Moayedi, Y. Bai, J. Magn. Magn. Mater. 381, 1-9 (2015).
13. V.Rives, O.Prieto, A.Dubey, S.Kannan, J. Catal. 220, 161-167 (2003).
14. F.Prinetto, D.Tichit, R.Teissier, B.Coq, Catal. Today. 55, 103-116 (2000).
15. Yu-jiaSuna, Yi-feiDiaob, Hui-gang Wanga, Guangju Chena, Mei Zhanga, Min Guoa, CeramicInternational. 43, 16474-16481 (2017).
16. H. Moradmard, S. Farjam, Shayestel, P Tohidi, Z Abbas, M. Khaleghi, J. alloys. Compd. 650, 116-122 (2015).
17. NallaSomaiah, T.V.Jayaraman, P.A.Joy, Dibakar Das, J. Magn. Magn. Mater. 324, 14, 2286-2291 (2012). ■

Antioxidant potential of aqueous extract of immature fruit and developing shoot of *Solanum torvum* (Swartz)

D. Shanthi, K. Raja and R. Saravanan

Post Graduate and Research Department of Zoology,

Dr Ambedkar Government Arts College, Vyasarpadi, Chennai 600039, Tamil Nadu

email: rsaravanan0268@gmail.com

Corresponding author: Dr R. Saravanan

ABSTRACT

The role played by free radicals which are highly reactive oxygen species has become increasingly relevant in various of degenerative diseases. Focus on natural antioxidants in medicinal plants are gaining momentum in present days reflecting the immense valuability of Ayurveda, Naturopathy and Siddha medicine in treatment of ailments. The present study will focus on the antioxidant potential of aqueous extract of *Solanum torvum* immature fruits and developing shoot which contribute to the scavenging of free radicals. *In vitro* antioxidant potential of aqueous extracts of *Solanum torvum* immature fruits and developing shoot was assessed by 1,1-Diphenyl-2-Picryl hydrazyl (DPPH), Superoxide anion and Nitric oxide (NO₂) at various concentrations (200 µg, 400 µg, 600 µg, 800 µg and 1000 µg). In the present study a significant antioxidant potential was found in the aqueous extracts of immature fruits.

Keywords: *Solanum torvum*, immature fruits, developing shoots, *in vitro* antioxidant activity, DPPH, Superoxide anion and nitric oxide.

Introduction

Herbals are considered to be promising source of medicine in the traditional healthcare system. The efficacy and safety of herbal medicine have turned the major pharmaceutical population towards medicinal plant research. There is a need for more effective, less toxic and cost effective antioxidants and antimicrobials from natural sources to treat various non-communicable and communicable diseases (Singh et al., 2002).

The role played by free radicals which are highly reactive oxygen species has become increasingly relevant. They initiate or propagate development of

diseases such as cancer, liver and cardiovascular disorders. The oxidation induced by reactive oxygen species can result in cell membrane disintegration, membrane protein damage and DNA mutation (Jayakumar et al., 2016a). Antioxidants with free radical scavenging activity play an important role in protecting damage by reactive oxygen species (Darkwah et al., 2018).

Solanum torvum (*S.torvum*) have revealed cytotoxic activities, antimicrobial, anti-viral activity, anti-inflammatory and anti-tumour (anticancer) activity (Joseph Sakah Kaunda and Ying-Jun Zhang, 2019). The present study will focus

on the free radical scavenging activity of aqueous extract of *S.torvum* immature fruits and developing shoots which contribute to the quenching of free radicals.

Materials and Methods

Collection and Identification of Plant Material

Immature fruits and developing shoots of *S.torvum* (Plate-1) used for the study were collected from in and around Kancheepuram District, Tamil Nadu during May 2018. Fresh plant specimens collected were authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, Tambaram, Chennai. Registration No. (PARC/2018/3855).



Plate 1: *Solanum torvum* plant

Processing and Preservation of Plant Materials

Immature fruits and developing shoots of *S.torvum* (Plate- 2) were separated from freshly collected plants, washed in running tap water and rinsed in distilled water. The plant materials were chopped into small pieces and were shade dried for two weeks for complete dryness. The dried plant materials were powdered, using mechanical

grinder. They were ground well to fine powder and then transferred into airtight containers until further use.

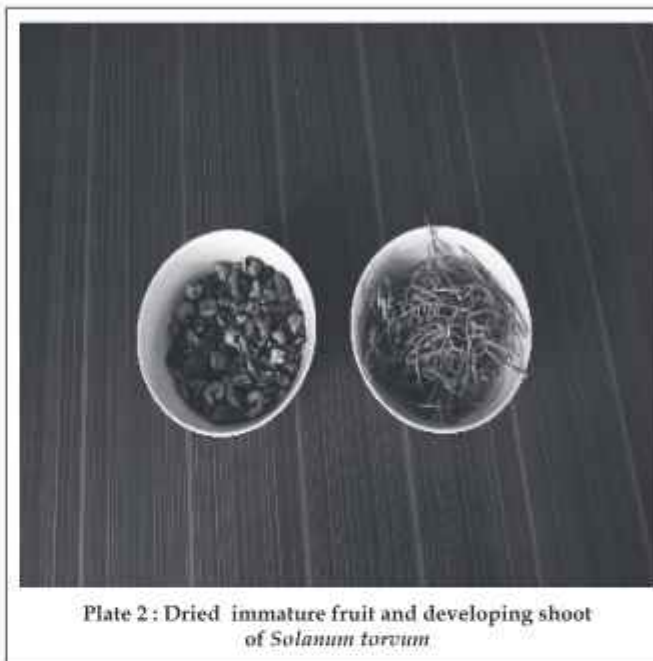


Plate 2 : Dried immature fruit and developing shoot of *Solanum torvum*

Preparation of Aqueous Extract: Cold Water Extraction

5 gm powdered samples of *S.torvum* dried immature fruits and developing shoots were soaked and dissolved in 50 ml of distilled water in a 250 ml conical flask. The flask was plugged with cotton wool and aluminium foil and was placed in a shaker for 24 hrs. The filtrate was concentrated in a Soxhlet apparatus to get the crude plant extracts. The extract was filtered using Whatman filter paper No1. The filtered extracts in the form of a concentrated paste was used for the study.

Determination of *in vitro* Antioxidant Activity of *Solanum torvum*

The aqueous extracts of dried immature fruits and developing shoots of *S.torvum* was assayed for antioxidant activity by the following methods. DPPH radical scavenging activity (Clarke et al., 2013), Superoxide anion radical scavenging assay (Nishimiki et al., 2002) and NO₂ radical scavenging assay (Garret,1974)

Results

The antioxidant potential of the aqueous extracts of immature fruits and developing shoots of *S.torvum* was assessed at various concentrations of 200 µg, 400 µg, 600 µg, 800 µg and 1000 µg by *in vitro* studies.

DPPH radical scavenging activity

The reaction capability of DPPH radical scavenging activity was determined by the decrease in its absorbance induced by antioxidants. At 200-1000 µg, the antioxidant activities of the aqueous extract of *S.torvum* immature fruits ranged from 25.85% to 49.43%, and for developing shoots from 20.84% to 39.83% and the standard BHT were 61.53% and 27.03 - 89.04%, respectively (Table -1, Figure- 1). Aqueous extract of *S.torvum* immature fruits show higher antioxidant activity when compared to the developing shoot extracts.

Concentration (µg/ml)	BHT	Immature Fruit	Developing Shoot
200	61.53 ± 0.01	25.85 ± 0.01	20.84 ± 0.02
400	67.62 ± 0.01	31.73 ± 0.02	22.72 ± 0.01
600	74.87 ± 0.02	39.82 ± 0.02	29.17 ± 0.01
800	82.57 ± 0.01	42.51 ± 0.01	35.71 ± 0.01
1000	89.04 ± 0.02	49.43 ± 0.01	39.83 ± 0.01

Values are Mean ± SD of three (n = 3) independent analysis of the extract

Values expressed as % radical scavenging activity

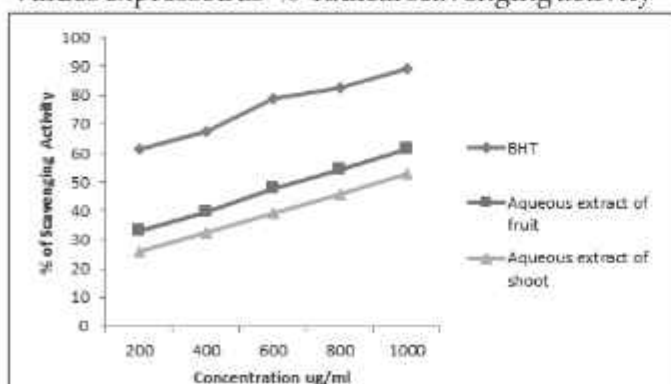


Fig. 1: DPPH radical scavenging activity of *Solanum torvum* aqueous extract of immature fruit and developing shoot

Superoxide anion radical scavenging activity

At 200-1000 µg, the superoxide radical scavenging activity of aqueous extracts of *S.torvum* immature fruits was varied from 31.41 % - 52.27% and that of the standard ascorbic acid was 57.29 % - 95.70 %. Radical scavenging activity of aqueous extracts of *S.torvum* developing shoots was 25.15 % at 200 µg concentration of the extract and at 1000 µg concentration of the extract it was found to be 41.73% (Table -2; Figure- 2). From 400 µg to 1000 µg superoxide anion radical scavenging activity was higher in the aqueous extracts of fruits.

Concentration (µg/ml)	Ascorbic acid	Immature Fruit	Developing Shoot
200	57.29 ± 0.12	31.41 ± 0.01	25.15 ± 0.01
400	67.83 ± 0.05	36.35 ± 0.01	32.61 ± 0.01
600	78.32 ± 0.01	44.73 ± 0.02	39.45 ± 0.03
800	85.92 ± 0.02	47.57 ± 0.06	40.45 ± 0.02
1000	95.76 ± 0.02	52.27 ± 0.01	41.73 ± 0.02

Values are Mean ± SD of three (n = 3) independent analysis of the extract

Values expressed as % radical scavenging activity

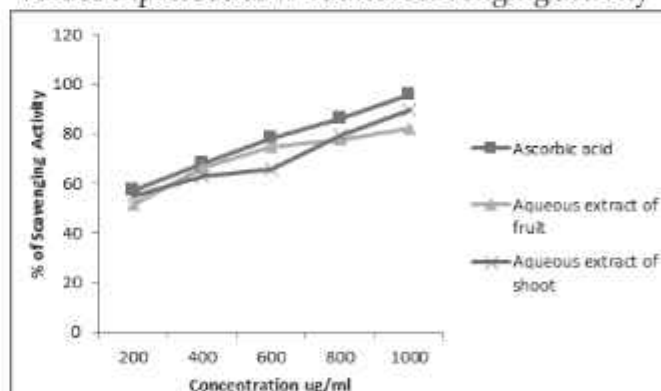


Fig. 2: Superoxide anion radical scavenging activity of *Solanum torvum* aqueous extract of immature fruit and developing shoot

NO₂ radical scavenging activity

The nitric oxide scavenging activity of aqueous extracts of *S.torvum* immature fruits was minimal at

29.57% at 200 µg concentration of the extract, whereas the maximum activity was 55.12% at 1000 µg concentration of the extract. *In vitro* nitric oxide scavenging activity of aqueous extracts of *S.torvum* developing shoots was 19.38% at 200 µg concentration of the extract and 41.21 % at 1000 µg concentration of the extract (Table -3; Figure- 3). The percentage antioxidant potential was higher in the aqueous extracts of *S.torvum* immature fruits when it was compared with the developing shoots.

Concentration (µg/ml)	Ascorbic acid	Immature Fruit	Developing Shoot
200	22.56 ± 0.02	29.57 ± 0.01	19.38 ± 0.01
400	34.41 ± 0.01	39.51 ± 0.05	25.19 ± 0.01
600	43.02 ± 0.01	44.41 ± 0.01	29.81 ± 0.01
800	54.84 ± 0.01	47.02 ± 0.05	35.51 ± 0.01
1000	66.56 ± 0.13	55.12 ± 0.01	41.21 ± 0.01

Values are Mean ± SD of three (n = 3) independent analysis of the extract; Values expressed as % radical scavenging activity

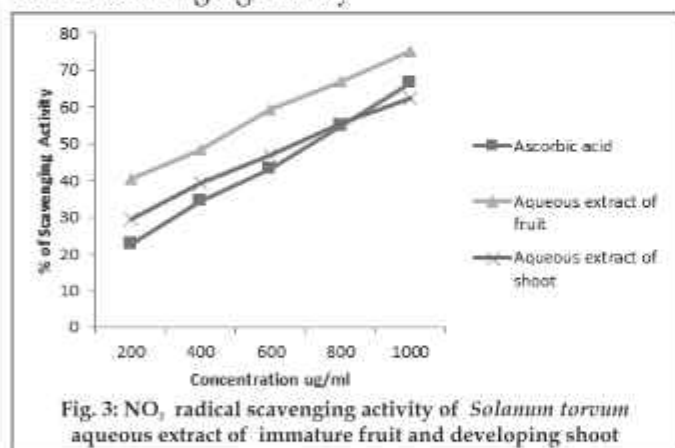


Fig. 3: NO₂ radical scavenging activity of *Solanum torvum* aqueous extract of immature fruit and developing shoot

Discussion

The results obtained from the present study indicates that the aqueous extracts of immature fruit and developing shoot of *S.torvum* possess antioxidant properties and could serve as free radical inhibitors or scavengers, or act as primary antioxidants (Kannan. et al., 2012; Abdul Aziz et al., 2016) DPPH is widely used to evaluate the free radical scavenging capacity (Abdul kadir et al.,

2015). The aqueous extracts of *S.torvum* immature fruit and developing shoot exhibited concentration-dependent radical scavenging activity. The antioxidant potential of aqueous extract and BHT as reference solution was evaluated for its ability to quench the synthetic DPPH radical in the present study.

The antioxidant potential increases with an increase in the concentration of the extracts. Dose-dependent DPPH scavenging activity of was reported. The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity (Fitriansyah et al., 2018). Earlier studies show hydro-alcoholic extract of *Desmodium gangeticum* have the proton-donating ability and can serve as free radical inhibitors or scavenger, acting possibly as primary antioxidant (Usha and Suriyavathana, 2012). The reaction of DPPH with aqueous extracts of *S.torvum* immature fruit and developing shoot was slower when compared to BHT.

The antioxidant potential of aqueous extract of *S.torvum* immature fruit and developing shoot was analysed for the extracts potential to scavenge superoxide anions. The aqueous extract of *Solanum torvum* immature fruit and developing shoot exhibited concentration-dependent radical scavenging activity, which show an increase in percentage inhibition with an increase in concentration, being compared with the standard ascorbic acid.

Superoxide anions is one of the potent reactive oxygen species which are produced from molecular oxygen. They are involved in the formation of hydrogen peroxide, hydroxyl radical and singlet oxygen (Hemmani and Parihar, 1998). These compounds induce oxidative damage to biomolecules such as lipids, protein and nucleic acids associated with cellular structures of the living organisms (Nishimiki et al., 2002). Results of this study clearly indicates that aqueous extract of *S.torvum* immature fruit and developing shoot is a potent scavenger of superoxide radicals.

The present study show an increase in percentage inhibition of free radical scavenging activity of NO₂ with increasing concentration of the aqueous extract of *S.torvum* immature fruit and developing shoot. The activity of synthetic antioxidant ascorbic acid was more pronounced than that of the extract which indicates the dose-dependent antioxidant potential of nitric oxide.

Nitric oxide is another free radical produced in mammalian cellular system involved in regulation of various physiological processes. Several degenerative diseases are associated with its increased production (Nathan,1992). The development of drugs to target and prevent its overproduction is gaining importance for treating various chronic inflammatory diseases (Shen et al., 2002). In the present study, the aqueous extract of *S.torvum* was assessed for its inhibitory effect on nitric oxide production. Nitric oxide radical generation was inhibited by immature fruit and developing shoot aqueous extract of *S.torvum*. The percentage inhibition of nitric oxide by the immature fruit extracts of *S.torvum* seems to be more predominant in the study.

Antioxidant activity of the alkaloids in plant *S mauritianum* has been reported against hydrogen peroxide induced oxidative damage in human erythrocytes (Jayakumar. et al ., 2016b). Flavonoid compounds which contain hydroxyl functional groups, are responsible for the antioxidant effect in plants (Kalita et al.,2012) Phytochemical compounds present in the aqueous extract of *S.torvum* immature fruit and developing shoot are likely to contribute to the antioxidant potential of aqueous extract (Gandhiappan and Rengasamy 2012; Thenmozhi, and Mahadeva Rao, 2012).

Conclusion

Plants are considered to be promising source of medicine in the traditional healthcare system. The present study indicates the antioxidant potential of aqueous extracts of unripe immature fruit and developing shoots of *S.torvum* which paves way to

test whether it serves as inhibitors, scavengers or act as primary antioxidants in prevention of free radical formation.

Acknowledgement

The authors are grateful to **Tamilnadu State Council For Higher Education (TANSCHÉ)**, Department of Higher Education, Government of Tamilnadu, for the grant of financial assistance under the Teacher's Minor Research Project Scheme during the year 2017-2018.

References

1. Abdul Aziz R, Abdul Kadir, Nashriyah Mat, Md M. Hasan and Md S. Jahan, 2016 *In vitro* antioxidant activity of the ethanolic extract from fruit, stem and leaf of *Solanum torvum*. *Sci Asia*; 42:184-189
2. Abdul kadir A R, D D Zawawi, A K Yunusa and M S Jahan 2015 *In vitro* anti-oxidant potential, total phenolic content and total flavonoid content of methanolic flower and seed extract of miracle tree (*Moringa Oleifera* Lam.). *Aust J Basic Appl Sci*, 9: 27-31
3. Clarke G, K N, Ting, C Wiart and J Fry 2013. High correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. *Antioxidants*; 2: 1-10.
4. Darkwah W K, M B Adinortey, A Weremfo, F K Abrokwah and E Afriyie, 2018 Total phenolic, flavonoid and alkaloid contents, oxidative DNA damage protective and antioxidant properties of methanol and aqueous extracts of *Dissotis rotundifolia* whole plant. *Free Radicals Antioxidants*; 8(2): 55-59
5. Fitriansyah SN, D L Aulifa, Y Febriani and E Sapitri. 2018 Correlation of total phenolic, flavonoid and carotenoid content of *Phyllanthus emblica* extract from bandung with DPPH scavenging activities. *Pharmacog J*, 10(3): 447-452

6. Gandhiappan J and R Rengasamy 2012 Comparative study on antioxidant activity of different species of Solanaceae family. *Adv Appl Sci Res*; 3:1538-1544.
7. Garret DC.1964 The quantitative analysis of drugs, In: Champman and Hall, Japan.1; 3: 456 - 458
8. Hemmani T and Parihar MS 1998 Reactive oxygen species and oxidative DNA damage. *Ind. J. Physiol. Pharmacol*; 42: 440-444.
9. Jayakumar K, VGM Krishnan and K Murugan, 2016a Evaluation of antioxidant and antihemolytic activities of purified caulophyllumine-A from *Solanum mauritianum*. *J Pharmacogn. Phytochem*; 5(2):195-199.
10. Jayakumar K, S S Sumayya and K Murugan. 2016b Evaluation of mitigating effect of purified solasodine from *Solanum mauritianum* against hydrogen peroxide induced oxidative damage in human erythrocytes. *Inter J Appl Biol Pharma Tech*; 2: 1-9
11. Joseph Sakah Kaunda and Ying-Jun Zhang, 2019 The Genus *Solanum*: An Ethnopharmacological, Phytochemical and Biological Properties Review. *Nat Prod Bioprospect.*, 9(2):77-137.
12. Kalita S, G Kumar, L Karthik and K V Rao. 2012 *In vitro* antioxidant and DNA damage inhibition activity of aqueous extract of *Lantana camara* (Verbenaceae) leaves. *Asian Pac J Trop Biomed*; 2(3):1675-1679
13. Kannan M, B. Dheeba S. Gurudevi and A.J A Ranjit Singh 2012 Phytochemical, antibacterial and antioxidant studies on medicinal plant *Solanum torvum* *J Pharma Res*; 5(5): 2418-2421
14. Nathan C.1992 Nitric oxide as a secretory product of mammalian cells. *FASEB J*, 6:305 - 3064.
15. Nishimiki M, N A Rao and K Yagi, 2002 The antioxidant activity, reducing power, superoxide anion radical scavenging. *J Ethnopharm*; 79: 325-332.
16. Shen SC, W R Lee, H Y Lin, H C Huang, Ch Ko and L L Yang 2002 *In vitro* and *in vivo* inhibitory activities of rutin, wogonin, and quercetin on lipopolysaccharide - induced nitric oxide and prostaglandin E(2) production. *Eur J Pharmacol*; 446 :187-194.
17. Singh R P, K N Chidambara Murthy and G K Jayaprakash, 2002 Studies on antioxidant activity of pomegranate peel and seed extracts using in vitro models. *J Agric Food Chem*; 50: 86-89
18. Thenmozhi, A. and U S Mahadeva Rao 2012 Comparative free radical scavenging potentials of different parts of *Solanum torvum*. *Free Rad. Antioxid*; 2: 24-29.
19. Usha V and M Suriyavathana 2012 Free radical scavenging activity of ethanolic extracts of *Desmodium gangeticum* *J Acute Med*; 2: 36-42



Government of Maharashtra **Ismail Yusuf College**
of Arts, Science and Commerce Jogeshwari (East), Mumbai -60.