

#### KISHINCHAND CHELLARAM COLLEGE

Vidyasagar Principal K.M. Kundnani Chowk, D.W. Road, Churchgate, Mumbai-20.





**VOLUME XIII - XIV** 

2016-19

FROM
LAB TO
LIFE

# THE SCIENCE OF TODAY IS THE TECHNOLOGYOF TOMORROW.

-- EDWARD VELLER--

#### From the Principals' Desk

'A good scientist is a person in whom the childhood quality of perennial curiosity lingers on. Once he gets an answer, he has other questions.'

~ Frederick Seitz, physicist



Questioning is key to development and progress. As a scientist, but also as an informed citizen of the nation, it is important for us to keep our curiosity alive. The mind of a curious person is 'active'. It is this crucial factor that enables the generation of new knowledge, discoveries and the development of solutions to various problems plaguing the world.

It gives me immense joy to present to you, yet another volume of 'Jigyaasa', a research magazine and an undergraduate Research endeavour of KCSHP'ians. Jigyaasa-volume XIV encapsulates the enriching journey of the Science Honours Programme Batch XIII and Batch XIV students who, over a period of eighteen months have conducted and presented their research. This is a unique opportunity for these curious undergraduate students to make an early entry into the research arena.

Jigyasa is important for excelling in any job and doing it better, because one keeps asking questions, learning from others, and looking for ways to do their job better. At K C College Jigyaasa is an important ingredient of the process of learning at every stage of our students journey.

I am grateful to the research scholars, research guides and members of the Nonteaching Staff involved in Jigyaasa for their dedication and untiring efforts in ensuring that this Science Honours Programme 'Jigyaasa' has run successfully for the past 15 years. I applaud the efforts of all the students, whose research work has been compiled in the form of informative reports, research articles and research papers in this volume. I wish all my students and teachers a life full of humility, gratitude, intellectual curiosity, and the drive to never stop learning. Choose curiosity over fear to live a fulfilled life. All the very best in your future endeavours, Team Jigyaasa!

Dr. Hemlata K. Bagla
Principal

### From the Conveners' Desk

#### Let us reach for the stars with our feet firmly rooted on the ground...

With every new batch of 'Jigyaasa- Science Honors Program', there are innumerable opportunities to interact with the young and inquisitive minds of our selected 'Jigyaasus'. With this unique undergraduate research program, KC College started a new precedence of nurturing young minds of undergraduate Science



classes, from 'Lab to Life', fifteen years ago. Since then, each year of Jigyaasa-SHP has been a joy ride for the faculty and students, having fun while they learn, much more than the prescribed science in their syllabus. The Shpians once enrolled, embark on a journey of science and self discovery. They learn to think beyond their own subject, work as a team, look at a problem with a scientific magnifying glass and try to find a solution to their research question.

The work presented during the Intercollegiate Research College Meet, gets published in this inhouse research magazine called 'Jigyaasa'. It gives me a great pleasure to present this volume of batch XIII and XIV, which contains research work completed by SHP students of both these batches, along with module reports that outline their eighteen months long journey of accomplishments. What makes us as mentors, more happy is a fact that, many of these projects try to address issues that are of social relevance. Because we at KC, have set a goal of 'Reaching the Unreachable' as our next mandate, under the DBT Star Status, granted to KC College during this year, by Department of Biotechnology (DBT), Govt of India. We stand committed to this goal through all our endeavors conducted by Shpians.

As a Convener of this program, I wish to express my gratitude towards our Principal, Dr. Hemlata K.Bagla for her guidance, Vice Principals Dr. Shalini Sinha and Prof. Smarjeet Padhi for their able support and partnership with CPCA that offers better opportunities to science students, to explore the world beyond science. I also thank the Registrar and office staff, non teaching staff for their cooperation in all our activities. I take this opportunity to say a big 'Thank You' to teachers and students from Computer Science Department for working tirelessly to bring out this great volume of Jigyaasa, year after year. Last but not the least, I wish to thank the Coordinator Dr. Sheela Valecha, all the faculty members and students, who are part of the KC SHP family, for keeping up the tradition of making each of the SHP activity, better than the best....

Wishing all readers, a Happy 'Jigyaasa' time!!!

Sagarika Damle Convener- 'Jigyaasa'- SHPKCC

### From the Co-Ordinator's Desk

# Success is achieved and maintained by those who try and keep trying.....

It is a matter of joy for me to express my sentiments towards the achievements of our Team Jigyaasa, started during the Golden Jubilee year of K.C. College (2004-05). Jigyaasa, Science Honors Program, an undergraduate endeavor attained the status of Best Innovative Practice by UGC in 2007.



The motto of SHP is to nurture young minds from Lab to life. Apart from the research projects, the young shpians are exposed to an array of sessions like soft skills of communication, Finance Gym to understand the money matters Gender sensitivity, Ethical Hacking or Cyber safety, Interactive sessions, Music and spirituality, Visual communications, Photoshop, CV writing, Critical Thinking, ICT tools in research, Poster and ppt making ,Environment awareness, Mangrove Walk, Dawat-e-shp the cooking delicious and nutritious healthy food, Research Methodology and Research paper writing.

The culmination of this 18 months program is the most popular "Felicitation Ceremony" where the successful candidates are awarded the certificates of merit. Over the years we have had many distinguished personalities such as

Dr. Vijay Khole (Vice Chancellor)

Dr. Naresh Chandra(Pro VC)

Dr. Maduri Pejawar(Dean-faculty of science Mumbai University)

Mr. Rakesh Gupta (MD Konark group)

Mr. Anil Chopra CEO, MD Hindustan lever (Lakme) and many more from Academia and Industry who have graced our SHP felicitation ceremony in the past. These stalwarts have applauded the efforts put in by K.C College in offering this unique opportunity to undergraduates, to have an early start in research arenas

The highlight of the ceremony is the release of research volume JIGYAASA. Thanks to the student volunteers and teachers of Computer science dept. The research papers published in this magazine, are the outcome of training received by the students from stalwarts in research and their mentors throughout their SHP journey. We as Team Jigyaasa re extremely grateful for the constant motivation and guidance given to us by our pragmatic Principle. Dr. Hemlata Bagla, in all our endeavors, we are also thankful to the vice principals Mr. Smarjeet Padhi and Dr Shalini Sinha for their support. A big heartfelt "Thank you." to all the teacher mentors for their active participation that has helped us in continuing this program for last fifteen years i also thank the non-teaching staff and office staff for their assistance. We are very proud of each and every participant of batch XIII and BATCH XIV for making this program a memorable one.

**DR Sheela Valecha** Co-ordinator SHP Jigyaasa

# **EDITORIAL**

# "Research is formalised curiosity. It is poking and prying with a purpose"

#### - Zora Neale Hurston

Jigyaasa Volume XIII-XIV is the compilation of adventures explored by the SHP Batch XIII-XIV students, and their research papers that exhibit their research work driven by passion, curiosity and guidance of their skilled mentors.. Undergraduate research encourages students to see themselves as invested in their academic community, which may be beneficial to forge a career in academia or can contribute to an individual's personal reinvention.

Jigyaasa is an attempt to raise the standard of our manuscripts at par with other academic journals. It covers the journey of the SHP Batch XIII and XIV students in the different sections. These include research papers written under the guidance of mentors, an illustrious account of the National and International publications earned by the members of Team Jigyaasa till date. It portrays the adventures of the students through the different modules, workshops, field trips, assignments and more. It reflects the lessons learned, the experience gained, the camaraderie and the spirit of being a community of young researchers.

We would like to to express our deep regards and gratitude towards Principal, Principal, Vice Principal, Chief mentor and the Convener, for their support and guidance in releasing this volume. We greatly appreciate the time and effort that all the students, teaching and the non-teaching staff members of each of the departments who have so willingly come forward to help. Thanks to team Jigyaasa in making it turn into a beautiful Research Journal that will ensure its journey to gain recognition in the years to come.

Wishing all the readers a Happy and astounding Reading...

# SHP BATCH XIII

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# LIFE SCIENCES

"Life Science research can be done on multiple platforms, Since we have very small number of people flying into space, the more people you have, the better"

LAUREL CLARK

# EFFECT OF COMMERCIALLY AVAILABLE FOOD COLOUR ON THE DEVELOPMENT OF CHICK EMBRYO

#### Bhurat Daksha, Ramesh Anuradha, Porbanderwala Huzaifa, Aashu Vajpai\* and Rai Shalini\*

\*Author for correspondence:

Department of Life Sciences, K.C.College, Churchgate, Mumbai 400 020.

Email:shalini.rai@kccollege.edu.in Email:ashvajpai23@gmail.com

#### ABSTRACT

The analysis of toxicants in commercially available food colours is an upcoming area of research. It is believed to have adverse effects on the health of humans and animals alike due to increased consumption and biomagnification. This study involves checking the effects that synthetic food colours have on the developing chick embryo, the choice of this model organism was unanimous as it shared considerable sequence homology with human beings in the early stages of development. The effects, positive or negative, that these commercially available food colours had on various morphological characteristics and biochemical parameters like protein content and total lipid content was the highlight of this study.

One of the major challenges was to establish a stable culture after the infusion of synthetic food colours. This proved to be quite daunting hence in order to reduce the mortality rate a pilot experiment of culturing shell less embryos was carried out which progressed to become a separate experiment in itself simultaneously. Shell less cultures were found to be useful in studying the various developmental stages of the developing embryo and various other developmental processes including organogenesis. Hence it was plausible to correlate the differences in development with the experimental shelled cultures or control shelled cultures.

Key Words: Food colour, chick embryo, toxicants, shell less cu

#### INTRODUCTION

The link between colour and taste is logical. A lot of natural foodstuffs are altered by the addition of food colour and various synthetic processes that eventually earn them a label called "processed food". These synthetic additions usually have harmful consequences. High concentrations of synthetic food colour

present in processed food usually cause abnormalities. The study of these harmful repercussions that prolonged consumption of synthetic food colours have on human health is gaining an impetus in recent times. The model organism used for this comparative study is the chick embryo. It is primarily preferred over all the other organisms as it is easy to access and observe and is therefore an attractive model to study developmental processes. In the early stages of development, chick embryo morphology is very similar to human, both are amniotes and their development is very similar. Also the chicken and human genomes share considerable homology. Experiments in chicken, which examine the function of have helped elucidate genes. underlying mechanisms of human genetic diseases and provide a basis for testing novel therapies.

Synthetic food colours often have hazardous chemicals or toxicants whose effects might not manifest at reduced concentrations but might have adverse consequences due to repeated consumption and increased retention of these toxicants. Effects of the popular food additive benzoate on neural development was studied in chick embryo. Neural tube obstruction was detected on microscopic examination in one embryo in one of the groups to which 20mg/kg of sodium benzoate was administered. (16). Effects of Monosodium glutamate on chick embryo development was observed after the treated eggs were infused with 0.1ml of MSG solution (0.75 MSGmg/gm, egg weight) in the air chamber before incubation. MSG is a natural excitatory neurotransmitter in the brain. The artificial form of MSG is used as a food additive and flavouring agent. Prolonged and increased consumption has found to cause intense bleeding, retardation of growth, subcutaneous bleeding, abnormal hernia and other congenital malformations in an average 10 day old embryo. (11)

Though shelled cultures form an ideal experimental set up to observe various malformations and developmental changes during the course multiple ministrations of chemicals, both toxic and non toxic, at the end of their development they don't quite allow one to observe the changes taking place during the course of development.

Shell less cultures on the other hand enable one to observe the normal development of the embryo outside the egg shell thereby providing an insight on how development proceeds in a egg.

Previous studies in establishing a shell less culture have been carried out for a variety of reasons. Shell less cultures act as an alternative in vitro model to study various induced malformations and to study the normal development that takes place within a shelled culture. Experimental studies on animal models play essential roles in the development of preventive, diagnostic and therapeutic procedures for diseases in a wide spectrum of fields (7)

Shell less chick embryos at the selected stages of development were treated with varying concentration of glucose in saline in order to observe the tolerance of the embryo towards increasing external supplementation. This aided in correlating the various glucose induced malformations observed to the effects it might have on larger mammals or humans on increased or repeated consumption.(21)

Culturing of embryos outside the shell is referred to by students as "chicks-in-plastic," "chick-in-a-cup," or "the omelette lab," allows contiguous observation of living chick embryos from day 3 to day 18 of incubation. Differentiation of organ systems and extra-embryonic structures as

well as pre hatching behaviour can be studied by students from grade school to college. (20)

A shell less chick embryo culturing technique reproduced under local circumstances achieved the goal of demonstrating shell less chick embryo culturing as a potential experimental model in the field of developmental anatomy. The embryos were found to survive for a period of 15 days after which their survival rates vary. The most advanced age reached in the project was day 19 of embryonic life. (9)

This field of research is gaining an impetus in recent times as the mass production commercially available food colours, especially a few that are not approved by the FDA is on the rise. This often causes the addition of various toxicants under the pretext of improving the shelf life, taste of flavour of a particular food item. This study aims at studying the effects that synthetic food colours have on the developing chick embryo and the main objectives of this study were to study the effect that synthetic food colours have on the developing chick embryo for a period of six days and maintenance of shell less cultures in order to study their survival rate.

In the past various model organisms have been used to study effects of teratogenic substances. The embryonic zebra fish was used to study the effects of environment toxicants on its behaviour (14). Human relevant sugar levels of added sugar consumption have shown to increase female mortality and lower the rate of male fitness in mice (12). These experiments suggest that model organisms

are manipulative, easy to work with and the effects of various toxicants and other nutritional supplementations can be correlated with the adverse effects they might have on humans.

#### MATERIALS AND METHODOLOGY

# Procurement and Acclimatization: Fertilized eggs which were a few hours old were bought from Aarey dairy farm, Goregaon. The eggs were then allowed to acclimatize at 40 degrees for a period of 24 hours. Thus the age of the eggs at the beginning of establishment of shell less cultures and infusion of synthetic food colours was approximately 32-36 hours.

The shell-less culture being a novel way of growing an embryo in the absence of the confined space containing all the essentials that an eggshell provides was cumbersome initially, involving reduced rates of survival and a generous amount contamination. Over time the survival rate of the shell less cultures improved along with a reduction in the amount of contamination.

#### Dosage of synthetic food colour

The dosage administered to the shelled cultures was 200 microlitres of both raspberry red and green food colour infused in 10ml of avian saline, which was then autoclaved in order to maintain sterility. 50 microlitres from this was administered on a daily basis over a period of four days to various batches of shelled cultures. The experimental sets of embryos were then compared with the control set in terms of growth rate, survival rate, morphological characters, total lipid content and protein content.

The morphological parameters which involved assessing the growth rate and developmental changes in the embryo were carried out manually whilst comparing it with the standard Hamburger Hamilton stages. Estimation of the total lipid content and protein content of shelled cultures was done with the help of Stern Shapiro method and Lowry's method respectively.

# Incubation and infusion of chick embryo

The synthetic food colour prepared in saline was administered by the pin hole method to the shelled cultures wherein post infusion the hole was sealed using sellotape in order to prevent contamination. The eggs were then incubated for a period of 24 hours at 40 degrees. This process spanned four days after which the eggs were broken on the 5th day and the contents were emptied into a sterile glass bowl with utmost caution. Post which the control and experimental sets were compared along with the standard Hamburger and Hamilton stages in order to assess the changes in development, morphological various biochemical assays were also carried out in order to assess the intricacies in the changes that might have been brought about by the infusion of synthetic food colour in the total lipid content and protein content of the shelled culture.

A batch of shell less cultures were also infused with green food colour but further analysis and experimental procedures were stalled due to the high mortality rate.

Sterilized glassware like glass bowls, petri plates, pipettes, micro pipette- tips were used to maintain shell less cultures and to infuse synthetic food colours into shelled cultures. These experiments were mandatorily carried out in a sterile environment which is the Animal Tissue Culture Lab under stringent measures in order to increase viability and reduce contamination.

#### Morphology

Once the shelled cultures were opened in a sterile glass bowl the embryo was carefully cut around the edges using a sharp forcep and a brush to wipe away the extra albumin and yolk stuck onto it. It was then scooped up using a spatula and gently transferred onto a clean slide and then onto the stage of a dissection microscope. The embryos were then analysed in order to correlate the developmental changes observed.

#### **Biochemical Assays**

#### Stern Shapiro Method (1952)

This assay is typical for estimation of total lipid content. Lipids form a major part of the yolk that supplements the nutrition of the embryo up until it hatches hence it was imperative to check the positive or negative effects it has on the uptake of the same and hence the development and survival rate of the embryo.

#### Folin Lowry (1951)

This is the most widely used assay for protein estimation. It is preferred over Biuret because it is sensitive to concentrations below 5 micrograms per millilitre and hence more accurate. It is necessary to quantitatively discern the

protein concentration as plausible correlation could be made with the toxicant concentration present in synthetic food colour.

#### **Graphical Representation**

There were two sets of shelled cultures each that were infused with commercially available food colour raspberry red and green obtained from a company called TAMCO. The varying values for absorbance that was obtained for protein and total lipid content for each of the sets was plotted using a bar graph. The deviations from the values of the control set were then correlated and discussed accordingly.

#### RESULTS

#### **Biometric Analysis**

Shelled Cultures: The Shelled Cultures infused with red and green food colours were seen to show a little stunted growth in size as compared to the control. The embryos infused with red food colour showed incomplete formation of flexure and it was disrupted easily as compared to green infused embryo and control in all sets. So it was inferred that the Red Infused embryos showed abnormality during the growth and also the structures were developing inefficiently.



Fig 1: Batch 1 of Shelled Culture Infused: Control (left), Red Infused (centre) with distinct loss of flexion, Green Infused (right). [Approx 6 days on comparing to HH Stages 27-29].

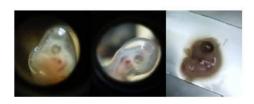


Fig 2: Batch 2 of Shelled Culture Infused: Control (left), Red Infused (centre) showing Stunted growth in brain development, Green Infused (right) [Approx 6 days on comparing to HH Stages 27-29].

Shell Less Culture: The Shell-less Culture was successfully established and was seen to show at par growth when compared to the Shelled Control through a period of six days. The survival rate was found to be vary in different sets. Initially the survival rate in the first few cultures was found to be 40% which gradually increased to a 60% survival rate.

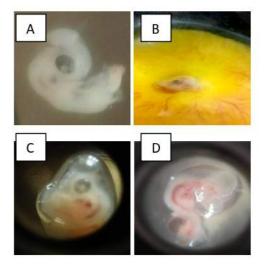


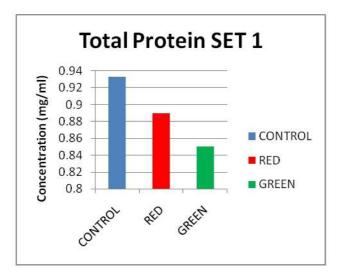
Fig 3:Shell-less Culture compared to Control: A&C:Control (left) approx 6 days B&D:Shell-less culture embryo approx 5.5-6 days.

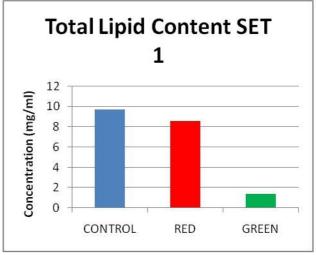
#### **Biochemical Analysis**

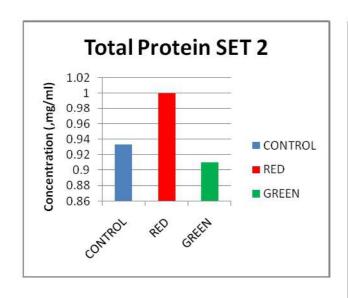
**Protein Estimation** was done from the albumen collected from each embryo broken 4 days after infusion. The absorbance was recorded and the

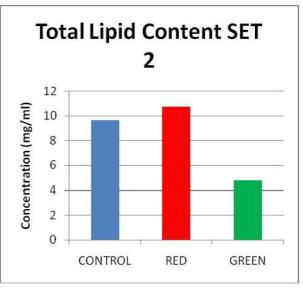
concentrations of the corresponding experimental value were found and plotted as a mean ( 0.772 & 0.863 experimental red) and (0.748 & 0.788 for experimental green). The Protein Concentrations found from the assay showed that there was a major deviation in the protein content in the experimental red (0.89 mg/ml)embryo & 1.0 mg/ml). Whereas the Green infused embryo showed slight deviation in the two sets.

results observed were known to deviate greatly in the experimental green of both sets (1.4 mg/ml & 4.79 mg/ml) with the Control (12.82 mg/ml). The experimental red was seen to show concentration of 8.56 mg/ml and 10.74 mg/ml. The inference from this assay was that lipid content showed a marginal deviation in the Green Infused embryos, as compared to the Control (9.645 mg/ml).









**Total Lipid Content** of the yolk that was extracted from the embryo when the eggs were broken 4 days after infusion. The

**DISCUSSIONS** 

Food Colours: As we all know that the synthetic food colours are harmful effects on all sorts of organisms. This study was basically our view of extrapolating the know facts at our level. There are specific ingredients in the synthetic dye which we have chosen and on which probably not much of the experiments are carried out. Like the tartrazine, brilliant blue, ponceau 4R and carmosine.

There are individual studies carried out on these chemicals but no morphological study could be found during our referencing.

Shell-less Cultures: In our study, we developed a shell less culture system for the chick embryos using a glass petri plate and a glass bowl (sterile). We choose this method since it does not require any complicated operations, special materials techniques. Also the use of a glass petri plate does offer a comparatively easy observation of the embryonic morphology from almost all angles as well as it is also easy to access the embryo for manipulations by removing the lid.

We transferred the embryos to the shell- less system after day 1 of preincubation period. There are certain papers which mention that transferring the embryo after 54h-55h is considered to be optimum in terms of its viability, but since our culture was supposed to last only for a period of 6 days, we decided to transfer it on the 2<sup>nd</sup> day itself.

Also, one of the reason for our retarded growth of the embryo could be the calcium content which was not provided in our experiments, but which the embryo would get from its shell (normal eggshell).

Another reason for the less viability or survival rate of the shell-less system would be that the aeration of the artificial vessel would have caused loss of moisture of the embryo via transpiration.

Kamihira et al. (1998) also pointed out the importance of oxygen supply in the later stage of embryo culture using surrogate eggshell. Hence, we had to maintain the oxygen content by rotating the petri plate at regular intervals.

The first 4-5 days of incubation in chicks correspond to 2-5 weeks of post conceptional age in human embryos. The shell-less culture system reported here, therefore, may also be used to help improve understanding of the pathophysiology in humans, although the technique has its own limitations.

#### CONCLUSION

- Speaking of infused shelled experimental sets, the experimental red set showed retarded growth in terms of morphological factors when compared with the experimental green set and the control set of shelled culture.
- For biochemical aspects, the protein content of both the experimental green and red sets (shelled) when compared to the control shelled culture did not show much deviation.
- The total lipid content of the experimental red set (shelled) was

found to be in range with the control shelled set, whereas, the experimental green set (shelled) showed exceedingly large deviation with respect to the control shelled set.

- Coming to shell less culture, we were successfully able to make it survive for a period of 6 days. It could have survived for a longer duration but due to lack of an ethical certificate, we had to stop it at the 6<sup>th</sup> day.
- The growth rate of the shell less culture when compared with the growth rate of the shelled culture showed retarded growth and development in terms of lens formation and the length and width of the embryo.
- The shell less culture when infused with the green synthetic food colour resulted in the blood island lysis.

Knowing the effects of the several ingredients of the synthetic food colour which we have used here on the human body from previous studies and literature review we could conclude that possibly the different ingredients would be the reason for difference in the observations. Like the presence of ingredients like Brilliant blue (known to cause refractory shock and muscle acidosis in humans) or tartrazine (known to cause DNA damage and cancer in humans) in the commercially available green food colour, could be responsible for the blood island lysis in the shell less culture and also for the deviation seen in the total lipid content analysis. And the ingredients like carmosine (known to cause breathing difficulties and cancer in humans) or ponceau 4R (known to cause ADHD, allergic responses and cancer in humans) present in the commercially available red food colour, could be responsible for the retarded growth in of morphological Creating a natural looking environment is a difficult task in itself and hence we faced a lot of challenges for establishing the shell less culture. The initial survival rate of the shell less culture was less than 40% but later by overcoming several challenges we were able to bring it up till 60%. The survival rate can still be increased making the exact replica of the withinenvironment. The retarded growth seen in the shell less culture could be because of the disturbances offered to the embryo during the transfer or rotation, either in terms of the environmental conditions or the manual errors.

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#### EXPRESSION OF STRESS RESISTANT GENE -HVA22 IN S.CEREVESIAE.

Heenal J. Panchal, Dr. Archana Thite\* and Dr. Suvarna Sharma\*

Department of Life Sciences, K.C. College, D.N. Road, Churchgate, Mumbai- 400020 India

#### Introduction:

- Aloe vera is a succulent plant which can withstand abiotic conditions like drought and extreme temperatures. It belongs to family Liliceae. It is a sturdy plant that with-stands abiotic stress such as extreme temperature and drought conditions. Drought and high salinity are the most important environmental factors that cause osmotic stress and dramatically limit plant growth and crop productivity (Boyer, 1982). Aloe is known to possess tolerance to cold stress and a DREB1-Drought Resistant Ethylene Binding Gene 1 has been isolated from Aloe vera and characterized (Yang-Meng Wang, 2007). Aloe has heat resistant genes hsp70, hsp100 and ubiquitin genes (Huerta C Freire M, 2012). Homologue of HVA22 is found in aloe vera.
- HVA22 -a highly inducible gene for Abscisic acid and environmental stress in Barley. Its yeast homolog, Yop1p, functions in vesicular trafficking and in the endoplasmic reticulum (ER) network in vivo.
  - The yeast Saccharomyces cerevisiae is the most sophisticated eukaryotic model for recombinant DNA technology. One of the main reasons is that the transmission genetics of yeast is well understood. In yeast, another important advantage is the availability of a circular 6.3-

- kb natural yeast plasmid. This plasmid, which has a circumference of 2 µm, known as the "2-micron" plasmid. It forms the basis for several sophisticated cloning vectors. This yeast plasmid is transmitted to the cellular products of meiosis and mitosis.
- Features such as a life cycle with isomorphic haploid and diploid phases, the formation of tetrads in meiosis, a short generation time, and the ability to grow in defined media forms the fundamentals of yeast methodology.

Considering the above characteristics, yeast is selected for learning about the expression of Yeast homolog gene HVA22 by carrying out yeast transformation.

#### Methods and Requirements

Lithium acetate method for yeast transformation

The lithium method is developed by Ito et al and was published in 1983.

- Lithium acetate is used to permeabilize the cell wall of yeast for use in DNA transformation
- One of the important findings was that monovalent cations such as Na+, K+, Cs+ and particularly, Li+ enhances the transformation efficiency of intact S. cerevisiae cells. It was found that incubation of intact cells with polyethylene

- glycol (PEG) and plasmid DNA is essential for transformation and short-term incubation of intact cells with PEG and plasmid DNA at 42°C (heat shock) enhances the transformation efficiency and transformation of the cells is most effective at the mid-log phase.
- Schiestl and Gietz increased the efficiency of the alkali cation protocol to 100 000 transformants / µg plasmid DNA by using singlestranded carrier DNA in the transformation mixture.

#### Requirements

Culture of Microorganisms - Yeast strains of Saccharomyces cerevisiae,

Plasmid DNA (HVA22 homolog from Aloe Vera)

**Equipments & apparatus -** Incubator, micropipettes, Test tubes, agar plates, etc.

Chemicals & Media - Lithium acetate, Tris-HCl ,EDTA,

Polyethylene glycol, Dimethylsulphoxide, YPD media

, Sabouraud's broth, Salmon sperm DNA.

- YPD media composition:

   For 1 L media,

   10gm yeast extract, 20gm peptone,
   20 gm dextrose, 1000ml distilled water.
  - Sabouraud's broth: o In mg/ L

40 gm dextrose, 10gm peptone, 15 gm agar, 1000ml distilled water

PERIOD OF STUDY	ACHIEVABLE TARGETS
Term 1 :- Oct 2015 – Feb 2016 :	Referencing.
(5 months)	
Term 2 :- Mar. 2016 – Sep. 2016:	
(6 months)	Tinding and the antiquel angula and distance for lab areas to the in-
	Finding out the optimal growth conditions for lab yeast strain.  Ordering for chemicals.
Term 3 :- Sept 2016 – Jan 2017:	Carrying out yeast transformation.
(5 months)	

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# STATISTICS

"A single death is a tragedy; A million deaths is a statistic"

JOSEPH STALIN

#### IMPACT OF IMPORTS & EXPORTS ON THE ECONOMY OF INDIA.

Yahya Sait ,Tanya Sharma, Mrs. Shailaja Rane\*
Department of Statistics, , K.C. College, D.W. Road, Churchgate, Mumbai 400020

#### ABSTRACT

"Export growth is important because of its effect on internal trade and economic stability. Even more, the rate of economic growth and the distribution of income and wealth in a country are closely related to export growth."

In a Developing country like ours, the role of Exports as well as Imports plays a major role on the development and Growth on the Economy of the nation.

The purpose of the following study is to study in detail the various aspects in Exports & Imports in India and how India has achieved Growth through it.

#### INTRODUCTION

For the past few years there has been a call for "Make In India" by the current government of our country. a new national program designed to transform India into a global manufacturing hub. It contains a raft of proposals designed to urge companies — local and foreign — to invest in India and make the country a manufacturing powerhouse.

As Narendra Modi in his speech very greatly said -

"My country's youth can resolve ... he would manufacture at least one such item (which is imported) so that we need not import the same in future. We should even advance to a situation wherein we are able to export such items. If each one of our millions of youngsters resolves to manufacture at least one such item, India can become a net exporter of goods,"

Exports of a country play an important role in the economy. A healthy balance, a sustainable development with trade and foreign exchange reserves to maintain the country's export growth should be a constant and high rate. Exports as a whole affect the industrial environment. To compete internationally, the industry standard for quality products, competitive price, good packaging, etc, which is important for overall industry. For export as a national priority for government and private sectors recognized by all agencies to export, export growth is to maintain high rates.

Taking this into consideration this study tries to examine the current state of India's Import and Export system and how it has changed over the years and also predict what it would be in the coming years. Hence the following parameters will be discussed and analysed throughout our study as we try to find ways to strengthen each aspect.

#### **OBJECTIVES AND AIMS**

 To find the Correlation between Imports and Exports and to Find the type of relation (linear or nonlinear).

- Study individual industries for Imports and Exports (Industrial I & E, Agricultural I & E)
- 3. Study the data for the Imports & Exports of the country Before and After the year 1991.
- 4. Measure the Growth in the economy & the different aspects in which Growth has taken place over the years (GDP etc)
- 5. Study Imports & Exports for both Air Freight as well as Sea Freight.
- Find ways through which Exports can be increased and Imports can be Decreased.
- 7. Identify the goods manufactured in India which has the maximum potential to Boost Exports.

# RESEARCH DESIGN & METHODOLOGY

Overview
Population and Study Sample
Sources of Data
Collection of Data
Data Analysis

- 1. First we identify and list the parameters on which we are going to be collecting and analysing data.
- Our second step would be to data collection and compilation. (EXIM BANK)
- 3. The compiled data should be thoroughly analysed and discussed.
- 4. By using method of sampling and various other statistical methods the data should be calculated and equation should be formed.
- 5. Explain through various graphical techniques the relation as well as growth parameters for the Imports and Exports of India
- 6. State the Conclusion and what the research has tried to achieve.

#### LITERATURE RIEVIEW

Some studies have claimed that there is no relation between exports and GDP or their growth rates. It can be expected that exports do not lead to growth; rather, economic growth (whether due to technological innovation, fiscal or monetary stimulus or other reasons) could lead to exports or the two may not be related (see, for example, Bhagwati 1978, 1988). Thus, we see that, analytically, these results are possible:

- export growth causes output (or GDP) growth;
- 2. output growth causes export growth;
- there is no long-run relation or causality between exports growth and output growth.

#### DISCUSSIONS AND CONCLUSION

Growth of an economy is directly related to exports. If exports increase at a faster pace as compared to imports, nothing can stop an economy from being a developed one. On the other hand, the instability in exports can adversely affects the process of economic development.

Since India is a fast developing country we predict that Imports of india is decreasing over the Years and Exports are increasing. Hence greater exports lead to a more competitive, technologically mature, productive and rapidly growing economy.

The period after 1991 has been marked by a substantial transformation in trade policy and the policy of trade is tilted towards export promotion and import

substitution/restriction to achieve higher growth rate as one of the objectives of existing trade policy. Therefore, through the present endeavour, we try to establish a causal relationship among these variables vis-à-vis growth rate, exports and imports.

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#### IMPACT OF SOCIAL MEDIA ON YOUTH OF MUMBAI

**Sonali Shah, Sanskriti Kapoor,** Mrs. Shailja Rane\*, Dr. R. B. Muley\* Statistics Department, K.C. College, D.W. Road, Churchgate, Mumbai 400020

#### Introduction

Social media is a platform to interact with people all over the world through texting, sharing images, audio-video sharing etc. It is one of the cheapest and fastest modes of communication on individual as well as mass level. It is something that is major part of all age group people. Its use is increasing day by day especially in the youth of the world. Social media has a great influence on the youth. This led us to think how much social media is governing decision of the generation and its positive and negative impacts on their lives. Here in this study Youth of Mumbai city is focused.

#### Statement of the Problem

Major issue today is how social media is becoming an essential need of the majority which in turn is influencing their lifestyle and opinion like political awareness, religion, trends, etc.

#### Relevance of the Study

This study will throw light on how social media has become an integral part of our lives and how one use that in their advantage and avoid damage caused by social media.

#### **Review of Literature**

There has been studies on social media. Social media are increasingly visible in higher education settings as instructors look to technology to mediate and enhance their instruction as well as promote active learning for students. Many scholars argue for the purposeful integration of social media as an educational tool. Empirical evidence, however, has lagged in supporting the claim.

#### Objectives of the study

- 1. To analyze the influence of social media on life of Mumbai youth.
- 2. To evaluate the purpose youth of Mumbai uses social media for.
- 3. To find the most preferred form of social media.
- 4. To assess the time spent on social media.
- 5. To analyze the dependency on social media
- To recommend some proper use of social media so one can benefit from it without being dependent.
- 7. To suggest how to channelize the use of Social media to our benefit.

#### Hypothesis

- It is likely to say that social media is major source of quick information and entertainment.
- It is likely to say that social media one of the easiest, quickest and most creative source of communication.
- 3. It is likely to say that youth is spending significant amount of their time on social media.
- 4. It is likely to say that social media is an necessary evil.
- Methodology

The descriptive method will be used to carry this study and a survey type research will be conducted through the questionnaire public opinion. Other statistical tools will be used to collect, distribute and analyze the study. Classifying as follows:

#### Conclusion

It is possible that social media will soon become an integral part of education and would consume most of our time.

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# BIOTECHNOLOGY

"Our world is built on biology and once we begin to understand it, then it becomes a technology"

RYAN BETHENCOURT

## STUDY OF ORAL MICROFLORA AND QUANTIFICATION OF SALIVARY BIOMAR KERS IN SMOKELESS TOBACCO CONSUMERS

Manali Kadu, Ajay Narayanan, Pranita Rane, Dr. Anupma Harshal\*, Ms. Nandini Desai\* Department of Biotechnology, K.C. College, D.N. Road, Churchgate, Mumbai- 400020, India

#### **ABSTRACT:**

**Objectives:** 1. Isolation and characterization of selected microbes from oral fluids of smokeless tobacco consumers.

2. Quantification of salivary biomarkers from saliva of consumers and non-consumers.

**Methods:** In a random population of 18 subjects, salivary biomarkers such as protein, malondialdehyde, thiocyanate and nitrite were assayed. Microbial analysis and Antibiotic Sensitivity Testing was also carried out on simple, selective and differential media for saliva samples.

**Results:** These results were tabulated and are analyzed by regression analysis. We report, an unusual ba cterial strain that was observed and further characterized. The Antibiotic Sensitivity Testing (AST) profile of the same is reported, it was found to be a plasmid bearing strain showing  $\beta$ -lactamase and Vancom yein resistance.

**Conclusion:** A significant increase in the levels of salivary biomarkers was observed in some saliva sam ple of SMT consumers as compared to non-consumers and hence can be used as effective biomarkers. U nderstanding the changes in the levels of these biomarkers and oral microbiota can help in preventing ch ronic diseases in their early stages.

**KEYWORDS:** Salivary cancer biomarkers, Smokeless Tobacco (SMT) consumers, Antibiotic Sensitivity Testing (AST)

**ABBREVATIONS:** SMT Consumers- Smokeless Tobacco Consumers AST- Antibiotic Sensitivity Testing

#### INTRODUCTION:

Betel quid chewing is an ancien t practice common in many countries of Asi a which was introduced by Europeans in 160 0s. India is next to China in both tobacco pro duction and consumption in the world. (Bod du Naveen- Kumar, 2016). Tobacco in India is used in various forms: chewing *pan* (mixt ure of lime, pieces of areca nut, tobacco and spices wrapped in betel leaf), chewing *gutkh a* or *pan masala* (scented tobacco mixed with lime and areca nut, in powder form), and *mishri* (a kind of toothpaste used for rubbing on gums) etc. Most of these products are

held in the mouth, cheek, or lip or chewed to allow absorption of nicotine in the lining of oral cavity. Tobacco contains variety of har mful chemicals and nitrogen compounds. Al ong with cancer, consumption of such produ cts may lead to other serious health problem s. Tobacco intake increases generation of fre e radicals and reactive species as well as so me constituents that can cause inflammation.

(S. Preethi, 2016). Thiocyanate, a metabol ic product of certain cyanide containing com pounds of tobacco has ability to cause chang es in epithelial cells. As saliva is in continuo us contact with the epithelial cells, it can be

used as a quantitative measure pertaining to tobacco exposure. Hence, Saliva can be a va luable source for periodontal diagnosis as it contains tobacco specific biomarkers.

Saliva is the first line of defence against oxidative stress, microorganisms. (F awaz Pullishery, 2015). Normal functions of saliva are critical for the maintenance of ora I health. The early diagnosis is key to the tre atment of any disease. (L. F. Mollashahi et a 1., 2016). Oral fluids provide an easily availa ble non-invasive method for the diagnosis of a wide range of diseases and clinical situati ons. The substances released due to chewin g of the product also disturb the normal oral microflora, certain molecules can also stimul ate or enhance the growth of pathogenic mic robes and saliva itself a nutrition rich mediu m which further enhances the growth. Hence , the study was undertaken to quantify certai n salivary biomarkers and to isolate and char acterize altered oral microflora from the sali va of smokeless tobacco consumers.

#### MATERIALS AND METHODS:

The study was carried out in the cit y of Mumbai between September 2016 and August 2017. A total of 18 people including three controls ranging from the age group of 20-60 years participated in the study.

A verbal consent was taken from the subjects after giving them the required information and answering any queries. Iml of saliva was collected in sterile centrifuge tubes which was the diluted with sterile distilled water in the ratio of 1:5.

The collected samples were then te sted for the presence of certain tobacco speci fic salivary biomarkers like thiocyanate, prot ein, nitrite and malondialdehyde.

Potassium thiocynate (concentration :  $0.4 \times 10^{-4}$  to  $2 \times ^{10-4}$  M) were used as standar ds and 0.2M ferrous nitrite was used as the working reagent. Sodium nitrite (Concentrat ion: 20 to  $100 \mu$ M) was used as standard for

estimation of nitrite. The reagent used was G reiss reagent prepared using 1% sulphanilam ide in 5% phosphoric acid and 0.1% naphthy lethylene diamine dihydrochloride (NED). P rotein estimation was carried out using Folin Lowry method using bovine serum albumin as standards. Malondialdehyde estimation i nvolved the use of thiobarbituric acid-trichlo roaceticacid reagent (TBA-TCA).

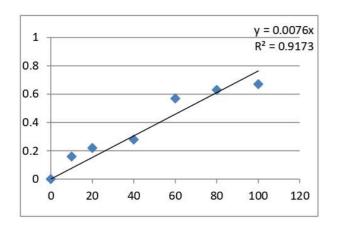
The saliva samples were analysed o n different media by streaking on sterile Nut rient Agar, Sabouraoud's Agar, Mac Conkey 's Agar and Veillonella Agar. The bacterial colonies observed on the sterile media plates were characterised by Hans Christian Gram 's staining method and observed under 100x oil immersion of the light microscope. The f ungal colonies were analysed by fungal stain ing using lactophenol cotton blue stain and o bserved under 45x of the microscope. The c olony characteristics were studied and noted down. Colonies showing unusual characteris tics were further analysed by other staining methods and biochemical tests. The resistan ce pattern of the organisms was studied and t hose showing resistance were further studied by determining the presence of a plasmid by extraction using cold alkaline lysis method f ollowed by Agarose Gel Electrophoresis (A GE).

#### **RESULT:**

The quantitative analysis of the unknown samples for protein, thiocyanate an d nitrite was done by correlation and regress ion analysis. The maximum value for nitrite, thiocyanate and protein for smokeless tobac co consumers was determined to be  $105.71\mu$  M,  $3.27\times10^{-4}$ M and  $120\times10^{-2}$  mg/ml respect ively. Malondialdehyde concentrations were determined using the extinction co efficient  $1.56\times10-5$  /M cm and the maximum value observed was  $4.3\times10^{-6}$ M. There was a significant increase in the concentration of these b

iomarkers for few samples when compared t o the control i.e. the non-consumers.

#### **Protein Estimation**

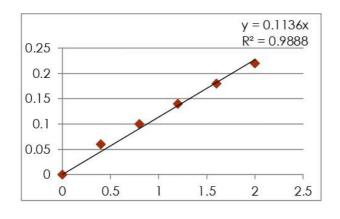


X AXIS: Thiocyanate concentration(M×10<sup>-4</sup>) Y AXIS: OD at 447nm

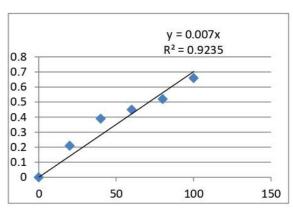
X AXIS: Protein concentration (mg/ml)

Y AXIS: OD at 660 nm

#### **Thiocyanate Estimation**



#### Nitrite Estimation



X AXIS: Nitrite concentration (mg/ml

Y AXIS: OD at 545 nm

#### **Concentrations of Biomarkers**

SAMPLE	CONCENTRATION	CONCENTRATION	CONCENTRATION	
	OF PROTEIN	OF	OF NITRITE	CONCENTRATION
	(mg/ml)	MALONDIALDEHYDE	(μg/ml)	OF
	\$6 (CONS.)	(M::10 C)	300000000000000000000000000000000000000	THIOCYANATE
		(M×10-6)		(M×10-4)
S1	32.85	1.4	97.14	0.88
S2	51.42	2.3	65.71	0.70
S3	32.85	1.4	25.71	0.79
S4	24.28	1.0	38.57	0.53
S5	81.35	0.8	18.57	1.41
S6	97.71	0.2	0.00	0.26
S7	120	0.19	0.00	1.59
S8	75.71	2.4	55.71	0.79
S9	84.28	2.6	90.00	2.83
S10	82.85	0.3	105.71	0.88
S11	95.45	4.3	40.00	3.27
S12	48.57	4.3	47.14	0.35
S13	105.71	0.7	28.57	0.97
S14	57.14	0.7	85.71	0.97
S15	48.57	1.2	10.00	0.70
C1	41.42	0.3	30.00	0.79
C2	20	0.12	4.28	0.26
C3	20	0.51	10.00	0.61

Key: S- Sample, C- Conrol

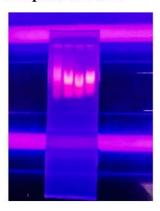
Microbiological analysis of the oral cavity r evealed the presence of Gram negative bacill i in one of the samples. Further biochemical tests were employed to identify the organism which was found to belong to the *Klebsiella spp*. Antibiotic sensitivity test of the same s howed resistance towards beta lactam drugs and glycopeptides (Vancomycin). The plasm id extracted from the organism displayed the presence of a supercoiled DNA when subjected to Agarose Gel Electrophoresis (AGE).

SR. NO.	TESTS	OBSERVED RESULTS	
1.	Capsule	Positive	
2.	Catalase	Positive	
3.	Indole Test	Negative	
4.	Methyl Red Test	Negative	
5.	Voges-Proskauer Test	Positive	
6. Citrate Utilization Test		Positive	
7.	Urease Test	Positive	
8.	Triple sugar Iron (TSI) Test	Yellow butt, red slant, gas production	

#### **Results for Antibiotic Sensitivity Test:**

Antibiotic	Concentration (mcg/disc)	Zone of inhibition (mm)	Sensitivity
Ciprofloxacin (CIP)	5	25	Sensitive
Vancomycin (VA)	30	0	Resistant
Oxacillin (OX)	1	0	Resistant
Imipenem (IPM)	10	27	Sensitive
Ticarcillin (TI)	75	0	Resistant
Gentamycin (GEN)	10	17	Sensitive
Tetracyclin (TE)	30	22	Sensitive
Neomycin (Ne)	30	13	Intermediate

### Agarose gel showing presence of Supercoi led plasmid DNA



KEY- (from left to right) LANE 1: *E.coli* DH5α (control),

LANE2-4: Resistant plasmid

#### DISCUSSION:

Saliva is a complex fluid secreted by oral cavity which is composed of a wide vari ety of constituents. Non-invasive parameter and easy availability of saliva serves diagnos is purposes. From literature survey it was kn own that tobacco intake increases generation of free radicles like thiocynate (SCN) and ni tric oxide (NO) which will lead to nitrosation process and lichenoid reactions respectivel y and lipid peroxidation is induced by reactive oxygen species. Thiocynate, nitric oxide and malondialdehyde are considered to be a nideal salivary biomarkers.

The study was conducted among random smokeless tobacco consumers li ke taxi drivers, support staff and construction workers by collection of their saliva. A significant increase was observed in the levels of salivary thiocynate, nitric oxide and malondialdehyde of smokeless tobacco consumers when compared to non-consumers. Elevated levels of biomarkers may be responsible for excessive cancer risk and disorders.

Oral health is influenced by oral micro flora. In our study, we reported difference in

oral flora of smokeless tobacco consumers a nd non-consumers. Gram negative rods are u nusual isolates found in normal flora of oral cavity, suggesting the presence of host defen ce mechanism. Change in the oral microbio me may reduce immunity. The Gram negati ve (rods) isolates from Veillonella agar sho wing resistance to glycopeptide (vancomyci n) was reported which showed resistance against beta-lactum drugs on further antibiotic sensitivity testing which was plasmid mediat ed. Hereby, understanding the alteration in the levels of biomarkers and oral microbiome will aid in prognostic diagnosis.

#### CONCLUSION:

Tobacco addiction in large number of adults develops during adolescence. In co untries with the high prevalence of smokeles s tobacco use, their prices are lowered, warn ing labels are weak and surveillance is not ef ficiently mediated. Health organization and government should therefore launch progra ms for creating awareness among tobacco co nsumers. Elevated values of thiocynate, prot eins, malondialdehyde and nitrite helps to di fferentiate between smokeless tobacco consu mers and non-consumers and therefore can b e used as effective biomarkers. The organis ms showing unusual characteristics should b e effectively analysed to study the effects on the oral cavity. The results obtained in our st udy highlights the alteration in the condition s of the oral cavity of SMT consumers when compared to non-consumers.

#### ACKNOWLEDGEMENT:

We, the students of Science Honors Program (S.H.P.), Biotechnology Department, K.C. Coll ege, would like to express our heartfelt gratitude to all the people who have given their support in making this project a magnificent experience. We express our deepest sense of gratitude to our Principal Madam, Dr. Hemlata Bagla, who has been the greatest source of inspiration This project wo

uldn't have been possible unless our S.H.P. Co-ordinator Dr. Sagarika Damle would have backed us throughout the entire course. Also, sincere thanks to the Department of Biotechnology. This disquisition could have not been possible without our mentor, Dr. Anupma Harshal and co-mentor Ms. Nandini Desai. We would like to convey our gratefulness to her for her exemplary guidance, monitoring and constant encouragement throug hout this project and also for motivating us at each and every step.

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#### EXTRACTION OF NATURAL DYES & ITS APPLICATIONS

**Bushra Khan, Ramya Sindhyan**, Anushi Divan\*, Sejal Rathod\*
Department of Biotechnology, K.C. College, D.N. Road, Churchgate, Mumbai- 400020, India .Jai Hind College, Churchgate, Mumbai, Maharashtra 400020, India.

#### ABSTRACT

There is a worldwide demand for natural dyes due to increased awareness on their therapeutic properties. Several artificial dyes are harmful and may be carcinogens. The current research aimed at extracting dyes of different colors from natural plant sources, carrying out its characterization & its applications. The dyes were tested for their antimicrobial ability and were found to be inhibitory to common organisms like *S. typhi, P. aeroginosa, C. diphtheriae* & *S. aureus*. Phytochemical tests were performed and they confirmed presence of several important metabolites like Proteins, Carbohydrates, Phenols, Tannins, Terpenoids, Flavonoids and many more. The dyes were tested for antioxidant activity by FRAP assay and antioxidant levels ranging from 0.62 to 2.00 were obtained. Preparation of cosmetics like lip balms, was done and dyes were also tested as natural food coloring agents. The results indicated that the dyes caused inhibition of wide range of bacteria and proved to be good sources of antioxidants. They departed a natural color to food items and hence can replace artificial food colorings. Thus the use of natural dyes should be promoted as they are cheap, safe and non-toxic to use.

**Key words:** natural dyes; antimicrobial; anti-oxidant; cosmetics; food coloring.

#### Introduction:

Dyes are natural or synthetic (artificial) substances used to add a color to or change the color of something. Artificial dyes have been used widely since its inception. They are used in everyday items like clothes, food products, cosmetics, plastics, etc. The harmful effects of these dyes, however, go unnoticed by the people. In the recent years, due to the scientific development, the adverse effects of these dyes are being discovered. They have been found to cause or contribute to various allergic reactions, toxicity, mental disorders like ADHD, tumor formations, etc. Recent studies show that seven of the artificial dyes contributed to cancer in lab animals, including brain and testicular tumors, colon cancer, and mutations.(4) These dyes not only pose a risk to the biotic components, but are also responsible for degradation of abiotic components like soil & water. The contamination of soils and waters by dye-containing effluents is of environmental concern. Water pollution can also result from manufacturing synthetic dyes when untreated dye effluent is dumped directly on bodies of water.

Dyes derived from natural materials such as plant leaves, roots, bark, insect secretions, and minerals were the only dyes available to mankind for the coloring of textiles & other products until the discovery of the first synthetic dye in 1856.(17) Rapid research strides in synthetic chemistry, supported by the industrialization led to the development of synthetic alternatives to

popular natural dyes and gradually pushed the natural dyes into oblivion.(17) However, environmental and health issues have once again revived interest in production & use of natural dyes. Natural dyes are considered eco-friendly as these are renewable and biodegradable; are skin friendly and may also provide health benefits to the wearer.(17) Natural dyes are harmonizing & soothing to the human eye.(18) Some of its constituents are anti-allergens, hence prove safe for skin contact and are mostly non-hazardous to human health.(18)

Beetroot (Beta vulgaris) is high in immune-boosting vitamins & minerals, it is also good for the bones, liver, kidneys, it reduces risk of heart disease & improves endurance and blood pressure.(3) The petals of Rose (Rosa) are beneficial to the skin & cure dry and patchy skin; they are also used in health tonics. Carrot (Daucus carota) is a rich source of vitamin A & helps in protecting eye health & vision. Marigold (Tagetes) has been used to make healing herbal ointments; it is a rich source of flavonoids and anti-oxidants. Spinach (Spinacia oleracea) is high in protein, zinc, fibre, vitamins, etc; its health benefits include skin care, eyesight, regulated blood pressure etc. Peppermint (Mentha piperita) soothes digestive issues, sore muscles, various respiratory illnesses, etc.(2) Henna (Lawsonia inermis) has the ability to relieve headaches, detoxification, skin protection, boost hair health, etc. Pomegranate peels(Punica granatum) are effective detoxifying agents, combat tool against heart diseases

in prevention of skin cancer, for coughs & sore throats, etc.(1)

Bioactive compounds in plants are known to be responsible for various bioactivities of the plant.(5) Phytochemical assays are often considered to be the first step for characterization of a plant sample depending on the presence of the various metabolites. A positive response of the plant sample for the presence of Phenols, Tannins, Terpenoids, Glycosides, etc indicates it potential use for defence against micro-organisms, since most of these metabolites impart the ability to inhibit the growth of organisms. Since natural dyes are obtained mostly from plant sources, they are a rich source of antioxidants, and thereby can replace the artificial antioxidant supplements available in the market today. The various advantages of natural dyes over artificial dyes help in promotion of use of these natural dyes in industries for textiles, foods & beverages, cosmetics, etc.

With regards to the increased scope of natural dyes, its study, production & usage; the present research is designed for the extraction of dyes from natural plant sources, its characterization & studying its applications.

#### Materials & Methods:

#### Selection & collection of plant materials-

8 different plant sources were chosen for extraction of dyes of 4 different colors. Beetroot & Rose for Red, Pomegranate peel & Henna for Brown, Spinach & Peppermint for Green, Carrot & Marigold for Orange.

#### Extraction of natural dyes-

Dyes were extracted using both water & organic solvents. Water-based dyes were prepared by blanching, grinding & filtering the plant material using Distilled water.(17)(18) These were further lyophilized for concentration. Organic solvents were used for extraction of the color-imparting phytochemical. Methanol was used for Beetroot, Rose, Marigold, Henna.(7)(8) Ethanol was used for Pom peel, while Petroleum ether was used for Carrot. Spinach & Peppermint were extracted using acetone.(9)(13)(14)

#### Qualitative phytochemical analysis-

The water-based dyes were used for phytochemical screening. Phytochemicals like Proetins, Glycosides, Terpenoids, Quinones, Coumarin & Saponins were analysed.(5) Phenols. Tannins, Carbohydrates, Flavonoids were also analysed.(6)(7)(10)

#### Antimicrobial ability-

The ability of the dyes to inhibit bacteria was tested using the Agar dilution method. 1g of concentrated dye mixed with 2ml 20% DMSO was incorporated into molten nutrient agar butts which were poured onto plates. 6 common pathogenic organisms, namely, *E. coli, S. typhi, P. aeroginosa, S. aureus, S. faecalis & C. diphtheriae.* were spot inoculated on the plates and incubated to check for results.(15)(16)

#### Anti-oxidant activity-

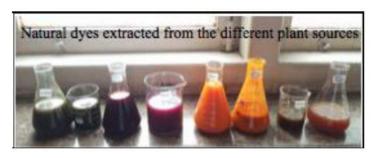
The ability of the dyes to scavenge free oxygen radicals was determined using the FRAP (Ferric Reducing Ability of Plasma) assay.(8)

#### Applications of the dyes-

- 1. Use as Food coloring agents- Noodles were colored by boiling them in water containing the dye for 5-10 minutes, while, doughs of white flour were prepared using dye mixed with water.
- 2. Cosmetic production (Lip-balm)- Natural ingredients like aloe gel, almond oil, beeswax, rice powder & cornstarch were mixed well. 5-6 drops of the dye was added and mixed until a good consistency was obtained. The prepared lip-balms were then stored at 4°C and RT.

#### Results:

The extracted dyes showed a good color imparting ability and were of the desirable concentration.

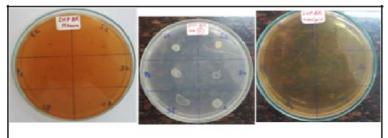


Phytochemical analysis revealed the presence of important metabolites like Proteins, Carbohydrates, Phenols, Tannins, Terpenoids, Glycosides. These are major metabolites present which are responsible for several additive properties of the plants. In the table given below, '+' and '-' indicates presence and absence of that phytochemical respectively.

Dyes Compound	Beetroot	Rose	Pom peel	Henna	Spinach	Pepper mint	Carrot	Marigold
Proteins	+	+	+	+	+	+	+	-
Carbohydrates	+	+	+	+	+	+	+	+
Flavonoids	-	+	+	•	+	+	+	+
Glycosides	+	+	+	+	+	+		+
Terpenoids	+	+	+	+		+	+	
Saponins	+	+	+	+	+	+	*	+
Phenols	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	-	+
Quinones	+	+	+	+	a	+	-	
Coumarin	+	+	+	•	-	-	7	+

The dyes were checked for their antimicrobial ability by the Agar Dilution method. Organisms like S. aureus, P. aeroginosa, were inhibited by all the dyes. While, S. typhi, E. coli, C. diphtheriae & S. faecalis were inhibited by most of the dyes. The following is a tabular representation of the antimicrobial ability of the dyes as observed, upon comparing with the controls.

Organism Dyes	E. coli	S. typhi	P. aeroginosa	S. aureus	S. faecalis	C. diphtheriae
Beetroot	**					
Rose	+			**	+	**
Pom peel	+	***	**	**	+	<del>(10</del> )
Henna			-	**		**:
Spinach	+			••	-	ā
Peppermint				**		
Carrot		+	+		+	+
Marigold	440					+
Media Control	+	+	+	+	+	+
DMS0 Control	+	+	+	+	+	+



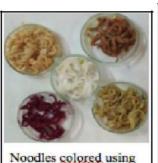
Inhibition showed by Henna dye(left) & Marigold dye(right) on comparison with Control(centre)

The anti-oxidant activity of the dyes was tested using FRAP assay & ascorbic acid as standard. Highest antioxidant activity was shown by Beetroot dye, followed by Henna, Rose, Carrot dye. Other dyes also showed a good anti-oxidant activity.

Dyes	FRAP value	2.05	S	tandard Asco	rbic acid gra	ph
Beetroot	2.00	2 -				_
Henna	1.99	1.95			A	
Rose	1.98	1.9 L		1		
Carrot	1.97	₩ 1.85 —	-			
Pom peel	1.94	1.8				
Spinach	1.86	1.75		,		
Marigold	0.90	1	50	100	200	300
Peppermint	0.62		Ç	one of ascorbi	e acid(ug/ml)	

The noodles & whilte flour dough that were prepared using the dyes retained a good color and no

considerable change in taste was imparted to the food products. The prepared lip-balms were found to be stable upon storage at RT & 4°C for upto 4 weeks. They had a good consistency and gave a vibrant color, Also, since the dyes were prepared using all natural ingredients, they gave a refreshing and soothing feel to the skin.







natural ingredients.

#### Discussion:

The dyes extracted from natural sources are rich sources of important bioactive compounds. Plants like Henna, Beetroot, Carrot, Spinach, etc are known to have therapeutic properties. The extracted dyes showed prominent and lucid colors.

Upon testing for presence of various phytochemicals, the dyes showed presence of important metabolites like Proteins, Tannins, Carbohydrates, Phenols, Glycosides and many more. These phytochemicals are known to have therapeutic properties like like protecting the cellular systems from oxidative damage, modulating immune system, preventing cancer and lowering the risk of chronic diseases like cardiovascular diseases, etc. The antimicrobial ability of the dyes was tested using the agar dilution method & the dyes showed inhibition to common pathogenic organisms like Staphylococcus aureus, which is a common skin infection causing bacterium; Pseudomonas aeroginosa, which is a frequent cause of various nosocomial infections. Organisms like Streptococcus faecalis, Salmonella typhi, Pseudomonas aeroginosa, and Corynebacterium diphtheria, that are the causatives of various afflictions were also inhibited by most of the dyes. Being extracted from natural plant sources, the dyes were found to be rich in anti-oxidants using the reduction based analysis-FRAP(Ferric Reducing Ability of the Plasma) assay. highest anti-oxidant level The spectrophotometrically was that in Beetroot Red dye (FRAP value-2.00). The other dyes obtained were also found to have a good anti-oxidant activity. The food products colored using the dyes showed no change in taste & retained a vibrant color. Also, the cosmetic (lipbalms) prepared using the dyes were

found to be stable upon storage & gave a refreshing and soothing feel to the skin.

Natural dyes are on the rise ever since the harmful effects of artificial dyes have begun to uncover. Presence of important metabolites and high anti-oxidant activity are an indication of the beneficial purposes these dyes will serve in food industries. The ability to inhibit commonly pathogenic organisms is proof of the medicinal & therapeutic properties of these natural dyes. Natural dyes can, thus, replace artificial dyes in the near future.

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#### PREDISPOSITION OF

# ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD) AMONG SECONDARY SCHOOL CHILDREN

**Mayuri Iyer, Samrin Shaikh,** Anushi Divan\*, Shailaja Rane\*, Dr. Sejal Rathod\* Department of Biotechnology, K.C. College, D.W. Road, Churchgate, Mumbai 400020

#### ABSTRACT

**Objective**: ADHD is a neurobehavioral disorder that develops during childhood and can lead to other mental health difficulties. This research survey aims at determining the number of secondary school children that are predisposed to ADHD and assessing the gender difference in the prevalence of ADHD while taking into account other factors like - locality in which the child lives, economic status, father and mothers occupation. The ultimate goal is to spread awareness about the prevalence of ADHD in the Indian population.

**Method**: 410 school children aged between 10-16 years were selected randomly from towns and suburbs of Mumbai and their parents were made to fill out a questionnaire containing 15 DSM-IV-TR questions.

**Result:** 40% of secondary school children showed symptoms highly consistent with ADHD out of which males were 46% and females were 35%.

**Conclusion**: Our study showed significant percentage of secondary school children predisposed to ADHD (40%) due to lack of awareness. Also males were more prone (46%) than females(35%) in all age groups considered (10-16yrs). 14-16yrs of age group was more predisposed to ADHD (50%).

**<u>KEYWORDS</u>**: ADHD, secondary school children, neurobehavioral disorder, DSM-IV-TR.

#### INTRODUCTION

ADHD is a neurological disorder that develops during childhood and can persist into adulthood. The "attention deficit" component of ADHD refers to inattention, or difficulty focusing for long periods and being easily distractible.

The "hyperactivity" portion of ADHD is used to describe behaviour that is restless,

agitated, and difficult to resist [1]. Symptoms of the disorder first become apparent in preschool years and persist into adulthood in approximately 60% of the cases. Attention deficit hyperactivity disorder (ADHD) is a complex disorder having multiple causes including genetics as impacted by one's environment. The condition is usually diagnosed in childhood, when difficulties arise during play and school, and it is marked by lack of concentration, short attention span, and physical restlessness [2][3]. ADHD often is blamed on bad parenting, or a "bad" attitude [4][5]. In ADHD patients, reductions in volume have been observed in total cerebral volume, the prefrontal cortex, the basal ganglia (striatum), the dorsal anterior cingulate cortex, the corpus callosum and the cerebellum [6]. Neuroimaging studies have also reported reduced white matter (WM) volumes, midsagittal corpus callosum (CC) areas, and cortical thickness in ADHD patients compared with controls [7][8]. Scientists are not sure what causes ADHD. Like many other illnesses, a number of factors can contribute to ADHD, such as:

#### **OBJECTIVES**

This study aims to:

 identify the percentage of secondary school children predisposed to ADHD.

- Genes
- Cigarette smoking, alcohol use, or drug use during pregnancy
- Exposure to environmental toxins during pregnancy
- Exposure to environmental toxins, such as high levels of lead, at a young age
- Low birth weight
- Brain injuries

#### SOCIAL RELEVANCE

ADHD influences the following :-

- Increase in drug abuse
- Increase in levels of depression
- Increase in anxiety disorder
- Increase in low self-esteem
- Increase in learning disabilities
- Increase in damaging of social & personal relationships
   Undiagnosed ADHD can lead to development of Learning disabilities,
   Tourette Syndrome, Oppositional defiant disorder, Conduct disorder, Anxiety and depression, Bipolar disorder.
  - identify the gender difference in the prevalence of ADHD.
  - compare the distribution of ADHD among different age groups.

 spread awareness about the prevalence of ADHD in the Indian population.

#### **METHODOLOGY**

This study aims to:

- identify the percentage of secondary school children predisposed to ADHD.
- identify the gender difference in the prevalence of ADHD.
- compare the distribution of ADHD among different age groups.
- spread awareness about the prevalence of ADHD in the Indian population.

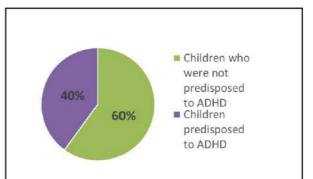
#### RESULTS AND DISCUSSIONS

This study reports results of 410 students within the age group of 10-16 yrs from various schools across Mumbai.

# 1) PREDISPOSITION OF ADHD AMONG SECONDARY SCHOOL CHILDREN

Amongst the 410 students 164 students were predisposed to ADHD and 246 students were not predisposed to ADHD. That is 40% of students were predisposed to ADHD.

At 95% confidence interval the percentage predisposition range is between 36%-44%. The percentage of students predisposed to ADHD is high.



<u>Figure 1:</u> Percentage predisposition of ADHD among secondary school children

# 2) PREDISPOSITION OF ADHD AMONG DIFFERENT AGE GROUPS

The above results suggest that 14-16yrs are more predisposed to ADHD than other age groups surveyed. The chi square test suggests that there is an association between predisposition of ADHD and age of the child. The Z test showed that there is a significant difference in the proportion of 10-13yrs and 14-16yrs students predisposed to ADHD. (The Z-Score is -2.3595. The p-value is 0.00914. The result is significant at p <0.05)

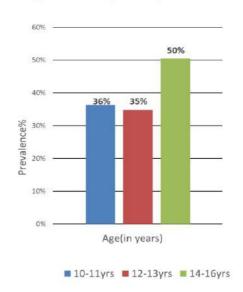
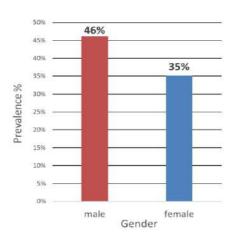


Figure 2: Percentage predisposition of ADHD among different age groups (10-16yrs).

### 3) PREDISPOSITION OF ADHD AMONG MALES AND FEMALES

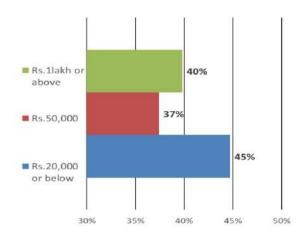
The above results suggest that males are more predisposed to ADHD than females. The chi square test suggests that there is an association between predisposition of ADHD and gender of the child. The Z test showed that there is a significant difference in the proportion of male and female students predisposed to ADHD. (The Z-Score is 2.2725. The p-value is 0.020137. The result is significant at p <0.05)



<u>Figure 3:</u> Prevalence of ADHD in males and females.

#### 4) ADHD AND SOCIO-ECONOMIC STATUS

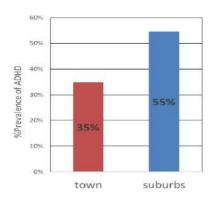
The chi square test suggests that there is no association between predisposition of ADHD in the child and the familys monthly income. (The p-value is 0.473459)



<u>Figure 4:</u> ADHD and socio-economic background.

#### 5) ADHD AND LOCALITY

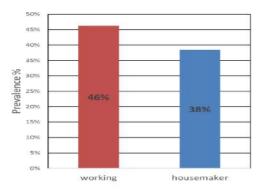
The above results suggest that children living in suburbs are more predisposed to ADHD than those in towns. The chi square test suggests that there is an association between predisposition of ADHD and the locality where the child lives. The Z test showed that there is a significant difference in the predisposition of ADHD in town and suburban children. (The Z-Score is -3.616. The p-value is 0.00015. The result is significant at p <0.05)



<u>Figure 5:</u> Relationship between ADHD and the locality in which the child lives.

# 6) ASSOCIATION OF ADHD WITH THE MOTHERS OCCUPATION

The chi square test suggests that there is no association between predisposition of ADHD in the child and the occupation of the childs mother. (The p-value is 0.207755)



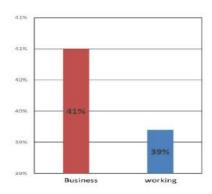
<u>Figure 6:</u> Association between ADHD and the occupation of the mother.

#### AWARENESS PROGRAMME

Due to the social stigma attached with mental health conditions in India, ADHD remains undiagnosed therefore only limited information is available regarding

# 7) ASSOCIATION OF ADHD WITH THE FATHERS OCCUPATION

The above results suggest that children whose fathers are in business are more predisposed to ADHD than those whose fathers are working. The chi square test suggests that there is an association between predisposition of ADHD and the fathers occupation. The Z test showed that there is a significant difference in the predisposition of ADHD among children whose fathers are working and those whose fathers are businessmen. (The Z-Score is -9.8231. The p-value is 0.020137. The result is significant at p <0.05)



<u>Figure 7</u>: Association between ADHD and the fathers occupation.

prevalence of ADHD in the India. Thus it becomes essential to undertake programmes to spread awareness about this disorder among the population, as it may lead to adverse effects and development of other mental health disorders. A seminar was conducted in R.B.K school, Mira Road, Mumbai to spread awareness about ADHD among school children and teachers.

#### **CONCLUSION**

Since the percentage Predisposition of ADHD is found to be significantly high (40%) more attention must be given towards spreading awareness about the prevalence of ADHD in secondary school students and abolishing the social stigma attached with mental health conditions in India. The factors influencing the predisposition of ADHD in children were age, gender, father's occupation, area of residence. Mother's occupation and the

monthly income does not affect predisposition of ADHD.

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#### ANTIMICROBIAL AND ENZYMATIC ACTIVITY OF ENDOPHYTIC FUNGI ISOLATED FROM LEAVES OF AVICENNIA MARINA AND CHARACTERIZATION OF MANGROVE SOIL

Prachi Pawar<sup>1</sup>, Saloni Samant<sup>1</sup>, Dr. Pratibha Shah<sup>2</sup>\*, Nandini Desai<sup>1</sup>\*

- 1. Department of Biotechnology, K.C. College, D.W. Road, Churchgate, Mumbai-400020
- 2. Department of Microbiology, K.C. College, D.W. Road, Churchgate, Mumbai-400020

#### **ABSTRACT**

Endophytic fungi from mangroves are increasingly recognized for production of various novel bioactive compounds and the soil properties influence the vegetation, species composition and structure of mangrove forest. In the present study, sampling of healthy leaves and rhizosphere soil of *Avicennia marina* was carried out. A fungal endophyte was isolated from the leaves and was found to be of the genera *Alternaria*. The endophyte showed strong antimicrobial activity against Gram positive bacteria, production of five extracellular enzymes was detected as well. The grayish-black, clay-loam nitrogen rich mangrove soil was found to be alkaline in nature and nutrient limited in potassium and phosphorous. Enzymatic and antimicrobial potential of the fungal endophyte offers a promising source of bioactive compounds. Also, the analysis of different properties of soil is important for mangrove establishment and conservation as they have become endangered. This study paves way to further developmental research as Mangroves act as a highly productive ecosystem of immense ecological value.

**Key Words:** Endophytic Fungi, Bioactive Compounds, *Avicennia marina*, *Alternaria*, Antimicrobial Potential

#### INTRODUCTION

Endophytes are micro-organisms that are present in the living tissues of various plants, establishing a mutual relationship without causing any symptoms of disease [1]. They have proven to be rich sources of natural compounds, showing variety pharmacological and biological activities [2]. There are many reports and studies on the biological activities of endophytes like anti microbial, anti neoplastic, antioxidant, antiviral, anti diabetic, immunosuppressive, anti thrombotic, anti-inflammatory, anti Alzheimer's activity etc. [3]. Most commonly isolated endophytes are fungi and bacteria, of which fungi form the dominant group. Recently fungal endophytes have been explored for diverse applications owing to their production of extracellular enzymes [4]. These enzymes are produced as a defense mechanism against pathogenic invasion and for obtaining nutrition from host [5]. Fungal enzymes have vast biotechnological

applications in agriculture, biomedical, food and beverage industries [6].

Association of endophytic fungi with mangrove plants is of prime importance. Mangrove forests act as very important tropical coastal tidal ecosystems and grow on nutrient-rich muddy substrates which are low in oxygen and undergo variations in salinity [7]. Mangrove plants have adapted to a unique habitat with muddy saline waters, anaerobic soil, brackish tidal activities and high microbial and faunal competition. Endophytic fungal association with mangrove plants confers protection from adverse environmental conditions and allows them to successfully compete with saprobic fungi decomposing senescent parts [8].

Mangrove ecosystems harbor diverse groups of microorganisms which perform an important role in nutrient recycling and regulate chemical environment of the ecosystem [9]. Various physical, chemical biological properties influence vegetation, species composition and structure of mangrove forest. Mangrove's soil condition is an essential factor for mangrove reforestation and coastal rehabilitation [10].

Thus considering the importance of mangrove endophytic fungi and the mangrove ecosystem as a whole, the current study focuses on the isolation and characterization of endophytic fungi and mangrove soil analysis.

#### MATERIALS AND METHODS

#### Sample Collection

Healthy leaves and rhizosphere soil sample of Avicennia marina were collected from Soonabai Pirojsha Godrej Marine Ecology Center, Mumbai, in month of September.

#### Isolation of endophytic fungi

The leaves were washed under running tap water and then cut into 1cm x 1cm pieces without midrib. Surface sterilization of leaves was carried out under aseptic conditions by agitating the leaf cuttings in sterile distilled water for 1 min, 70% ethanol for 1 min, 2.5% sodium hypochlorite for 4 min, 70% ethanol for 30 sec and washing with sterile distilled water thrice [11]. Leaf cuttings were then inoculated on Potato Dextrose Agar supplemented with 100µg/ml Streptomycin. The inoculated petri plates were sealed with parafilm and incubated at 27°C for 1 week in dark conditions.

#### Identification of the fungal endophyte

The isolated endophytic fungus was identified up to genus level based upon colony morphology, growth pattern and microscopic observation by Lacto Phenol Cotton Blue staining.

#### Preparation of fungal extract

Secondary metabolite extraction was carried out as described with minor modifications [12]. Endophytic fungal isolates were further inoculated in 100 ml Potato Dextrose Broth and incubated at room temperature for one week under stationary conditions with intermittent shaking for enrichment of the fungus. The broth culture was filtered to separate the mycelia and filtrate. The filtrate was then transferred into separating funnel with equal volume of ethyl acetate. After mixing it well for 10 min, the filtrate was kept still for 5 min till two clear immiscible

layers were formed. The upper layer of ethyl acetate containing the extracted compounds was separated using separating funnel. The extract was concentrated by removing the solvent using rotary evaporator.

#### Antimicrobial Assay

Antimicrobial potency of the fungal metabolites was examined by Agar Cup Diffusion method against five test micro organisms. Sterile Muller Hinton agar was bulk seeded with 0.3 ml of test culture. Wells were carved in the agar using a cork borer and 200µl of the fungal extract was added into the wells. The plates were incubated at 37°C for 24 hours and the zone of inhibition was measured and compared with DMSO as control.

#### **Enzymatic Activity**

of the Preliminary screening fungal endophyte for six extracellular enzymes was carried out by spot inoculation on solid media with specific substrates for respective enzymes- Starch agar for Amylase, Cellulose Congo red agar for Cellulase, Tributyrin agar for Lipase, McCartney's agar for Pectinase, Fraziers agar for Protease and Christensen's Urea agar for Urease. The plates were incubated for 48 hours. After incubation, Starch agar and Fraziers agar were flooded with 1% iodine and 1% HgCl respectively. All the plates were examined for zone of inhibition.

#### Soil Analysis

Characterization of mangrove soil was carried out with respect to physical, chemical and biological properties.

- Pyhsical Parameters- Color of the soil was determined by visual observation and soil texture was analyzed by sieve method.
- Chemical Parameters- Determination of pH and Electrical Conductance of the soil were performed using pH meter and conductometer respectively. Macronutrients like Nitrogen, Phosphorus and Potassium were quantified [13].
- Biological Parameters- Nitrogen Fixing bacteria were isolated using Ashby's mannitol broth and Ashby's mannitol agar.

#### RESULTS AND DISCUSSION

Endophytic was isolated fungus by inoculating surface sterilized leaf cuttings on Potato Dextrose Agar which showed fungal growth around its surface indicating the presence of fungal endophyte. The isolated endophyte was subjected to Lacto Phenol Cotton Blue Staining and was observed microscopically at 400x magnification. Based on the morphology and growth pattern the endophyte was identified as Alternaria. Alternaria is an important fungus due to its different life style from saprophytes to endophytes which depends upon the host susceptibility. It acts as an important endosymbiont which can stimulate host defence mechanism against various pathogens



Fig. 1: Fungal endophyte on leaf cutting

The fungal ethyl acetate extract was checked for its antimicrobial activity against five test organisms. Maximum antimicrobial action was observed against the Gram positive test organisms- *Staphylococcus aureus* and *Corynebacterium diphtheriae*. The extract

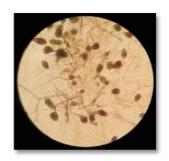


Fig. 2: Endophytic fungus at 400X

showed some inhibition against Gram negative bacteria *Escherichia coli* and fungal strain *Candida albicans* as well but no activity was observed against *Salmonella typhi*.

MICRO	Zone of Inhibition(mm)			
ORGANISMS	Control (DMSO)	Fungal Extract		
S. aureus	0	25		
C. diphthariae	0	18		
E. coli	13	16		
S. typhi	12	12		
C. albicans	17	19		

Fig. 3: Table showing results of antimicrobial activity.

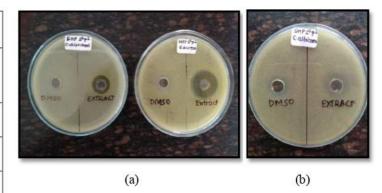




Fig 4: Antimicrobial activity against (a) S. aureus & C. diphthariae, (b) C. albicans, (c) E. coli & S. Typhi

Production of fungal enzymes was detected using various selective media and the endophyte showed presence of enzymes Amylase, Lipase, Protease, Pectinase, Urease.

However, Cellulase was absent since no decolourization was observed around the colony.

OBSERVATION
+
+
+
+
+

KEY: + = Positive

- = Negative

Fig. 5: Table showing results of enzymatic characterization.

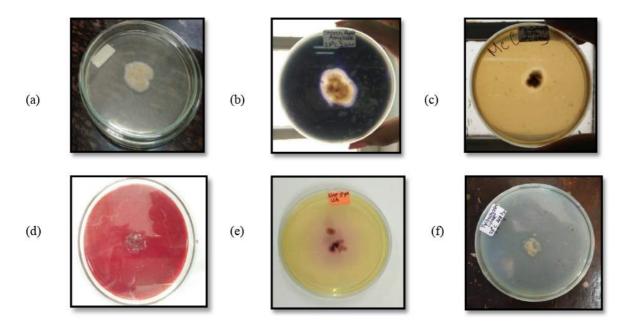


Fig. 6: (a) Protease activity, (b) Amylase activity, (c) Pectinase activity, (d) No Cellulase activity, (e) Urease activity, (f) Lipase activity

The greyish clay loam mangrove soil was found to be alkaline in nature and showed presence of a melanin producing free living nitrogen fixing organism *Azotobacter*, with a low generation time, proving to be an advantage for quick culturing of the organism. Generally, growth of *Azotobacter* requires about 8-12 days but this strain of organism showed growth in about 3-4 days. The soil was rich in nitrogen content while poor levels of Potassium and Phosphorus were seen.

Parameters	Results
PHYSICAL  Color Texture	Grey Clay loam
pH Electrical Conductance Total N Total K Total P	8.3 11.24 mS/cm 57.4% 6.4 mg/litre 0.0897 mg/litre
BIOLOGICAL  Nitrogen fixers	Free living Melanin- producing Azotobacter having low Generation time



Fig 7: Melanin-producing Azotobacter colonies on Ashby's Mannitol Agar

Mangroves grow in unique swamp regions which are water logged and hence soil plays a major role in the plant development. Since the mangrove environment is under high stress conditions, the micro organisms growing in it can have great potential for bioremediation [14]. Mangroves provide a unique ecological environment for diverse bacterial communities [15]. Various soil parameters are important drivers for the establishment and persistence of mangrove ecosystem.

#### CONCLUSION

The Endophytic fungi are one of the most unexplored and diverse group organisms that make symbiotic associations with higher life forms and may produce beneficial substances for host [16]. This study shows the presence of endophytic fungus in the leaves of mangrove plant Avicennia marina. The enzymatic and antimicrobial potential of the fungal endophyte offers promising source of bioactive natural products that can be used to satisfy demands of pharmaceutical, medical agriculture and industries [17]. The discovery of novel antimicrobial metabolites is an important alternative to overcome the increasing levels of drug resistance by plant and human pathogens. Our results contribute the understanding to mangrove plant Avicennia marina as an important source of endophytic fungi and the scope for future investigations on the endophytes for their bioactive potential and industrial applications [18]. The study of mangroves soil plays an important role understanding mangroves as important ecosystem with immense ecological value and helps in conservation

of this highly reproductive ecosystem at local and global scale.

#### ACKNOWLEDGEMENT

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STUDY OF PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY FROM DIFFERENT

PLANTS BELONGING TO MALVACEAE FAMILY.

**Dhanashri B<sup>1</sup>, Samta V<sup>1</sup>,** Dr. Sagarika Damle<sup>2,\*</sup>, Sharon K<sup>3,\*</sup>

<sup>1,3</sup> Department of Biotechnology, K.C. College, D.W. Road, Churchgate, Mumbai-400020<sup>2,\*</sup>

Department of Life Sciences, K.C. College, D.W. Road, Churchgate, Mumbai-400020

**ABSTRACT** 

Objective: Hibiscus species have been documented for treatment of high blood pressure,

bacterial infections etc. in tribal medicines. We aimed at analyzing the phytochemical profile of

Hibiscus rosa-sinesis L. and Hibiscus fragilis L., to select plant with potential medicinal use.

Method: Total Flavonoid and Phenol Content was determined Spectophotometrically. The

antioxidant activity was determined by DPPH assay. HPTLC was performed on TLC F60 plates.

Antimicrobial activity was determined by agar cup method. HPLC was performed using C-18

column. Silver Nanoparticles were synthesized using 1mM silver nitrate.

**Result:** Comparable quantities of flavonoid and phenolic were found in all samples along with

good antioxidant activity. HPTLC revealed many compounds and antimicrobial activity was not

observed even at concentrations up to 1g/ml. HPLC showed presence of Gallic acid in HRL and

HFS sample whereas presence of Quercetin in HRL sample. Silver nanoparticles were

synthesized and their size were estimated using NTA.

Conclusion: Hibiscus fragilis L., stem showed highest antioxidant activity and presence of 14

metabolites in its chloroform extract by TLC. HPTLC confirms the presence of Quercetin in

Leaves, which, along with good flavonoid and phenolic content can be correlated to its

antioxidant activity.

Keywords: Hibiscus, Flavonoid, Phenolic, HPTLC, Antioxidant.

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#### INTRODUCTION

Hibiscus is a genus of flowering plant in the family Malvaceae, native to East Asia. Malvaceae is one of the important family consisting of 82 genera and species with over 200 1500 species of Hibiscus(U.A. Essiett, E. I. ,2014). Hibiscus rosasinesis is a bushy, evergreen shrub with glossy leaves and solitary, vibrant color flowers ranging from white through yellow and orange to scarlet and shades of pink, with both single and double sets of petals. The study of Hibiscus rosasinesis have been carried out worldwide which showed that the leaves of the plant are used as emollient, anodyne and laxative in Ayurveda. In South-Asian traditional medicine, various parts of the plant are used in the preparation of variety of foods. Different preparations such as tea, decoctions from different parts of the Hibiscus plant have widely used in folk medicine. Hibiscus leaf tea has been found to contain lots of Vitamin C which when consumed helps to build immunity over cough and cold .Leaf tea also helps in regulating the proper menstrual cycle by lowering the levels of estrogens and thus helps to maintain a balanced hormonal level. Hibiscus leaf tea has also been found to be effective in reducing the blood pressure and lowering the LDL Cholesterol levels. The plant has been known to have several pharmacological properties including anti anti-implantation, estrogenic abortifacient, antidiabetic, hypotensive, antiinflammatory activities.

Studies have revealed the presence of important phytoconstituents in the plant .The most important class which is the polyphenols. Polyphenols are plant metabolites characterized by the presence of several phenol groups such as hydrolysable tannins, lignans, strilbenes and Flavonoids. Flavonoids include flavonols. isoflavones. flavones. anthocyanidins, among which flavonols are most commonly known for their anti-oxidant and antimicrobial biological activities. The importance of plant phenolic compounds from plant materials is a raising interest due to their redox properties which allow them acting as reducing agents hydrogen donors, and singlet oxygen quenchers, .These compounds have also been reported to have multiple functions to counteract the free radicals and also to inhibit different types of oxidizing enzymes Lalita Subedi, S. T. (2014).

Nowadays, there is a growing need to develop ecofriendly processes, which do not use toxic chemicals in the synthesis protocols. Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. Selection of solvent medium and selection of eco-friendly nontoxic reducing and stabilizing agents are the most important issues which must be considered in green synthesis of NPs.

Silver NPs are of interest because of the unique properties which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic super-conducting materials, cosmetic products, and electronic components (Iravani,et.al, 2014).

The aim of the present study is to evaluate the antioxidant activity and the presence of different phytoconstituents present in Hibiscus rosasinesis .L and Hibiscus fragilis .L along with the quantitative determination of phenol and flavonoid content in the plants.

#### MATERIALS AND METHODS:

#### Selection and collection of plant Materials:

Garden hybrids of *H* .rosa sinensis *L*. and *H*. fragilis *L*. were taken for this study. Leaves and stems of the above plants were collected from Mumbai, India.

#### **Preparation of Powdered Extract:**

Leaves and stem of the plants were washed in distilled water to remove any dirt or dust. The samples were dried in an oven at 45°C, till a constant weight was obtained. The dried samples were powdered with the help of a mixer grinder (Hadi Ud Zaman, 2017).

#### Preparation of Ethanolic & Methanolic Extract:

Extraction of powdered samples was carried out at 4°C. About 10 g of sample was extracted using 100 mL of cold solvent for 30 minutes in rotatory shaker. Then, the extract was filtered using

Whatman filter paper no. 1 to obtain a liquefied extract (MOHAMMED, 2016).

#### **Preparation of Aqueous Extract:**

About 50mL of above solvent extract was evaporated to dryness using hot air oven at 60°C. The evaporated extract was suspended in 50mL of sterile distilled water (Satheesh Kumar Bhandary, 2012).

#### **Phytochemical Screening:**

The preliminary phytochemical analysis of powdered extract was according to methods described by Godghate (2013) & N. Savithramma (2011).

#### **Determination of Total Flavonoid Content:**

Total Flavonoid Content of ethanolic extract was estimated by aluminum chloride method. Amount of flavonoid is expressed in QE mg/g using Quercetin as standard (Rajalakshmi Padma Vairavasundaram1., 2002).

#### **Determination of Total Phenolic Content:**

Total Phenolic Content of methanolic extract was estimated by Folin ciocalteu method. Amount of phenol is expressed in GAE mg/g using Gallic acid as standard (Anusha Bhaskar Nithya, 2011).

#### **Antioxidant Analysis:**

Free radical scavenging activity of methanolic extracts was determined by DPPH (2,2-Diphenyl-1-

picrylhydrazyl) assay . Dilutions of ascorbic acid were taken from 20-140~mg/ml . Volume in each tube was made up to 4mL using 1mM DPPH solution. Ability of plant to scavenge free radicals is expressed as % inhibition in mg/mL of plant extract. The scavenging activity was calculated from control sample OD using the following equation.

% of radical scavenging activity =

Absorbance of control – Absorbance of sample

\_\_\_\_\_

×100

#### Absorbance of control

Finally, IC50 value was calculated for each sample extract where dilutions of Ascorbic acid were used as standards. High IC50 value indicates less antioxidant capacity (Prasad, 2014).

#### Antimicrobial activity:

Antimicrobial activity of aqueous extract was determined using Agar cup diffusion assay. Samples of concentration 100mg/mL, 500mg/mL & 1g/mL were used for antimicrobial assay. (Olaleye, 2007), (Al-Hashimi, 2012).

#### HPTLC analysis:

HPTLC analysis was conducted to study the number of metabolites present in the ethanolic extracts. Stationary and mobile phase used were TLC F60 Plates (10x10) and Toluene: chloroform: ethanol (4:4:1) respectively (Bhradwaj, 2017), (C. Reetesh, 2015).

#### **HPLC** analysis:

HPLC was performed using JASCO BINARY HPLC. Separation was achieved using C-18 reversed phase chromatographic column. Mobile phase used for separation of phenols is water: acetonitrile (80:20) and for separation of flavonoids is acetonitrile: 2% acetic acid (40:60). Standard used for phenol and flavonoid is Gallic acid and Quercetin respectively (Seal, 2016).

#### Green synthesis of silver nanoparticle:

10mL of Aqueous extract(1g in 10ml of Distilled water heated at 60 °C for 20 minutes and filtered using whatman filter paper no 1) assorted with 1mM of 90 ml of silver nitrate solution and was heated at 50 °C. A change in the color of solution was observed during the heating process. The size of the nanoparticle synthesized was estimated using nanoparticle tracking analysis (Gnanadhas Gnanajobitha, 2013), (EK.Elumalai, 2010).

#### **RESULTS:**

Samples are abbreviated as HRL and HRS for *H.* rosa sinensis *L.* leaf and stem and as HFL and HFS for *H. fragilis L.* leaf and stem respectively.

#### **Phytochemical Screening:**

Preliminary phytochemical analysis revealed the presence of flavonoids, tannins, phenols, diterpenes, & alkaloids in all samples. The results of phytochemical analysis are showed in table below where "+" indicates presence of phytochemical and "-" indicates absence of phytochemical.

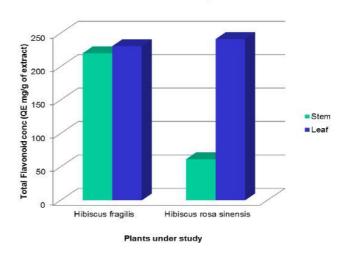
PHYTOCHEMICAL	HFL	HRL	HFS	HRS
Saponins	743	- 1	-	7-
Tannins	+	+		27
Phenols	+	+	+	+
Phytosterol	12	9	+	+
Phlobatannins	(2)	:	=	3
Diterpenes	+	+	+	+
Leu coantho cyanin	12	: [		1 %
Steroid	R#1		ē.	85
Anthocyanin	828	a	-	12
Anthraquinone	+	+	+	+
Carbohydrate (Molish test)	+	+	+	+
Proteins - Ninhydrin	+	+	+	+
- Xanthoproteic	+	+	+	+
Alkaloids	+	+	+	+
Coumarin	+	+	+	+
Flavonoids				
- NaOH test	+	+	+	+
- NH40H test	+	+	+	+
Cardial glycoside	(4)	-	-	25

#### **Total Flavonoid Content:**

Total Flavonoid Content was found to be highest in *H. rosa sinensis L.* leaf sample. Amount of flavonoid present in all samples is:

	Hibiscus fragilis (ug/g)	Hibiscus rosa Sinensis L. (ug/g)
Stem	220.04959	61.39432007
Leaf	230.62661	241.2036281

# Comparative estimation of Total Flavonoid content between two *Hibiscus* sp.

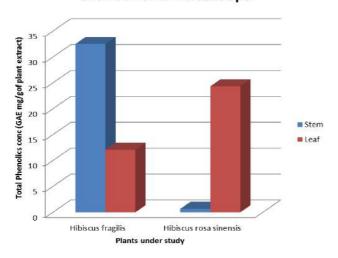


#### **Total Phenolic Content:**

Total Phenolic Content was found to be highest in *H. fragilis L.* stem sample. Amount of phenol present in all samples is:

	Hibiscus fragilis L. (GAE mg/g of extract)	
Stem	32.50557	0.677321478
Leaf	12.06254	24.32835614

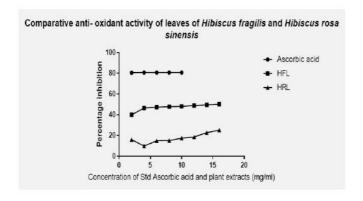
# Comparative estimation of Total Phenol content by Folin's method between two *Hibiscus* sps.

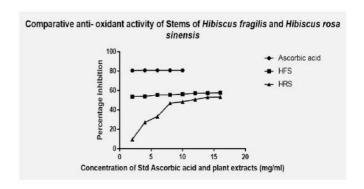


#### **Antioxidant Analysis:**

Presence of Flavonoids and Phenols affirms the invitro antioxidant potential of the different parts of two species of Hibiscus. The highest antioxidant property was observed in *H. fragilis L.* stem sample. Anti-oxidant property of samples is:

Sample	Ascorbic acid	HFL	HRL	HFS	HRS
IC50	<2	13.9	>20	= 2</td <td>11.4</td>	11.4
value	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml





#### Antimicrobial activity:

Aqueous extract of plant up to 1g/ml were not able to inhibit the growth the test organism (*Escherichia coli & Staphylococcus aureus*). No inhibition is indicated as "-".

Concentr	HRL	HRS	HFL	HFL	Test
ation					organ-
					ism
100	=	-		-	E. coli
(mg/mL)					
500	=	186	-	1-1	E. coli
(mg/mL)					
1.00	=	XIII	-	-	E. coli
(g/mL)					
100	<b></b>	/E	<b></b>	-	S.
(mg/mL)					aureus
500	-	8±	=:	S=1	S.
(mg/mL)					aureus
1.00		-	<b>(</b>	-	S.
(g/mL)					aureus
1.00	Ξ	-		-	S.

Positive control: Ampicillin 10mg/ml; Zone of inhibition: 10.1mm

#### HPTLC analysis:

Metabolites present in the plant samples were separated and studied using HPTLC.

Sample	Solvent	No.	of	peaks	Peak
		observed		corres-	
		25.4	266	L 4 G	ponding
		254	366	After	to
		nm	nm	derivit-	std.
				ization.	quer-
					cetin
					Rf
					values.
HFL	Ethanol	4	4	6	-
HRL	Ethanol	11	11	11	0.23
					(254nm)
					,
					0.24
					(366nm)
HFS	Ethanol	6	3	5	-
Quer-	Ethanol	4	3	2	0.23
cetin					(254nm)
					,
					0.24
					(366 nm)

#### **HPLC** analysis:

Gallic acid and Quercetin are distributed in different parts of plants. Plants samples were subjected for the estimation of Gallic acid and Quercetin by HPLC analysis.

#### > Flavonoids.

Standard (Quercetin)	HRL	HFS	
No. of peaks	4	4	
Retention time similar to that of quercetin	4.875	-	
Retention time of Quercetin	4.975 [ Area under peak= 1186162uV-sec]		
Area under peak	28393uV-sec	-1	

#### > Phenols.

Standard ( Gallic acid)	HRL	HFS	
No. of peaks	4	4	
Retention time similar to that of gallic acid	2.975	2.992	
Retention time of Gallic acid	3.133 [ Area under peak= 2851758 uV-sec]		
Area under peak	356672 uV-sec	141958uV- sec	

#### Green synthesis of silver nanoparticle:

The color change is the preliminary observation which shows the presence of silver nanoparticle whose size was determined using nanoparticle tracking analysis. The size of silver nanoparticle for *H. rosa sinensis L.* leaves was found out to be 306nm and that of *H. fragilis L.* stem was found out to be 179nm.

#### **CONCLUSION:**

The results of this study reveal the presence of important phytoconstituents such as the flavonoids, phenols, alkaloids, tannins to be present in different parts of the plant. The Total Flavonoid Content was estimated using Aluminum chloride method. The flavonoid content was found to be highest in the HRL sample (Hibiscus rosasinesis. L leaves) .Also the Total Phenolic Content estimation was carried out using Folin Cicolteau method and was found to be highest in the HFS sample( Hibiscus fragilis .L. stem). The presence of these phytochemicals affirms the in vitro anti-oxidant potential of the Plant. The anti-oxidant potential of the plant extracts was determined using DPPH assay. The results obtained confirm the in vitro anti-oxidant potential of the samples under study.

High Performance Thin Layer Chromatography was also performed to identify and characterize other phytoconstituents of the plant. The following analysis revealed the presence of maximum number of compounds in the Hibiscus rosasinesis leaves sample. Further Quantitative estimation of the phytoconstituents was done using High Performance Liquid Chromatography. It showed the

presence of quercetin and gallic acid in the Hibiscus rosasinesis L leaves and the highest amount of gallic acid was found to be present in the Hibiscus fragilis L. stem. The anti-microbial activity of the plant extract was also tested using agar well diffusion method against Escherichia coli (Gram negative) and Staphylococcus aureus (Gram Positive) using Ampicillin as a standard .Green synthesis of silver nanoparticles of two plant extracts was carried out using 1mM AgNO3.

Phytochemicals are potent anti-oxidants, metal chelators or free radical scavengers and hence they own health promoting properties.

In this study the in vitro antioxidant assays indicate that due to the higher in vitro anti-oxidant activity and higher phenol and flavonoid contents, these phytochemicals could be significant source of natural natural antioxidants which might be helpful in preventing the progress of various oxidative stress and stress related disorders. Thus the use of natural anti-oxidants would help prevent several carcinogenic effects occurring due to the use of synthetic anti-oxidants. Thus further isolation of therapeutically active constituents is essential for the formulation of new herbal drug.

H.fragilis L. stem showed promising results as it has high amount of phenol and highest anti-oxidant property. The size of silver nanoparticle sythesied using H.fragilis L. stem was of size 179nm which can further be applied in drug delivery system with further purification.

**AKNOWLEDGEMENT:** We express our sincere thanks to the Principal of Kishinchand Chellaram College

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### **MICROBIOLOGY**

"Always trust a microbiologist because they have the best chance of predicting when the world will end"

TEDDIE RAHUBE

# SCREENING OF BETA LACTAM RESISTANT ORGANISMS FROM RAW AND DRINKING WATER SOURCES IN MUMBAI

Urvi Shah, Sharmila Choudhary, Akanksha Singh, Dr. Pratibha Shah\*, Prof. Vijay Thigle\*

Department of Microbiology, K.C. College, D.W.Road, Churchgate, Mumbai 400020, India.

\*Author for correspondence (shahpratij@gmail.com)

#### ABSTRACT

The spread of antibiotic resistant bacteria (ARB) in the environment is a major public health issue. Aquatic ecosystems is a major source of ARB, which have entered from human, animal and industrial sources. Beta-lactams are a class of most widely used broad spectrum antibiotics in therapeutics. In the current study, 7samples from various raw and treated drinking water sources in Mumbai were screened for the presence of beta-lactam resistant Gram negative bacteria by filtering through a cellulose nitrate membrane and using MacConkey's agar. The isolates were identified by studying the morphological and biochemical characteristics. The resistance of the bacteria towards the beta-lactam antibiotics-Ampicillin, Aztreonam, Ceftazidime, Cefmetazole and Imipenem was evaluated using Kirby Bauer's disc diffusion method.87.5% of the isolates were found to be resistant to at least one of the beta-lactam antibiotics. The highest resistance rate was found against 3<sup>rd</sup> generation penams (87.5%) and the least was found against carbapenems (12.5%). Thus, the study indicates that aquatic ecosystems in Mumbai can act as reservoir of ARBs, which could potentially be transferred to susceptible bacterial pathogens.

**<u>KEYWORDS</u>**-Beta-lactam resistant organisms, Kirby bauer's method, antibiotic susceptibility test.

#### INTRODUCTION

Access to safe drinking water is essential for human health[1]. Water resources are sources of water that are useful or potentially useful. Only 3% of earth's water is fresh water. Most of it in icecaps and glaciers (69%) and ground water (30%) while all lakes, rivers and Swamps combined only account for a small fraction (0.3%) of the earth's total fresh water reserves. 97% of the water on the earth is salt water. Water is the natural resource which gets easily polluted by recreational activities and sewage disposal. Studies have been done by various researchers to measure the chemical and microbial contaminants in the natural water bodies. [2] Beta-lactam antibiotics are most commonly used for treatment of bacterial infections world wide.

The most clinically important antibiotic resistance genes are those that encode enzymes that hydrolyze lactams (bla genes) [4]. These traits confer high level resistance to -lactam antibiotics, which are the most widely used antibiotics in clinical veterinary practice[5,6].Extended Spectrum -Lactamase (ESBL) is group of enzymes that can hydrolyze a variety of lactams including cephalosporins ceftazidime, cefotaxime, and ceftriaxone and monobactams like aztreonam in addition to but does not hvdrolvze cephamycins like cefoxitin.Most of the ESBLalso have the ability to hydrolyze fourth-generation cephalosporins including cefepime[7]. The aim of the study was to screen the water samples from different areas in Mumbai for the presence of antibiotic resistant bacteria against five betalactam antibiotics by Kirby bauer's disc

#### MATERIALS AND METHODS

diffusion method.

Collection Of Water Samples: Clean plastic bottles were used for water sample collection from the site and the samples were transferred to the laboratory for microbiological analysis[3]. The water samples were processed within 2-4 hours of collections, in case of delay the samples were refrigerated at 4 degree Celsius.

Isolation by Membrane Filtration. For all the samples, three volumes of 100Ml were filtered through 0.45 m pore sized filter (cellulose nitrate membranes, Hi-media) using a water pump .These membranes were aseptically placed upon plates with Macconkey's agar media to select out gram

negative organism ensuring that no air bubbles were trapped.

Identification Of Bacterial Isolates—Cultural, Morphological Characters: Subculturing was carried out on distinct colonies until pure cultures were obtained and were preserved. Individual colonies were purified and identified by morphological and biochemical techniques and further identified by biochemical examination and VITEK 2 compact.[8]

Antibiotic Susceptibility Test: Antibiotic Susceptibility testing was performed by Kirby-Bauer test also called as Disc Diffusion test using standard procedure of the Clinical and Laboratory Standards Institute. Beta lactam group of antibiotic discs of standard concentrations were placed

on agar plates swabbed with isolated organisms and the susceptibilities of the organisms to the antibiotics were determined. The antibiotics used were Ampicillin(AMP10),Aztreonam(AT30), Ceftazidime(CAZ30),Cefinetazole(CMZ30), Imipenem (IPM10).

**RESULT AND DISCUSSION** All the water sample showed a very high bacterial contamination. The cellulose membrane filter placed on selective media was used for isolation and identification of organisms. Table 1 shows the result of identification of the organism. The antibiotic resistance pattern was studied using disc diffusion method. Table 2 shows the results of antibiotic susceptibility test. The graph shows that the 87.5% of the isolate were resistant to atleast one of the beta-lactam antibiotics. The highest resistance rate was found against third generation penams (87.5%) and the least resistance was found against carbapenams(12.25%). Thus study showed the presence of several gram negative bacteria in the raw source water that were highly resistant to many beta-lactam antibiotics like Ampicillin and some were also found to be resistant to third generation penams. Also highly resistant bacteria were also isolated from treated drinking water samples. The sensitivity was also found to be in the intermediate range for a number of antibiotics for all strains studied, raising concerns that there might be a shift towards resistance in those organisms. organisms pose a grave risk to humans coming in contact with them as an infection caused by such organisms would be virtually untreatable and eventually fatal. This can further compound the problem of antibiotic resistance due to the horizontal intra and inter-genus transfer of plasmids. presence of antibiotic resistant bacteria in the treated water is possible because water treatment could

increase antibiotic resistance and induce transfer of antibiotic resistant genes among bacterial population. certain [9]Water distribution systems could serve as a reservoir for the spread antibiotic resistance. Further work should identify the presence of biofilms in the distribution lines and develop methods to reduce the ARBs in drinking water[10]. Further studies should be done on the resistant and intermediate resistant strains of organisms to check whether the resistance conferred upon the organisms was plasmid borne or chromosomal, identify the presence of biofilms in the distribution lines and develop methods to reduce the ARBs in drinking water. There is paucity of information regarding beta-lactam resistance

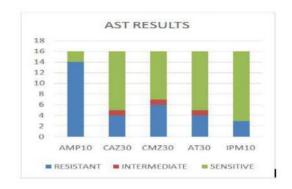
Table 1

CULTURE	ISOLATED ORGANISM BY		
NO.	VITEK 2 COMPACT		
1	Comamonas testosteroni		
2	Panto ea spp		
3	Pseudomonas fluorescens		
4	Enterobacter cloacae		
5	Klebsiella pneumoniae		
6	Pseudomonas oleovorans		
7	Acinetobacter haemolyticus		
8	Klebsiella pneumoniae		
9	Klebsiella pneumoniae		
10	Acinetobacter baumanii		
11	Klebsiella pneumonia		
12	Klebsiella pneumonia		
13	Klebsiella pneumonia		
14			
15	Acinetobacter baumanii		
16	Acinetobacter lwoffii		

Table 2

Culture no.	Resistance pattern
1	(AMP),(CAZ),(AT)
2	(AMP)
3	(AMP),( CAZ)
4	(AMP),(CMZ),(IPM)
5	(CAZ), (IPM)
6	(AMP), (CMZ)
7	(AMP) ,(CAZ)
8	(AMP),(CAZ), (CMZ)
9	(AMP)
10	(AMP),(CAZ), (AT)
11	(AMP)
12	(AMP)
13	(AMP),(CAZ),(CMZ), (AT)
14	(CAZ)
15	(AMP),(CAZ),(CMZ), (AT)
16	(AMP),(CAZ), (CMZ), (IPM)

in drinking water sources from Mumbai. To the best of our knowledge, this is the first study reporting beta-lactam resistance in water samples from Mumbai.



#### CONCLUSION

The study indicates that aquatic ecosystems in Mumbai can act as reservoirs of antibiotic resistant bactertia, which could potentially be transferred to susceptible pathogens. It is a health risk to a large mass of population consuming water from these sources. Unmonitored usage of antibiotics should be avoided.

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#### STUDY OF DRUG RESISTANCE ORGANISMS FROM FOMITES

Minu Agarwal, Shradha Gore, Janvi Shah, Sejal Rathod\*

Department of Microbiology, K.C. College, D.W. Road, Churchgate, Mumbai 400020

#### **ABSTRACT**

Fomites are inanimate objects that can serve as vehicle for pathogen transfer. Antibiotic resistance of micro-organisms is one of the grievous challenges of the 21st century. The life-saving drugs have ceased to work because of the increasing emergence of microbial strains resistant to them. The aim of this study is to establish the possible presence of antibiotic resistant bacteria on fomites in the trains (western railway) and evaluate their antibiotic susceptibility pattern.48 samples from various areas of train compartments were used for the study. Antibiotic susceptibility testing of all the isolates was performed using Disc diffusion method. A variety of organisms were isolated including a few pathogens. Most of the isolated organisms were found to be resistant to multiple antibiotics. The effect of essential oils like cinnamon and clove buds were tested on the isolated organisms and were found to inhibit them at a concentration of 0.25 to 1%. To prevent the spread of such resistant organisms proper sanitation and hygiene awareness has to be brought about as they pose a threat to public health.

**KEYWORDS**: Antibiotic Resistant organisms, antibiotic susceptibility, fomites, train, essential oils.

#### INTRODUCTION:

Fomites consist of either porous or nonporous surfaces that when contaminated can act as a vehicle for transmission of pathogens. Fomites can be contaminated with aerosolized bacteria, contact with fluids etc. Many common places are shared by people increasing the risk of transmission and infection. Public transport can lead to bacteriological, viral or fungal infections. [4]. Contributing to the complexity is an incomplete understanding of indirect contact transmission. Indirect contact transmission refers to person-to-person transmission of disease via an intermediate fomite (i.e., inanimate object acting as a carrier of infec tious disease). Indirect contact, or fomitemediated contact, is poorly understood due, in part, to the nature of the transmission route. There are a number of ways fomites can be contaminated with infectious disease, including contact with bodily fluids,

body parts, or other fomites and settling from airborne particles by talking, sneezing, coughing, or vomiting [5]. Contamination of a fomite may provide no obvious or visible evidence of infectious disease presence. Additionally, the routes by which an infectious agent contaminates a fomite are equally able to infect a susceptible individual without the intermediate fomite. Therefore, it is often difficult to determine whether a transmission event occurred directly between an infected host and a susceptible host, or the event occurred indirectly via a fomite.

This study focuses on the detection of drug resistant organisms.

#### **OBJECTIVES:**

- 1. Collection of samples from various parts of the train.
- 2. Isolation and identification of the organisms.
- 3. To determine the antibiotic susceptibility of an organism.
- 4. To characterize the resistance prevalence of an antibiotic.
- 5. To formulate a herbal disinfectant spray.
- 6. To circulate the project findings to the clinical and scientific communities and to the general public.

#### MATERIAL AND METHODS:

Collection of samples: Samples were collected from different parts of train (handle, rod, seat and window) by swab method. [1].

**Isolation:** Isolation was carried out on sterile Mac Conkey (selective) and Nutrient agar (Hi - Media).

Identification of Bacterial Isolates— Cultural, Morphological Characters: Subculturing was carried out on distinct colonies until pure cultures were obtained and were preserved. Individual colonies were purified and identified by morphological and

biochemical techniques and further identified by biochemical examination and VITEK method.

Antibiotic Susceptibility Test: Antibiotic Susceptibility testing was performed by Kirby-Bauer test also called as Disc Diffusion test on sterile Muller-Hinton's media (Hi - media) and using standard procedure of the Clinical and Laboratory Standards Institute. Antibiotic discs of standard concentrations were placed on agar plates swabbed with isolated organisms and

the susceptibilities of the organisms to the antibiotics were determined. The antibiotics used were as follows: Ampicillin (AMP<sup>10</sup>), Amoxyclav (AMC<sup>30</sup>), Cefotaxime (CTX<sup>30</sup>), Chloramphenicol (C<sup>30</sup>), Ciprofloxacin (CIP<sup>5</sup>), Gentamicin (GEN<sup>10</sup>), Tetracycline (TE<sup>10</sup>) and Ticarcillin (TI<sup>75</sup>). [Rached Ismaïl, et al.]

**Determination** of MIC: Antimicrobial activity of herbal essential oils cinnamon (Cinnamomum zeylanicum) and clove (Syzygium aromaticum) was tested by Agar dilution method. The suspensions of the 14 isolated cultures were adjusted to a turbidity of 0.5 McFarland. The Nutrient agar was used in order to obtain concentrations from 50μl (0.25%) to 200μl (1%) and plates were poured. Saline suspensions of the 14 isolates were spot inoculated on to the plates. The lowest concentration was capable of inhibiting visible growth after 24 h of incubation at 37°C was then recorded as the MIC [2].

Formulation of the herbal disinfectant spray was done.

#### RESULTS AND DICUSSIONS:

- 1. A total of 48 samples were collected from trains at peak timings.
- 2. 13 Gram negative and 1 Gram positive isolates were selected.
- Identification revealed a variety of microorganisms, most of them were opportunistic pathogens.
- The minimum inhibitory concentration (MIC) of cinnamon and clove bud was found to be 0.46% (92μl) and 0.25% (50μl) respectively.

Table 1 - Identification of isolates by Vitek method:

SR. NO.	ISOLATE S	ORGANISM S	SITES
1	4	Enterobacter cloacae complex	Shelf
2	5	Enterobacter cloacae complex	Window
3	6	Pantoea spp	Seat
4	9	Enterobacter cloacae complex	Seat
5	10	Rhizobium radiobacter	Handle
6	11	Cronobacter sakazakii group	Shelf
7	12	Acinetibacter baumannii complex	Seat
8	13	Enterobacter cloacae complex	Window
9	14	Pseudomonas stutzeri	Window
10	16	Kocuria rosea	Shelf
11	17	Alcaligenes faecalis spp faecalis	Seat
12	18	Pseudomonas stutzeri	Window
13	20	Acinetibacter lwoffii	Shelf
14	21	Acinetibacter baumannii complex	Window

Table 2 - Antibiotic resistance pattern of bacterial isolates from trains.

SR. NO.	ISOLATE S	RESISTANCE PATTERN
1	4	AMC
2	5	AMP, AMC
3	6	AMP, CTX, TI

4	9	TE, TI
5	10	TI
6	11	AMP
7	12	AMP , AMC,
		CTX, C, TI
8	13	AMC
9	14	25
10	16	AMP, CTX, CIP
11	17	AMP, CTX, C, TI
12	18	GEN
13	20	CTX, CIP
14	21	AMP, AMC, CTX

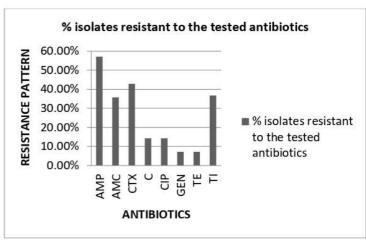
**KEY** - Ampicillin(AMP<sup>10</sup>), Amoxyclav(AMC<sup>30</sup>), Cefotaxime(CTX<sup>30</sup>),

Chloramphenicol(C<sup>30</sup>),

Ciprofloxacin(CIP<sup>5</sup>), Gentamicin(GEN<sup>10</sup>), Tetracycline(TE<sup>10</sup>) and Ticarcillin(TI<sup>75</sup>).

Multi drug resistant organisms: - Isolate 6, 12, 16, 17, 21.

#### GRAPH 1 -



KEY -

ANTIBIOTICS	RESISTANCE (%)
Ampicillin(AMP <sup>10</sup> )	57.14%
Amoxyclav(AMC <sup>30</sup> )	35.71%
Cefotaxime(CTX <sup>30</sup> )	42.86%
Chloramphenicol(C <sup>30</sup> )	14.28%
Ciprofloxacin(CIP <sup>5</sup> )	14.28%
Gentamicin(GEN <sup>10</sup> )	7.14%
Tetracycline(TE <sup>10</sup> )	7.14%

Ticarcillin(TI<sup>75</sup>)

35.71%

#### CONCLUSION:

- Multiple drug resistant organisms were found from various regions of the train compartments.
- 2. Risk of commuters being exposed and infected by the resistant organisms.
- Possibility of normal flora acquiring resistance.
- 4. Proper cleaning and sanitization is required as thousands of people travel regularly by trains.
- 5. Health of public is important and therefore, the trains should be disinfected regularly.
- 6. After train travel sanitize yours hands.

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#### A STUDY ON BACTERIAL DECOLORIZATION OF AZO DYES

Aditee Ashar, Runali Jagtap, Diksha Sawant, Dr. Pratibha Shah\*
Department of Microbiology, Kishinchand Chellaram College, Mumbai 400020,
Maharashtra, India
\*Corresponding author

#### ABSTRACT

#### KEYWORDS

Azo dye, Crystal violet, dye degrading bacteria, textile effluent

Azo dyes are extensively used in textile industries to impart colour to the material. Disposal of dyes in the environment causes damage to the ecosystem and biodiversity. Microbial degradation and decolourization of azo dyes has gained more attention recently because it is an eco-friendly and economical process. In the present study, crystal violet degrading bacteria were isolated from textile effluent on Nutrient agar amended with the dye. 11 potent bacterial the selected which exhibited were decolourization in 48 hour. Kinetic studies were performed to analyse the influence of different dye concentrations, pH and temperature on degradation rate, under static conditions. Thus, the study emphasizes the potential role of microbes for rapid and safe biological treatment of textile effluent.

#### INTRODUCTION

Over the last few decades, globalization and industrialization has drastically increased which has led to an increase in environmental pollution. Among various industries, the textile dying industries discharge large volume of waste water after dyeing process [M. Sudha et.al, 2014]. It is estimated that around 10 -15% of the dyes are lost in the effluent during the dyeing processes [M. Sudha et.al, 2014]. This wastewater contains different types of dyes such as azo, diazo, acidic, reactive, basic, disperse, anthraquinone dyes.

Azo dyes are the largest group of dyes which are extensively used in industries to impart colour to the material. They have a synthetic origin and a complex aromatic structure (N=N group). Depending upon the number of azo-groups, azo dyes are divided into monazo, disazo, trisazo, tetrakisazo and polyazo. These dyes account for around

60-70% of all dyes used in food and textile manufacture [S. Kannan et.al, 2013]. Crystal violet is a type of azo dye which has extensive uses in human and veterinary medicine as a biological stain and as a textile dye [S.Adeeb Mujtaba Ali et.al, 2014].

Discharge of azo dyes in the environment causes serious health and environmental problems. It may affect photosynthetic activity in aquatic life by reducing light penetration [S. Kannan et.al, 2013]. This alters the pH, increases biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and gives the rivers intense colourations [S. Kannan et.al, 2013]. It greatly affects the water quality. The high concentration of dyes is known to cause ulceration of skin, and mucous membrane, dermatitis, perforation of nasal septum, severe irritation of respiratory tract and on ingestion may cause vomiting, pain, haemorrhage and sharp diarrhoea [C. Lavanya et.al., 2014].

Various physico-chemical methods are employed for the decolourization of azo dyes. Although they can remove dyes partially, various limitations prevent them to be economical and thus cannot be used widely and economically [S. Kannan et.al, 2013]. Biological methods has gained more attention recently because it is eco-friendly and economically feasible. Microbes have also shown to completely decolourize the azo dyes. A number of microorganisms have been found to be able to decolourize textile dyes including bacteria, fungi, and yeasts [S. Kannan et.al, 2013].

The present study focused on isolation and screening of degraders from dye textile effluent for decolourizing Crystal Violet (Azo dye) and optimize its culture parameters to maximizing the decolourization.

#### MATERIALS AND METHODS

Sample collection and screening for dye degraders: Textile dye effluent sample was collected in clean container from GENESIS INDUSTRY, TARAPUR, MAHARASHTRA. The effluent was spread plated on Nutrient Agar medium amended with 30PPM Crystal Violet dye and incubated at 37°C for 24 hours. Strains that showed high decolourizing ability were chosen for further studies.

Media and Dye: Nutrient Agar media was used for studies. (Media composition: Peptone-5gms/l, Sodium chloride-5gms/l, Beef extract-1.5gms/l, Yeast extract-1.5gms/l, Agar-1.5gms/l, pH (at 25°C) -7.4 ±0.2 [Hi-Media Laboratories Pvt. Ltd., Mumbai,India].

To the above media, Crystal Violet Dye was added in various concentration and volumes as shown below.

Dye	500	750	1000
Concentration	PPM	PPM	PPM
Dye Volume	2.50	3.75	5.00
	ML	ML	ML

Stock - 1g Crystal Violet dissolved in 100ML Distilled Water.

Total volume made up to 5 ML using Sterile Distilled Water.

**Purification of bacterial colonies:** 25 isolates were obtained by initial isolation and screening which were streaked on Sterile Nutrient Agar with 30PPM and 100 PPM Crystal violet at 37°C for 24hrs. 11 isolates that gave best degradation at 500PPM concentration of Crystal violet after 48hrs were selected.

#### **Kinetic Study:**

Effect of dye concentration: To determine the effect of different concentrations of Crystal violet dye decolourization, media was amended with different concentrations such as 500, 750 and 1000PPM and incubated for 48hrs at 37°C.

Effect of pH: Media pH was adjusted to 4, 6, 7.4 and 9 by addition of either 1N NaOH or 1N HCl in order to study its effect on decolourization of Crystal violet (500 PPM concentration) at 37°C for 48hrs.

Effect of temperature: To study the effect of temperature on decolourization of Crystal violet dye, the media with 500 PPM concentration was adjusted to RT and 37°C for 48 hrs.

**Consortium study:** The best five dye degraders were mixed in various combinations for the consortium study and incubated for 48hrs at 37°C.

**Decolourization assay:** Samples were withdrawn and centrifuged at 10,000 rpm for 5min and collected supernatant was read at 540nm by colorimeter. The percentage of decolourization was calculated by formula-

PERCENT DECOLOURIZATION = (Initial absorbance value – Final absorbance value / Initial absorbance value) × 100.

**Identification of the dye degraders:** Identification of the dye degraders was done by VITEK METHOD and by studying the morphological characteristics.

#### RESULTS

Plate Assay: The pure cultures of the 11 isolates were inoculated on Nutrient Agar plates amended with 500PPM of crystal violet. Plates were incubated at temperatures in light for 24hrs. The Agar assay was carried out to detect the decolourization activity of the organisms. After incubation, zone of clearance around the colonies was observed.

Broth Assay: The liquid cultures of the 11 isolates were collected, centrifuged and their optical density was measured at 540nm. The broth assay was carried out to detect the optimum conditions for decolourization by the organisms. The Decolourization % was calculated using O.D Values and the graphs were plotted.

Effect of Dye Concentration: All the 11 isolates could decolourize crystal violet in a concentration range of 500PPM to 1000PPM. Maximum decolourization was observed for all isolated at a dye concentration of 500PPM. A maximum decolourization percentage of 99.24% was observed within 48 hours by isolate 5 followed by 97.72 by isolate 2. All the 11 isolates could decolourize dve concentration of 1000PPM in 48hrs. Maximum decolourization of 51.40% by isolate 11 within 48 hours. (Figure 1)

Effect of pH: A pH range of 4 to 9 was used for kinetic study. Optimum pH for all isolates for found out to be 7.4 followed by pH 6. The decolourization activity showed

a decrease at pH below 6 due to acidity nature of the effluent. (Figure 2)

Effect of temperature: Kinetics study for temperature was carried out at RT and 37°C. The optimum temperature for all isolates was found out to be 37°C. The decolourization activity decreased at room temperature. With change in temperature, the enzyme can start losing its structural integrity and become inactive ultimately affecting bacterial growth. (Figure 3)

Consortium Study: The 5 best isolates which gave maximum decolourization where used for consortium studies in different combinations. However the decolourization activity of the consortium combinations did not show efficient decolourization as compared to the decolourization activity of the individual isolates. In case of consortium a maximum decolourization of 64.39% was observed for 500PPM within 48 hours.

**Identification of the dye degraders:** The colony characteristics and biochemical characteristics indicated that the organisms were *Stenotrophomonas maltophilia* and *Enterobacter cloacae complex*.

#### DISCUSSION

From the previous studies, it was found that the bacterial dye degraders could decolourize 97.12% dye of 300PPM concentration in 24 hours. [S. Kannan et. al., 2013].

Comparatively, from the present study, a degradation of 99.24% was observed for 500PPM concentration in 48 hours.

Also, another study showed that the bacterial dye degrader gave a decolourization of 70% for a concentration of 900PPM in 48 hours [Jannatul F. D., et al., 2013], which is comparable to our study that showed a maximum decolourization of 51.40% at 1000PPM in 48 hours.

#### CONCLUSION

In the light of the above discussion, it can be concluded that, Azo dye Crystal Violet rapidly decolourize under static aerobic conditions. The isolated dye degraders can be used for effective biological treatment of Azo dyes. Further work in this direction is in progress.

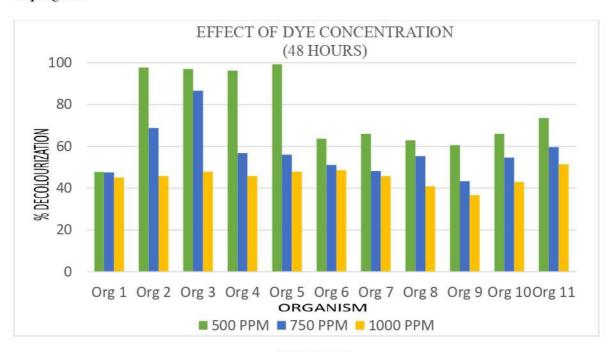


FIGURE 1

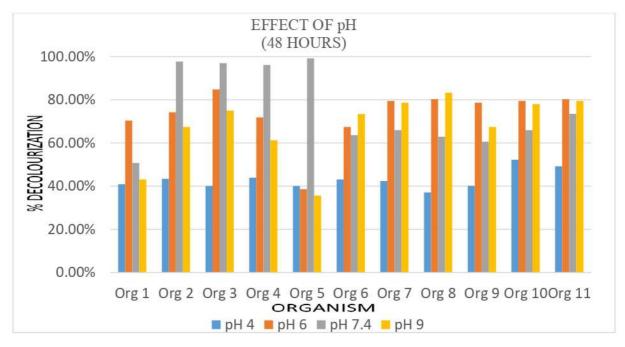
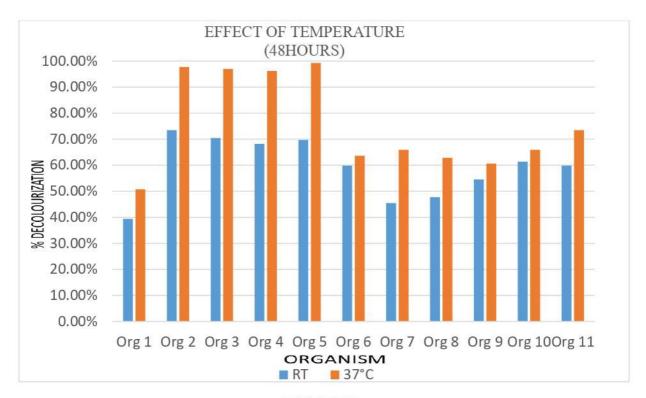


FIGURE 2



#### FIGURE 3

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# **PHYSICS**

"Look up at the stars and not down at your feet, try to make sense of what you see and wonder about what makes the universe exist."

STEPHEN HAWKING Hydrogen as replacement to Carbon based Energy Infrastructure
Tanmay Chandane, T.S., Dr Pandey\*, J. & Dr Wagle, S\*.

Department of Biotechnology, K.C. College, D.N. Road, Churchgate, Mumbai- 400020, India

#### Abstract:

Carbon has been used to produce energy for a long way now. From fossil fuels to wood and biogas, there are different ways to produce energy. While these different processes are quite efficient, they aren't environment friendly and we are currently seeing all sorts of effects of pollution caused by this. Be it global warming or ozone depletion in atmosphere, we are harming ourselves by this current situation. To change that, Hydrogen based Energy System is suggested. With latest developments in different fields regarding hydrogen, there is a need to put all the knowledge from various fields together and utilize it to make clean energy and essentially save ourselves from doom.

#### Introduction:

For thousands of years, Carbon has been circulating in various forms in the atmosphere or essentially the surface of our planet from Carbon dioxide gas to become Carbohydrate in plants to various living or dead biomasses etc. Humans started using more and more Fossil fuels after the Industrial Revolution.

As you can see, there are large spikes in the concentration of the gases after the 18th Century.

The Carbon Cycle was running its due course properly until the modern times.

What we have done is we added extra carbon to this cycle. We dug up the carbon from carbon reservoir which were deep underground and threw it up in atmosphere.

So right now we are living in a carbon based economy. Most of the energy sources we know, they have their roots in the element carbon. According to the US Energy Information Administration's 2006 estimate, the estimated 471.8 EJ total consumption in 2004 had fossil fuels supplying 86% of the world's energy.

The end product of almost all the energy production from carbon contains different compounds of carbon, mainly the oxides of carbon. Carbon Monoxide gas is a known pollutant and Carbon Dioxide is a greenhouse gas and is the cause of Global Warming. Plus there is always a chance of us using up all the available fossil fuels.

The end of this energy production system will be in two ways: One is we will eventually run out of all the fossil fuel. Provided we don't destroy ourselves before reaching this point. Then we will be forced to move to a new energy system. This seems quite chaotic. Or second way is that, before the fossil fuels deplete we can find a new, viable energy system which is environment friendly and can completely replace current energy production system.

#### **Review of Literature:**

Hydrogen is the first element that formed after the Big Bang. It is the simplest and most abundant element in the universe. Of all the baryonic matter in the universe, hydrogen is about 75%. [2] Finding efficient ways of using the element is also very useful for extraterrestrial expeditions since most of the gases in space are Hydrogen and Helium.

On earth, in the atmosphere hydrogen gas is scarce, about 1 ppm by volume. But on Earth's surface, hydrogen is the third most abundant element [3], mainly in forms of chemical compounds. Exploiting this property, we can produce hydrogen gas for different energy mechanisms.

In a study done in year 2000, around 95% of hydrogen gas was prepared by Steam

Reforming of hydrocarbons. [3] The final product of this process is hydrogen gas and oxides of carbon. This is where the problem lies. Using such hydrogen gas in processes even if they are clean will not entirely be environment friendly. One of the easiest methods of producing hydrogen is electrolysis of water. It is considered very less energy efficient hence steam reforming is preferred over it. But it is the cleanest source of hydrogen provided the electricity used was produced by clean methods. Photocatalytic splitting of water to produce hydrogen gas is also a very clean technique. Research is going on to increase the efficiency.

Solar energy is a good alternative but the problem with that are one needs a large charge carrying battery on which research is going on and it's not quite mobile for certain applications. Instead of waiting for your car's battery to charge up, one can simply refill hydrogen gas and hydrogen fuel cells or hydrogen internal combustion engines can produce the required energy on the go.

#### Methodology:

We have to find replacement for carbon energy production system. The suggested candidate is Hydrogen.

- ☐ To investigate all possible mechanisms of production of hydrogen, their efficiency and how much environmental friendly these processes are. Processes in consideration are Electrolysis of Water, Photocatalytic Splitting of Water.
  - ☐ To study possible storage mechanisms of Hydrogen.
- ☐ Energy is produced from hydrogen through certain processes. The aim is to study various energy conversion processes, their efficiencies, their costs and their environmental friendliness. Processes in consideration are Hydrogen Internal Combustion Engine and Hydrogen Fuel Cell.
- ☐ Find drawbacks if any, which will be helpful for future researches.

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# USE OF ULTRASONIC SENSORS IN BLIND CANE TO DETECT OBSTACLES IN FRONT OF IT AND AVOID COLLOSION.

#### ROHAN GAUTAM, JOYTSANA MAM \*& SHAILA MAM\*

Department of PHYSICS DEPARTMENT, K.C. College, D.N. Road, Churchgate, Mumbai-400020, India

#### **ABSTRACT**

A white cane is used by many people who are blind or visually impaired. Its primary uses are as a mobility tool and as a courtesy to others, but there are at least five varieties, each serving a slightly different need. There are different types of cane like guied cane, identification cane or symbol cane, support cane, kiddie cane, green cane.

Blind people have use canes as mobility tools for centuries, but it was not until after world war I that the white cane was introduced.

#### INTRODUCTION

White cane is used by many people who are blind or visually impaired. These canes are made from aluminium, graphite – reinforced plastic or other fiber – reinforced plastic and come with wide variety of tips depending upon user preference.

The white cane is commonly accepted as a "symbol of blindness" different countries still have different rules concerning what constitutes a "cane for a blind".



ULTRASONIC DISTANCE
MEASURMENT PRINCIPLE

Ultrasonic transmitter emitted an ultrasonic wave in one direction and started timing when it launched ultrasonic spread in the air and would return immediately when it encountered obstacles on the way at last the ultrasonic receiver would stop timing when it received the reflected wave. As ultrasonic spread velocity is 340 m/s in the air, based on the timing record(t) we can calculated the distance(s) between the obstacles and transmitter, namely: S=340t/2 which is so difference called time distance measurement principle.

### **LITERATURE**

#### **REVIEW**

When an object moves the motion can be detected by relatively simple motion sensors which is design to detect the change in luminance at one point on retina and corelate it with a delayed change in luminance at a neighbouring point on retina.

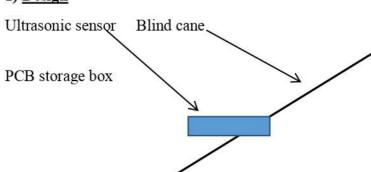
#### (Reichardt, 1961)

Ultrasonic method involves beam of high frequency sound wave that are introduced into material for detection of surface and internal defect. It includes resonance thro transmission and pulsed echo. Ultrasound has ability to detect split, checks, shake, pitch, decay, and knots.

(Mc Donald, 1980)

#### Methods

#### 1) Design



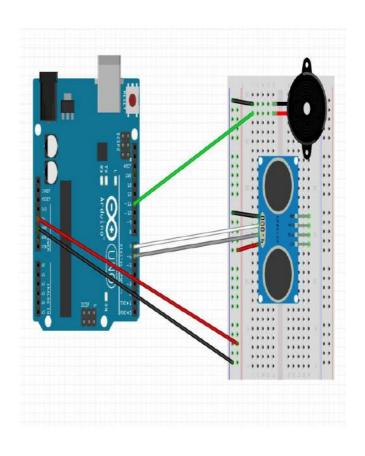
## Block diagram of blind cane

## 2) Instruments required -

- 1) Ultrasonic sensor HC SR04
- 2) Arduino UNO ATmega32
- 3) Connecting wires
- 4) Soldering apparatus
- 5) Plastic pipe
- 6) Glue
- 7) Cutter
- 8) Breadboard
- 9) Vibration motors
- 10) LED
- 11) 470 ohm resistors
- 12) 9v batteries

### Procedure

We connect ultrasonic sensors with Arudino UNO ATmeag32 as shown in figure.



#### Ultrasonic sensor HC - SR04



## Conclusion

This model is helpful to blind people. It consist of ultrasonic sensors which

continuously emits ultrasonic waves from transmitter which when returns back when it encountered with the obstacles in the way.

If there is any obstacle near sensor the vibration motor start vibrating and the blind person knows easily that there is an obstacle in front of it.

nuary 2012

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# MODULE REPORT BATCH XIII

# **PREAMBLE**

<u>The Jigyaasa Science Honours Programme</u> provides students at the undergraduate level with an opportunity to pursue an independent research project in an area of interest under the supervision of a mentor teacher. This program aims at "Nurturing young minds from lab to life." It has given us a chance to further consolidate our communication and research skills, hone our project and time management as well as problem solving ability.

As a part of this program we had various sessions which helped us understand about research on another level and teach us the importance of team work. As a result these modules have overall helped us develop our personalities and explore new horizons.

Research is to see what everybody else has seen, and to think what nobody else has thought.

-Albert Szent-Gyorgyi

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## **MODULE 1: Interactive session**

RESOURCE PERSON: Dr. Sagarika Damle & Dr. Anupama Harshal

DATE: 05/04/16

This session with no doubt was the most enjoyable session of all. The main aim of this session was to transfer the focus from teacher-based lectures to learner centric participation. This session encouraged us all to participate and interact more with each other. Not only did we understand the importance of teamwork but we also understood the importance of being attentive at all times. The beginning of the session was a wake up call for all of us. We were given a paper having a list of simple tasks we all were to complete. At first it seemed very simple and easy, we all were in a hurry to finish up first. What we did not do was that we didn't do what we were told. The first task was to read everything before doing anything which we didn't do. At the end we realised we were to do only a few tasks and we ended doing all and got nothing right. The main aim was to teach us all to understand before we act. This was an eye opener for us all.

Later, as the session moved on we realised the essence of teamwork and leadership. In the end we all got to know each other better as well as ourselves.

The average adult attention span is approximately 20 minutes. However allowing the brain to break from a lecture by participating in experiential learning extends concentration through practical application of new knowledge. Interactive sessions make it impossible to sleep through class. This session was impossible to sleep through and a much needed one.

# MODULE 2: Time Management

RESOURCE PERSON: Principal Ms. Manju Nichani

DATE: 06/04/16

"Either you run the day, or the day runs you" -Jim Rohn

Managing ones time and making the most of it is a difficult task many people fail at. There could have been no one better to enlighten us on how to utilise our time as one resource in organising, prioritising, and succeeding in studies. The question we need to ask ourselves is How do I spend my time each day? The reason many of us are unable to cope up with everyday work and assignments is because of our poor time management skills. We do not value time and take it for granted. The process of developing time management skills is a journey that requires ones dedication, needs practise and other guidance along the way.

The first step to efficiently manage time is to make a schedule of how we are to spend the day. We need to prioritise our assignment which are important and have a due date. Until that is done all unnecessary activities are best to be suspended. Last moment work is always a disaster and never up to the mark. Time should be allotted for studying with regular breaks in between as it is impossible to continuously study, a study break of a few minutes is vital.

Mainly using ones free time wisely is important. Think of times when we can study "bits" as when walking, riding the train, etc. The bottom line is to put our time to good use and not waste it. Once the time of doing something has passed there is no getting it back.

# **MODULE 3: Lab Techniques**

RESOURCE PERSON: Mr. Tiwari, Mr. Sitaramji & Mr. Ankush

DATE: 06/04/16

As we all are mainly focusing on lab based research work, it is important for us to understand and learn basic lab based skills and techniques. Our lab helpers Mr. Tiwari, Mr. Sitaramji and Mr. Ankush taught us how to handle basic lab instruments and how to dispose them when not required. They taught us how to wrap glassware and pipettes when they are to be autoclaved, how to make cotton plugs and how to seal flasks when they are to be stored. We even learned how to prepare media used in everyday lab work. This module was very helpful as it taught us the basic lab techniques which are essential if we were to work independently on our own project.

# MODULE 4: Writing a research proposal

RESOURCE PERSON: Ms. Pratiksha Palahe

DATE: 07/04/16

"Ones research is only as good as ones proposal."

Responsibility and relevance are required for research. A research proposal is a coherent and concise summary of the proposed research. This session on how to write a research proposal was the most important one as a research proposal is intended to convince others that you have a worthwhile research project and that you have the competence and the work plan to complete it.

A research proposal should contain all the key elements involved in the research process and include sufficient information for the readers to evaluate the proposed study. Most importantly it should convince the reader that you have a good grasp of the relevant literature and the major issues and that your

methodology is sound. All research proposals should address questions like: What you plan to accomplish? Why you want to do it? How are you going to do it?.

A good and well written research proposal should contain a title page, an abstract, an introduction, a literature review, methodology, expected results and discussions.

An abstract is of approximately 300 words and should include the research question, the rationale for the study, the hypothesis, the method and main findings.

The main purpose of the introduction is to provide the necessary background or context for your research problem. How to frame the research problem is perhaps the biggest problem in proposal writing.

A literature review gives credits to those who have laid the groundwork for your research. A literature review is important as it shows your ability to critically evaluate relevant literature information and indicates your ability to synthesise and integrate the existing literature.

The methods section should convey to the reader how you plan to tackle your research problem. It will provide your work plan and describe the activities necessary for the completion of your project.

The first step at writing a research proposal is to systematically plan what you are going to do and ask the right questions, only then can you accomplish your goal.



# **MODULE 5: Reference and Literature survey**

RESOURCE PERSON: Ms. Sharon K.

DATE: 07/04/16

Referencing is a mention or citation of a source of information in a book or article. Referencing should be done throughout the project to study the protocols and methodology and to avoid duplicity. A reference should include: Author's/Editor's name, Publication date/year, Title of the paper/book/article, Publication information. It is necessary to include the Authors name in a reference as we have obtained information from their research and hence they need to be acknowledged, if not, it becomes a crime- Plagiarism(failure to acknowledge). Good referencing demonstrates the depth of your reading, supports and strengthens your argument and enables the reader to locate the sources referred in your paper.

A Literature review can be a summary(recap) or synthesis(reorganisation) of information. It can be a new interpretation of old data or can be a trace of the intellectual progression of the field. The main aim of a literature review is to discuss published information within a certain time period. Doing a careful and thorough literature review is essential as it tells the reader that you haven't neglected the basics of research. A literature review needs a clear line of argument and should define and limit the problem you are working on.

# **MODULE 6: Conservation of resources**

RESOURCE PERSON: Dr. Archana Thite

DATE: 11/04/16

"Children of a culture born in a water rich environment, we have never really learned how important water is to us. We understand it but we do not respect it." -William Ashworth

Conservation is the ethical use and protection of valuable resources, such as trees, minerals, wildlife, water and others. The Earth has several non renewable natural resources which we humans depend on to survive. Therefore sustainable use and conservation of resources ensures the ability of a society to thrive. Natural resources are of two types; Biological and Nonbiological resources. Biological resources include fossil fuels, plants and animals. Nonbiological resources include water, sunlight, minerals and air. Biological resources depend on nonbiological resources.

There is a growing need for conservation of water all over the globe. Water forms the basis of all life. It many parts of the world, especially India it is scarce, people in these places crave for a drop of water while in the developed regions where water is abundant, it is generally wasted. Conservation of water is essential as it is needed by humans for survival, by plants for growth, by all animals for sustaining life. Without water there would be no life. Water decides the type of food chain and diversity of flora in a region. Water makes up 65% of the human body.

Water is a vital natural resource as without water there would be no life. Some measures to conserve water include Rain water harvesting, Drip irrigation, checking taps for leakages and recycling of used water.

# MODULE 7: Live stress free

RESOURCE PERSON: Mr. Vijay Thigle

DATE: 12/04/16

"Much of the stress people feel doesn't come from having too much to do. It comes from not finishing what they have started" -David Allen

Stress is a physical and emotional reaction that everyone experiences as they encounter changes in life. In today's time, everyone experiences stress in some or the other form. Stress has positive as well as negative effects. Stress has a positive effect on us when it makes us deal constructively with daily problems and meet the challenges. This is called eustress. Stress has negative effects when it becomes continuous. This is called distress.

As students we are always stressed about our studies. Mr. Thigles session on living life stress free was an interactive one. His methods to combat stress by controlling our breathing and relaxing our muscles was were helpful and aided in attaining a clear mindset. We understood the connection between the body and mind, how physical relaxation can cause our mind to relax. Mr. Thigle taught us many breathing exercises as well as some stretching exercises to loosen up our body and have a peaceful mind. We even learned that shouting is a way of releasing the stress which has built up in our body. Overall this module was very helpful for us students as we learnt about many different ways to deal with a stressful situation, especially when it comes to exams.



### **MODULE 8: Statistics: Introduction**

RESOURCE PERSON: Dr. Sonal Dasani

DATE: 12/04/16

Statistics is the collection, classification/tabulation, analysis and presentation of data. Statistical methods and analyses are often used to communicate research findings and to support hypotheses and give credibility to research methodology and conclusions. Statistics is most commonly used in survey based projects. It plays an important role in research of almost any kind because they deal with easily-quantified data. The study of statistics enables researchers to look at large sets of data and condense it into meaningful information.

In this session we learned about some of the most commonly used statistical tools which include, frequency distribution, chi square table, correlation analysis, regression analysis, hypothesis testing, degree of freedom, z-test, students t-test, level of significance and ANOVA (Analysis of variants).

Key aspects of statistics include finding mean, determining outliers on either end of a bell curve, and finding the range of quantities for set points. Statistics comes into action especially when the size of the sample is to be selected.



# MODULE 9: Dawaat -E- SHP

RESOURCE PERSON: Mr. Prateek S.

DATE: 18/04/16

This was less of a session and more of a party for us all. We got to explore our inner master chef in this session. We were put into random groups and were made to prepare a few dishes with a limited amount of ingredients. This was a challenge for us as many of us haven't had much experience in the kitchen but well we did it and it was amazing. The dishes we all prepared were a 100% healthy and delicious too. It was the most enjoyable experience in the course of the entire module and definitely something to look forward to again.



MODULE 10: Chai & Why

DATE: 20/04/16

This session was mainly for our seniors for troubleshooting the problems that had arised with respect to their proposed research project. It was a learning experience for us as we got to see various research projects that were undertaken, which gave us an idea of what we were to look forward to. It was a problem solving session where the teachers and students had an open discussion regarding their projects. Ideas were exchanged and suggestions were

given by the various teachers so as to how the project could be improved furthermore.



MODULE 11: Micro-pipetting

RESOURCE PERSON: Ms. Nandini Desai

DATE: 25/04/16

A micropipette is a vey fine pipettes used for measuring, transferring, or injecting very small quantities of liquid. A micropipette can dispense volume of liquids ranging from 1 to 1000 micro litre. They are mainly used in biopharma companies for transferring micro quantities of a liquid. Micropipettes use a disposable pipette tip to aspire liquid. The basic parts of a micro pipette include a plunger button, tip ejector button, volume adjustment dial, volume display, tip ejector, and shaft.

In this session we were taught the technique of handling a pipette with care and how to efficiently use a micropipette. Micro-pipetting is again a frequently used lab technique during recombinant DNA technology.

# MODULE 12: Bhabha Atomic Research Centre (BARC)

DATE: 29/04/16

We students were given a unique opportunity to attend the 'Swachh Bharat Abhiyan' at the Bhabha Atomic Research centre organised by the Department of atomic energy (DAE). The main objective of this seminar was to achieve the target of cleanliness, develop new technologies to treat waste materials and to make people aware of such technologies. Many accomplished scientists put forward their research proposals and plans and enlightened us on their research. Indigenous water purification technology which uses the Pressure Driven Membrane process can be used to improv the quality of water in small villages as well as large cities. Environment friendly Plasma Technology is used for treatment of non biodegradable waste materials such as plastics and rubber tyres as well as carcass. Multistage Biological Treatment Solution can be implemented on existing STP which are not able to process sewage to optimum efficiency. Radiation Hygienization of municipal sewage sludge was one of the most interesting proposed project.

These upcoming technologies that we got to know about can be of great help in the treatment of water and solid waste management.

# MODULE 13: Gender sensitization

RESOURCE PERSON: Prof. Vijendra

DATE: 20/03/17

Gender sensitization refers to the modification of behavior by raising awareness of gender equality concerns. Gender sensitization is all about changing behavior and instilling empathy into the views that we hold about our own and other sex. It is important for every individual to understand the feelings of the opposite sex so that the person can empathize regarding the views of others. This module involved discussion on various factors responsible for gender biases. The major ones are stereotypes, discrimination, feminism and sexual harassment. Stereotypes are assumptions that people make about members of a group based on an image .Traditional and current stereotypes tend to assign women's roles to private spheres and men's to more public ones. Gender discrimination is a state where a bias exists between people based on their sex. Preference for a boy child is the major cause of gender discrimination. According to the Supreme Court guideline, Sexual harassment can be defined as "unwelcome" sexually determined behaviour (whether directly or by implication) as: Physical contact and advances, Demand or request for sexual favors, Sexually colored remarks ,Showing pornography and Any other unwelcome physical, verbal or non-verbal conduct of a sexual nature. Stringent laws exist against sexual harassment as it is considered a major crime. This module was an important one as it is essential to educate the youth of our country about this subject to reduce crimes and also to make people aware of their sexual rights.

# MODULE 14: Time Management

RESOURCE PERSON: Ms. Natasha Advani

DATE: 20/03/17

Time management is an integral part of managing our work and efficiently complete our important tasks. Efficient time management involves setting time bound goals, being punctual, reducing procrastination and giving more of our time and effort in performing the task which is of greater priority first. This module highlighted the fact that one should not commit to help anyone in their work unless it would be possible to manage his/her own work and achieve his/her goals within the time constraint. The module was interesting and involved various activities which made us understand the importance of time and its management.



# **MODULE 15: Advanced Communication Skills**

RESOURCE PERSON: Ms. Freyaz Shroff

DATE: 21/03/17

Communication is the process of sending and receiving information among people. Communication is more than speaking or writing. To be effective, communication requires the creation of a common understanding of ideas, desires and observations among people. Communication is a two-way exchange that involves both a presenter and an audience. Communication is classified into 2 types: Verbal and Non-verbal.

This module mainly focused on non verbal communication . Non-verbal communication is defined as the communication among people by sending and receiving wordless cues. It includes apparent behaviors such as facial expressions, eyes, touching, and tone of voice, as well as less obvious messages such as dress, posture and spatial distance between two or more people. Even silence speaks. Non-verbal communication can have a great impact on the listener and the outcome of the communication. People tend to have much less conscious control over their non-verbal messages than of what they're actually saying. Non-verbal communication greatly helps to deliver a message supported by an emotional insight thus strengthening the overall gravity of the message. Various activities were performed which involved communication by non-verbal means to accomplish a given task thus aiding us in improving our non-verbal communication skills.



### MODULE 16: Media and Youth

RESOURCE PERSON: Dr. Sanjay Ranade

DATE: 22/03/17

Media is a term which can be explained as a system created by the people through which mass communication becomes possible to entertain and inform the mass audience. A major stepping stone in the path of contemporary youth is that of the media and it's all to often fatalistic, unfavourable and adverse representation of today's youth. The media primarily are modes of communication in contemporary culture that are often created by teams of people to entertain, inform, persuade and provide avenues of expression for mass audiences. As this is the case the media plays a pivotal and integral part in our lives, affecting where we go, what we do and what we think, ultimately dictating a large part of our existence. This module focused on impact of social media on people and the transition in the use of media, comparing the present and the past situation and influence of media on people. Social media helps an individual to express one's thoughts, develop social skills, develop awareness, global awareness. Social media has both positive and negative impact. It's an individual responsibility to be influenced by positive aspect of social media.

**MODULE 17: Corporate Etiquette** 

RESOURCE PERSON: Ms. Manisha Mishra

DATE: 22/03/17

Etiquette is a code of behavior that delineates expectations for social behavior according to contemporary conventional norms within a society, social class, or group. To Exhibit Professionalism and develop a polished image and instill a perception of trustworthiness, we need corporate etiquette. This module focused on visual communication i.e., appearance and international level style

scale. This module explained the way one should present themselves in the corporate world. For us being undergraduate students the module was more about the dress code and body language one should have in an interview. The module stressed on the 4A rule: Be appropriately dressed, Be attractive, Stay authentic, The attire should be affordable.

A technique called the Johari Window was explained which helps people to better understand their relationship with themselves and other; used in corporate settings and self help groups.

MODULE 18: CV Writing

RESOURCE PERSON: Ms. Kavita Peter

DATE: 22/03/17

A CV is the most flexible and convenient way to make applications. Curriculum Vitae or CV is a short account of one's career and qualification prepared typically by an applicant for a position. The module highlighted the difference between CV and resume and the format for writing a resume. A resume is a written compilation of your education, work experience, credentials, and accomplishments that is used to apply for jobs. There are three types of resume: 1) Skill oriented 2) Chronological 3) Functional. A resume is designed to provide a detailed summary of an applicant's qualifications for a particular job - it is not usually meant to provide a complete picture whereas CVs are thus much longer than resumes, and include more information, particularly related to academic background.

Components/Format of a CV:

- -Name & amp; Contact info
- -Objective or areas of interest (one line purpose)
- -Education (level of degree, institute, GPA, dates of courses)

- -Skill Set
- -Grants, Honors, Awards
- -Publications, Presentations
- -Experience/Employment (name of company, dates, position title, action + skills used)
- -Scholarly or Professional Membership
- -References (name & amp; contact info of 2 people min.)

https://www.myperfectresume.com/ can be used for resume designing.

**MODULE 19: Motivating Young Adults** 

RESOURCE PERSON: Ms. Aarti Gupta

DATE: 23/03/17

Young adults that lack motivation and feel as though they aren't good enough majorly as a result of experiencing failure. A majority of these young men and women take on each day with a self-worth of mediocrity, at best.In todays competitive world people subconsciously compare themselves to other people and feel less about themselves and most of the time they give up. The importance of motivating young adults is a crucial, yet nearly unthinkable task. This module focused on student empowerment . A few exercise involving meditation were organized to accomplish empowerment of students during this module. The main points outlined in this module were:

Our personal beliefs create our personal reality

Your mind becomes more creative as you learn to think and speak in new patterns

Each of us are connected to a divine wisdom that guides us.

# **MODULE 20: Cyber Security**

RESOURCE PERSON: Mr. Sachin Dedhia

DATE: 23/03/17

Cyber security or information technology security are the techniques of protecting computers, networks, programs and data from unauthorized access or attacks that are aimed for exploitation. This module explained the technique which is used for hacking and also provided certain basic solutions to protect such hacking. The concept of ethical hacking was introduced and the courses one can undertake to learn the same. With the sudden rise in the Internet usage across the globe over the past few years, there has also been a rise in the amount of online scams and frauds. The importance of using a secured server {https(secured)} was explained to prevent any phishing attacks. Use of proxy servers {eg: www.crazycall.net} for calling people by some other number was demonstrated which made us realise just how easily one could be scammed by using simple computer technology. Overall this module was very informative as it instilled in us the importance of being aware of the threats we could possibly face on the internet which occupies most of our time.



MODULE 21: Veganism

RESOURCE PERSON: Mr. Arvind Kanna

DATE: 24/03/17

Veganism is a lifestyle which seeks to abstain, as far as its possible and practicable, from all forms of exploitation of, and cruelty to, animals for food, clothing or any other purpose. This module explains the concept of speciesism and difference between a vegan and a vegetarian. Vegetarians do not eat meat, fish, or poultry. Vegans, in addition to being vegetarian, do not use other animal products and by-products such as eggs, dairy products, honey, leather, fur, silk, wool, cosmetics, and soaps derived from animal products. Speciesism, in applied ethics and the philosophy of animal rights, the practice of treating members of one species as morally more important than members of other species; also, the belief that this practice is justified. The speaker had great knowledge about the topic and also shared his experience of becoming a vegan. Various alternatives to dairy based products were suggested such as soy milk or rice milk and tofu. A video on the life of animals inside a butcher shop was shown which displays just how cruel man can be. In the end being a vegan, vegetarian or nonvegetarian is an individuals personal choice but if we want to stop animal cruelty we should first change our lifestyle then think of changing others perspective.



# **MODULE 22: Digitisation**

RESOURCE PERSON: Mr. Abhijeet Gole

DATE: 24/03/17

Digitization is the process of converting information from written form into a digital format This module focused on Digital India plan of government. The Digital India program is a flagship program of the Government of India with a vision to transform India into a digitally empowered society and knowledge economy and was initiated and supported by our Prime Minister. The speaker also talked about various infrastructure related digital services. Some of the important tools mentioned were-:

- Non-Internet Banking Unified Payment Interface(UPI)
- o AIDC- Automatic Identification and Data Control
- o GIS- Geographic Information System
- o RFID- Radio Frequency Identification
- National Skill Development Authority
- o MOOCs- Massive Online Open Courses



# MODULE 23: Edelweiss Financial Group Talk on GST

RESOURCE PERSON: Commissioner (Tax)

DATE: 30/03/17

Goods & Services Tax Law in India is a **comprehensive**, **multi-stage**, **destination-based tax** that is levied on every **value addition**. In simple words, Goods and Service Tax is an indirect tax levied on the supply of goods and services. GST Law has replaced many indirect tax laws that previously existed in India. GST is **one indirect tax** for the **entire country**. The GST is governed by GST Council and its Chairman is Union Finance Minister of India - Arun Jaitley.

There are 3 taxes applicable under GST:

- **CGST- Central Goods and Services Tax:** Collected by the Central Government on an intra-state sale (Eg: Within Maharashtra)
- **SGST- State Goods and Services Tax:** Collected by the State Government on an intra-state sale (Eg: Within Mahaashtra)
- IGST- Integrated Goods and Services Tax: Collected by the Central Government for inter-state sale (Eg: Maharashtra to Tamil Nadu)

GST will combine the following taxes which will now be charged as a single tax: Corruption Tax, Value Added Tax (VAT), Food Tax, Central Sales Tax (CST), Octroi, Entertainment Tax, Purchase Tax, Luxury Tax.

This Module aimed at making us aware of this new taxing system and highlighting the advantages of having one single tax rather than various taxes charged separately.



# MODULE 24: Colour Healing

RESOURCE PERSON: Dr. Tejas Lokhande

DATE: 30/03/17

Color therapy and healing (also known as chromotherapy or light therapy) is a type of holistic healing that uses the visible spectrum of light and color to affect a person's mood and physical or mental health. Each color falls into a specific frequency and vibration, which many believe contribute to specific properties that can be used to affect the energy and frequencies within our bodies. This session highlighted how different colours can change our moods and was followed by Rangoli making.





# **MODULE 25: Interactive Session**

RESOURCE PERSON: Dr. Anupama Harshal & Ms. Amruta

DATE: 31/03/17

Empowerment is the promotion of self-actualization. This module was based on team spirit and team dynamics while performing assigned tasks. The module addressed various solutions for many behavioral problems which people face. Group tasks were given to evaluate and improve ones problem solving skills, inter personal skills, communication skills and technical skills. Emphasis was given to "Think Inside The Box" rather than the common notion to think outside the box. Some of the main notable points gained from this module were:

There is always a need for evaluation

A message can be conveyed even with minimal resources as long as its strong

Silence is never a good mode of communication

Lateral Thinking

One of the major barriers to communication is being judgmental

A persons Ringtone reflects their personality

Every group activity has to be inclusive for efficient team work



### MODULE 26: Visit to Mint Museum

DATE: 31/03/17

The RBI Monetary Museum aims at documenting and preserving the wide array of Indian currency that has been used since ancient times. The Museum proposes to put in place permanent, temporary and itinerant exhibits of the representative coinage of India, paper currency, gold bars as well as financial instruments and curiosities down the ages. A multimedia presentation on the Story of Indian Paper Money was first shown followed by a tour of the museum which displayed Indian coinage dating back to the 6<sup>th</sup> century BC and the various Indian currencies used till date. The museum has a representative collection of over 10,000 exhibits of Indian coinage, paper currency, financial instruments and monetary curiosities. The journey from coins to paper money reveals how society started accepting tokens of money in lieu of money in terms of the value of the metal. The entire visit was carried out in a systematic order and no one missed out on anything.

# MODULE 27: Ethics in Research

RESOURCE PERSON: Mr. Vivek Patkar

DATE: 03/4/17

Research involving animals or human subjects or participants raises ethical, legal, social and political issues. This module addressed the importance of ethics in research which is a very broad term used to define all the factors determining whats right or wrong. Concept of Research related to ethics in practice i.e., meta ethics, normative ethics and applied ethics were also introduced. There are three broad type of ethical theory: Consequentialist theories and Nonconsequentialist theories are act centered while Agent-centered theories are virtue and moral character. The main focus of this module was the various types of Research Misconducts- Plagiarism, Fabrication and Falsification. The importance of giving credit to the researchers whose data has been used in your study is essential. This module was essential to us undergraduate students as it helped us understanding the right way in which research is to be done.



# MODULE 28: Research Paper Writing

RESOURCE PERSON: Dr. Radhika & Dr. Maya

DATE: 03/4/17

A research paper is the culmination and final product of an involved process of research, critical thinking, source evaluation, organization, and composition of the work a researcher has done over the due course of time written in a prescribed universal format. This module explained the commonly used format of a research proposal- the APA[ American psychological association] format. The main components of a Scientific Research Paper are:

Abstract

Introduction

Materials and Methods

Results

Discussions

### References

During this module the importance of writing a research paper in the correct format and publication of a research paper along with the mode of publication was explained.



# **END NOTE**

The science honours programme was a unique opportunity given to us, undergraduate students to pursue research. The modules held were not only educative, but also taught us that values were important for a successful life. I'm really grateful to SHP for the wonderful modules organized. . I thank our principal ma'am Dr. Hemlata Bagla for providing this opportunity to us of experiencing research at the undergraduate level.

If we knew what it was we were doing, it would not be called research, would it?

-Albert Einstein

# THANK YOU!

# SHP BATCH XIV

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# LIFE SCIENCES

"Life Science research can be done on multiple platforms, Since we have very small number of people flying into space, the more people you have, the better"

LAUREL CLARK

# Extraction of Bromelain from pineapple waste and creating a formulation against acne causing pathogens.

Sukaina Abbas, Maitri Trivedi, Tejashree Shanbhag, Amruta Kothare Department of Life Sciences, K.C. College, Churchgate, Mumbai 400 020

#### ABSTRACT

**Objective:** To effectively extract Bromelain from waste parts of the pineapple such as stem, crown and peel and study its properties. Bromelain is considerably absorbable in the body without losing its proteolytic activity or producing any major side effects. We evaluated the potential of pineapple waste extracts towards its antimicrobial activity. This study also aims to check the antibacterial activity of commercial facewash against acne causing pathogens and draw a comparison

Methods: The antimicrobial activity of Bromelain and face washes was studied by disc diffusion and ditch-plate method against E. coli, S. aureus, P. aeruginosa, C. diphtheria and P. acne. Bromelain extracted was subjected to purification by ammonium precipitation and dialysis. Antioxidant activity was analyzed by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Bromelain enzyme was estimated by employing Folin-Lowry method

**Result:** E. coli and S. aureus showed high sensitivity towards Bromelain whereas P. aeruginosa and C. diphtheria exhibited lower sensitivity. Bromelain was also effective against the growth of P. acne. Bromelain also exhibited antioxidant properties as it could decolorize DPPH.

**Conclusion:** Pineapple can be used as a treatment or preventive measure against acne. Its easy purification and extraction from waste also prove its economic feasibility.

Keywords: Bromelain, Pineapple, antimicrobial, antioxidant, protein purification

#### Introduction

Pineapple (Ananas comosus) has been used as a traditional medicine by several cultures throughout time and Bromelain had been established in 1876. Bromelain gets its properties mainly due to the presence of its sulfhydryl proteolytic enzymes. Bromelain is classified as stem bromelain or either firuit bromelain depending on the origin of the protease. (Rowan et al, 1990). Bromelain is present throughout the pineapple plant however the concentration and composition may vary depending on the part of the fruit and its variety. (Gautam S. 2010). Extraction of Bromelain makes it feasible as its bioavailability rises during maturity of fruit rather than during fruit development. (Maurer, 2001). Proving to be a better candidate than ficain and papain whose levels are highest during fruit development. (Bartholomew, 2003).

Extensive studies have been done with Bromelain to establish its properties and to be explored for its clinical characteristics. Therefore, finding feasible and effective extraction is necessary. Bromelain even though in reduced quantities is still present in pineapple wastes such as core, peel and leaves. (Sriwatanapongse et al, 2000). Various purification strategies have been discussed and developed for extraction of Bromelain. Bromelain precipitates at 20% to 60% with ammonium sulfate showing highest recovery at 20%. Dialysis to be followed after ammonium precipitation to concentrate and purify bromelain further by a cost-effective manner. (Vinal Pardhi et al, 2016).

Skin problems like wrinkles, acne and dry skin seemed to be solved using proteases such as papain and bromelain. (Ozlen and Chatsworth). Yong ker Loon observed sensitivity of S. aureus using it against Pineapple extract

Partially purified bromelain from the pineapple core also inhibited the growth of S. epidermidis and P. acne (Y. Hidayat et al, 2018). Bromelain also been extensively used as a line of treatment for periodontal gingivitis and found to be effective against periodontal pathogens. namely, S. mutans, A. actinomycetemcomitans, P. gingivalis. (Praveen et al, 2014). Anjos et al studied the combined antibacterial activity of papain and bromelain on *Alicyclobacillus spp*. Edema caused by post-surgical trauma also has been effectively treated by Bromelain. (MacKay et al, 2003).

The antimicrobial action of Bromelain against several bacteria motivated its study against acne causing pathogens.

Acne is a skin condition affecting the skin's oil glands is a very prominent condition. It can create a lot of psychological disturbances and stress on the individual that it effects. P. acne is an opportunistic pathogen that plays an important role in the growth and cause of acne.

S. aureus is a part of the normal skin microbiota and is associated with skin conditions such as folliculitis and also reported to enhance the effect of other microbes in acne lesions. (B. Kumar, et all, 2006).

Based on this background study, we decided to explore the possible effects of Bromelain as a treatment plan for acne.

#### Materials:

Biological material: Pineapple (Ananas comosus), Aloe vera, Turmeric (Curcuma aromatica), S. aureus, E. coli,

P. aeruginosa, P. acne (MTCC: 1951) and C. diphtheria.

Chemicals: Sodium acetate trihydrate, Acetic Acid, ammonium sulphate, sodium carbonate, Sodium Potassium tartrate, Folin- Ciocalteu reagent, DPPH (2,2 diphenyl-1-picrylhydrazyl), Sodium Stearate, NaOH, SLES, Propylene glycol, Ethanol, Tris buffer

Media: Nutrient agar and Nutrient Broth: Peptone,

Beef extract, Yeast Extract, NaCl, Agar

### Methodology:

- 1. Sample preparation:
  - 1.1 Crude Extract Preparation: The pineapple parts were categorized as fruit, stem, peel,





core and crown. Small pieces were made and they were homogenized and ground with

Fig1: Waste parts of the pineapple

- 0.1M of sodium acetate buffer (pH 7.0). The juice obtained was filtered and centrifuged at 6000 rpm for 20 mins at 4°C. This extract was used as the crude Bromelain.
- 1.2 Ammonium Sulphate Precipitation: The crude was subjected extract Ammonium sulphate at 40% saturation. The crude extract was placed in a beaker over ice at a magnetic stirrer. Ammonium sulphate was gradually added and then allowed to settle for one night in the fridge. The solution was then centrifuged at 6000 rpm for 10 mins at 4°C. The pellet was later dislodged in 10mM tris, pH 7.0 and subjected to dialysis. The supernatant was later again subjected to 60% saturation and then dialyzed.

#### 1.3 Dialysis:

- 1.3.1 Activation of dialysis membrane.

  7cm of dialysis membrane was added into boiling distilled water with 2% sodium carbonate for 30
- 1.3.2 minutes. The membrane was

transferred and boiled again for 30 minutes in distilled water.

Dialysis: The fractionate from 1.3.3 ammonium precipitation was loaded into dialysis bag and tagged. It was loaded into a beaker filled with dialysis buffer. The process of dialysis carried was out in cold conditions by keeping it over ice and using a magnetic stirrer.

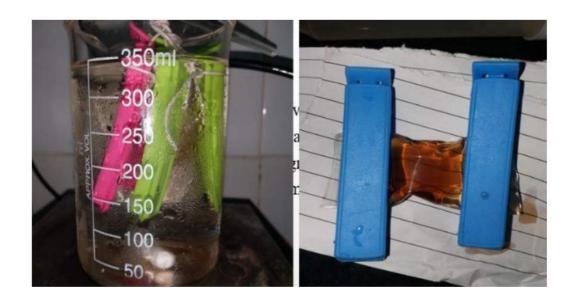


Fig2: Dialysis chamber and Dialysis bag loaded with sample (left to right)

- Measurement of Protein content: Crude bromelain and fraction protein content was diluted appropriately and was determined by using the Folin-Lowry method. BSA was used as a standard for protein estimation. The optical density was read at 660nm.
- 3. Antibacterial activity assay:
  - 3.1 Bacterial maintenance: S. aureus, C. diphtheriae, E. coli, Pseudomonas aeruginosa, P. acne (MTCC: 1951) were maintained on Nutrient Agar slants and incubated at 37°C.
  - 3.2 Ditch plate technique: Antimicrobial susceptibility of the organisms towards the crude extract was observed by using the ditch plate technique. 50% (v/v) of the sample mixed with molten agar was poured into ditch created of 1.5cm in width and loopful of bacteria was streaked across. The plates were left overnight to incubate at 37°C. The Zone of Inhibition was measured.



Fig3: Ditch plate against core crude

- 4. Antioxidant activity (DPPH free radical scavenging activity): The antioxidant activity of the extracts and standards was based on the radical scavenging effects of 2,2 diphenyl-1- picrylhydrazyl (DPPH). The diluted working solutions of the samples were made in water. Ascorbic was used 10-100ug/ml. 0.002% DPPH was prepared in ethanol. The test sample/standard was mixed with DPPH in 1:1 ratio and incubated in the dark for 30 minutes. The solution mixtures were read at 517nm. The OD was recorded and percentage inhibition was calculated by the formula below. Ethanol with DPPH (1:1) is used as a blank. Percent inhibition = 100 where A is OD of blank and B is OD of sample. (Khalaf, 2008; Lee- Fong, 2014)
- Formulation: Three different face wash formulations were made the only difference being with the source of bromelain in each, stem bromelain, crown bromelain and peel bromelain.

Material	Function		
Bromelain	Antimicrobial/Antioxidant		
Turmeric	Antioxidant		
Aloe Vera Gel	Moisturizer		
Xanthum gum	Thickener		
Tea tree oil	Essential oil/ Preservative		
NaOH, SLES, Sodium stearat e, Propylene	Soap Base		
glycol			

Fig4: Formulation created

5.1 Physical characteristics of the facewash were studied such as the color, pH, washability, foamability and consistency.



- 5.2 Antibacterial testing of formulation: The antimicrobial activity of the crude extract was determined using the disc diffusion method. Nutrient agar plates were cotton swabbed with the cultures. Sterile Whatman discs impregnated with the test samples were placed. samples were tested in four concentrations of (v/v) percentage at 25%, 50%, 75% and 100%. They were left overnight to incubate at 37°C. The Zone of Inhibition was measured.
- Comparison of commercial products: Four herbal facewashes were selected at random and their antimicrobial action against bacterium were tested at four (v/v) % concentration levels (i.e. 25%, 50%, 75%, 100%)
- Statistical comparison of commercial products against Bromelain formulations: ANOVA testing was done at 5% level of significance using Excel Data toolpak.

#### **Observation and Result:**

- 1. Estimation of protein content: Fruit contained the highest concentration of Bromelain as crude extract and even after purification. The protein yield in stem increased with purification. However, in peel bromelain yield decreased purification leading to the possibility of other protein contaminants present in the peel. The results are depicted in Fig 6.
- Antimicrobial studies of Crude Bromelain by ditch plate method: The results are graphically depicted in Fig 7. P. acne growth was considerably inhibited by Crude Bromelain, highest by Fruit extract.
- Antioxidant activity of crude Bromelain extracts: natural antioxidants are present in

Bromelain that contribute to preventing oxidative stress. By statistical analysis, there is clear difference between activity of Ascorbic acid and crude bromelain extracts. While lesser than ascorbic acid, there is still considerable amount of activity. As we can see (Fig.8), above 20ug/ml the activity

is comparable to higher range of concentrations also.

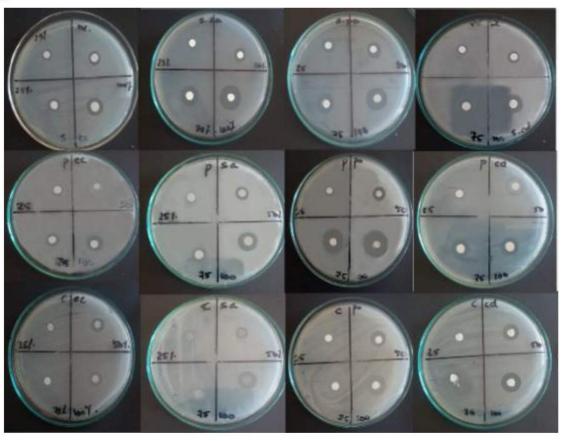
### 4. Physical characteristics of formulation:

ictor	PEEL FORMULATION	STEM FORMULATION	CROWN FORMU
Н	8.5	8.9	8.3
olor	Golden yellow	Brownish yellow	Pale gree
istency	Semi-solid	Semi-solid	Semi-soli
ability	Good	Good	Good

5. Antimicrobial studies of the formulation: the formulation was tested by disc diffusion against P. acne, E. coli, C. diphtheriae, S. aureus. The zone of inhibition is graphically represented and tabulated. By statistical analysis (ANOVA) it is proven that the three formulations are similar to each other.

### Fig5: AST plates of formulation

- Comparison of commercial products: all the facewashes were found to be quite effective against P. acne. However Brand 2 and brand 3 showed no inhibition against E. coli.
- 7. Statistical Analysis of data: For S. aureus and P. acne the formulations were found to have comparable and similar effects to the commercially available formulations. For E. coli the bromelain formulations were statistically better. For, C. diphtheria the commercial formulations proved to be more effective



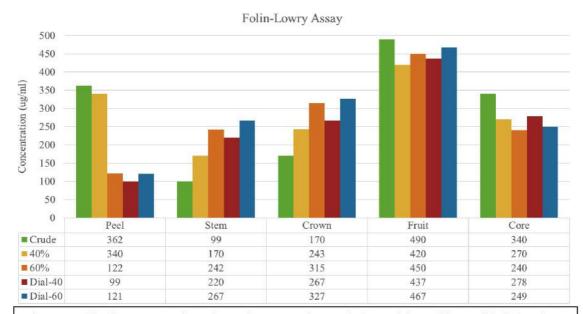


Fig6: Graphical representation of protein content in Crude bromelain and its purified fractions

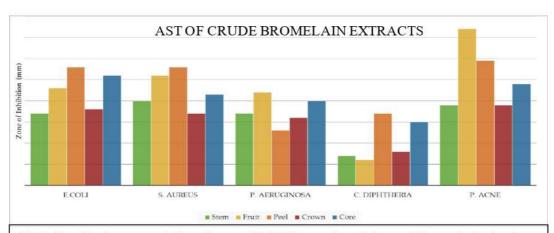


Fig7: Graphical representation of zone of inhibitions of crude bromelain against microbes

	E. coli	S. aureus	P. aeruginosa	C. diphtheria	P. acne
Stem	17 <u>+</u> 3mm	20 <u>+</u> 2mm	17 <u>+</u> 2mm	7 <u>+</u> 2mm	19 <u>+</u> 2mm
Fruit	23 <u>+</u> 5mm	26 <u>+</u> 4mm	22 <u>+</u> 2mm	6 <u>+</u> 1mm	37 <u>+</u> 4mm
Peel	28 <u>+</u> 6mm	28 <u>+</u> 2mm	13 <u>+</u> 4mm	17 <u>+</u> 5mm	29 <u>+</u> 5mm
Crown	18 <u>+</u> 3mm	17 <u>+</u> 2mm	16 <u>+</u> 4mm	8 <u>+</u> 2mm	19 <u>+</u> 2mm
Core	26 <u>+</u> 2mm	21 <u>+</u> 4mm	20+2mm	15 <u>+</u> 2mm	32 <u>+</u> 3mm
Table 1: Tabu	lar representatio	n of zone of in	hibitions of crud	e bromelain agai	nst microbes

### ANTIOXIDANT ASSAY

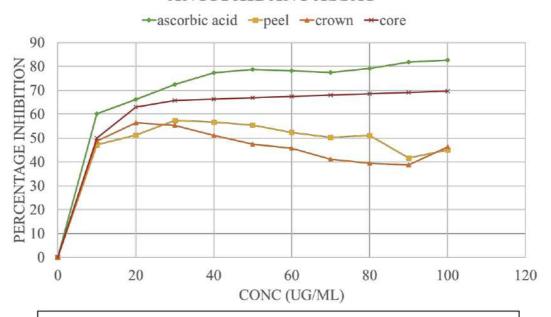


Fig8: Graphical representation of antioxidant assay performed by DPPH assay

	Percentage Inhibition(%)					
Concentration (ug/ml)	Ascorbic acid	Peel	Crown	Core		
0	0	0	0	0		
10	60.12	47.23	48.76	50		
20	66.201814	51.183673	56.43	63		
30	72.421769	57.43	55.22	65.78		
40	77.324263	56.718821	51.11	66.342		
50	78.684807	55.4	47.44	66.904		
60	78.231293	52.380952	45.77	67.466		
70	77.55102	50.3	41.1	68.028		
80	79.138322	51.147392	39.43	68.59		
90	81.777778	41.67	38.76	69.152		
100	82.684807	45.12	46.32	69.714		

Table2: Tabular representation antioxidant assay

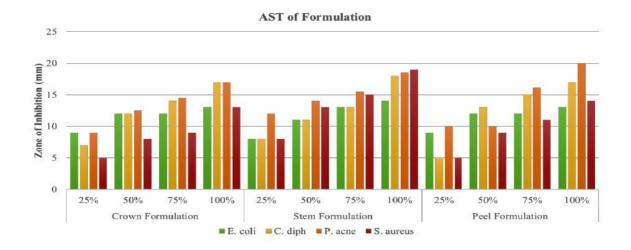


Fig9: Graphical representation of zone of inhibitions of bromelain formulation against microbes

	Crown Formulation					Stem Formulation				Peel Formulation			
	25%	50%	75%	100	25%	50%	75%	100	25%	50%	75%	100%	
	(mm)	(mm	(mm	(mm	(mm	(mm	(mm	(mm	(mm	(mm	(mm	(mm)	
E. coli	9	12	12	13	8	11	13	14	9	12	12	1 3	
C. diph	7	12	14	17	8	11	13	18	5	13	15	1 7	
P. acne	9	12.5	14.5	17	12	14	15.5	18.5	10	10	16.1	2 0	
S. aureus	5	8	9	13	8	13	15	19	5	9	11	1 4	

Table3: Tabular representation of zone of inhibitions of bromelain formulation against microbes

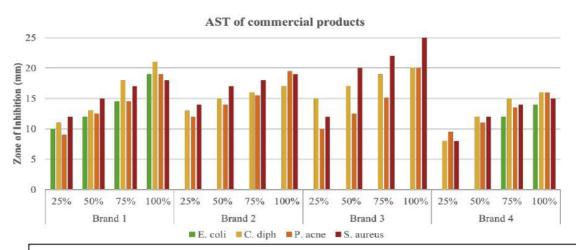


Fig10: Graphical representation of zone of inhibitions of commercial products against microbes

		Brand 1			Brand 2			Brand 3			Brand 4					
	25%	50%	75%	100 %	25%	50%	75%	100 %	25%	50%	75%	100 %	25%	50%	75%	100 %
	(mm	(mm )	(mm )	(mm )	(mm	(mm	(mm )	(mm	(mm)	(mm)	(mm )	(mm	(mm)	(mm )	(mm)	(mm
E. coli	10	12	14.5	19	0	0	0	0	0	0	0	0	0	0	12	14
C. diph	11	13	18	21	13	15	16	17	15	17	19	20	8	12	15	16
P. acne	9	12.5	14.5	19	12	14	15.5	19.5	10	12.5	15.1	20	9.5	11	13.5	16
S. aureus	12	15	17	18	14	17	18	19	12	20	22	25	8	12	14	15

Table4: Tabular representation of zone of inhibitions of commercial products against microbes

#### Discussion:

Pineapple is an age-old fruit, consumed and used traditionally for many of its medicinal properties. It has been used for debridement of skin (Houck JC et al, 1983). Bromelain is also used as a replacement during deficiency of pepsin and trypsin due to its activity and stability over a wide pH range. (Balakrishnan et al, 1981). It is also a potent treatment for periodontitis due to its antimicrobial effects against periodontal pathogens such as P. gingivalis (Praveen et al. 2014). Bromelain also nullifies the effects of enterotoxins produced by E. coli and V. cholerae and thereby prevents Diarrhea. (Mynott et al, 1997). Research also suggests that purification of Bromelain also increases its activity due to less interference of co-compounds. Therefore, cost effective methods need to be explored for extraction of Bromelain. Hence, why we decided to explore extraction of Bromelain from waste parts of Pineapple. Pineapple wastes are usually discarded and then allowed to biodegrade. Initially, we considered the fruit as the only edible member and treated the rest as waste i.e. the peel, the core, crown and the stem. But the core while usually not eaten is utilized in juice making, resulting in lesser easier availability of core. Usually most of the studies of Bromelain have been carried out using the fruit and the stem bromelain. The Bromelain concentration in peel decreased upon purification, however the crown and stem turned out to be good potential sources, the crown especially due to its abundant nature. We used ammonium sulphate precipitation and dialysis due to the ease of usage and also due to its lesser requirements making the procedure feasible. Bromelain was stored at 4\*C as it has been studied and reported that Bromelain starts to lose more than half its activity at 60°C and is completely degraded when the temperature hits 70°C. (Hale, 2005). Due to its antimicrobial properties, we decided to explore bromelain as a potential line of treatment against acne. We tested it out with S. aureus and P. acne, the bacterium most commonly associated as causative agents of acne. (B. Kumar et al, 2006) We initially tested it out with ditch plate method as easy to test it out against multiple microorganisms at once. We saw positive results as the microorganisms proved to be sensitive against crude bromelain particularly P. acne. Once its efficacy against P. acne had been established, formulation was a key task. Using proteins as a key 147 ingredient is a dicey process due to the physical and chemical stresses that it is subjected to may cause conformational and activity changes. The formulation was kept herbal and natural to be less harsh on the skin. Tea tree oil was used a natural preservative and Xanthan gum powder was used as a thickener instead of using the standard sodium benzoates and other thickeners. The formulation proved to be effective in terms of its

antimicrobial activity. Once we got the formulation to work, our key focus was to check if it was a viable market option in terms of cost and efficacy. We randomly chose four herbal facewashes acclaimed to be used for acne and anti-pimple and compared the antimicrobial action of the formulations we created and the commercially available one to establish its competitiveness. We created three different formulations owing to three different sources of Bromelain since we also wanted to compare the difference in activity of bromelain depending on its source. However, the activity remained statistically comparable to each other. So, whilst their molecular nature may or not be different, their effect and biochemical nature seems to be similar. The formulation as a facewash is more of a preventive measure rather than a direct treatment as it prevents the growth and proliferation of the bacterium. Bromela in can also be further explored to study it as a bactericidal gel with direct treatment for acne, however it's interaction with other skin flora may also need to be studied to see if it is a selective protease or not. Bromelain interactions with other skin proteases and antimicrobial peptides such as psoriasin can also be explored.

#### Conclusions:

Acne has always been a cause of great physical and social distress and due to its very sensitive and irritative nature, one has to be very cautious with poking around acne to prevent another outbreak or more distress to the skin. Bromelain has proved to be a potential protease that can be used clinically for treatment of acne owing to its antimicrobial activity and ease of purification and extraction from waste parts of the pineapple. Since the purified protease is more active and potent than the crude and the extraction is cost-effective, it makes a good candidate. Bromelain also has a wide stability range in terms of temperature and pH. The antioxidant property of bromelain also contributes to the benefit of skin in preventing oxidative stress.

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## Phytochemical Analysis and Antioxidant Analysis of fruit rind of *Couroupita guianensis* Aubl.

Sagarika Damle, Aravind Venkatasubramanian, Atmaya Vartak Department of Life Sciences, K.C. College, Churchgate, Mumbai 400 020

#### Abstract:-

Couroupita guianensis Aubl. also known as Kailashpati or Cannonball tree is a plant which belongs to family Lecythidaceae and is of common occurrence in Mumbai city. Leaves, flowers and fruit pulp of this plant are known to have anti-microbial, anti-fungal, anti-septic properties. However, no records are found on the use of shell or rind of the fruit which is considered as waste. This project elucidates different phytochemicals present in rind of the fruit and checks its antioxidant activity. The preliminary phytochemical studies revealed the presence of tannins such as phlobatannins, triterpenes, flavonoids, and alkaloids. The antioxidant activity was detected using DPPH assay, which showed decolourisation of sample. Isolation of phytoconstituents were done by two different methods viz, Thin Layer Chromatography and Column Chromatography. It was observed that Column chromatography can yield pure isolates, it is more time consuming than TLC. Thin Layer Chromatography allows to test multiple samples with different solvent systems within less time. The TLC plate can also be used to perform antioxidant test and anti-microbial test by bioautography.

Keywords:- Shell, Phytochemicals, Antioxidants, TLC, Colum Chromatography.

#### Introduction

Cannonball tree, also known as 'Kailashpati' is a tree that belongs to family Lecythidaceae and is native to Central and South America. The tree is religiously and culturally significant in The Republic of India. The flowers of the tree appear to resemble a 'Shivling' which is protected by a hooded Naga.

Due to this characteristic feature and the sweet fragrance that it produces, these trees are often seen planted near Shiva Temples. The tree is commonly spotted in Mumbai and is seen flowering throughout the year. The fruits serve as the identification mark for the tree. They appear like cannonballs hanging from the low hanging branches. The fruits

have a hard woody shell. The sour pulp has many seeds and contains Carbohydrates, Alkaloids, Glycosides, Phytosterols, Flavonoids, Tannins, etc (Pandurangan, Sahadeven, Sunkar, Krishna, & Mohana, 2018). The pulps are used to make a beverage from the pulp. The fruit has a characteristic foul odour which may be due to the presence of sulphur compounds in the fruit pulp (Nelson & Wheeler, 2005). The phytochemical analysis of a plant gives us insight into the various properties that the plant may possess like antioxidant, antimicrobial, etc properties. The shell of the plant is found to be separable into an inner and an outer shell. The comparative account of the inner and outer shell remains unexplored.

## **Materials and Methods**

### Collection of Plant material

Fresh fruits of Couroupita quianensis were collected from a tree near Chhatrapati Shivaji Maharaj Terminus, Mumbai. The fruits were washed and then broken apart and the fruit pulp was scooped out. The shells were then sundried and then dried in the hot air oven at 60°C and weighed. The drying was continued till three constant dry weight readings were obtained. The shells were then broken into small pieces using a mortar and pestle and then powdered in a mixer grinder. The powders of inner and outer shell were stored separately inside coloured bottles to avoid light degradation.

## Preparation of Extract

and then it was tied into a thimble using new sheet of muslin cloth. The thimbles were inserted into the Soxhlet extractor(Hot continuos extraction). The extraction was carried out at 80°C using ethanol (Cooper-Driver & Harborne, 2007). The extraction was carried out for 10 cycles and then the sample was sample was concentrated using a rotatory evaporator. The extracts obtained were subjected to phytochemical tests, and antioxidant assay by DPPH method.

## Phytochemical Analysis

The phytochemical analysis helps us to know the phytochemical composition of a given sample. This can be done by qualitative experiments for the detection of various compounds.

## Test for Carbohydrates

To 0.5ml of filtrate, 0.5ml of Benedict's reagent is added. The mixture is then heated on a boiling water bath for 2 min. A characteristic colored precipitate indicates the presence of sugar. Test for Alkaloids To a few drops of plant sample extract, 2 drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

## Test for Alkaloids

To a few drops of plant sample extract, 2 drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

## Test for Saponins

The extract is diluted with distilled water and made up to 20 ml. The suspension is shaken for 15 min. Formation of foam indicates the presence of Saponins.

## Test for Flavonoids

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

## Test for Tannins

To a few drops of extract, 2ml of dilute ferric chloride solution (5%) is added. Formation of violet color indicates the presence of tannins.

## Test for Terpenoids

To a 2 ml of extract, few drops of saturated solution of trichloro acetic acid are used. Formation of colored precipitate confirms the presence of terpenoids.

## Thin Layer Chromatography

TLC studies were carried out to detect the presence of various phytochemicals (Cooper-Driver & Harborne, 2007). It is a mode of chromatography in which the extract is spotted on end of a thin sorbent layer supported on a glass plate. The plate is prepared by dipping a clean glass plate into a slurry of silica gel made with chloroform. The plates are dried in the hot air oven at 110°C to activate the silica. The sample is spotted with a capillary tube. A solvents like Petroleum Ether, Acetone, Benzene, and Methanol were used. The plates were kept in pre-

saturated chromatography chamber and allowed to run. The best separation was observed in 8:3(Pet Ether:Acetone). The spots were visualized in Iodine gas chamber and yellow to brown spots were observed.

## Column Chromatography

Column Chromatography is a chromatographic technique which can be used to isolate phytochemicals based on their solubility in a given solvent system. The column chromatography of the samples were carried out. The solvent system used was Pet Ether: Acetone was used in increasing concentrations of Acetone (9:1, 8:2, 7:3, and so on). Bands of various colors like Pink, Yellow, Orange, etc were eluded from the column. Each fraction was collected in separate amber colored bottles and stored away from direct sunlight to avoid degradation by light.

## Antioxidant Assay by DPPH Method

The antioxidant activity of the extracts and standards was based on the radical scavenging effects of 2,2 diphenyl-1-picrylhydrazyl (DPPH) by modified method (Braca, Sortino, Politi, Morelli, & Mendez, 2002). The diluted working solutions of the samples were made in water. Ascorbic was used 10-100ug/ml. 0.002% DPPH was prepared in ethanol. The test sample/standard was mixed with DPPH in 1:1 ratio and incubated in the dark for 30 minutes. The solution mixtures were read at 517nm. The OD was recorded and percentage inhibition was calculated by the formula below. Ethanol with DPPH (1:1) is used as a blank. Percent

inhibition= $\frac{A-B}{A}$  x100 where A is OD of blank and B is OD of sample.

#### Results and Discussion

The preliminary phytochemical analysis of the sample showed the presence of Tannins, Terpenoids, and Flavonoids in the Inner shell, and the presence of Triterpenes, Terpenoids, Flavonoids, Tannins, Phlobatannins in the Outer shell. This indicates that there is a difference in the phytochemical composition of the Inner and Outer shell. Natural antioxidants present in herbs, spices, etc are responsible for the reduction or prevention of negative consequences caused by oxidative stress. They contain free radical scavenging

Table 1:- Detection of Phytochemicals

Phytochemical	Outer Shell	Inner Shell
Alkaloids	No	No
Saponins	No	No
Tannins	Yes	Yes
Flavonoids	Yes	Yes
Triterpenes	Yes	No
Terpenoids	Yes	Yes

Phlobatannins Yes No

compounds like flavonoids, phenols, and polyphenolic compounds. In this paper, we have evaluated the free radical scavenging activity of the powdered samples of inner and outer shells of Couroupita quianensis Aubl. The ethanolic extracts of the inner and outer shells showed free radical scavenging activity with IC<sub>50</sub> (µg/ml, Mean±SD) values of 9.2±0.1, and 10.3±0.3 respectively. These values are comparable to the IC<sub>5</sub> ovalues of the standard Ascorbic acid which was 8.9±0.1. However, the chemical constituents present in the extract and which cause this activity need to be investigated, it is probable that the activity is due to the presence of compounds like tannins, flavonoids, carbohydrates, etc which happen to be present in the samples. Several of such compounds are known to have potent antioxidant property and the ability to scavenge free radicals (Lee, Koo, & Min, 2004). As a result, the fact that the plant shows good antioxidant property, it may be used in drugs against cancer, hepatic diseases, etc.

#### Conclusion

The phytochemical analysis was carried out by TLC, and column chromatography. The composition of the inner and the outer shell were compared using these techniques. The antioxidant property of both samples were carried and it was found that they show DPPH

scavenging activity comparable to ascorbic acid. The work therefore has shown that it is potentially usable against diseases. Furthermore, the results of the study also support the use of the plant in traditional medicine and the knowledge can be used to assist in the provision of healthcare.

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# CHEMISTRY

"Chemistry begins in the stars, the stars are the source of chemical elements, which are building blocks of matter and core of our subject"

**ANONYMOUS** 

### EFFECT OF A.C. WATER ON KOTA TILE

## Pooja Jain

Email: jainpooja1245@gmail.com

Department of chemistry, K.C. College, Churchgate, Mumbai-20.

Author for correspondence\* Dr. Satish S. Kolte

## ABSTRACT

Kota tiles are preferred for flooring and wall cladding, and paving and facades of buildings. It is very tough, non water-absorbent, non-slip and non porous. Chemically Kota blue tiles are siliceous calcium carbonate rocks. It is acid sensitive. Air-conditioning water is essentially the same as distilled water; mineral free and a total dissolved solid level near zero. It may contain heavy metals from contact with the cooling coils and other HVAC equipment. Literature review shows that when AC water falls continuously drop by drop on Kota tiles, it results in erosion of Kota tiles. In the current research activity conductance and PH of AC water was measured before its fall on Kota tiles & after it is drain from for several days. The effect on pH & Conductance indicates the effect of erosion.

#### INTRODUCTION

### KOTA TILE:

- Kota tiles are fine-grained variety of limestone, quarried at Kota district, Rajasthan, India.
- Kota stone is hard, tough, oil resistant, and non-water absorbent, non-slippery and have excellent stone resolvability.
- Kota tiles as is better known in building stone terminology, is basically a calcareous sedimentary rock available in a number of colours (blue, green, brown, grey etc) and textures

- Literature studies reveals that the AC water is same as distilled water chemically.
- AC takes the hot air from our room, passes it over the pipes in which refrigerant flows, now the heat from air is taken by the refrigerant liquid and the air is cooled.
- Air contains moisture and this moisture condenses as the air cools.
- The water condensed on the pipes is then sent out using drain tube

#### BACKGROUND

 Observation of the effect of AC water on Kota tiles during passed three year.

#### A.C. WATER:

Tile	Weight of Tile(gms)	Type of Water	Amount in cm
1	18.249	AC Water	100
2	17.280	AC Water	100
3	18.375	AC Water	100
4	18.520	Concentrated AC Water	25
5	16.619	Distilled Water	50
6	15.788	Concentrated AC Water	100

- ► Kota tile becomes rough indicating erosion .
- ► Rough surface also accelerate algal growth
- 1: Procedure:- (Replicate analysis)
- 1. Collection of water from AC.
- 2. Take 6 beakers containing piece of Kota tile of dimension 1cm x 1cm

## <u>AIM</u>

> To investigate the causes of erosion of Kota tiles due to AC water.

## RESULTS

## **OBJECTIVES**

- Identify the cause behind erosion of Kota tiles.
- > Study the component of AC water.
- > Remedial measure

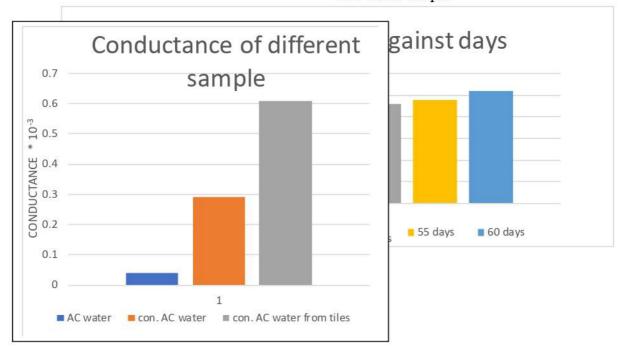
## **TOOLS**

> pH and Conductance

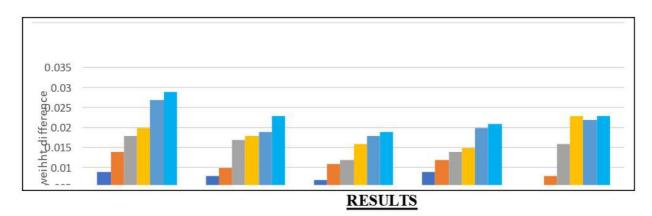
## **METHEDOLOGY**

Graph:

CASE 2 : Procedure:- concentration of A.C water sample.



- Creation of Situation of AC water interaction with Tile.
- ➤ 20 Litres water was allowed to drip on new Kota tile (Flow rate 1ml/min).
- Water collected was analysed for pH, Conductance.
- ➤ Concentration of water to 250 cm<sup>3</sup>
- Analysis of concentrated water for pH, Conductance and Hardness.
- > Observe the change in texture of tile.



## **METHEDOLOGY**

To determine calcium content of con.

AC water sample after flowing through

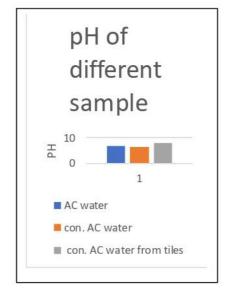
Kota tile by flame photometry.

## Ion Exchange Chromatography

- To determine calcium content of con. A/C water sample after flowing through Kota tile by ion exchange chromatography.
- ➤ **Result**: 5.7 ppm of calcium ions found in above con. Sample
- To determine calcium content of con. A/C water sample by ion exchange chromatography.
- ➤ **Result:** 3 ppm of calcium ions found in above con. A/C sample.

## Hardness comparison

- ➤ Hardness of con. A/C water is 22 ppm.
- ➤ Hardness of con. A/C water drain from Kota tile is 85 ppm.



**Result**: Hardness of con. A/C water is less than Hardness of con. A/C water drain from Kota tile

Analysis of A.C. sample which is concentrated after drain from Kota tile and diluted Kota tile sample using Inductively Coupled Plasma Atomic Emission Spectroscopy.

Vol. Flask no.	Vol. Of 1000ppm ca2+ sol.	Total volume	Con. Of ca2+ mg/l	Emission intensity
1	20	100	20	17.7
2	40	100	40	40.1, 41
3	60	100	60	65.4
4	80	100	80	80.5
5	100	100	100	89.4
6	Unknown 1%	100	1%	0.9, 0.0
7	Unknown 5%	100	5%	35.2, 22.5, 17, 22.5
8	Unknown 10%	100	10%	91.6, 49.7, 52.2, 58

## ❖ RESULTS

❖ The AC water become alkaline after flowing from Kota tiles as pH is

Sample	Si	Ca	Pb	Cl
	Ppm	ppm	ppm	Ppm
BLANK-AC WATER	1	1		
SAMPLE	7.053	10.2694	ND	1163.19
BLANK-KOTA TILE	1.5543	11.9402	0.0617	1099.14
DILUTED KOTA TILE	İ			
SAMPLE	147.257	2629.95	0.7502	962.459
CON AC WATER	8.153	131.743	ND	674.422

## CONCLUSION

- The release of Calcium ion from tile in the water.
- ❖ Increase in conductance of AC water.

increase.

- \* Erosion of Kota tiles due to AC water.
- Hardness of con. Ac water which is drain from Kota tile is more than con. A/C water.

## **FUTURE GOALS**

► Rate of erosion determination .

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## STUDY OF REACTIVE DYES AND THEIR BIODEGRADATION

## Shireen Siddiqui

Email siddiquishireenl@gmail.com

Department of Chemistry, K.C. College, Churchgate, Mumbai-20.

Author of correspondence\* Dr. Sheela Valecha

#### ABSTRACT

Coming from a background which deals with the dyeing and processing of the textile, the disposal of the wastewater of dyeing is a big concern.

This Wastewater from the industries which are toxic in nature is released directly in the environment. They are released in the surface waters through drainage system, seeps into the ground water and soil too near the water bodies.

Hence, cheaper method like Biodegradation of the dyes is to be carried out.

KEYWORDS- Dyes, Textile, Reactive, Biodegradation, Disposal

## **INTRODUCTION**

A dye is a natural or synthetic substance used to add color or change the color of something. There are different uses of dyes, one of which is textile dying.

There are various classes and types of dyes that are used for textiles such as:

- Acid Dyes
- Basic Dyes
- Synthetic Dyes
- Direct Dyes
- Natural Dyes
- Reactive Dyes
- Azo Dyes

Amongst many other dyes.

Certain Azo dyes are carcinogenic in nature and hence are banned all over the world. There are 112 Azo and Benzidine containing dyes which are banned in India.

The Dyes that are most commonly used in textile industries are Reactive Dyes.

The Reactive Dyes can be classified into further types:

- Vinyl Sulfone Based reactive dyes.
- · Cyanuric Chloride Based Cold dyes.
- Cyanuric Chloride Based Hot dyes.
- High Exhaust dyes.
- Bifunctional dyes.

<u>ADVANTAGES</u>: Reactive dyes have various advantages over other dyes and hence are most commonly used.

- Permanency in color: Reactive Dyes can be easily said to be most permanent of all dyes.
   This is because of the unique quality of the dye to form a covalent bond with the substrate of the cellulose.
- Easy washing: The fibre that are dyed can be safely washed with white garments without danger of coloring it.

- Chemical bonding: Chemical bond significantly improve product's color, stability and wash ability
- Wide range of color: These dyes provide a large range of colors.

It undergoes Nucleophilic substitution or addition.

General formula is S-F-T-X

Where, S: Solubalizing Group,

F: Chromophore,

T: Bridging group;

X: Reactive system.

It requires pH range of 11-11.5 and is water soluble.

**PROBLEM TO THE ENVIRONMENT:** The wastewater from the industries mostly from the small scale which are not treated before releasing in the environment.

Although it has many advantages and is most commonly used, in most case almost 50% of thedye is lost through hydrolysis during the dyeing process and therefore, appears as wastewater.

The textile industries are considered one of the most important industries all over the world and are considered as 5<sup>th</sup> largest source of foreign currency, but are also considered as main source of water pollution. They are also not easily scale industries have softener plants that treats this wastewater. Small scale industries do not have it as it is expensive and hence they cannot afford it.

<u>AIM AND OBJECTIVE</u>: As the other method being expensive, there should be an alternative affordable to these small scale industries.

Therefore, the idea of Biodegradation can be used for the degradation and decolorisation of the dyes. This can also be reuse the water if the degradation occurs.

#### PROCESS:

• Lower Cost: They are comparatively cheaper than the other dyes.

<u>COVALANCY</u>: Reactive Dyes are the only class of dyes which makes covalent bond with the fibre and becomes part of it

The process of fungal dye degradation consists of two stages. The first stage involves the reductive cleavage of the dyes' azo bond (-N=N-), resulting in the formation of aromatic amines, that is generally colorless but potentially hazardous. The second stage involves degradation of the aromatic amines under aerobic conditions.

There are factors affecting the decolorision of dyes.

Various Microorganism that decolorize reactive dyes.

The dye here used is reactive dye Navy Blue HE2R.

Organisms	Dyes	Reference	
<u>Fungi:</u> Trametes villosa Pycnoporus sanguineus	Drimaren Brilliant Blue	Machado et al., 2006	
Pleurotus ostreatus Funalia trogii Aspergillus ochraaceus	Remazol Brilliant Blue R Remazol Brilliant Blue R Reactive Blue - 25	Palmieri et al., 2005 Deveci., 2004 Parshetti et al., 2007	
Bacteria: Rhizobium radiobacter Pseudomonas luteola Citrobacter sp., CK3 Pseudomonas sp.	Reactive Red 141 Reactive azo dyes Reactive Red 180 Reactive Red 2	Telke st al ., 2008 Hu ., 1994 Wang et al ., 2009 Kalyani et al ., 2009	
Algae Synechocystis sp. Phormidium sp.	Reactive Red Remazol Blue, Reactive Black B	Karacakaya et al., 2009	
Yeast: Kluyveromyces marxianus IMB3	Remazol Black B	Meehan et al., 2000	

100 80 60 40 20 0 30 40 50 decolrization

This contains diazo group. It has structure as follows:

Further study of degradation of this and other reactive dye is carried out.

## **DECOLORIZATION OF NAVY BLUE HE2R**:

Biodegradation was carried out by the White Rot fungi. The strain was maintained on the Potato dextrose agar plate at 30°C and stored at 4°C.

Decolorization experiment was conducted in 250ml conical flask. This fungi was tested for its ability to decolorize selected Navy Blue HE2R dye over a period of 24 hours.

DECOLOURISATION%=<u>INITIAL</u>-

FINALx100

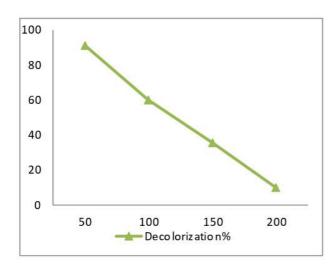
**INITIAL** 

EFFECT OF VARIOUS CONCENTRATIONS:

METHOD: To study the effect of different initial dye concentrations on decolorizing process, measured quantities of dyes as 50, 100, 150 and 200 ppm were added to the culture flask. These flasks were observed for decolourisation in 24 hours.

**OBSERVATION:** The rate and extent of decolourization was affected by the ranging concentration.

For the Navy Blue HE2R at concentration, 50 ppm, the decolorization was 90.75%, for 100ppm it was found out to be 59.90%, followed by 150 ppm (35.25%) and 200ppm (9.90%)



## EFFECT OF DIFFERENT TEMPERATURES:

METHOD: The effect of temperature on decolorisation process was studied by keeping the flask of the concentration of 50ppm of dye with the fungi at different temperatures of 30, 40 and 50°C.

**OBSERVATION:** The decolorisation was found to differ at different temperatures. It was maximum at 40°C i.e. 91.90.%, then 82.50% at 30°C and 73.33% at 50°C

TOXICITY TEST: Microbial toxicity test was further conducted to check the effect of the dye before and after decolorization on the growth of microorganisms with Escherichia Coli.

**OBSERVATION:** It was observed that the dye before decolorization was toxic to the microbial cells hence it showed inhibition on

the microbial growth. Whereas, the decolorized product showed no inhibition in the growth of the microbial cells and hence were not toxic to the microorganisms.

So, it was concluded that dye Navy Blue HE2R, which is toxic to the microbial cells, were converted into a non toxic product by decolorisation by the fungi.

<u>CONCLUSION</u>: In conclusion, discharge of effluent into water bodies is a serious environmental problem. Reactive dyes are azo based dyes and they are recalcitrant to degrade by conventional treatment method. The biological treatment is an effective alternate method to decolorize and mineralize the dyes in effluent without leaving harmful byproducts.

**FUTURE ASPECTS:** Other toxicity tests like phytotoxicity can be done. Futher, different parameters can also be taken into consideration with more range of dyes and microbes.

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## QUALITATIVE AND QUANTITATIVE ANALYSIS OF ORGANIC JUICES AND INDUSTRIAL BEVERAGES

#### Sohrab Feroze Sethna

Email sethnasohrab97@gmail.com

Department of Chemistry, K.C. College, Chruchgate, Mumbai - 20

Author of correspondence\* Mr. Karun Sodah

## **ABSTRACT**

## Introduction (Significance of the topic):

The topic for this research has been chosen very strategically. Coming from a business background which deals with manufacturing of concentrates of various flavors for use in the Food and Beverage industry, this is the best opportunity for me to get a broader perspective about Food Technology which will provide me with a first hand experience of what I am about to pursue in the future.

#### Components and aspects of the Research:

This research basically deals with the detailed analysis of various commercial beverages, which we all consume on a daily basis. It also deals with the analytical study of natural fruit juices for comparison with the commercial juices. The scope of this research includes estimation of the basic preservatives used, types and concentration of sugars added (Brick Levels) and to check the quality aspects in the manufacturing of these juices. The same scope extends to certain aerated beverages as well. An important factor taken up in this research, are the effects of these beverages on human health and metabolism. When this particular area of research is explored two very important questions arise in our minds

- 1. Are these commercial juices really "FRUIT" JUICES?
- 2. Are the juices really healthy and beneficial for human consumption as claimed?

After exploring the various aspects of this research certain, very important inferences have been drawn which are sure to shock us!

Keywords - Fruit Juices, Carbonated Drinks, Preservatives, Sugar Levels, Human Health.

## INTRODUCTION

A preservative is a substance or a chemical that is added to products such as food, beverages, pharmaceutical drugs, paints, biological samples, cosmetics, wood, and many other products to prevent decomposition by microbial growth or by undesirable chemical changes. In general, preservation is implemented in two modes, chemical and physical Chemical preservation entails adding chemical compounds to the product. Physical preservation entails processes such as refrigeration or drying. Preservative food additives reduce the risk of foodborne infections, decrease microbial spoilage, and preserve fresh attributes and nutritional quality. Some physical techniques for food preservation include dehydration, UV-C radiation, freeze-drying, and refrigeration. Chemical preservation and physical preservation techniques are sometimes combined.

In addition to basic food items, people also store frozen or preserved garden-grown fruits, vegetables and freeze- dried or canned produce.

Food can be classified as fast food, junk food, whole food, organic food out of these whole food is unprocessed, unrefined so it has very short shelf life. Nowadays Mostly all food products have food preservatives. The purpose is generally to preserve the natural characteristics of food and to increase the shelf life of food, and inhibit natural ageing and discoloration that can occur during food preparation such as the enzymatic browning reaction in apples after they are cut.

Natural methods of preservation usually aim to exclude air, moisture, and microorganisms, or to provide environments in which organisms that might cause spoilage cannot survive.

Natural way of Food Preservation can be done by Boiling, freezing, pasteurizing, dehydrating, smoking, pickling. Adding Sugar, sometimes sugar is combined with alcohol for preservation of luxury products such as fruit in brandy or other spirits, salt, alcohol, vinegar are also often used as food preservatives. They very efficiently drop the growth of bacteria in food. Coffee powder and soup are dehydrated, dried for preservation.

Artificial way of food preservation can be done by nuclear radiation, vacuum packing and hypobaric packing Nowadays certain synthetic Chemical are used as food preservatives. They are the most effective for a longer shelf life and stop or delay the growth of bacteria, suppress the reaction when food comes in contact with oxygen or heat, they also prevent the loss of some essential amino-acids and some vitamins enhance the food flavours and colours.

Sodium benzoate, Benzoic acid, Sodium sorbate, Potassium sorbate, Sodium nitrite are used as Antimicrobial agents they inhibit the growth of bacteria, molds, insects and other microorganisms.

Some substances used as Antioxidants (that act as free radical scavengers) are Vitamin E, Vitamin C, Pine Bark Extract, Grape Seed Extract, Sodium Erythorbate Sodium Diacetate, Sodium Succinate, Sodium Dehydro Acetate, Succinic Acid and Ascorbic Acid, Parabens, Erythorbic Acid, Proplyphenols.

Also some Chelating agents work as preservatives for example Disodium ethylenedia minete traacetic acid (EDTA), Polyphosphates, Citric acid and Ascorbic

Harmful Effects of Preservatives

There are certain harmful effects of using chemicals for preservation such as; Sulfites are common preservatives used in various fruits, may have side effects in form of headaches, palpitations, allergies, and even cancer.

Nitrates and Nitrites: These additives are used as curing agents in meat products it gets converted into nitrous acid when consumed and is suspected of causing stomach cancer

Benzoates are used in foods as antimicrobial preservatives, and have been suspected to cause allergies, asthma and skin rashes.

Sorbates /sorbic acid are added to foods as antimicrobial preservatives. Reactions to sorbates are rare, but have included reports of urticaria and contact dermatitis.

Also a nuclear radiation when used for preservation does not make foods radioactive, but may cause changes in food color or texture.

After consuming certain foods if it causes allergy that can be noticed but some people develop the symptoms of allergy day or two later, so it is difficult to know what is causing the problem. People consume variety of foods so it is difficult to find out the exact substance which causes allergy. For this reason people have to go on an elimination diet. They stop eating all foods that might be problematic and introduce one at a time to see if side reaction occurs. Side reactions of these preservatives can be immediate or build up in the body over time. Only in recent years have researchers

acid Monosodium Glutamate (MSG) Disodium Guanylate and Disodium Inosinate are used as food flavouring agents.

seriously considered the physical impact of these additives over the long term.

## OBJECTIVS/SCOPE OF THE RESEARCH

- To check the compliance of Orange Juice and other fruit juices with standard requirements.
- Estimation of different preservatives in different juices and other aerated drinks sold in the market.
- Estimation of Sugar Levels (Brick Levels) in these beverages.
- Estimation of other additives in different commercial beverages.
- Effects of different preservatives on human health and metabolism.
- To check the packaging and other parameters in the commercially sold fruit juices.

## PRATICAL RESEARCH UNDERTAKEN

## $\underline{\textbf{1.}} \ \textbf{PRESERVATIV}\underline{\textbf{ES}} \ \textbf{US}\underline{\textbf{ED}} \ \textbf{IN} \ \textbf{DIFFERENT} \ \textbf{COMMERCIAL} \ \textbf{BEVERAGES}.$

	ACCEPTABLE DAILY INTAKE (ADI)
PRESERVATIVE	(Mg/kg BODY WEIGHT)
Sodium Benzoate	5
Sodium Propionate	10
Potassium Sorbate	25
Sodium Diacetate	15
Sorbic Acid	25
Benzoic Acid	5
Sulphur Dioxide	0.7
Sodium Nitrate	0.2
Lactic Acid, Citric Acid and Acetic Acid	No Limit

## 2. LEVELS OF BENZOIC ACID IN SOFT DRINKS AND FRUIT JUICES.

#### I. Introduction;

The use of chemical preservatives has become important in food industries in INDIA. Benzoic acid is one of the commonly used chemical preservatives in the food and beverage industry. India has become a middle income country and as a result the production of fast, processed and convenient products especially in food and beverage industry by indigenous manufacturers has increased over the past decade due to lifestyle changes and improved standard of living. This has led to a lot of proliferation of soft and fruit drink industries.

A survey conducted showed that some manufacturers prepared the products with low quality assurance and few accredited laboratories. The use of benzoic acid as an antimicrobial agent has been observed to have adverse effects such as metabolic acidosis, convulsion, hyperactive and hyperpnoea in experimental animals and humans given very high doses of benzoic acid. The development of allergic reactions to benzoates in humans, such as urticaria, non-immunological contact urticaria and asthma, has also been reported in some studies. Benzene which is carcinogenic can be formed at very low level (ppb level) in soft and fruit drinks containing both benzoates and ascorbic acid. Exposure to heat and light further stimulate the reaction. The use of food additives is limited by specific regulations. India, as many countries, follows the

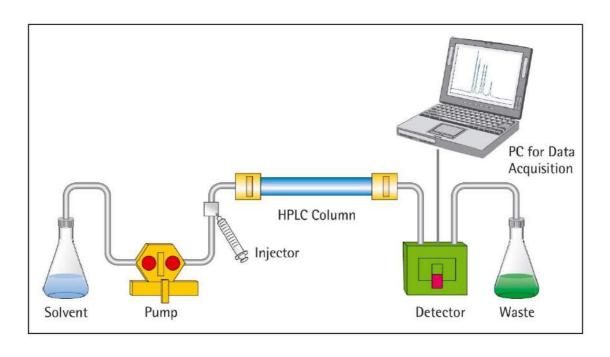
recommendations by the joint FAO/WHO Expert Committee on Food Additives on the safe use of food additives and limit of 150 mg/L benzoic acid has been established.

Although there are several analytical methods for the determination of levels of benzoic acid, such as spectrophotometric and gas chromatographic (GC) method, in this study we used high performance liquid chromatography (HPLC), which has a high specificity and minimum sample preparation and does not need derivatization.

In this study, the concentration of benzoic acid was measured in both soft and fiuit drinks. The results from this study will provide information for consumers' safety and health protection. The information obtained combined with consumption rate can also help to estimate the acceptable daily intake (ADI) of benzoic acid by consumers.

## II. Materials and Methods;

## HIGH PERFORMANCE LIQUID CHROMATOGRAPHY



### 2.1 Standards and Chemicals;

Benzoic acid, extra pure, Acetonitrile, (HPLC grade), Ammonium acetate, 97%, Acetic acid (AR) and Water (HPLC grade).

## 2.2 Chromatographic Conditions;

The chromatographic analysis was carried out by high -performance liquid chromatography. The analytical column was  $4\mu$  polar -RP 80A  $150\times2mm$  4 micron column with 325 UV detector.

The mobile phase contained a mixture of acetonitrile-ammonium acetate buffer adjusted to pH 4.4. The chromatographic separation was achieved with isocratic elution condition at a flow rate of 0.4 mL/min and 20 µL of sample were injected. The UV-spectrophotometer was used to determine the wavelength of maximum absorption of the benzoic acid prior to the analysis.

## 2.1 Calibration Curve of Standard of Benzoic Acid Solutions;

Standard solutions containing 1.0, 5.0, 15.0, 25.0, and 50.0 mg/L of pure benzoic acid were prepared. 20 µl of each standard was injected. The peak areas were measured and those of the analytes were plotted against the concentration (mg/L) of the benzoic acid. Least square linear regression analysis was used to determine the slope, y-intercept and the correlation coefficients of the standards plots. The quantification of the samples was based on comparison of the peaks of the standards with those of the samples.

### 2.3 Sample Preparation;

Five different brands of soft drinks (COCA-COLA, PEPSI, SPRITE, THUMBS-UP AND

MIRINDA) and 5 fruit juices (ORANGE, APPLE, CRANBERRY AND MIXED FRUIT

JUICES OF REAL BRAND AND TROPICANA ORANGE JUICE) were purchased from the open markets in three batches. No extraction was done, but the samples were filtered and centrifuged at 3000 rpm for 30 minutes and the supernatant taken. Samples were diluted prior to the analysis.

#### III. Results and Discussion;

The external standard method was used to calibrate the chromatographic system for benzoic acid quantification. Peak identification of the benzoic acid in various soft drinks and fruit juices were based on the comparison between the retention times of standard compounds and was confirmed by spiking known standard compounds to the sample. For that purpose standard solution prepared with pure benzoic acid ranging from 1.0 to 50.0 mg/L were used. The UV absorbance at 238 nm was obtained. The HPLC system used presented optimum separation with minimal bandwidth with elution time of  $7.5 \pm 0.27$  min for benzoic acid and 10 min was set for the run of each analysis. A total of 5 (n = 5) soft drinks and 5 (n = 5) fruit juices were analysed.

For the soft drink analysis, the concentration of benzoic acid detected in the samples ranged from not detected (nd) to 564.00 mg/L. Out of the 5 soft drink samples analysed only one sample had no levels of benzoic acid, 1 sample showed levels above the set standard for benzoic acid or benzoates in soft drinks (150 mg/L) with the rest within the range of zero to 150 mg/L.

# Thumbs-up had a level of Benzoic Acid of 152 mg/L.

The total estimate of intake of benzoic acid for soft and fruit drink for adults and children was 0.19 and 1.14 mg/L respectively (Table 5). Soft drinks contributed substantially to the intake of benzoic acid or benzoates with 0.19 mg/L for adults and 1.14 mg/L for children. The intake of benzoic acid for fruit juice was 0.00072 and 0.0043 mg/L for adults and children respectively. This also shows that the intake of benzoic acid is higher in soft drinks than fruit juices. This is because most of the fruit juices producers use pasteurization as the method in preserving their products. The high level of the per capita consumption of fruit juice shows that Indians take in much of fruit juice. This may be due to the abundance of natural fruits in the country. The potential daily intake of benzoic acid calculated are within the range of 0-5mg/L body weight per day.

5 samples of fruit juices were analysed for the levels of benzoic acid. The concentration of the samples ranged from not detected to 148 mg/L with a mean of 31.00 mg/L. One product had no level of benzoic acid. Most of the fruit juices that had labels which displayed as 'no preservatives' actually had some levels of benzoic acid in them.

## IV. Conclusion;

The analytical determination of benzoic acid as preservative is not only important for quality assurance purposes but also for consumer interest and protection. This work used a simple, fast HPLC method for the determination of benzoic acid in soft and fruit drinks samples in India. The results showed that the benzoic acid concentration varied between different kinds of soft drink samples, with some levels above the maximum values established by national and international legislation and authorities. Most of the products that declared "no preservatives" on some of the fruit juice were not in accordance with their label claims. There was a great significant difference between the levels of benzoic acid in the soft drinks and fruit juice as carried out by the one way analysis of variance

at a significant level of 5% with the soft drinks having high levels than the fruit juice. This may be attributed to the fact that most of the fruit juice manufacturers pasteurized their products and therefore tend to add little of the benzoic acid. In conclusion, for producers whose products exceed the legislation limit, it is recommended that whenever possible there should be judicious use of additives and benzoic acid levels should be regulated and only used in the control of yeast and bacteria in food products. A regular monitoring and education should be organized for soft drinks and fruit juice manufacturers in India. This work has given us a first hand information to the levels of benzoic acid in soft and fruit drinks on the Indian market and also the daily intake of benzoic acid by children and adults.

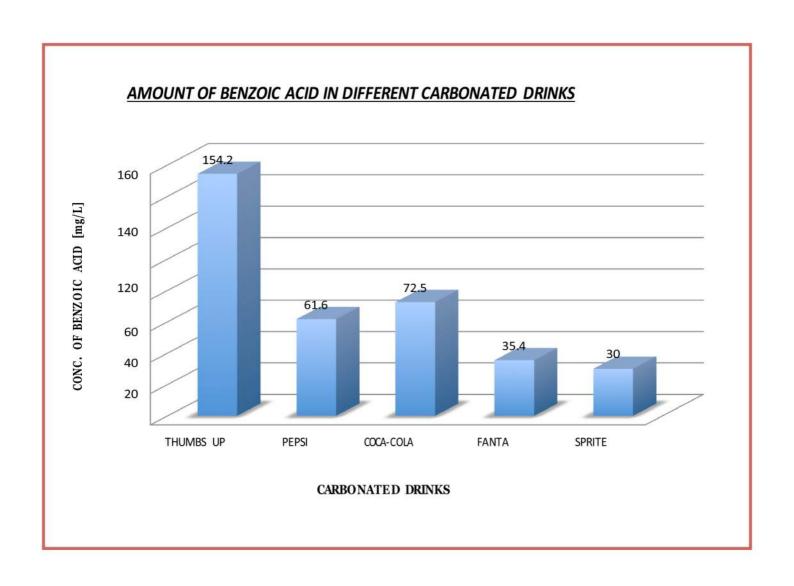
## ESTIMATED DAILY INTAKE (EDI) OF BENZOIC ACID

Category	Concentration [mg/L]	EDI for Adults [mg/L]	EDI for Children [mg/L]
Soft Drinks	70.48	0.19	1.14
Fruit Juice	31.00	0.00072	0.0043
		Total = 0.19072	Total = 1.1443

#### EFFECTS OF BENZOIC ACID

- The use of Benzoic acid as an antimicrobial agent has been observed to have adverse effects such as metabolic hyperactivity acidosis, humans with high doses.
- The development to allergic reactions to benzoates such as asthma has also been reported in some studies.
- When benzoates are mixed with Vitamin C (Ascorbic Acid), they form benzene, a known carcinogen and DNA damager.
- Like most chemicals that have toxic properties, the effects of benzoic acid depend on its concentration. At the low concentrations found in plants and foods, the chemical isn't dangerous for most people. Once it's eaten or produced, benzoic acid is absorbed through the lining of the digestive tract and eventually converted into Hippuric acid, which is excreted in the urine. 177

· Benzoic acid can irritate the eyes, skin, lungs, and digestive tract. Most people aren't high enough exposed to concentrations to experience this irritation, but people who work with the pure chemical or concentrated mixtures need to be careful. Benzoic acid can enter the body through the skin as well as the digestive and respiratory tracts.



# <u>DETERMINATION OF PRESERVATIVE SODIUM BENZOATE IN SELECTED SAMPLES OF BEVERAGES.</u>

### I. Introduction;

Fruit juices are consumed globally, not merely owing to their flavor, taste, and freshness, but also because of their valuable wellbeing properties while consumed on regular basis. Several people are becoming aware of the significance of consuming fruits in their every day diet as fruit juices are appropriate and suitable sources of carotenoids and polyphenolic compound exerts antioxidative, immunomodulatory and antimicrobial effect.

The most preferred oldest chemical preservative used in cosmetic and food industries is sodium benzoate which is soluble in water, a white granular or crystalline powder in appearance. Sodium benzoate is used as a common food preservative in salad dressings, carbonated drinks, jams, fruit juices and other condiments and is labeled by the code E211, classified "Generally Recognized As Safe"

(GRAS) and regulates the concentration of sodium benzoate to 0.1% by weight in food products by The US Food and Drug Administration (FDA). Its content must be declared and must not exceed the established limits by legislation. In season, the fruit is preserved to make it available for further use in o season. During preparation of packed juices, more preservative is added, thus increasing its amount in the finished product. Benzoates and sorbates are the most commonly used preservatives in food stuffs and are analyzed by using anion exchange high-performance liquid chromatography (RP-HPLC), capable of determining microgram quantities of preservatives.

# Determination of Sodium Benzoate in Mango Fruit Drink;

Mango is one of the main tropical fruit and is considered to be among one of the most consumed fresh fruits in the world. Mango is a unique fruit in terms of diverse products processed from it especially from its immature to fully ripe stages. Due to antimicrobial effect of potassium meta bisulfite and sodium benzoate, mango pulp is effectively preserved using these chemicals at suitable concentration. Mango nectar a ready to drink beverage is prepared by diluting single-strength mango pulp with the addition of sugar, water and preservatives to make product that has a certain percentage of mango pulp/ puree. Mango nectar can be made either from mangoes directly or from canned, aseptically packaged or frozen puree. The percentage of pulp in the beverage must be noted on the label. To be labeled as a fruit juice, the US Food and Drug Administration (FDA) mandate that a product be 100% fruit juice. In general, juice drinks contain between 10% and 99% juice and added sweeteners, flavors, and sometimes fortifiers, such as vitamin C or calcium. According to FDA regulations, these ingredients must be listed on the label. Some juices have naturally occurring high contents of potassium, vitamin A, and vitamin C. In addition, some juices and juice drinks are fortified with vitamin C, vitamin D and calcium. Juice contains no fat or cholesterol, and unless the pulp is included, it contains no fiber.

In a study conducted, Different brands of Mango soft drinks, available in the market were taken. These were FROOTI, MAAZA AND SLICE. Benzoic and Sorbic acids were analyzed by high performance liquid chromatography (HPLC). Only one sample presented a preservative level above that permitted by the legislation that was SLICE. This study was conducted so that awareness could be created among consumers regarding safety of processed fruit juices and as far as the use of sodium benzoate is concerned.

### II. Methods;

Quantitative research was undertaken to determine the amount of preservative sodium benzoate in selected samples of fruit juices. Various brands of fruit juices available in the market were taken for analysis. Fruit juice flavor included was mango where as brands included were FROOTI, SLICE and MAAZA. Six samples were subjected to determination of sodium benzoate content as preservative in fruit juices. The samples were analyzed for sodium benzoate content twice. All of the samples of the drinks were procured from the market in the month of March for analysis of sodium benzoate content. Titrimetric method was used to determine amount of sodium benzoate as a preservative in samples of drinks as described in A.O.A.C (Association of Official Analytical Chemists) Manual.

#### Sodium benzoate was calculated as:

1ml of 0.05 N NaOH = 0.0072 gm of anhydrous sodium benzoate.

That is titrated value multiplied by 0.0072. Sodium Benzoate content is obtained in grams. To obtain sodium benzoate content in milligrams multiply it with 1000. The data collected was compiled and tabulated using computerized data base management programs. MS Excel was used for the interpretation and analysis of the data. Sodium benzoate values of the sample were compared with the standards i.e. 0.1% as set by International Standards of Health.

## III. Results;

Sodium benzoate as a preservative was extracted in six samples of mango juice of the above brands. Sodium benzoate was extracted in very minute quantities in the mango juice of MAAZA brand. The analysis of mango juice samples showed that sodium benzoate extraction varied from one season to another

due to seasonal availability of fruit. It is evident from the table that mango juice of SLICE brand contained 0.102% sodium benzoate which slightly exceeded the standard value of 0.1% maximum during winter season due to non availability of fresh fruit in the market. It was further observed that pack of the mango juice was labeled as "Preservative" without specifying its proper name. In mango juice of FROOTI and MAAZA brands both values were found to be within limits i.e. 0.092% in winter season and 0.083% in summer season. The label of mango juice FROOTI brand provided no information about any preservative. Determination of sodium benzoate in samples showed that sodium benzoate was extracted in very less amounts in the brands of MAAZA. On label of FROOTI brand of mango juice the preservative "sodium benzoate E211" was mentioned but it certainly did not contain sodium benzoate, whereas on label of mango juice of SLICE brand word "permitted preservative" was mentioned but sodium benzoate of mango juice showed the mango juice exceeded the standard value in winter season that is 0.102%.

Brand Name	Winter Season Value	% Sodium Benzoate	Summer Season Value	% sodium Benzoate	Standard Value 0.1%
	mg/100ml		Mg/100ml		
Maaza	92.16	0.092	83.52	0.083	Within Limit
Frooti	93.34	0.096	92.21	0.092	Within Limit
Slice	102.24	0.102	98.64	0.098	Beyond Limit in Winter Season

### Determination of Sodium Benzoate Preservative in Orange Juice;

Different samples of Orange Fruit Juice were taken. These were: REAL ORANGE JUICE, TROPICANA ORANGE JUICE, MINUTE MAID PULPY ORANGE and RAW Orange Juice.

The same analysis procedure was carried out as done for Mango Drinks and certain results were drawn.

Sodium benzoate as a preservative was extracted to safe level in four samples of orange juice of above brands. Sodium benzoate was not extracted in the orange juice of RAW brand. The analysis of orange juice samples of REAL and TROPICANA showed that sodium benzoate extraction varied from one season to another due to seasonal availability of fruit. It is evident from the table that sodium benzoate was extracted at higher amount in summer season and relatively at a lower amount in winter season due to non availability of fruit in the market. The values found from the analysis conducted during winter and summer season

lied within the limit as set by International standards of Health to 0.1% maximum. It was further observed that pack of the orange juice was labeled as "Preservative" without specifying its proper name in TROPICANA brand. In orange juice of REAL brand "All Pure No Preservative" was written but sample analyzed during winter and summer season indicate that sodium benzoate was extracted below 0.1% maximum. On label of PULPY ORANGE brand of orange juice the preservative "sodium benzoate E211" was mentioned. On label of orange juice of RAW brand "100% Pure No Preservative" was mentioned and it did not contain preservative.

Brand Name	Winter Season Value mg/100ml	% Sodium Benzoate	Summer Season Value mg/100ml	% Sodium Benzoate	Standard Value 0.1%
RAW	ND	ND	ND	ND	ND
TROPICANA	75.6	0.075	82.8	0.082	Within Limit
REAL	82.8	0.082	87.4	0.087	Within Limit
PULPY ORANGE	92.8	0.092	95.4	0.095	Within Limit

ND = Not Detected

#### IV. Discussion;

According to the results there is a seasonal variation in the amount of sodium benzoate. Mostly the levels of sodium benzoate in mango and orange juices of all brands during summers and winters were within the limits approved by FDA (i.e. 0.1%), except a slightly increased value of sodium benzoate in mango flavor of SLICE during winters (0.102%). The presence of sodium benzoate in finit juices available at local market, it was observed that in two one sample of fruit juice was found to be deviated from FDA approved range of addition of sodium benzoate. Results of this study cannot be compared with previous studies, as different method of detection (HPLC) was used in them while in current study the method of detection used was titrimetric.

### V. Conclusion;

Results of the current study showed the presence of sodium benzoate in fruit juices and 183 squashes available at local market. This study was attempted to provide information about amount of preservative added to fruit juices and squashes. So that awareness could be created among consumers regarding safety of processed fruit juices, as far as the use of sodium benzoate is concerned.

### EFFECTS OF SODIUM BENZOATE ON HUMAN HEALTH

Studies suggest that acceptable amount of sodium benzoate is around 10-15g in a month. Consumption of sodium benzoate more than 12g has adverse effects on human health which include the following:

- Severe headache, fainting and blurred vision.
- Chest pains and slow heart rate.
- Asthma attacks and high blood pressure

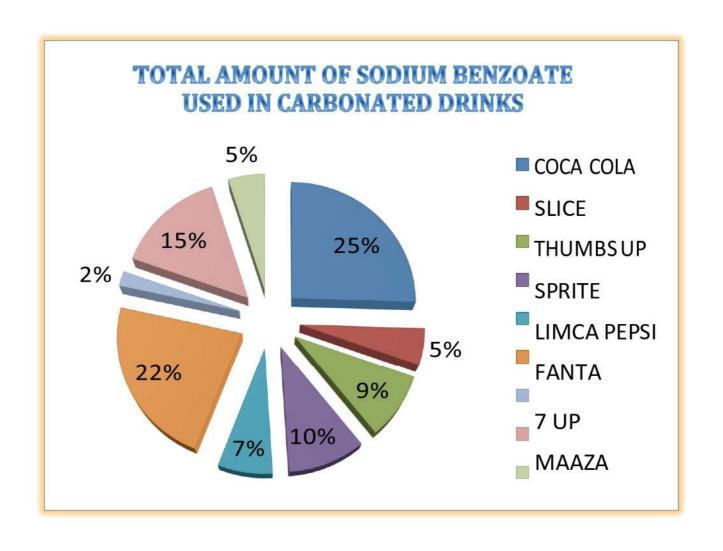
 Causes damage to important parts of DNA.

### FACTS;

• AVERAGE INTAKE OF SODIUM BENZOATE DUE TO JUICES IS 13g.

- A PERSON DRINKING ONLY ONE GLASS OF JUICE EVERYDAY CONSUME 1.3g OF SODIUM BENZOATE IN JUST 3 DAYS.
- CERTAIN FRUIT JUICES CONTAIN AROUND 1.5g/L OF SODIUM BENZOATE RATHER THAN 1.3g/L.

HENCE DUE TO EXCESSIVE CONSUMPTION OF COMMERCIALLY SOLD FRUIT JUICES AND DRINKS THERE ARE VARIOUS HARMFUL EFFECTS ON HUMAN HEALTH AND METABOLISM.



### <u>DETECTION OF SODIUM BENZOATE</u> IN DIFFERENT CARBONATED DRINKS.

A Comparative analysis and study was conducted for the amount and quantification of Sodium Benzoate in different commercially sold aerated drinks available in the market. Certain results and inferences have been drawn out and given above in a chart form.

vessel and stir to mix. Press the START button of the Reflectometer and at the same time immerse both reaction zones of the test strip in the measurement sample for approx. 2 s. Allow excess liquid to run off via the long edge of the strip onto an absorbent paper towel. Immediately insert the test strip into the strip adapter. After 10 minutes the strip is measured in the Reflectometer. The result [mg/l] is automatically stored.

### III. Serial Measurements;

### ESTIMATION OF TOTAL SUGARS IN APPLE JUICE

### I. Sample Preparation;

Dilute the fruit-juice sample with distilled water (dilution factor 250). To achieve this dilution pipette 0.2 ml of sample into a 50-ml volumetric flask and make up to the mark with distilled water.

### II. Analysis;

Place 10 ml of distilled water, and 1 ml of the correspondingly diluted sample in the test

After the first measurement, any number of further measurements can be made by pressing the START button. This, however, requires the use of a stopwatch, since the countdown function of the Reflectometer is available only for the first measurement. The detection zones of the test strip must be appropriately protected from light during the reaction time.

### IV. Calculation Scheme;

Total sugar content [mg/l] = result [mg/l] x dilution factor Total sugar content [g/l] = result [mg/l] x dilution factor: 1000

### V. Results;

Sample	Total Sugar [g/l]		
Real Apple Juice	62.5		
Tropicana Apple Juice	60.0		
RAW Apple Juice	54.8		

The content of total sugar in fruit juice is essentially made up of Glucose, Fructose and Sucrose. Hence the total sugar test including the amount of Sucrose is given below:

Sample	Stated on label	Total sugar	Sucrose	
	[g/l]	[g/l]	[g/l]	
Real Apple Juice	100.0	60.0	35.6	
RAW Apple Juice	90.0	54.8	28.8	
Tropicana Apple Juice	89.0	60.0	32.6	

### Contents of Total Sugars in other fruit Juices;

Fruit Juices	Glucose	Fructose	Sucrose
	[g/l]	[g/l]	[g/l]
Real Orange Juice	24.0	64.0	17.0
Tropicana Grape Juice	81.0	83.0	2.0
Real Cranberry Juice	26.0	29.0	45.1
Real Mixed Fruit Juice	26.6	26.7	68.3

From the above tests, experiments and studies carried out, one thing is clear that every commercially sold fiuit juices, and carbonated soft drinks do contain heavy quantities of numerous chemical preservatives in them. Though these preservatives do increase the shelf-life of the beverages and are commercially feasible, health wise, excessive consumption of these beverages in harmful for human health and metabolism.

Coming back to the 2 important questions; Whether these commercial juices are really "FRUIT JUICES"? The answer is NO. These are chemical drinks which contain less than 50% of natural pulps and natural additives and the major composition of these beverages are just chemicals. Also, Are these juices really beneficial for consumption as claimed? The answer lies in the research itself.

### **FUTURE ASPECTS**

- To study the packaging schemes of beverages and to see their effects on the concentrations of preservatives and additives.
- To estimate the amounts of different additives in commercial beverages.
- To study the quantification of Sorbic Acid, Sodium Propionate and Sulphur Dioxide Preservatives in different beverages.
- To carry out a comparative study of the commercial juices with natural fruit juices in terms of Brix Levels and Preservative Concentrations.
- To study about the different types of colour additives added in different beverages.

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# BIOTECHNOLOGY

"Our world is built on biology and once we begin to understand it, then it becomes a technology"

RYAN BETHENCOURT

### PRODUCTION OF LACTIC ACID VIA FERMENTATION OF LEAVES OF AMARANTHUS SPECIES

#### Shubhankar Dube

Biotechnology Department, Kishanchand Chellaram College, Churchgate, Mumbai, India.

\*Author for correspondence Dr. Pratibha Shah, Hajra.AS.Gupta

### ABSTRACT

Amaranthus is a genus of cosmopolitan plants which consists of annual as well as short lived perennial plants. These plants generally are weeds which grow and spread rapidly owing to high rates of seed production and herbicide resistance causing trouble for farmers all over the world. Most of the farmers end up burning these weeds to get rid of them, this study provides a commercial alternative to the current trend. A consortia of *Streptococci and Lactobacilli* obtained from freshly prepared curd was used to conduct fermentation of hydrolysed leaves of cowpea. Cowpea plant was easily accessible and was used as a representative of entire amaranth. Fermentation was conducted for 48hrs and precipitation technique was used to separate out calcium lactate salts. Upon acidification using sulphuric acid, the calcium is precipitated out and crude lactic acid was obtained. Since this study focuses on its application in agricultural field, simpler and cheaper techniques were used to ensure that this avenue is commercially viable. The production of lactic acid with this substrate and the extraction techniques used, can be used by small laboratory set up by farmers providing much less economic strain and supervision.

Key Words: Lactic acid, Amaranthus, fermentation, precipitation.

### INTRODUCTION

In this modern age, we highly rely on use of polymers in our day to day life, be it meagre packaging materials or exceptional auto ancillaries. Lactic acid is a widely used monomer for making organic polymers, also known as poly-lactic acid (PLA)1. It is an optically active organic acid with three carbon atoms. This hygroscopic compound is colourless in its liquid state and is miscible in water and ethanol. The market for this organic acid is exponentially increasing with the growth of industrial sector. It is mainly used in food industries making also is its way into pharmaceuticals, cosmeceuticals. healthcare and biodegradables<sup>2</sup>. worldwide production on year 2005 was approximately 120000 tonnes per year<sup>3</sup>. Asia-Pacific region is projected to be the fastest growing market for the same. Naturally, this calls for better and cheaper techniques to meet the ever-growing requirements.

Lactic acid can be produced both naturally and synthetically. Chemical synthesis being a more crude method, mainly produces racemic lactic acid is eclipsed by the biological synthesis which offers the advantage of enantiopure products.

This study proposes use of leaves of Amaranthus spp. as a substrate for lactic acid bacteria (LAB) to metabolise and produce lactic acid in the process. The amaranth primarily consists of wild, rapidly growing and spreading weeds. These weeds have been known to be a big problem for farmers and they eventually burn the entire growth to get rid of it. The study provides a commercial alternative to that.

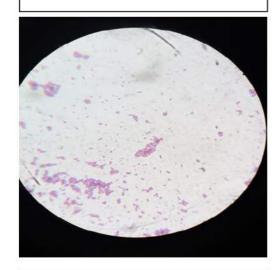
### **MATERIALS & METHODS**

### <u>Isolation of LAB</u>:

Dairy products are a great source for LAB, freshly prepared curd was used. The sample was streaked onto Rogosa agar & incubated in anaerobic chamber for 72hrs at room temperature. Lactobacilli & Streptococci strains were isolated by this method. The strains were found to be Gram positive, catalase negative & microaerophilic.



Lactobacilli



Streptococci

### Hydrolysis of sugars in leaves:

Cowpea leaves were used as a representative of amaranth due to its availability. The leaves contain higher carbohydrates which cannot be metabolized by microbes so acid hydrolysis is conducted to break them down into simpler pentose & hexose sugars.

The leaves were powdered, dried & mixed in 1% H<sub>2</sub>SO<sub>4</sub>. The mixture was then autoclaved for 40-60 minutes & then the centrifuged to remove leaf powder. The solution now contains hydrolysed simpler sugars.<sup>4</sup>

### Fermentation:

The inoculum was prepared by introducing both the strains into Rogosa broth (pH 5-6) & incubating it anaerobically at room temperature for 72 hrs. The broth will show presence of thick yellowish layer at the bottom which was pipetted out and transferred aseptically to the fermentation broth. The fermentation broth was Rogosa broth with the hydrolysed sugar sample used instead of glucose.

The fermentation was conducted for 24-48hrs & calcium carbonate was added to the fermentation broth after this period. This causes formation of calcium lactate salts from the lactic acid present in broth.

### Extraction of lactic acid:

The calcium lactate salts settle down at the bottom of broth which are then centrifuged & removed from the broth. It is then given multiple washes with water & 70% ethanol. The salt is then resuspended in water. To this solution, conc. Sulphuric acid is added. This causes a displacement reaction to take

place which leads to formation of lactic acid & calcium sulphate as salt.

Due to presence of impurities from fermentation broth, the solution formed after acidification process was distilled through a mini distillation apparatus. A clear colourless solution was removed at 94°C. A brackish black solution was left behind which showed to have hygroscopic properties. Lactic acid is known to be hygroscopic in nature so it is suspected that the solution contains lactic acid.

After about 45 minutes, the chromatogram was developed with bromocresol purple.



Acidified solution



Black hygroscopic solution



Clear solution obtained at 94°C

### Detection of lactic acid:

The lactic acid can be detected easily by using paper chromatography technique. The hygroscopic solution & lactic acid standard was spotted onto paper. The solvent system consisted of ethanol:liquor ammonia:water (80:4:16). The paper was dipped into solvent system and allow to run.

### RESULT

Lactic acid was produced via fermentation of hydrolysed cowpea leaves. Paper chromatography was conducted to detect the presence of lactic acid. The chromatogram showed the spot same as standard solution indicating that the solution contains lactic acid. However this

solution still contains impurities from the fermentation broth.

### **DISCUSSION**

The hydrolysis method used here was nonenzymatic hence inefficient at breaking down the cellulose into glucose<sup>5</sup>. It also consisted of larger quantities of pentose sugars which won't yield lactic acid when metabolized. However in order to mainta in simplicity of the procedure for practical application in farms, this procedure was used despite of its shortcomings.

Another study focused on using rice straws as a substrate for fermentation due to its mass production in China<sup>6</sup>. It involved use of LAB community with capability of lactic acid production. enhanced emphasized on quality of product over quantity since substrate was ample & cheap. This study tackles a similar problem pertaining weeds. however due simplicity of the procedure, quality of product obtained is not as good, however quantity is not an issue. Provided more research is done in developing a more efficient and suitable consortia is done, the issue with the quality of product can be dealt with.

### CONCLUSION

The crude lactic acid produced by this procedure can be another source of income for farmers while also being an incentive to prevent burning weeds and causing air pollution. Farmers with large fields can set up a small laboratory & with minimum investment.

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# COMPARISON OF CULTURE INDEPENDENT AND CULTURE DEPENDENT TECHNIQUES TO ENUMERATE THE NUMBER OF MICROORGANISMS FROM WATER SAMPLES IN MUMBAI

### JANAV ANANMAY M PANCHAL1

Department of Biotechnology, Kishinchand Chellaram College, Mumbai, Maharashtra, India

Author of correspondence\* Dr. Sejal Rathod2, Amina Dholkawala3

### **ABSTRACT**

Culture-dependent techniques have gained much popularity in determining the microbial load of samples. But there are other techniques termed as Culture-independent techniques, which are just as effective in determining the same. The latter exploits the use of extraction of nucleic acids from the microorganisms present in that sample, and then correlating the quantified nucleic acids with standard data. Thereby giving rise to the number of microorganisms that would have been present in the sample. This study compares methods under both culture-dependent and culture-independent techniques, and discusses the level of similarities and deviations seen while carrying out the above mentioned techniques on water samples collected from Mumbai. India.

**Keywords:** Culture independent techniques, DNA extraction, Millipore filtration, Enumeration, Water sample study, Mumbai.

#### INTRODUCTION

Microorganisms are extremely tiny but yet can be highly pathogenic, they are present all over the biosphere and are impossible to eradicate completely. Some organisms maybe also be present as the normal flora on and inside our body systems.

Water is an integral part of a developing country like India, it is therefore essential to test the microbial load of the water, assess the nature of the microbes within it and correlate it to the type of diseases they cause. The vast majority of microbes are keeched into our water systems due to industrial and public activities; like unchecked effluent dumping, non-compliance to effluent treatment and irresponsible citizen activities. Unchecked hospital and municipal wastewaters may contain pathogenic viruses, multiple drug resistant mutants, yeasts, protozoa, parasite eggs and a host of other deadly microorganisms.

This study assesses the microbial load of water samples collected from various sites in Mumbai which is important for the people who come in contact with these waters on a daily basis directly or indirectly on a sanitation standpoint.

The use of simple techniques for both Culture Dependent and Culture independent techniques are advocated, for they serve as economical and yet reliable options for assessing the water quality. Culture Independent techniques especially are further elaborated and prioritized for the simple reason being that they are superior and accurate for organisms that fail to be cultured on laboratory based media, such as VBNC (Viable But Non Culturable) bacteria.

### MATERIALS AND METHODS

The water samples for this study have been grouped into two "study-sets" viz. Natural and Commercial. Samples collected from Chowpatty (South Mumbai), Vasai creek, Vihar Lake (Powai) serve as Natural Sources for this study-set. And samples from household's and college's tap water system serve as Commercial sources for this study-set.

The techniques employed for this study involve two categories namely – Culture Dependent methods and Culture Independent methods.

Firstly, the Culture Dependent methods used here are the Viable Count Methods which includes both Spread plate and Pour plate techniques.

Secondly, the Culture Independent method used here is the complete DNA Extraction via the TRIZOL Method followed by Spectrophotometric analysis and comparison with standard data from research papers.

The Culture Dependent technique for Viable Counts involves –

- (i) Spread plate technique This technique involves serial dilution of the water sample in concentrations ranging 10-1 to 10-8. Followed by spreading a 0.1ml aliquot aseptically onto Nutrient Agar plates with a sterile glass spreader, until completely dry. Incubation of the plates at 37°C for 24h. Postincubation, calculation of the CFU (Colony Forming Units)/ml is done. [1]
- (ii) Pour plate technique This technique also involves serial dilutions in a similar 10-1 to 10-8 fashion. Followed by the addition of 0.3ml aliquot into sterile molten Nutrient Agar butts, at a favorable temperature with caution. Next, these butts are poured into sterile glass petri plates and are allowed to solidify. Incubation succeeds this step at 37°C for 24h. Post-incubation, calculation of the CFU (Colony Forming Units)/ml is done and Results data is documented. [2]

Simultaneously, for the same given water sample being studied, Culture Independent techniques are also carried out.

The Culture Independent technique involves -

Millipore Filtration - of the water sample with Cellulose Nitrate Paper for a volume of litre.

Carried out in sterile conditions aided by a vacuum pump and Millipore Filtration Assembly.

Transfer of the filter paper to eppendorf tubes – after cutting the filter paper in a 1cm x 1cm fragment.

DNA extraction with the TRIZOL Reagent (1ml) – which contains phenol and guanidine isothic ynate as two of the most crucial chemicals for the lysis and extraction of the DNA.

Also, 200µl chloroform (Centrifuge for 15mins at 12,000 rpm at 4°C) and 300µl absolute ethanol (Centrifuge for 5min at 3,000 rpm at 4°C). Pelleting and resuspending DNA (in DEPC water) [3]

Spectrophotometric analysis of the DNA pelleted with DEPC Water (Diethyl pyrocarbonate) (serves as blank) at 260nm.

Enumerating the total number of cells present for the given sample using appropriate formulae and calculations. (As discussed further in the Results section)

The above two techniques are carried out on the same day for a given sample to avoid any discrepancies in and during result data evaluation.

### **RESULTS**

The data obtained from carrying out the above mentioned techniques are categorized as per their sample sources and tabulated as observed below.

Formulae employed for Calculation -

Culture Dependent techniques:

$$CFU/ml = \frac{\text{Number of colonies x Dilution Factor}}{\text{Volume of Culture Plate}}$$

Culture Independent techniques:

Conc of DNA =  $A260 \times Dilution$  factor x 50

Where, 50 = A260 (1.0 for pure DNA)

No. of cells = 
$$\frac{\text{Conc of DNA}}{2.5 \times 10^{-9}}$$
 [4]

For Natural Sources – the average number of microbial cells per ml are as follows:

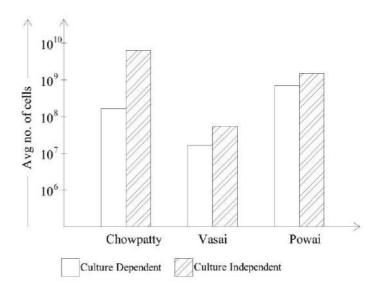
Source of	Culture	Culture
Origin	Dependent	Independent
	(CFU/ml)	(cells/ml)
Chowpatty	3.60 x 108	8.50 x 109
Vasai	2.13 x 107	7.00 x 107
Powai	9.25 x 108	1.50 x 109

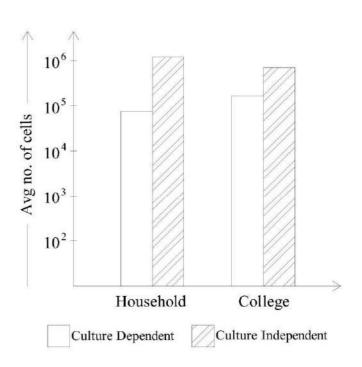
The graph provided below shows the relation between the Culture Dependent and Culture Independent techniques for the various water samples of natural origin. A higher count can be observed in Culture Independent readings owing to the fact that both viable and dead cells; culturable and non-culturable cells are accounted for here.

For Commercial Sources – the average number of microbial cells per ml are as follows:

Source of	Culture	Culture
Origin	Dependent	Independent
(Tap water)	(CFU/ml)	(cells/ml)
Households	9.70 x 104	1.05 x 106
Colleges	1.50 x 105	9.70 x 105

The graph provided below shows the relation between the Culture Dependent and Culture Independent techniques for the various water samples of commercial origin. The overall water quality can also be considered "poor" according to the U.S. Environmental Protection Agency's (EPA) Standards. [5]





These water samples are not as contaminated as the natural sources for they have been treated by the general municipal treatment facilities before being supplied to the houses and colleges. However it surely will require sufficient in-house treatment using appropriate water filtration systems.

### DISCUSSION

Culture Dependent and Culture Independent techniques comparison has been demonstrated for water samples of natural and commercial origin collected from Mumbai. The Significance of this study – Unconventional culture-independent techniques have been brought into the limelight along with their comparison to culture dependent techniques. Culture dependent techniques have also been proven to be more efficient and quick in terms of delivering quality results. They may or may not be economical in certain circumstances.

Some microbes cannot be cultured in laboratories, they can be enumerated by Culture Independent techniques. Eg: VBNC (Viable But Non Culturable bacteria) which are alive, but they do not grow or divide hence cannot be cultured. Eg – Vibrio spp, Listeria spp, etc. [6]

On a sanitation standpoint, a large number on individuals (children and workers) come in contact with these waters directly or even indirectly. It is important to assess the microbial load of the water samples for this reason. Large number of industries being close to the water bodies dump their semi- or un-treated wastes into the water bodies. These can both harbor pathogenic microbes as well as destroy the natural marine flora and fauna.

Future Prospects include but are not limited to the following:

Widening the sample study-set by surveying other water bodies in and around Mumbai.

Employing the use of other Culture Dependent methods.

Employing the use of other Culture Independent techniques such as Direct Microscopic Count, Flow Cytometry, qPCR (real time quantification PCR), RT-PCR (Reverse Transcriptase PCR), FISH (Fluorescent In Situ Hybridization), and triplicate PCR reactions of partial 16S rDNA.[7][8]

Phenotyping the microorganisms enumerated and correlating it to the probable diseases they cause.

Determining the level of antibiotic resistance of the microorganisms enumerated.

Determining the sources of intrusion of the microorganisms into the water bodies.

#### ACKNOWLEDGEMENT

I would like to conclude by extending my warm thanks to my mentors – Dr. Sejal Rathod and Ms. Amina Dholkawala for their undivided attention and constant support. Also, my parents for them being there for me during the ups and downs along the course of this research experiment.

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# **MICROBIOLOGY**

"Always trust a microbiologist because they have the best chance of predicting when the world will end"

TEDDIE RAHUBE

### ISOLATION AND CHARACTERIZATION OF LYTIC COLIPHAGE AND ITS APPLICATION AS BIOCONTROL AGENTS IN FOOD INDUSTRY.

\*Chonkar Purva<sup>1</sup>, Jain Siddhi<sup>2</sup>, Devkate Radhika<sup>3</sup>, Thigle Vijay<sup>4</sup>, Satish Rajitha<sup>5</sup>

1, 2, 3, 4, 5 Department of Microbiology, K.C. College, Churchgate.

purvachonkar@gmailcom\*

### ABSTRACT:

Bacteriophages are viruses that infect bacterial cells. Bacteriophages infecting *E.coli* are called coliphages. In this study we have isolated coliphage from sewage sample, using *E.coli* as the host. The presence of coliphage was checked by qualitative plaque method. After enrichment, the phages were isolated and enumerated by quantitative plaque method. The phage titre was found to be  $7.1 \times 10^{15}$  pfiv/ml. The isolated coliphage was characterized by studying its host diversity, the effect of pH, temperature and cations by qualitative and quantitative plaque method. It showed narrow host range and could only infect *E.coli* and *E.coli* 113-3D. The coliphage remained stable at a wide pH (3–12) and temperature range (4–45°C). Studies were conducted to determine the effect of Ca<sup>+2</sup>, Mg<sup>+2</sup>, K<sup>+</sup> cations (1mM) on coliphage propagation. In the presence of K+ ions, the phage showed the highest rate of infectivity. The objective of this study was to isolate and characterize a coliphage to effectively control food contamination due to *E.coli*. The coliphage was found to be effective in controlling *E.coli* contamination on meat products. This could be the first step towards exploiting the potential of coliphages as biocontrol agents against *E.coli*.

Keywords: Coliphage, Biocontrol agent, cations.

### **INTRODUCTION:**

Bacteriophages are viruses that infect bacteria bacteria eater) (12) (13). (bacteriophage means Bacteriophage, do not attack bacteria indiscriminately; they usually attack only one specific host. Most phages are 24-200 nm in length. Every phage has a head structure, which can vary in size and shape. Some are icosahedral (20 sides), others are filamentous. The head (or capsid) is composed of many copies of one or more type of protein, and it contains the phage's genetic material (i.e. nucleic acid). The genetic material can be ssRNA, dsRNA, ssDNA, or dsDNA between 5 and 500-kilo base pairs (kbp) long in either a circular or linear arrangement(14)(15)(16). Many, but not all, phages have tails attached to the phage head, the tail is a hollow tube through which the nucleic acid passes during infection, and its size can vary considerably. In the more complex phages, like T4, the tail is

surrounded by a contractile sheath which contracts during infection of the bacterium, at the end of the tail, some phages have a base plate and one or more tail fibers attached to it; these structures are involved in the attachment of the phage to the bacterial cell (17) to enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins or even flagella. This specificity means that a bacteriophage can only enter bacteria that bear the certain types of receptors that they can bind to, and it is these portals of entry that determine the phage's 'host range'. Bacteriophage that infects E. coli sometimes are referred to coliphage generally, the bacteriophage is referred to simply as phage. As is true for all viruses, the phage can replicate only within host cells. In other words, coliphage can replicate only within E.coli. Phage must attach to a receptor on the surface of a bacterial cell in order to initiate an infection. This interaction between the phage and receptor is very specific - a given phage type only will bind to a

specific receptor molecule. Thus, all phage are not alike. The aim of the present work was characterization of *E.coli* phage isolated from sewage and studies its characteristics like host density, temperature, pH, and the effect of cations by qualitative and quantitative method. We also studied the use of coliphage as a biocontrol agent in reducing the load of *E.coli* on raw meat.

### **MATERIALS AND METHODS:**

### 1. <u>Isolation of phage from sewage</u> sample.

Three samples were taken from different places in Mumbai. All three samples were subjected to enrichment. One gram of sample was mixed with 10ml phage buffer (10mM Tris, pH7.5, 10mM MgCl2, 68mM NaCl, and 1ml CaCl2) and centrifuged at 3000rpm for 10 min. The syringe filtered (nylon supernatant was membrane, 0.2μ). 10 ml of above filtrate was added to E.coli (host) grown in 100 ml nutrient broth & further incubated at 48hours. Aliquots of the enriched sample were taken, centrifuged at 3000 rpm for 10mins and the supernatant was syringe filtered. The filtrate obtained was tested for the presence of coliphage against E.coli by qualitative plaque assay. One of the samples showed a zone of clearance indicating the presence of coliphage.

### 2. Isolation of phages:

Isolated phages were obtained from the above sample using quantitative plaque method. One of the lytic plaques was picked up and was enriched by the previous procedure. Aliquots from incubated media were again centrifuged and syringe filtered. The filtrate was again tested by qualitative phage assay against *E.coli* to confirm the presence of coliphage. The phage filtrate was stored at 4°C, in the presence of chloroform and was then used for further characterization study.

### 3. Enumeration of phages

The above enriched coliphage sample was enumerated by quantitative plaque assay using dilutions 10<sup>-1</sup> to 10<sup>-13</sup> of which 10<sup>-11</sup>, 10<sup>-12</sup>,10<sup>-13</sup> were plated to determine phage titre.

### 4. Study of Host diversity

Isolated coliphage was enriched with the different bacterial host (E.coli, E.coli 113-3D, Klebsiellae, S.typhi, S.paratyphi B) for 48 hours and then checked for infectivity by qualitative plaque assay with the same host organism.

### 5. Effect of pH

A range of pH from pH2 to pH12 was adjusted in nutrient broth. 0.1ml of coliphage was added into the broth & incubated at 37°C. Aliquots were collected after every interval of 2 hours up to 8 hours and 24 hours. The aliquots were checked for the presence of coliphage by qualitative plaque assay.

### 6. Effect of Temperature

Sterile tubes with 10 ml of nutrient broth were inoculated with coliphage and incubated at various temperatures (4°C, RT, 37°C, 45°C, 80°C). Tested for the viability of coliphage by qualitative plaque as say at intervals of 2 hours up to 8 hours.

### 7. Qualitative and Quantitative effect of cations

Different types of cations i.e. lmM of Ca<sup>+2</sup>, Mg<sup>+2</sup>, K<sup>+</sup> each were added to sterile nutrient agar butts. Qualitative and quantitative plaque assay was performed to check the presence of phage with cations. For quantitative plaque method, the dilutions used were 10<sup>-5</sup>, 10<sup>-6</sup> and 10<sup>-7</sup> and was compared with control (absence of cation).

### 8. Application of coliphage as bio control agent.

3 meat pieces approx (2cm x 2cm) were cut and used as a sample. In CONTROL 1 sample was inoculated with 10<sup>6</sup> suspension of *E.coli*, which was used as a host control. CONTROL 2 was inoculated with 0.1ml isolated phage (10<sup>-4</sup>), which was used as a phage control. The TEST was inoculated with 10<sup>6</sup> suspension of *E.coli* + 0.1ml of isolated phage (10<sup>-4</sup>). Samples were incubated at 37°C for 6 hours. Further processing of samples was carried out by addition of 10ml sterile saline to the tubes followed by vigorous vortexing. (Phage control: chloroform was added to kill bacterial cells). All the 3 tubes were

serially diluted and enumeration of host control and test was done by spread plate technique on sterile Mac Conkey's Agar plate. The phage control was enumerated by Quantitative Plaque assay technique.

### **OBSERVATIONS:**

Table 1: Determination of host range.

HOST	RESULTS
E.coli	+
E.coli 113-3D	+
S.typhi	-
S.paratyphi B	N=
Klebsiella	(1 <u>4</u> )

Key:

'+' presence of plaque

'-'absence of plaque

Table 2: Effect of pH.

pН	TIME						
pii	0 hrs.	2hrs	4hrs	6hrs	8hrs	24 hrs.	
2	+	( <b>=</b> )	(4)	<u> </u>	=	75 <b>2</b>	
3	+	+	12	<u> </u>	2	048	
4	+	+	+	s <del>Ť</del>	- <del>+</del> -	+	
5	+	+	+	+	+	+	
6	+	+	+	Æ	+	+	
7.4	+	+	+	+	+	+	
9	+	+	+	÷	+	+	
10	+	+	+	+	+	+	
11	+	+	+	÷	+	÷	
12	+	+	£	+	+	+	

Key:

'+' presence of plaque

'-'absence of plaque

**Table 3: Effect of Temperature** 

TIP (DIP) ( TIP) F	TIME					
TEMPERA TURE	0hrs	2hrs	4hrs	6hrs	8hrs	
4°C	+	+	+	+	+	
RT	+	<del></del>	#	+	#	
37°C	+	+	+	+	+	
45°C	+	+	+	+	+	
80°C	+	19	(-)	-	-	

Key:

'+' presence of plaque

'-'absence of plaque

Table 4: Qualitative effect of Cations

CATIONS	RESULT
Ca+2	+
Mg+2	+
K+	+

Key:

'+' presence of plaque

'-'absence of plaque

Table 5: Quantitative effect of cations

CATIONS	PHAGE COUNT pfu/ml	DIFFERENCE IN PHAGE COUNT
Ca <sup>+2</sup>	6.35x10 <sup>15</sup>	10.56% ↓
Mg <sup>+2</sup>	9.84x10 <sup>15</sup>	27.84% ↑
K <sup>+</sup>	1.7x10 16	58.23% ↑

Without ions	$7.1 \times 10^{15}$	-
	7.1 A 10	

Table 6: Phage as bio control agent

SR.NO	TUBE	COUNT (0 HOURS)	COUNT (6 HOURS)
1.	Host control	10 <sup>6</sup> cfu/ml	1.6×10 <sup>11</sup> cfu/ml
2.	Phage control	$10^3 \mathrm{pfu/ml}$	$3.1\times10^6$ pfu/ml
3.	Test	10 <sup>6</sup> c fu/ml ( <i>E.coli</i> ) + 10 <sup>3</sup> p fu/ml (Phage)	5.5×10 <sup>7</sup> cfu/ml ( <i>E.coli</i> )

temperatures. (4°C, RT, 37°C, 45°C, 80°C) The phage was viable at 4°C, RT, 37°C, 45°C up to 8 hours and lost its viability at 80°C (table 3)

### **Results and Conclusion:**

Three sewage samples were enriched for the presence of coliphage out of which 1 showed the presence of coliphage. This sample was further subjected to quantitative plaque method and one clear plaque (phage) was picked up for further study. The phage was once again enriched and its lytic ability was detected by qualitative plaque method.

Enumeration of the isolated phage was performed by quantitative plaque assay technique using dilutions  $10^{-1}$  to  $10^{-13}$  of which  $10^{-11}$ ,  $10^{-12}$  and  $10^{-13}$  were plated to determine phage titre which was found to be  $7.1 \times 10^{15}$  pfu/ml.

Characterization of the isolated coliphage was done by checking host range, the effect of pH, effect of temperature, qualitative and quantitative effect of cations on the isolated phage.

Qualitative plaque assay was performed with different bacterial hosts (E.coli, E.coli 113-3D, Klebsiellae, S.typhi, S.paratyphi B) to determine the host range of isolated coliphage. The isolated phage could infect only E.coli and E.coli113-3D whereas it was unable to infect Klebsiellae, S.typhi and S.paratyphi B (refer table1). This indicates that the phage is highly host-specific and has a narrow range of infectivity.

The isolated phage was tested for its viability using qualitative plaque assay at pH ranging from pH2 to pH12. It was observed that the phage was viable from pH 4 to pH 12 up to 24hours and lost its viability at pH 2 and at pH3 after 2 hours. (table 2)

Similarly, the isolated phage was tested for its viability using qualitative plaque assay at different

Effect of different cations (Ca<sup>+2</sup>, Mg<sup>+2</sup>, K<sup>+</sup>) on infectivity of isolated coliphage was tested with using Qualitative plaque assay technique. Plaques were obtained in presence of calcium, magnesium and potassium ions (table 4)

Quantitative phage assay was performed in presence of  $(Ca^{+2}, Mg^{+2}, K^+)$  using dilutions  $10^{-5}, 10^{-6}$  and  $10^{-7}$ . The phage titre obtained in presence of Calcium, magnesium, and potassium was  $6.35 \times 10^{15}$  pfu/ml,  $9.84 \times 10^{15}$  pfu/ml and  $1.7 \times 10^{16}$  pfu/ml respectively. An increase in phage titer by 58.23% was observed in presence of potassium ions and calcium showed a decrease in phage titer by 10.56%. (Refer table 5)

The count obtained in host control and phage control was  $1.6 \times 10^{11}$  cfu/ml and  $3.1 \times 10^6$  pfu/ml and test showed a count of  $5.5 \times 10^7$  cfu/ml. On comparing the host control and test a decrease in host count from  $10^{11}$  to  $10^7$  cfu/ml was recorded.

### **DISCUSSION:**

The objective of this study was to isolate and enumerate coliphage against *E.coli*, its characterization, and application as a biocontrol agent in the food industry. Stagnant sewage outlet sample was found to be the best source for coliphage. Coliphage was isolated from one of the totals of 3 sewage samples tested. The phage titer was found to

be 7.1×10<sup>15</sup> pfu/ml.The coliphage has narrow range infecting only *E.coli* and *E.coli* 113-3D.

The phage was stable at a wide range of pH from pH 4 to pH 12. Phages are often quite sensitive to protein denaturation in an acidic environment, which may result in a loss of viability of the phage (Hazen, 2002). Maximum phage viability was seen between pH4 to pH12. Isolated coliphage was found to be stable between temperatures 4°C to 45°C. Phage viability was lost at 80°C. Phage viability can be checked between 45°C to 80°C to check the thermal inactivation temperature. Loss of viability due to high temperature is due to disruption of head proteins and subsequent release of nucleic acid.

Isolated coliphage showed lytic activity in presence of Ca<sup>+2</sup>, Mg<sup>+2</sup>, K<sup>+</sup> ions. However, maximum infectivity of coliphage was observed in presence of K<sup>+</sup> ions i.e. 58.23% increase, whereas Ca<sup>+2</sup> ions showed a decrease in phage titer by 10.56%. An increase in plaque forming units was observed in presence of K<sup>+</sup> ions, maybe due to the enhancement of phage adsorption on host cells due to the presence of K<sup>+</sup> ions.

Isolated coliphage was found to be an effective biocontrol agent with respect to raw meat as the *E.coli* count was decreased after phage treatment on infected meat. We also need to test whether the isolated coliphage is capable of lysing against food pathogens such as *E. coli O157:H7* using same techniques. Observing the structure of coliphage by transmission electron microscopy can be done. This will help us in identify the family of the phage. Also further genomic analysis needs to be done for the identification of isolated coliphage to know if the phage is a novel or known. Isolation of coliphages against other species of coliform group can be done in order to formulate coliphage spray to decrease the load of pathogenic coliforms in local butcher shops.

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### Screening of Antimicrobial and Antioxidant properties of Plant extracts for the formulation of Cosmetic products

Aesha Agarwal, Anagha Nair, Pratiksha Chavan, Sejal Rathod\*

Department of Microbiology, K. C. College, Vidyasagar Principal K.M. Kundnani Chowk, D.W. Road, Churchgate, Mumbai 400020, India

**Abstract:** Plants are rich source of phytochemicals that have been used as the ingredients in traditional systems of medicines, nutraceuticals, herbal cosmetic and bioactive principles in pharmaceuticals. The chemicals used in skin care products are often very harsh on the skin and too abrasive for people with sensitive skin. Skincare product containing plant extract can nourish and help treat skin problems. The research aims to select a few commonly available plants as a source of Antimicrobial and Antioxidant agent for formulation of a cosmetic product. The plant sources selected are Solanum tuberosum, Coriandrum sativum, Murraya koenigii. Processing involved drying of the plant sources, followed by preparation of methanol and aqueous extract. These extracts were subjected to various Antimicrobial analysis by disc diffusion against Staphylococcus Streptococcus pyogenes, Corynebacterium diphtheriae Minimum Concentration (MIC) to study the individual activity, while the Agar dilution was used to study the synergistic activity and Antioxidant analysis such as DPPH and FRAP. The Solanum tuberosum failed to show any antimicrobial activity MIC of Coriandrum sativum was observed to be 2% and for Murraya koenigii it was 0.5%. Good Anti-oxidant properties were observed for both the extracts and were used in preparation of an herbal skin cosmetic.

**Keywords**- Antioxidant, Antimicrobial, Plant extract, Skin

### INTRODUCTION

Cosmetic products are used to cleanse and enhance the external appearance of the skin. The key ingredients include: water, emulsifier, preservative, humectant etc. ingredients of cosmetic products can be synthetic or natural in nature. (1)

Industrialization made availability and affordability to cosmetic products with synthetic components easy. Our ancestors trusted in the naturally derived components to boost and maintain good skin health. After learning the environmental and health issues associated with synthetic product, the belief in natural products is regenerated.

The peels *Solanum tuberosum* contain vitamin B, Vitamin C, calcium and iron. <sup>(2)</sup> *Coriandrum sativum* leaves are loaded with

Vitamin C and antimicrobial compounds. *Murraya koenigii* contains calcium, vitamin A, vitamin B, vitamin C and is known to have antifungal and antibacterial properties. (3) Oxygen is essential to life, but as the body uses oxygen generate product known as Reactive Oxygen Species and common free radical Radical called Reactive oxygen species (ROS) have important cell functions like cell signaling, ion transportation, apoptosis etc. However, excess of ROS attacks the nucleic acid, amino acid side chains, proteins etc. and cause DNA, RNA, protein and lipids resulting in increased risk

of cancer, cardiovascular disease and other disease. (4) (5) Antioxidants counteract or neutralize the effect of free radical.

Antimicrobial is an agent that kills the organism or stops the growth <sup>(6)</sup> and are required to decrease the burden of microbial disease.

### MATERIALS AND METHODS

Selection and collection of plant materials: Three plant sources were chosen for evaluation of antimicrobial and antioxidant source such as *Solanum tuberosum* (potato peels), *Coriandrum sativum* (leaves), *Murraya koenigii* (curry leaves).

**Preparation of Sample**: The samples was washed, air dried followed by heating in the hot air oven at 60°C, then ground to obtain the powder.

Preparation of the Extract: Hot water, cold water, methanol and oil extracts were prepared. Methanol extract was obtained by continues hot percolation technique using Soxhlet apparatus. Hot water extract was prepared by boiling the sample in distilled water. Cold water extract was prepared using sterile distilled water and kept in shaker for 8 hours. Oil extract was made similarly as hot water extract but instead distilled water is replaced by sterile paraffin oil.

Antimicrobial ability: Methanol extract obtain by extraction is dissolved in 50% DMSO and incorporated into molten Nutrient agar. Antimicrobial activity was studied in two ways individually and in combination. Individual activities were studied using methods like Disc diffusion, Agar well (7) and Agar dilution (8). Combined activity was performed by Agar dilution and filter paper strip method. MIC was determined by Agar dilution using different concentration. 3

Gram positive skin pathogenic organisms namely Staphylococcus aureus, Streptococcus pyogenes, and Corynebacterium diphtheriae was used in the analysis.

Antioxidant activity: The ability to scavenge free oxygen radical was determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) antioxidant assay and FRAP (Ferric Reducing Antioxidant Power) assay.

Formulation of a Cosmetic Products: Three cosmetic products were formulated namely face wash, Scrub and cream.

• Cream<sup>(3)</sup>: An Oil-in-water (O/W) emulsion-based cream formulated. The formulation

components for the preparation such as

Stearic acid, Lanolin, Mineral oil were taken in porcelain dish and melted in boiling water bath. To that Potassium Sorbate (Preservative) and the oil mixture is slowly mixed with water. The oil extract of curry leaves and coriander leaves was added in equal quantity. When it is in the cream form essential oil is added.

Wash (9). Face A cosmetic preparation made was using components such as Xanthum gum. Propylene glycerol, Sodium lauryl ether sulfate, Sodium Chloride. All these components are mixed making it a Soap Base and then water extracts of curry leaves and coriander leaves are mixed. When the facewash is ready then Preservative and Essential oil is added.

• Scrub (9): Scrub a semi-abrasive cosmetic lotion includes components such as Beeswax, Borax, Almond oil. The ingredients are mixed in proper proportion and water extract and powdered sample of curry leaves and coriander leaves was added. After the formulation is ready Preservative and Essential oil is added to the preparation.

### Physical Analysis of Cosmetic Products: PHYSICAL ANALYSIS OF COSMETIC PRODUCTS

These formulations were subjected to various analysis such as physical analysis which include color, odor, appearance, pH, washability, etc.

### RESULTS

### ANTIMICROBIAL ACTIVITY

### Individual analysis:

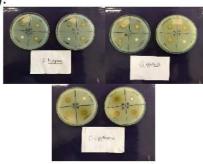
By disc diffusion method: The samples when analyzed for the antimicrobial activity, Solanum tuberosum peels were observed to inhibit S. aureus and Corynebacterium diphtheriae, but no inhibition was observed against S. pyogenes, whereas the other extracts of Coriandrum sativum and Murraya koenigii inhibited all the three test organisms.

**Table. 1:** Tabular representation of the antimicrobial ability of the plant extract in terms of zone size

HOT (mm)	(mm)	METHAN OL (mm)	CONTR OL (mm)
Coriand	rum sa	tivum	
10.2 5	11.5	10.5	0
9	11.7 5	9.75	0
8	11.2 5	10.75	0
	(mm) Coriand 10.2 5	(mm) (mm)  Coriandrum sa  10.2 11.5 5 11.7 5 8 11.2	(mm)         (mm)         OL (mm)           Coriandrum sativum         10.2         11.5         10.5           5         9         11.7         9.75           5         5         11.2         10.75

C.diphther iae	13	13.3	14	0
S. aureus			13.5	0
S. pyogenes			14	0
	Solanur	n tubei	rosum	
C.diphther iae	11.2 5	12.1 6	13.6	0
S. aureus	7.5	0	12.5	0
S. pyogenes	0	0	0	0

**Fig. 1:** Activity in terms of zone size, shown by *Coriandrum sativum* against *S. pyogenes* (left), *S. aurues* (center) *and C. diphtheriae* (right).



 By agar dilution method: The MIC of C. sativum was found to be 2% and that of M. koenigii was found to be 0.5%.

**Table 2:** tabular representation of the antimicrobial ability by Agar dilution (mic determination)

ORG.		CON	CENT	RAT	ON
	0.1	0.5	1	2	CONTR
	%	%	%	%	OL
	Mu	rraya l	koeni	gii	
S. aureus	+	824			(25)
S. pyogenes	+	-	-	-	=

C. diphtheri ae	+	-	8	170	\$1700 E
	Coria	ndrum	sativ	um	
S. aureus	+	+	+	-	8 <b>4</b> 8
S. pyogenes	+	+	+	-	*
C. diphtheri ae	+	+	+	-	-

Key: (+) growth observed; (-) no growth observed

**Fig. 2:** Inhibition shown by *Coriandrum* sativum extract of 1% concentration (left) and 2% (right).



### Combined analysis:

 By filter paper strip method: The M. koenigii and M. koenigii showed synergistic activity.

**Table 3:** tabular representation of combined activity of *C. sativum* and *M. koenigii*.

PLANT EXTRACT	SIZE OF ZONE OF INHIBITION
C. sativum	12mm
M. koenigii	12mm
C. sativum + M. koenigii	26mm

**Fig. 3:** Combined Antimicrobial Activity shown by *Coriandrum sativum* and *Murraya koenigii* against *S. pyogenes* (right) and *S. aureus* (left).



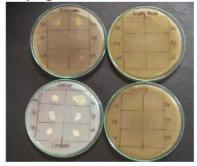
• By Agar Dilution method: 0.25% of C. sativum and 0.25% of M. koenigii together where observed to inhibit S. aureus, S. pyogenes, C. diphtheriae.

**Table 4:** tabular representation of combined activity of *C. sativum* and *M. koeniqii* by Agar Dilution method.

organisms	0.5% C. sativum	0.5% M. koenigii	0.25% C. sativum+0.25% M. koenigii
S. aureus	+	-	15
S. pyogenes	3 <del>5</del>	-	15
C. diphtheriae	+	=	15

Key: (+) growth observed; (-) no growth observed

**Fig.4:** Combined inhibitory Activity shown by *Coriandrum sativum* and *Murraya koenigii*, by Agar Dilution Method.



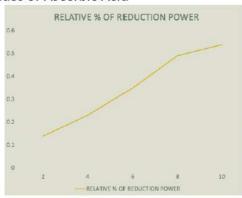
ANTIOXIDANT ACTIVITY

### • FRAP Assay:

**Table 4:** FRAP values of dyes in ascending order.

EXTRACT	O.D. (700nm)	ACTIVITY OBS.
M. koenigii	0.29	1000
C. sativum	0.40	1380

Fig 5: Graphical representation of FRAP values of Ascorbic Acid



### COSMETIC PRODUCTION

- Evaluation of the formulated cosmetic products: the result of formulation of scrub is presented in Table 5. The overall evaluation report reveals an ideal tropical formulation containing extracts of Coriandrum sativum and Murraya koeniaii.
- Table 5: evaluation of the formulated cosmetics

Parameter	Observation	Observation	Observation
	for scrub	for facewash	for cream
Appearanc	Coarse	Homogeneou	Semisolid
e	semisolid	s, smooth	
Color	Dark green	Pale green	Cream color
Odor	Pleasant	Pleasant	Pleasant
рН	8	7.6	8
After feel	No residue left	No residue	No residue
	behind	left behind	left behind,

			nostaining of skin
Washability	Easily washed off when	Easily washed off when	Easilywashed off when
	placed under running water	placed under running water	placed under running water

•

### DISCUSSION

• Discussion

- The plants extracted from natural sources are rich in phytochemicals and phenolic compound. Plants like Coriander sativum leaves, Solanum tuberosum (potato peel), Murraya koeniggi (curry leaves) known to have good antimicrobial and antioxidants properties. They also have antioxidant, anti-fungal, antiseptic and disinfectant properties that help in clearing up your skin from many problems. They are rich in Vitamin C, Vitamin B, Vitamin A, Vitamin E, folate and beta carotene. It helps in maintaining smooth skin, prevent in from acne and also in getting rid of dead skin cells and makes the skin healthy. It also helps in cures cut, burns, and rashes
- The antimicrobial activity of plant extracts were tested against Staphylococcus aureus, Streptococci pyogenes, Corynebacterium diphtheria using agar dilution, Agar well and Disc diffusion methods. The plant extracts showed inhibition to common pathogenic organisms like Staphylococcus aureus, Streptococci pyogenes, Corynebacterium diphtheria but Solanum tuberosum did not show appreciable antimicrob ia l activity. The antioxidants activity was by FRAP assay studied using spectrophotometrically that as coriander sativum was 1380. The Other extracts also showed good antioxidants activity. Cosmetic products such as face wash, face cream, face scrub were found to be refreshing, soothing and

- glowing effect to the skin. Organic beauty products have
- advantages over conventional cosmetics Naturally scented. as environmentally friendly, Avoid irritants, no side effects. Good antioxidants activity was shown indicates that prevent it from oxidative damage of skin. The ability to inhibit the test organisms and prevent it from any skin disease and improves the skin glowing. Soothing and prevent from acne. Thus, use of natural extracts cosmetics plant over conventional cosmetics is of future benefit.

According to study by Hanan Al Harbi, Dr. Uma Irfan and Dr. Sarah Ali of European journal of pharmaceutical and medical research show antimicrobial activity is showing more inhibition of Staphylococcus aureus is show and our is showing less inhibition to methanol extract of curry leaves. According to study by Krutika Patel and Mita Vakilwala shows that antimicrobial activity is showing more and our study is showing less zone if inhibition to methanol extract of coriander sativum.

### ACKNOW LEDGMENT

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#### ANALYSIS OF BIODEGRADATION OF AZO DYES

Gaurav Sriram, Uzma Shaikh, Vaidehi Shrestha, Dr. Pratibha Shah\*

Department of Microbiology, K.C. College, Vidyasagar Principal K.M. Kundnani Chowk, D.W. Road, Churchgate, Mumbai 400020, India

\*E-mail address for correspondence (shahpratij@gmail.com)

#### ABSTRACT:

Azo dyes, very commonly found in various industrial effluents, have a deleterious effect on the environment. In the current study, antibiotic susceptibility test (AST) against 12 antibiotics was carried out for 11 potent strains of azo dye (crystal violet) degrading bacteria previously isolated from textile effluent using the Kirby Bauer's disc diffusion method. Two best dye degrading isolates were screened for plasmid by alkaline lysis followed by agarose gel electrophoresis, wherein one of the strains was found to possess a plasmid. Their AST results indicated that they were both resistant to beta-lactam antibiotics. Plasmid curing by sodium dodecyl sulphate (confirmed by replica plate technique) was followed by dye degradation assay and AST of the cured strain. Antibiotic susceptibility increased but dye degradation remained unaffected, indicating that the ability to decolorize the azo dye probably did not originate from the plasmid DNA. Furthermore, cell-free crude enzyme assays indicated the presence of an extracellular dye degrading enzyme complex with similar degradation rate as that of whole cells; a good prospect for commercial manufacturing of a cell-free dye degrading system Moreover, immobilization of the enzyme could be an influential future prospect in large-scale bioremediation and treatment of industrial effluents.

KEYWORDS: azo dye, degradation, enzyme, plasmid

### INTRODUCTION:

Synthetic dyes are extensively used in textile, paper, food, colour photography, paper printing, plastic, cosmetics, pharmaceutical; leather and toy industries [1, 22]. Azo dyes, considered to be the most prevalent group of synthetic dyes, are electron-deficient xenobiotic compounds due to the presence of azo (N=N) and sulfonic (SO3) electron-withdrawing groups. Hence, azo dyes are prone to endure oxidative bacterial catabolism under aerobic environmental conditions [16].

Thousands of azo dyes are available in the commercial market, out of which, at least 500 have carcinogenic aromatic amines as part of their chemical structure [15]. After entering the human body through ingestion or skin contact, these compounds are metabolized to aromatic amines by azoreductases in the gastrointestinal tract and in the mammalian liver [4, 15]. Approximately 90% of 4,000 dyes analyzed in a survey had LD50 values greater than 2000 mg/kg with highest toxicities found among basic and diazo groups

of dyes [18] Azo dyes are considered to be rather persistent pollutants for the reason that they are not readily degraded under aerobic conditions [11, 21]. Under anaerobic conditions, azo dyes can be reduced by intestinal bacteria and various environmental microorganisms to colourless amines, which however, may be toxic, mutagenic, and carcinogenic to humans and animals [3,5].

The textile industry is one of the most chemically intensive industries and the major source of contamination of potable water. The direct discharge of large quantities of unused dyestuff having complex chemical properties into water bodies affects their ecological environment [12]. The treatment of textile wastewater is essential before discharging the wastewater into a receiving water body [13]. During the past two decades, several physicochemical decolorization techniques have been reported; however, few have been accepted by the textile industries [14]. Biological methods are generally considered environmentally friendly as they can lead to complete mineralization of organic pollutants at low

cost. Moreover, there is a need to develop novel biological decolorization processes leading to more effective cleaning up of azo dyes using cell-free systems that are as efficient as whole cells.

Thus, this work aims to (I) screen for pathogenicity of azo dye degrading isolates, (II) study the potential of these isolates in azo dye degradation, (III) detection of plasmids associated with degradation. (IV) determination of dye degradation efficiency of cell-free extracts.

#### MATERIALS AND METHODS:

Azo dye decolorization assay: To study the decolorization efficiency of 11 strains previously isolated and characterized from a textile effluent [25], 0.1 mL of the culture (24 hours old culture of density 0.1 O.D. at 540nm) was inoculated into 3 different concentrations of Crystal Violet [16] (azo dye), viz. 50ppm, 75ppm and 100ppm, prepared in Luria Bertani (LB) broth. Uninoculated medium without the dye was used as control. 1 mL aliquots, withdrawn after 48 hours of incubation at 37°C, were centrifuged at 10,000 rpm for 15 minutes. The absorbance of the centrifuged, 1:10 diluted supernatant and the control was read at 540nm using a colorimeter. The decolorization efficiency (%) of different is olates was calculated as follows:

% Decolorization = Ablank - Atest X 100

 $A_{blank}$ 

where, Ablank: Absorbance of blank

and Atest : Absorbance of test

Antibiotic Susceptibility Test (AST): All the 11 strains previously isolated from the textile effluent [25] were screened for their susceptibility towards 12 antibiotics belonging to different groups by Kirby Bauer's disc diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [8]. The 12 commercially available antimicrobial discs used, obtained from HI-Media, were from the beta lactam (Amikacin 30µg/ml, Cefepime 30µg/ml, Cefotaxime 30µg/ml, Aztreonam  $30\mu g/ml$ , Imipenem  $10\mu g/ml$ ), carboxyfluoroquinoline (Ciproflaxin), polymyxin (Colistin  $10\mu g/ml$ ), nitrofuran (Nitrofuratoin

300µg/ml), sulfonamide (Trimethoprim 30µg/ml), polyketide (Tetracycline 30µg/ml), aminoglycoside (Streptomycin 10 µg/ml) and glycopeptide (Vancomycin 30µg/ml) groups. Post 24 hours of incubation at 37°C, the zone sizes were measured in mm and were interpreted on the basis of the standard CLSI chart for AST.

Plasmid Extraction: On the basis of their decolorization efficiency at 100ppm, 2 most potent dye degrading strains were selected and screened for plasmid using Alkaline Lysis Method [7]. This was done to detect whether the dye degradation gene was present on plasmid or was chromosomally mediated. 24 hour old cultures grown on LB Agar slants containing 300μg/mL of ampicillin were used. The plasmids were separated using 1% Agarose Gel Electrophoresis; visualization was done under a UV Trans-illuminator [2].

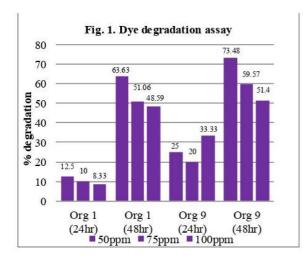
Plasmid Curing: Plasmid curing of the isolate was carried out using Sodium Dodecyl Sulphate (SDS) [6, 19] at its highest sub-lethal concentration i.e. 0.5%. Curing was confirmed using Replica Plate Technique on LB agar. Colonies that failed to grow on the replica plate (LB agar having 300µg/mL of Ampicillin) but exhibited growth on the master plate (LB agar without Ampicillin) were postulated to be cured of plasmid.

Preparation of Cell-Free Extract and Comparative Dye Degradation Assay: To study the dye degradation efficiency of the cell free extract, 1mL of 24 hour old culture (density 0.1 O.D. at 540nm) was inoculated in 100mL of LB Broth (having 50ppm of Crystal Violet) for 48 hours under shaker conditions. The broth was then divided into 2 parts of 50 ml each; one part was treated with 30 drops of Toluene (which would lyse the cells and release intracellular enzymes) [20] while the remainder was kept untreated. Both parts were centrifuged at 10,000 rpm for 15 minutes and the supernatants were used as cell-free extracts. These were used instead of whole cells for a comparative dye degradation assay.

#### RESULTS:

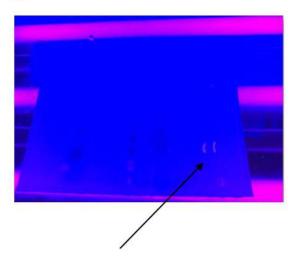
Azo dye decolorization assay: The rate of degradation against different concentrations (50 ppm, 75 ppm, 100 ppm) of crystal violet by the 11 isolated organisms from textile effluents was estimated at 48

hrs. Isolate no. 1 and isolate no. 9 showed maximu m degradation at a concentration of 100 ppm of crystal violet. Rate of degradation was calculated as % decolorization. (refer fig. 1.)



Antibiotic Susceptibility Test (AST): The susceptibility of the 11 isolates towards 12 different antibiotics was checked by the Kirby Bauer's method. The zone sizes were measured and interpreted by comparing with the CLSI Standard chart for AST. Isolates no. 1 and 9 exhibited resistance towards a wide range of antibiotics, especially those belonging to the  $\beta$ -lactam group.

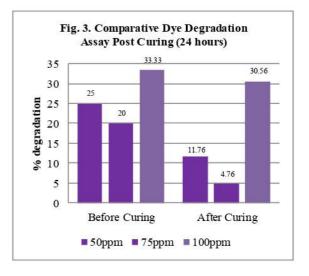
Plasmid isolation, visualization & curing: Isolates no. 1 and 9 were screened for plasmid. Visualization was done using a UV trans-illuminator. Isolate 9 showed a super-coiled plasmid on the agarose gel medium. Isolate 1 did not show any plasmid. (refer fig. 2.)



Organism 9 Supercoiled Plasmid (Observed Under UV Trans-illuminator)

Fig. 2.

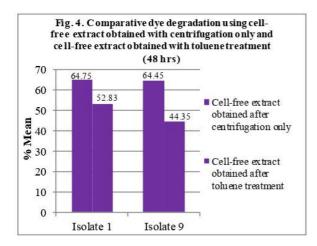
Degradation assay post-curing for isolate no. 9: Dye degradation rate checked for the cured colonies of isolate 9 showed no significant difference in the rate of decolorization. T test was carried out and the difference in dye degradation before and after curing was found to be statistically insignificant; (paired t test, P > 0.05). This indicates that the factor responsible for degradation was not plasmid mediated. (refer fig. 3.)



Antibiotic Susceptibility Testing for the plasmid cured isolate no.9: Susceptibility of the plasmid cured isolate 9 against 12 antibiotics was checked by Kirby-Bauer method and zone sizes were measured and interpreted by comparison with CLSI standard chart. The isolate no.9 exhibited susceptibility against all the antibiotics to which it was resistant. This indicated that the antibiotic resistance was plasmid mediated.

Comparative Dye Degradation Assay of cell free extract: The dye degradation assay carried out for the cell free extract of isolates 1 and 9 was carried out and the effect of toluene on the cells was studied. There was no significant difference in the rate of degradation in the cell-free extract post centrifugation; however degradation rates for the cells treated with toluene decreased indicating a probable interference of toluene with enzyme activity. (refer fig. 4.)

Statistical t test was carried out and the mean difference was found to be statistically significant (unpaired t test, P < 0.05). This suggests that the enzyme responsible for degradation could be extracellular.



#### **DISCUSSION:**

In the current study, the cell-free crude extract (obtained from strain 9) exhibited a degradation rate of 73.06% for 100ppm crystal violet dye concentration in 48 hours. From a previous study by Lade et al., it was found that bacterial dye degraders could decolourize 12-22% of 100ppm concentration of azo dye in 48 hours, which is comparatively lower than the present study. Similarly, another study [23] with a Gram Positive bacterium gave a maximum dye degradation rate of 40% in 96 hours, which too is lower than the degradation efficiency observed in the present study. In addition to this, it was also found that azo dye degradation (for strains 1 and 9) is mediated by extracellular enzymes, which is similar to a study [24]. Since industrial effluents usually contain a complex mixture of dyes, dye degradation efficiency of the cellfree crude extract for a combination of recalcitrant dyes needs to be analysed for their effective in situ bioremediation. Moreover, optimization immobilization of the cell-free extract could be an influential prospect future in large-scale bioremediation and treatment of industrial effluents. Phytotoxicity studies of the azo dye degradation products can help determine the dye mineralization efficiency of the cell-free extract and whether the bioremediation process is generating any toxic end products. Lastly, strain improvement of the other nine

isolates followed by strain stability studies could help generate safer, more stable and better choices for large scale manufacture of dye degrading enzyme(s).

#### **CONCLUSION:**

From 11 is olates, two best dye degrading is olates were found to be antibiotic resistant ( $\beta$ -lactam, polyketide and glycopeptide groups). On plasmid is olation, strain 9 was found to contain plasmid, whereas it was absent in strain 1. Upon curing of plasmid of strain 9, it was analyzed that the dye degradation was not plasmid mediated but antibiotic resistance was plasmid mediated. By the present study, it was found that the enzyme(s) responsible for degradation were extracellular. The degradation efficiency was retained with crude cell-free extracts when compared with whole cells; a good prospect for commercial manufacturing of a cell free dye degrading system.

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# COMPUTER SCIENCE

"Computer Science is no more about computers than Astronomy is about telescopes"

EDSGER W. DJIKSTRA

#### IoT BASED SMART HOME USING RASPBERRY PI

#### Rohan Biswal, Mahenoor Mansuri, and Mrs Vimala Rani. R\*

Department of Computer Science, K.C. College, D.W. Road, Churchgate, Mumbai 400020 \*Author for Correspondence (vimalayes@gmail.coms)

#### **ABSTRACT**

In this paper, our objective is to implement the Internet of Things Technology in making a smart home. Internet of Things (IoT) allows us to control devices through the internet. The Main propose is to use Raspberry Pi in order to control home appliances, thus automating modern homes via mobile or computer. Internet of Things (IoT) allows us to control devices through the internet. We will be using Raspberry Pi for controlling the devices and various sensors for data collection. The working can be monitored using a GUI (Graphical User Interface) would be used to monitor and control the elements. The Automation of the house is observed. The user was able to control various house elements, a smart home for smart people and the smart city is being achieved. This system saves time and manpower as it can be accessed and controlled by the authorized user from anywhere and makes our life a lot easier.

**<u>KEYWORDS</u>**: Smart home, IoT, automation, Raspberry Pi, Sensors, Pi camera, GUI, wireless control.

#### INTRODUCTION

IoT or Internet of things is an up-coming technology that allows us to control hardware devices through the internet. So here we propose to use IoT in order to control home appliances, thus automating modern homes via mobile or computer. It involves controlling the lighting, ventilation, fan, AC and security systems in-house as well some of the other home appliances. This is a step towards smart homes or smart house. Smart homes are a very board term and home automation is a step towards it. These ways save time as it can be accessed and controlled by the authorized user from anywhere and make our life a little easier. To detect changes in the background using different sensors (light sensor, temperature sensor, motion sensor, gas sensor, moisture sensors etc.)

#### METHODOLOGY

In this, the automation is done by using Raspberry Pi (a packet sized single-board computer) In this there will be an application that will control all the system of the house we can switch on and off the devices(lights, fans, etc.) and other devices through it. We can power on and off a device from another location through remote control. The device (lights, fans, etc.) is connected to raspberry pi which can be controlled wirelessly. Whether you give that command by voice, remote control, tablet or smartphone, the home reacts.

The device is controlled via a Node-Mcu Module. NodeMCU is an open source IoT platform. It includes firmware which runs on the ESP8266 Wi-Fi SoC from Espressif

Systems, and hardware which is based on the ESP-12 module.

- The user sets up and Node Mcu that is and the server.
- The auth token helps the server to distinguish between different devices in a large network pool
- When the command is given the Node Mcu receives the command and then works on the next part of the circuit with help of a Relay channel.
- Relays are switches that open and close circuits electromechanically or electronically. Relays control one electrical circuit by opening and closing contacts in another circuit.

#### ANALYSIS AND CODES

#### Auth Token

A security token (sometimes called an authentication token) is a small hardware device that the owner carries to authorize access to a network service. The device may be in the form of a smart card or may be embedded in a commonly used object such as a key fob.

Python GUI (Graphical User Interface)

Helps in controlling the system (Raspberry Pi)

Google Assistant

Google Assistant is an artificial intelligencepowered virtual assistant developed by Google that is primarily available on mobile and smart home devices. The automation is purely based on voice command.

• Intruder Detection System

An intrusion detection system (IDS) is a device that monitors a smart house systems for intruders.

#### CONCLUSION

There are many home automation companies that promise to automate your house, but they do charge a lot of money. Our aim is to automate the house with much cheaper products so that everyone will be able to afford this home automation system. Raspberry Pi is used in this version of the product. We also used a Node Mcu that replaces raspberry pi in network based communication.

I have successfully built a cheap Smart home model which controls the full house and we have also used Google assistant as a medium to send updates to the relay channel to turn on and off the home lights.

For future enhancement and improvising the use of product, we are using IFTTT. We will also using different other sensors.

#### LIMITATIONS

- The application doesn't support other voice assistants.
- The Scope of control is only limited to few devices.
- Few sensors have been only tested.

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#### SMART MIRROR AND AUTOMATION

#### Yagnesh Vakharia

Department of Computer Science, K. C. College, Churchgate, Mumbai - 400020 \*Author for Correspondence Geeta N. Brijwani \* (geetabrijwani@yahoo.com)

#### **ABSTRACT**

In this paper, Smart Mirror displays date and time, weather forecast and reports, headlines with respect to the device location with power saving feature. Acrylic mirror sheet will be used for object reflection. The program will automatically detect the device location and displays various information based on its location accordingly. It can be accessed remotely by the user from an Android application. The remote application allows user to register and activate their product. Also, it can be used to turn off Smart Mirror and change location, country news headlines and weather. Along with that AR (Augmented Reality) technology has been integrated, application named as "Virtual Newspaper" that superimposes a media file on a user's view of the real world. To keep track on user's activity, an admin panel is there which displays the user's activity performed over Mirror or Application. It will display the registered customer details and their activity records. Administrator is only allowed to modify the customer details record. This system will reduce the human efforts, saves energy and time.

**<u>KEYWORDS</u>**: Raspberry Pi, security module application, acrylic mirror sheet, android app, web application.

#### INTRODUCTION

A mirror is an object found in most people's homes. In mirrors we see our reflections. But what happens when you combine the idea of a mirror with technology? How smart a mirror could be? A Smart Mirror usually consists of a one-way mirror with a screen attached to it that displays a static user interface. The display can show the viewer different kinds of information in the form of widgets, such as weather, time, date, and news updates. This product would be useful for busy individuals that want to multitask and stay informed while on the go. Instead of constantly pulling out a device, one could get informed while finishing daily grooming task. Lots of Smart Mirror includes various hardware components combined with different technologies. This version of Smart Mirror uses less and essential hardware components and displays maximum information which can be controlled by a remote application.

#### METHODOLOGY

Smart Mirror uses only two hardware components i.e. electronic display and raspberry pi. Raspberry Pi is a microcontroller It displays information from API hosted on AWS (Amazon Web Services) which assembles and provides data from other services. The information on Smart Mirror can be modify using a remote application namely 'Smart Controller'. The modification performed from Smart Controller reflects the database. An AR (Augmented Reality) program is also it which integrated in enhances the experience of reading newspaper. Smart Mirror uses polling technique to check the updated values in database. A Smart Admin Dashboard is also introduced in order to keep a track on user statistics.

- The user sign-ups into the Smart Controller using unique product key displayed by the Smart Mirror.
- User interface gets displayed on the Smart Mirror which will show date, time, location, weather, last updated time of weather, weather description and news updates.
- The user logins into Smart Controller which allows to modify data such as location, news type, country code, news category. It also allows to turn off the mirror.
- The second panel in Smart Controller displays a list of news updates with description and link to the source. It also includes button to start AR program named as 'Virtual Newspaper'.
- The admin logins into the Smart Admin Dashboard to view user details and statistics of usage of device and application.

#### ANALYSIS AND CODES

Product Key

Generates a random and unique product key of 16 characters assigned to each product.

 Python GUI (Graphical User Interface)

Gets the data from API and displays in form of widgets.

#### Login

The login form restricts unauthorized user from accessing controls.

#### Signup

The signup form registers the product key of the Smart Mirror.

#### Home

Home panel in Smart Controller includes Location, News Type (top-headlines or specific category), Country Code (country specific news updates) and Mirror Off button (turn off mirror).

#### News

News panel in Smart Controller includes list of top-headlines with description and links to the source website and AR (Virtual Newspaper) application.

#### Virtual Newspaper

Superimposes a streaming video relative to the images printed on newspaper.

#### Admin Dashboard

Requires Login and displays user details which can be modify and user activities.

#### CONCLUSION

This product has been made at cheaper price using only essential components required to build a Smart Mirror. An Acrylic Smoke Mirror Sheet is used for reflection rather than two-way thin Acrylic Mirror Sheet which has reduced the cost price. Raspberry Pi is used in this version of the product but it can be

replaced with Arduino which will save 35% of the total cost price.

I have successfully built a cheap Smart Mirror which displays basic information without use of any external sensors or hardware component. Also, it ensures that no unauthenticated user can access the system. It is remotely accessible and uses less packet data. Also, Virtual Newspaper is developed using AR which is integrated to the remote application, enhances the way of reading newspaper by playing a video over the targeted image printed on newspaper.

For future enhancement and improvising the use of product, an Admin Dashboard is created to display all the user records and application statistics in tabular format.

#### LIMITATIONS

- The application doesn't support encryption methods while transmitting data to the API.
- The product cannot be turned on from remote application.
- Admin Dashboard doesn't display user details in confidential way.

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# **STATISTICS**

"A single death is a tragedy; A million deaths is a statistic"

JOSEPH STALIN

#### STUDY OF EMPOWERMENT OF WOMEN IN URBAN AND RURAL AREAS

Sumeeta Dwivedi, Sayyed Zaiba,

Mentor: Shailaja Rane\*

Department of Statistics, K.C. College, Churchgate, Mumbai 20.

mail2 zaiba@gmail.com, sumeetadrivedi2@gmail.com

#### Abstract

Gender equality is one of the principles in the preamble of Indian constitution. Constitution not only grants equality to women but also empowers state to adopt measures to improve the status of women. From fifth five year plan to eighth five year plan emphasis was always on the advancement of women in different spheres. Even after introducing many policies for the development of women we are aware that position of women is still low in the society as compared to their counterparts. United nation has shown concern about the empowerment of women. Nation will be known as a developed nation if women are included in the development and planning. Participation of women in social, political and economic decisions is as important as their participation in household decisions. This study aims to determine the empowerment of women in rural and urban areas. A questionnaire was designed for data collection on parameters relating to women empowerment and data was collected through google forms and personal surveys. Statistical techniques like Descriptive statistics, Chi-square test, z-test, marascuillo test and Mann Whitney Wilcoxon test were applied for statistical analysis.

Study included 135 married women, 83 are from urban region and 52 are from rural region of India. Women selected from urban region has mean age 38.73 ± 9.95 sd., with 4.82% educated below 10th std, 93.98% of women studied above 10th std and 1.2% of women are illiterate. 54.22% of women are working in urban region while 45.78% are not working. 37.35% of women have taken loan while 62.65% of women had not opted for any kind of loan. 84.34% of women have an individual bank account and 6.02% of women do not have any bank account while 9.64% of women have joint account. In the matter of family expenses 9.64% of women takes the decision all alone, on the other hand 16.87% has said that husbands take the decision , 71.08% said both husband and wife mutually takes the decision and 2.41% of women have not answered the question regarding decision making. 36% of women living in urban region doesn't have any medical insurance. 42% of women didn't invest for their future in urban areas.

Women selected from rural region has mean age  $36.058 \pm 10.95$  sd., 36.54% of women are educated below 10th std, 36.54% of women studied above 10th std and 26.92% of women are illiterate. 67.3% of women are working in rural region while 32.69% are not working. 26.92% of women have taken loan while 73.07% of women had not opted for any kind of loan. 3.85% of women have an individual bank account and 75% of women do not have any bank account while 21.15% of women have joint account. In the matter of family expenses 23.08% of women take decisions all alone, on the other hand 40.38% of the women said husbands take the decision all alone and 36.53% of the women said that husband and wife both mutually takes the decision. Majority (83%) of women living in rural region doesn't have any medical insurance. 73% of women didn't invest for their future in rural areas.

Key words: Women Empowerment, household decisions, health, literacy, family planning.

#### INTRODUCTION

"Women empowerment can be defined in very simple words that it is making women powerful so that they can take their own decisions regarding their lives and wellbeing in the family and society"[1]. "Women Empowerment refers to increasing and improving the social, economic, political and legal strength of the women, to ensure equal-right to women''[2] Women Empowerment is a current issue for developed as well as developing countries. Women empowerment is all about their economic and social status. Empowerment of women can be achieved by motivating them to find their right place in the society and encourage them to participate in social. economic, political aspects. An empowered woman not only brings bright future to her family but also will be a reason for the brightest future of the nation. In brief we can define the term "Women Empowerment" as empowering women in external qualities such as education, employment, decision making, health and internal qualities such as selfawareness and self-confidence. Since India is considered as male dominating country. women are not given much importance in decision making. During Vedic period the condition of women was better but got deteriorated during medieval period. To empower women The Constitution gave equal status to both men and women and also Government has introduced policies and schemes to empower women. Reservations and quotas were introduced by the Government wherever there is imbalance.

#### AIM

The aim of this study is to check whether the policies introduced to uplift the status of women are implemented properly or not and to find out whether women still feel that they are treated lower than their male counterparts in the society.

#### METHODOLOGY

A Questionnaire was designed using google form[3]. It was distributed through email in urban areas and a person to person survey was conducted in rural areas. Primary data was collected from a sample of 135 married women selected using stratified random sampling. Sample of size of 135 was calculated by using 8.5% margin of error with 95% confidence level. Measure of empowerment involves a study of different parameters such as age, place of residence, education, occupation, age at marriage, number of children, owning medical insurance, financial status and decision making status.

MS Excel was used for sorting, filtering and plotting graphs. R software was used for testing multiple proportions, SPSS was used to test the normality of the data. Statistical techniques like Descriptive statistics, Chi-square test, z-test, Marascuillo test and Mann Whitney Wilcoxon test were applied for statistical analysis.

#### ANALYSIS AND RESULT

#### Statistics:

Total 135 women with age  $39 \pm 10$  years from urban areas and women with age 36  $\pm$  11 years from rural areas.

Characteristic	Frequency(%)	
Age in years		
16-25	10(7.41)	
26-35	61(41.19)	
36-45	28(20.74)	
46 and above	36(26.67)	
Residency		
Rural	52(38.52)	
Urban	83(61.48)	

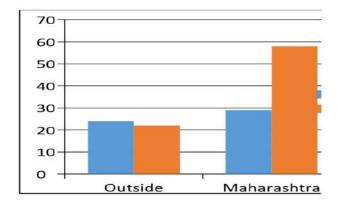
Education  Illiteracy  Below 10 std  Above 10 std	16(11.85) 96(71.11) 23(17.04)	Conclusion: Moliving within Mowork in comparoutside Mahara	Iaharashtra rison with	are allowed	
Occupation		2.i Employmen	nt status in	Rural Area	
Working Non-Working	73(54.07) 62(45.93)			Working b Marriage	e fore
Age at Marriage				Yes	No
Below 18	29(21.48)	Working	Yes	24	09
18-25	67(49.63)	After Marriage			
26-33	38(28.15)	8			
35 and above	2(1.48)		No	07	12

Table 1

Table 2

Ho: P = 0.5 against H1:P > 0.5

#### 1. Maharashtra Vs outside Maharashtra



Graph 1

From Graph 1it is noticed that more number of women living within Maharashtra are allowed to work in comparison with women living outside Maharashtra

Ho:P1 = P2 vs H1: P1 > P2

P1 = Proportion of women allowed to work after marriage within Maharashtra

P2 = Proportion of women allowed to work from outside Maharashtra

P value = 0.01659

Reject H0.

P: Proportion of women who are allowed to work in Rural areas.

$$p=24/31=0..77$$
 (From **Table 2**)  $q=0.23$ ,  $\alpha=0.05$ 

Z=3 P value is 0.00135. Very small P value indicates rejection of Ho and accept H1 and conclude that in Rural areas proportion of women who are allowed to work is more than the proportion who are not allowed to work.

#### 2. ii. Employment status in Urban Region

		Working before Marriage	
		Yes	No
Working After Marriage	Yes	39	06
	No	12	26

Table 3

Ho: P = 0.5 against H1:P > 0.5

p = 0.7647 (From **Table 3**),  $\alpha = 0.05$ , Z = 3.78 P value is 0.00011.

Very small p value indicates rejection of Ho

Conclusion: In urban areas proportion of women who are allowed to work is more than the proportion who are not allowed to work.

2.iii.Employment status in Urban and Rural

	Working after marriage
	Yes
Urban	45(0.54)
Rural	34(0.6538)

Table 4

P1: Proportion of women who are allowed to work in urban areas

P2: Proportion of Women who are allowed to work in rural areas

Ho: P1=P2 against H1:P1<P2

 $p_1 = 0.54$   $p_2 = 0.6538$  (From **Table 4**),  $\alpha = 0.05$ 

P= 0.5852, Q=0.4148

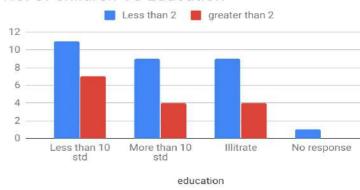
Z = -1.306 P value is 0.0951. Large p value indicates that we do not reject Ho

Conclusion: Proportion of women who are allowed to work in urban areas are equal to the proportion of Women allowed to work in rural areas.

Discussion: Hence from the hypothesis testing for employment status we can conclude that large number of women are allowed to earn money either by doing small entrepreneurship at home or going outside for work.

#### 3. i. No. of Children Vs Education (Rural)





Graph 2

Conclusion Graph 3 indicates that education does not affect childbearing in Rural area.

#### 3. ii.No.of Children Vs Education (Urban)

No of children Vs Education

70
60
40
40
40
10
0
Less than 10 std education

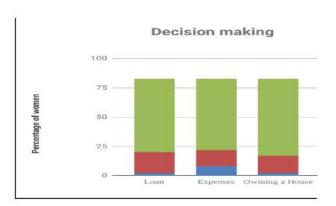
No. of children Less than 2
No. of children greater than 2

#### Graph 3

Conclusion The Graph 3 indicates that education does not affect childbearing in Urban area.

Discussion: Hence data suggest that education does not affect the size of the family (From Graph 3 and Graph 4)

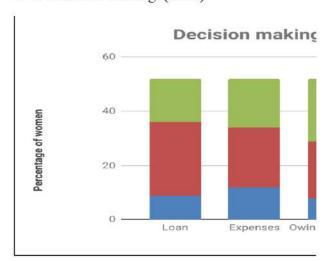
4. i. Decision Making (Urban)



Graph 4

Conclusion: From Graph 4 it is concluded that in Urban area the participation of women independently in decision making of loan and owning a house is less than that of expenses.

#### 4. ii. Decision Making (Rural)



Grap h 5

Conclusion From Graph 5 it is concluded that in Rural area the participation of women independently in decision making about taking loans and owning a house is less than that of expenses.

**Discussion** Hence data suggests that women are given an opportunity to participate in the decision making only in the situations where household expenses are to be decided and they are not given any importance if a loan has to be taken or in purchasing a house.

4.iii.Proportion of women involve in decision making in Rural and Urban Area

p1: proportion of women involve in decision making of loan

p2: proportion of women involve in decision making of household expenses

p3: proportion of women involve in decision making of buying a house

p1	0.666667
p2	0.735294
р3	0.733333

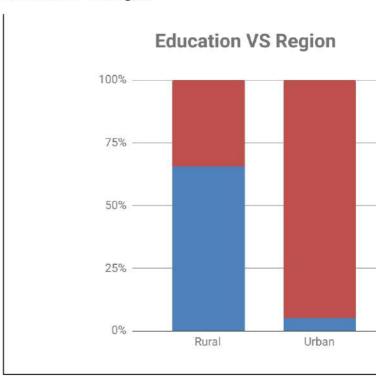
R Output to test for proportions(**Table 5**) using Marascuillo procedure is:

value critical.range

[1,] 0	0.07	0.135
[2,] 0	0.03	0.138
[3,] 0	0.04	0.134

Conclusion: There is no enough evidence to conclude any particular difference of proportions with respect to decision making in Loan, Household Expenses and buying a house is significant.

#### 5. Education Vs Region

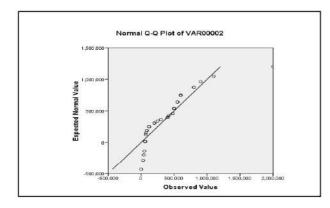


#### Graph 6

Conclusion It is concluded that the women in Rural area who are educated below 10 std are more than that of Urban area. While there are less number of women who are educated 10 std and above in Rural area than that of Urban area (from Graph 6).

Discussion: More women should be encouraged to improve their education levels.

#### 6.i. Test for normality of salaries



Graph 7

Graph 7 shows that salaries are not normally distributed. Therefore a non-parametric test (Mann Whitney Wilcoxon test) is used for testing the hypothesis.

6. ii. Mann Whitney Wilcoxon test for education and salaries earned

Ho: The incomes of two populations of women with education below 10th STD and 10th STD and above are identically distributed.

H1: The income of women with education below 10<sup>th</sup> STD is less than income of population with education 10<sup>th</sup> STD and above.

#### Z=-3.00628

Since  $Z_{statistic} < Z_{tab}$  that is -3.00628 <-1.96 .We reject Ho and conclude that the income of women with qualification below  $10^{th}$  standard is less than the income of women with qualification  $10^{th}$  standard and above.

#### 7.iLoan(Urban)

	Living with Husband	Not Living with Husband
Having a Loan	25	3
Not Having a loan	50	4

Table 6.1

 33% of the women who live with husband have taken loans, whereas 43% of the single women had taken loan in urban areas.(from Table 6.1)

outstanding	
	N
Yes	0
	1
8	8
1	2
	Yes

Table 6.2

 31% of the women who live with husband and who had taken loan are not able to clear their loan.
 Whereas 33% of single women

# could not clear their loan debt.(from Table 6.2)

#### 7. ii. Loan (Rural)

	Living with Husband	Not Living with Husband
Having a Loan	13	1
Not Having a loan	32	6

Table 7.1

 29% of the women who live with husband have taken loans, whereas 14% of the single women had taken loan in rural areas (from Table 7.1).

	Loan outstanding	
		N
	Yes	0
Living with		
Husband	4	9
Not living with		
Husband	0	1
Widow	0	1

Table 7.2

 31% of the women who live with husband and who had taken loan are not able to clear their loan.
 Whereas all single women could clear their loan debt (from Table 7.2).

#### **Conclusion and Suggestions**

More number of women living within Maharashtra are allowed to work in comparison with women living outside Maharashtra. Rural areas are competing with urban areas in allowing women to earn, even though it may be small

entrepreneurship at home or going outside for work. Data suggests that women are given an opportunity to participate in the decision making only in the situations where household expenses are to be decided and they are not given any importance if a loan has to be taken or in purchasing a house. Women are not given priority for owning a medical insurance and also they are not encouraged to invest for future in rural areas. Almost 96% of rural women and 94% of urban women suggested that there is a need of government policies for their upliftment.

In conclusion, it can be said that women in India, through their own unrelenting efforts and with the help of Constitutional and other legal provisions and also with the aid of Government's various welfare schemes, are trying to find their own place under the sun. And it is a heartening sign that their participation in employment-government as well as private and also their presence at the highest decision making bodies is improving day by day.

In rural areas more women should be encouraged to improve their education levels, own a medical insurance and invest for their future.

Women should be allowed to participate in the decision making at all levels.

There should be more awareness about government policies for women upliftment in Rural as well as urban areas.

#### Acknowledgement

I would like to express my special thanks of gratitude to my mentor Ms Shailaja Rane to guide us through all this research with patience, enthusiasm, immense knowledge and to motivate us to keep moving further towards accomplishment of our goal. We also thank Department of statistics for providing us with all the facilities needed. Also we would like to thank Principal Dr.Hemlata Bagla for giving us a platform to showcase our knowledge.

Last but not the least we would like to thank Convener of Jigyaasa SHP Dr. Sagarika Damle and Coordinator Dr. Sheela Valecha for providing us this opportunity. We would also like to thank our friends and family members for their support.

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#### #10YEARCHALLENGE

## COMPARISON AND ANALYSIS OF BOLLYWOOD MOVIES OF YEAR 2008 AND 2018.

Dhara Sankhla, Divya Srivastav, Anam Fatima Shaikh, Pratiksha Kadam\*

Department of Statistics, K.C College, D.W Road, Churchgate, Mumbai – 400020

\*Author of Correspondence: dharasankhla7@gmail.com

divyasrivastav20@gmail.com

anamfatimashaikh@gmail.com

pratiksha.kadam@kccollege.edu.in

#### ABSTRACT:

This research project focuses on comparison and analysis of Bollywood (Hindi) movies of 2008 and 2018 inspired by #10yearchallenge. Which year had better and more diverse collection of movies? Are the ratings of 2008 better than 2018? How much has the Bollywood industry changed? What kind of Bollywood movies do people prefer to watch? Etc. Such type of questions will be answered from this research.

The aim of this research project is to know how much Bollywood has changed in past 10 years.

Primary data was collected using Google forms to know the view of public on Bollywood movies. Secondary data was taken from IMDb sites and google.

A comparison of 2008 with 2018 Bollywood movies was done using statistical techniques like Welsch t-test, two-sample test for equality of proportions with continuity correction, regression and sentiment analysis. This project also includes interview analysis of people who are working in Bollywood Industry.

The data shows that there has been changes in Bollywood movies produced in 2008 and 2018. Movies of 2018 are better as compared to movies of 2018 according to the analysis.

Keywords: Comparing, Change, 10 years, Bollywood, IMDb.

#### INTRODUCTION:

#10yearchallenge was trending on social media recently. This project is inspired by this challenge, 'How Hard Did Aging Hit You' challenge was started on Facebook. It is believed that the first prominent user to partake in the challenge was Damon Lane, chief meteorologist for KOCO News 5 in Oklahoma. The post appeared on his Facebook page on January 11th, 2019. This challenge has several names: 2009 vs. 2019, How Did Age Hit You or 10 year challenge.

Bollywood is a major entertainment industry. Most of the people in India watch Bollywood movies for entertainment. Bollywood is a word given by Indian Media, it refers to the Hindi language movie industry in India. The word is often used to describe Indian cinema as a whole, but more precisely it means Hindi language movies only. These movies are made with different concept and ideas, some movies are hit and some are flop at box office.

As the years have passed, people and their mind set has changed. Since India is a secular country with people from diverse backgrounds Bollywood has to produce movies which majority of people will like to watch. This task is very hard as no one exactly knows what kind of movies may entice people.

#### METHODOLOGY

- **GOOGLE FORMS:** 
  - 1. Google analysis
  - Visualization
- \* MS EXCEL:
  - 1. Sort and Filter
    - a. Sorting

- b. Classification
- c. Cluster analysis
- 2. Graphical Analysis
- 3. T-test from Analysis Add-in

#### **R-PROGRAMMING:**

- Two sample test for equality of proportions with continuity correction.
- 2. Regression analysis

#### **SENTIMENT ANALYSIS:**

Using the following online resource we calculated the sentiment score:

https://www.danielsoper.com/sent imentanalysis/default.aspx

#### DATA COLLECTION

#### **❖ PRIMARY DATA:**

- 1. INTERVIEW
  - a. S. Ashwin (Director)
  - b. Omkar Shirgaoukar (Script writer )
  - c. Anand Naidu (Producer)

#### 2. QUESTIONNAIRE

a. Google Form
(https://docs.google.co
m/document/d/1UG2Y1
gQ4kQTh1kD7uUxylm
7jWG3jysksswwm3Q9I
fLo/edit?usp=sharing)

#### **SECONDARY DATA:**

IMDb (Internet Movie Database)

IMDb offers a rating scale that allows users to rate films on a scale of one to ten. IMDb indicates that submitted ratings are filtered and weighted in various ways in order to produce a weighted mean that is displayed for each film, series, and so on.

#### 2. GOOGLE RATING

It calculates an overall rating based on user ratings and a variety of other signals to ensure that the overall score best reflects the quality of the establishment.

#### **DATA ANALYSIS**

# 1) WELSCH t-TEST: TWO-SAMPLE ASSUMING UNEQUAL VARIANCES

Using Analysis Add-in of MS-Excel we obtained the following result:

(Gupta V., 2002) (Lu, 2010)

## ❖ For AVG RUNTIME of 2008 and 2018

 $\mu 1 = mean runtime of$  2008

 $\mu 2 = mean runtime of 2018.$ 

- H<sub>0</sub>: μ1=μ2 (there is no significant difference)
- o H<sub>1</sub>:  $\mu$ l  $\neq \mu$ 2 (there is significant difference)
- o  $H_2$ :  $\mu 1 > \mu 2$
- o p value(two tail)= **0.000183258**
- p value(one tail)=9.16289E-05

#### Reject Ho if p value < 0.05

o  $H_1$ :  $\mu 1 \neq \mu 2$  (there is significant difference)

H<sub>2</sub>: μ1<μ2</li>

p value(two tail)=0.00309192

p value(one tail)=0.00154596

#### Reject Ho if p value < 0.05

**Conclusion:** IMDb ratings of 2018 movies is mor of 2008 movies

# ❖ For GOOGLE ratings of 2008 and 2018

 $\mu 1 = mean of GOOGLE$ ratings of 2008

 $\mu 2 = mean of GOOGLE$ ratings of 2018

- H<sub>0</sub>: μ1=μ2 (there is no significant difference)
- O H<sub>1</sub>:  $\mu$ 1  $\neq$   $\mu$ 2 (there is significant difference)
- H<sub>2</sub>: μ1<μ2</li>
- p value (two tail)=0.011076615
- p value (one tail)=0.005538308

#### Reject Ho if p value < 0.05

**Conclusion:** GOOGLE ratings of 2018 movies is mor ratings of 2008 movies.

# ❖ For GOOGLE ratings and IMDb ratings of 2008 Movies.

Conclusion: Average run time of 2018 movies is less as contrared and of senage

runtime of 2008 movies.

#### For IMDb ratings of 2008 and 2018

 $\mu 1 = mean of IMDb$  ratings of 2008  $\mu 2 = mean of IMDb$ ratings of 2018

o  $H_0$ :  $\mu l = \mu 2$  (there is no significant difference)

ratings  $\mu 2 = mean \ of \ IMDb$  ratings.

- o  $H_0$ :  $\mu l = \mu 2$  (there is no significant difference)
- o H<sub>1</sub>:  $\mu$ l  $\neq \mu$ 2 (there is significant difference)
- H<sub>2</sub>: μ1>μ2

o p value(two tail)= 7.2773E-

21

p value(one tail)=3.63866E-21

Reject Ho if p value < 0.05

o  $H_0$ :  $\mu l = \mu 2$  (there is no significant difference)

O H<sub>1</sub>:  $\mu$ 1  $\neq$   $\mu$ 2 (there is significant difference)

H<sub>2</sub>: μ1>μ2

p value(two tail)=1.27243E-26

Conclusion: GOOGLE Ratings are more than IMDb ratings loval to the conclusion.

6.36215E-27

❖ For GOOGLE ratings and IMDb ratings of 2018 Movies.

 $\mu 1 = mean of google ratings$ 

 $\mu 2 = mean of IMDb ratings.$ 

Reject Ho if p value < 0.05

Conclusion: GOOGLE Ratings are more than IMDb 1

#### 2) TWO-SAMPLE TEST FOR EQUALITY OF PROPORTIONS WITH

CONTINUITY CORRECTION (Gupta V., 2002)

To compare the genres of movies released in 2008 and 2018.

1. TWO TAIL TEST

P1= Proportion of a particular genre in 2008 movies.

P2=Proportion of a particular genre in 2018 movies.

H<sub>0</sub>: P1=P2 (The proportion of movies for a particular genre is equal in 2008 as well as in 2018)

H₁: P1≠P2 (The proportion of movies for a particular genre is not equal in proportion in 2008 as well as in 2018)

Conclusion: There is no significant difference in the proportion of above

given genre in 2008 and 2018 respectively, except for the genre of

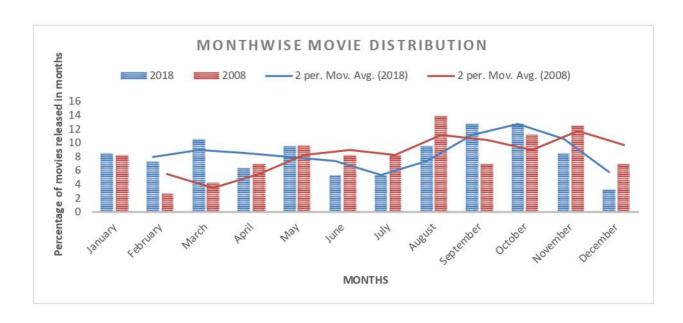
Romance and others.

ROPORTION	Decision criteria  Reject H <sub>0</sub> when p  value <0.05			
Genre	prop 1	prop 2	p-value	Conclusion
Comedy	0.221374	0.213333	0.9856	Do not reject H <sub>0</sub>
Horror	0.229008	0.12	0.4523	Do not reject H <sub>0</sub>
Biopic	0.007634	0.026667	1	Do not reject H <sub>0</sub>
Thriller	0.053435	0.08	0.4385	Do not reject H <sub>0</sub>
Drama	0.045802	0.006667	1	Do not reject H <sub>0</sub>
Adventure	0.038168	0.02	0.08614	Do not reject H <sub>0</sub>
Crime	0.022901	0.113333	0.5796	Do not reject H <sub>0</sub>
Romance	0.229008	0.12	0.02362	Reject H <sub>0</sub>
Others	0.022901	0.113333	0.006756	Reject H <sub>0</sub>

		Decision Criteria
iled test	Hypothesis	Reject H <sub>0</sub> if p value < 0.05
p-value	H <sub>0</sub> :P1=P2	Conclusion
0.02362	H <sub>1</sub> : P1>P2	Reject H <sub>0</sub>
0.9966	H <sub>1</sub> : P1 <p2< td=""><td>Reject H<sub>0</sub></td></p2<>	Reject H <sub>0</sub>
	<b>p-value</b> 0.02362	p-value         H <sub>0</sub> :P1=P2           0.02362         H <sub>1</sub> : P1>P2

**Conclusion:** More romantic movies were released in 2008 as compared to 2018 and others i.e. Psychological, Social, Political, Historical etc. movies were more in 2018.

#### 3) Visual Analysis of month wise movie distribution.



Conclusion: 2018

Maximum number of movie released in month of September and October.

Minimum number of movies released in the month of December.

2008

Maximum number of movie released in month of August.

Minimum number of movies released in the month of February

#### 5) SENTIMENT ANALYSIS (Sentiment Analyzer n.d.)

Q) What you see and hear affects your manners and how you react . Is that true in case of movies?

<u>Interpretation</u>: This text has a sentiment score of -67.5. This means that the overall sentiment or tone of This text is quite negative / serious.

**Conclusion**: Mannerism is not affected by what we hear and see in movies.

Q) What will be the scenario of Bollywood after 10 years?

<u>Interpretation</u>: This text has a sentiment score of -26.2. This means that the overall sentiment or tone of this text is somewhat negative / serious.

**Conclusion**: Scenario of Bollywood will not be good in coming years.

Most preferred movies of Bollywood

Favourite Artists of Bollywood movies

Actor	Actress	Director
-------	---------	----------

	Movie 2008	Movie 2018
IMDb	A Wednesday	Andhadhun
Survey	A Wednesday	Andhadhun
Interview	A Wednesday	Newton

IMDb	Amitabh	Deepika	Rajkumar
	Bacchan	Padukone	Hirani
Survey	Ranveer	Deepika	Rohit
	Singh	Padukone	Shetty
Interview	Shahrukh Khan	_	Imtiaz Ali

#### **ANALYSIS**

#### \* SECONDARY DATA (GOOGLE AND IMDb RATINGS)

IMDb 2018 rating are better than IMDb 2008. Google 2018 rating are better than 2008. Avg run time of 2018 movies is less as compared to average runtime of 2008 movies. Google has comparatively rated more than IMDb ratings in 2008 as well as in 2018. In 2008 and 2018 the proportion of genres (movies) are similar for comedy, action, drama, etc. except for the genre of romance are less and movies with new concept are more in 2018.

#### \* ANALYSIS BASED ON QUESTIONAIRE (MADE ON GOOGLE FORMS)

Comedy is most preferred genre in Bollywood movies with 69% which implies that the box office collection of these movies will be high and the chances of comedy movies going flop are less.29.1% respondents like watching movies in evening that means theatres will be more filled during evening time and least preferred is night

(16.4%).Rarely respondents go to watch movies in theatre (42.7%).Movies do affect mentality of respondents\but not much according to 29% respondents who rated 7/10.
60% respondents prefer to watch movies with friends. Movies do

have a strong impact on people according to 58% respondents.54% of respondents voted saying that the censor board should not have rights to decide what the respondents want to see in movies. 75.5% of respondents do not want to enter Bollywood. 40% of respondents think that movies collect good money because of storyline.72.7% of respondents feels that their community is represented in Bollywood movies. 61.8% of respondents prefer to watch Bollywood movies in theatre. 23.6% of respondents have rated 7/10 for changes observed in mentality of people because of movies.64.5 % of respondents think there is improvement in selection of movies since 2008. 42.7% of respondents prefer to watch Bollywood movies on Netflix.86.4% respondents said that more movies should be made on social causes. 20% respondents rated Bollywood 6/10.

#### ❖ INTERVIEW ANALYSIS

#### • S.ASHWIN (ASST.DIR. OF PINK)

Favourite movies are
Newton, Lunchbox and
Masaan Favourite actor is
Shahrukh Khan. Perception
building is 20% from
Bollywood movies.
Preferred genre romcom.

# OMKAR SHIRGAOUKAR SCRIPT WRITER(FOR DENE MAI KYA HARZ HAI)

Favourite movie: Dil Chahta Hai. In his opinion masala movies work more on screen. Movies are more preferable than web series. His preferred genre is reality depicting human emotions.

#### o <u>ANAND</u> NAIDU(PRODUCTION)

According to him storyline is the most important factor for a movies success.2018 movies are going in wrong direction. Most preferred genre is action.

#### CONCLUSION

People's perception and preference towards Bollywood has improved.People have rated more on Google than IMDB for movie ratings

.Major change what our generation wants is that more social movies should be made. Storyline is the most important factor for a movie's success. Most preferred genre in the coming years will be biopic and comedy is the most watched genre of movie with drama being constant in 2008 and 2018. Mostly people check reviews before going for a movie. Box office collection of movies mostly depend on the trailer and review .People like Bollywood movies which has good storyline. Mannerism is not affected by what we hear and see in movies. Scenario of Bollywood will not be good in coming years.

#### ACKNOWLEDGEMENT

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Mrs.Shailaja Rane and Mr.Shubham
Niphadkar, our interviewee Mr.S.Ashwin,
Mr.Omkar Shirgaoukar, and Mr.Anand
Naidu for giving us their valuable time.
We would also like to thank our
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# MODULE REPORT BATCH XIV

# **Module Report**

# For 'Jigyaasa'

Science Honors Program Batch XIV (2017-2018)



#### Preamble:

Let us start from begining when I got introduced to this course I knew nothing about it. But as it is an Honors program and I was always interested to do BSC with honors so this course became my attraction like a gravity.

Through out this 18 months course I have seen and learned a lot from each and every activity that this course has provided. I am sure am never going stop being a researcher and surely make my findings fruitful for masses like It has been taught to us in this program.

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Sr. No.	Modules	Date	Resource person.
1.	Inauguration Ceremony of SHP batch XIV		principal
2.	Mind and Mastery		Kush Panchal
3.	Advance Communication Skills	9 <sup>th</sup> Oct 2017	Mr.Martin Ebnazer Dhas
4.	Research Methodology	9 <sup>th</sup> Oct 2017	Mr.V.N Patkar
5.	Finance Gym	10 <sup>th</sup> Oct 2017	Ms Binal Ghandhi
6.	Review of Literature	11 <sup>th</sup> Oct 2017	Ms.Amruta
7.			Ms.Binal Ghandhi
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			Pujari
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13.	Importance of Visual Communication on an Impactful 1 <sup>st</sup> Impression	16 <sup>th</sup> March 2018	Ms Manisha Mishra
14.	Meeting the Convener & News letter Making	17 <sup>th</sup> March 2018	Dr.Sagarika Damle
15.	Dabbawala-A success story of management	20 <sup>th</sup> March 2018	Mr.Raghu Medge
16.	Photoshop	28 <sup>th</sup> March 2018	Mr.Paresh Pandya
17.	Writing an Academic Essay	2 <sup>nd</sup> April 2018	Ms.Kavita Peter
18.	Persisting Discrimination on against women	2 <sup>nd</sup> April 2018	Ms Srinidhi Population 1 <sup>st</sup> NGO
19.	Critical Thinking	2 <sup>nd</sup> April 2018	Dr.Anupma H Ms.Amruta Kothare
20.	ICT tools in Research	3 <sup>rd</sup> April 2018	Prof.Abhijit Gole (ruia college)
21.	Poster Making	3 <sup>rd</sup> April 2018	Ms.Aruta kothare
24.	Mangrove Walk	5 <sup>th</sup> April 2018	Mr.Amar Deshpande
25.	Dawat-e-SHP		

### 1. Inauguration Ceremony of SHP batch XIV

<u>Venue</u>: Seminar Hall, 4<sup>th</sup> floor, K. C. College <u>Resource Person</u>: Dr.Hemlata Bagla (Principal)

The event gave us many useful tips to start of the program with lots of enthusiasm. Dr. Hemlata Bagla, principal of K. C. College also addressed the ceremony with her always supporting words. She gave us guidelines for a more successful working experience. Their words have been with me throughout the year & I am keen to follow them for a glorious career. She very succinctly explained us what research is & reminded us of the requirements for a great scientist:

#### Inquisitive Skills, Hard work & should have Excitement.

A significant pointer he gave us, which I personally use, is that we should always keep a notebook handy to note down any ideas that strike us. "Opportunities' are plenty, to recognize it at the right time is important"

another helper that I really liked.

#### 2. Mind and Mastery

Venue: MMR, Ground floor, KC college

Resource person: Kush Panchal

This module as the name Mind and Mastery was really mindblowing. The resource person Kush Panchal has proved us that our hardwork in our desired field would definetly lead us to success. He taught us that the yoga is very important of every healthy body and we being student should exercise yoga for healthy mind. The best part was the bollywood yoga that we got to learn from him.

#### 3. Advanced Communication Skills

Date: 9th October 2017

Venue:Seminar hall,4<sup>th</sup> floor, KC college Resource person : Mr. Martin Ebnazer Dhas The advanced communication skills was taken by resource person Mr. Martin Ebnazer Dhas. He sarted his session in very unique manner. The uniqueness about his ppt was it was black and white. His session really taught me how important is to have strong communication skill. In his session the sheet he distributed and the way he communicate and explained the preceedings were very interesting. in that session I learned that how blindly we read things and our mind is trained to pick only what we want, which is not so good as a good communicator. So my learning from this session is that we should not blindly read, we should read things fully and with concentration.

#### 4. Research Methodology

Date: 9th October 2017

Venue:Seminar hall,4th floor, KC college

Resource person: Mr.V.N Patkar

This module was the most practical at the time as we were all looking for a perfect way to present our research for the coming SHP session. Dr. Patkar started of by telling us the starting point for any research:

Curiosity, Cause ↔ Effect study, Generalization of Available Results & to test an orthodox idea.

He provided us with the details of the elements of a research proposal, which in general include Abstract, Introduction, Methods, Results and Conclusions. He also provided us with a proper format for the proposal.

It was a very useful session for students like me who would want to make a carrier in research as it gave me the whole process of writing the proposal very handy. Thank you!

#### 5. Finance Gym

Date: 10th October 2017

Venue: Seminar hall, 4th floor, KC college

Resource person: Ms. Binal Ghandhi

This module was taken by Ms.Binal Ghandhi. This module was very informative . all got to learn a lot from this module.

### 6. Review of Literature

Date: 11th October 2017

Venue: MMR, Ground floor, KC college

Resource person: Ms. Amruta

When I got to know about literature is something that we have to do a lot before we actually start the research. So this module by Ms. Amruta has been boon to our start of research. We have learned how to read and understand any research paer. And which research paper will help us and how to find the correct research paper was clear as this module.

### 7. Finance Gym (activity)

Date: 11th October 2017

Venue: Seminar hall, 4th floor, KC college

Resource person: Ms. Binal Ghandhi

This finance gym activity was interesting as we have never done before. I do enjoyed a bit. And it may prove helpful for us in near future. The best part of this module is we got to know new which we never had idea before.

### 8. Gender Sensitivity

Date: 12th October 2017

Venue: Seminar hall, 4th floor, KC college

Resource person: Dr. Leena Pujari

World being the beautifull place yet remained biased to gender. My question has always been to this world that why don't we accept the differences easily, what is so difficult for all of us?

In this module I got chance to speak and keep my poin of view to the people seating and best part was many of us has same idea or I would say same mentality.

### 9. Ethical Hacking

Date: 13th October 2017

Venue: Seminar hall, 4th floor, KC college

Resource person: Sachin Dedhia

Hacking in more better manner it is ethical hacking. Who does not like to know hacking and when this module is ethically introduced to you the very next thing you know is you are interested in it.i love this session most because of my interest to it. And the resource person has proved to be very helpful. Though I don't remember every part of it but I am sure in near future I will be learning the ethical hacking as I got to my interest in it.

### 10. Spoken Word Competition

Date: 14th October 2017

Venue: Seminar hall, 4th floor, KC college

Resource person: Roohd

Spoken word by ROOHD, in this we got to hear few different and decent poetry by competitors and too took part and this is also one the session I like the most cause I got appreciated by them for my piece of art with the same prize as the other participant got.

### 11. Music And Spirituality

Date: 14th October 2017

Venue: Seminar hall, 4th floor, KC college

Resource person: Dr.Radha Kumar

The classical music the best of Indian form and our very own teachers dance on music is most memorable module for me. I got to know about music and we had something out of the box. The music was quite soothing at some point and the feel that we got is really memorable one. Also the table artist was very much perfect.

### 12. Interactive Session

Date: 16th October 2017

Venue: Seminar hall, 4th floor, KC college

Resource person: Dr. Anupma Harshal

This session taken by our very own Anupma Ma'am. Here we were told to form groups and we were not aware of who will be our group members but we all participated very well and we got to know how we can make best out of little that we own. The was handled very nicely. And has proved great icebreaker.

# 13. Importance of Visual Communication on an Impactful 1st Impression

Date: 16th March 2018

Venue: Seminar hall, 4<sup>th</sup> floor, KC college Resource person: Ms. Manisha Mishra

Ms. Manisha Mishra explained us very nicely about importance of visual communication on an impactful 1<sup>st</sup> impression. Usually we are taught to believe the saying '1<sup>st</sup> impression is last impression' but the importance of this words were clear after this session. The world out there will judge you with what we overall look at 1<sup>st</sup> time. And it is true cause we all know that we all has done this at some of time in life. This session was really helpful.

### 14. Meeting the Convener & News Letter Making

Date: 20th March 2018

Venue: Department of LifeScience, KC college

Resource person: Dr. Sagarika Damle

Newsletter that we made was after this session. This session guided well for the different kind of newsletter we referred. It was helpful for making of newsletter. Also we won one of the best newsletter tag.

### 15. Dabbawala- A success story of Management

Date: 20th March 2018

Venue: Seminar hall, 4<sup>th</sup> floor, KC college Resource person: Mr. Raghunath Medge

Six sigma the perfect example of six sigma is this dabbawala who proves how one can optimize its value of work by minimizing the errors. One of the best inspirational session.

### 16. Photoshop

Date: 28th March 2018

Venue: Computer Science Lab, KC college

Resource person: Mr. Parekh Pandya

Basics Photoshop learning by Mr. parekh pandya was interesting as we got practice practically which was lovely experience. This session has helped me to understand photoshop a little better than before. The resource person was very knowledgeable and up to date with the new softwares and apps used for the same.

Also we edited different celebrity picture and made different faces with vegetables.

### 17. Writing an Academic Essay

Date: 2<sup>nd</sup> April 2018

Venue: Seminar hall, 4th floor, KC college

Resource person: Ms. Kavita Peter

Ms. Kavita Peters session has really helped us as she said that writing disciplines our mind and peer review is very important. For the writing better academic assay she has also given us some pointers which is really helpful.

### 18. Persisting discrimination on against Women

Date: 2<sup>nd</sup> April 2018

Venue: Seminar hall, 4th floor, KC college

Resource person: Ms. Srinidhi Population first NGO

Ms. Srinidhi has made this session very much interactive where she made us speak about our own stories and the solution to it, how we faced and how the things are meant to be faced by real world. Also she share few cases what she has faced and her interaction with different type of harassed women and how she deal with it. Her session has taught me the importance of listening. Cause sometime person doesnot share problem that we give solution to it but listening to it is itself the solution.

### 19. Critical Thinking

Date: 2<sup>nd</sup> April 2018

Venue: Seminar hall, 4th floor, KC college

Resource person: Dr.Anupma H, Ms.Amruta Kothare

This session by our Anupma Ma'am and Amruta Ma'am has taught us to understand the simplicity of things and not make it complex by our experienced observation. We often have an habit of making things complicated. So in this session we were asked to perform certain

experiment for example melody was given and we were asked to eat and note down each and every observation, etc.

### 20. ICT Tools in Research

Date: 3rd April 2018

Venue: Seminar hall, 4th floor, KC college

Resource person: Prof. Abhijit Gole

Prof. Abhijit gole taught us the more advanced method of using excel. We have previously used and also worked on it by how to record a video over it was an interesting part taught by him.

### 21. Poster Making

Date: 3<sup>rd</sup> April 2018

Venue: Life Science Lab, KC college Resource person: Ms.Amruta Kothare

Poster making the most crucial part of presenting in Avishkar. Ms. Amruta has covered this session properly. In this session the different method of poster making was explained. We were also asked to practice making poster on the spot by the help of research we had completed till then.

### 22. Mangrove Walk

Date: 5th April 2018

Venue: Godrej Mangrove Park, Vikroli Resource person: Mr. Amar Deshpande

Most enjoyable session and the nature we got to explore is also one of the best module. The resource person Mr.Amar Deshpande explained each and everything very well. We got to know different types of mangroves also we had a lot of fun with our this year batch of SHP.

### 23. Dawat-e-SHP

Date: 2<sup>nd</sup> April 2018

Venue: LifeScience Lab, 5th floor, KC college

The most favorite session of them all. We were given fixed set of ingredient and we have to make the best out of that given ingredient. We were grouped randomly. We were group of 5 and our group name was **Appetite** and we won 3<sup>rd</sup> price too in it. Also we all eat the dishes made by our friends. The lesson I learned from this module is we can do best with limited material too.

### The End Note:

The modules held throughout the 'Jigyaasa' journey, were really educative, interesting and varied, the ones which taught us that values were the most important ingredient for the charter of a successful life. I'm really grateful to SHP for the wonderful modules organized. The efforts put in by the SHP mentors and professors associated with Jigyaasa- SHP to get quality resource persons are really commendable. The experience was really wonderful and I learnt much more than I ever thought I would through SHP.

Over all this 18 months journey has taught us life long lessons we I am never going to forget.

Thank you for everything that made us different and better from before.

# THANKYOU.



# SHP BATCH XIII





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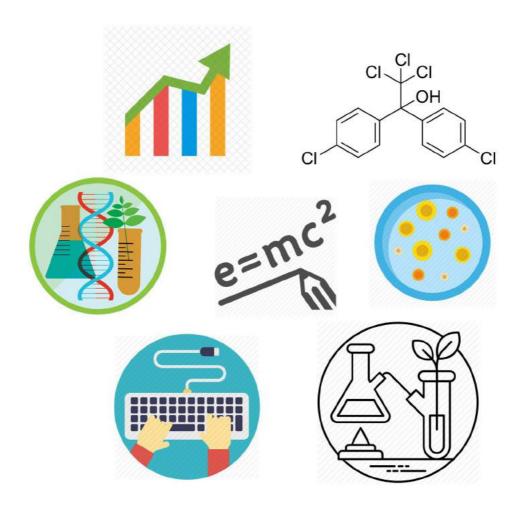
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# JIGYAASA VOLUME VIII VIV

**VOLUME XIII - XIV 2016-19** 



## Kishinchand Chellaram College

Vidyasagar Principal K.M. Kundnani Chowk, 124, D.W. Road, Churchgate, Mumbai - 400 020. Tel: +91-22-66981000 Fax: +91-22-2202 9092

E-mail: office@kccollege.edu.in • www.kccollege.edu.in