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

JIGYAASA

2015-16

VOLUME XII

FROM LAB TO life

From the Principal's Desk

Winston Churchill said, 'To improve is to change; to be perfect is to change often.' Although we are well aware that perfection is idealistic. K.C. works towards coming as close as it is possible to attain perfection. To achieve this, change essential and I believe that it is you, dear students, and the young fresh minds that are ready to embark on the journey from the lab to real life, with new and innovative ideas, helping bring change in the world.

It gives me immense joy to present yet another volume of 'Jigyaasa', a research magazine and an undergraduate Research endeavour of KCSHP'ians. Jigyaasa-volume XIII encapsulates the wonderful journey of Science Honours Programme Batch XIII students over a period of eighteen months offering a unique opportunity to the undergraduate students to have an early start in the research arena.

I extend my heartfelt gratitude to each and every research scholar, research guide, and members of the Non teaching Staff involved in Jigyaasa for their untiring efforts, keeping this Science Honours Programme 'Jigyaasa' running successfully for the past 13 years.
I applaud the efforts of all the students, whose research work has been compiled in the form of informative reports, research communications, research articles and research papers in this volume.

I wish Team 'Jigyaasa' all the very best in all their future endeavours.

Dr. Hemlata K. Bagla

I/c Principal

A Tribute to our Super Mentor

Dr. Manju Nichani completely exemplifies this saying, "Strength and Growth come only through continuous effort and struggle." She is a person who believes in tireless efforts and gains strength from a job that is well done.

Dr. Nichani wears multiple hats effortlessly and gracefully. Currently she is much immersed in the work of Sadhu Vaswani Institute of Learning Pan India that was her heart's calling but is also tackling the newer academic challenges with ease as a Director of Hiranandani Institute of Learning. Dr. Nichani was the former Principal of K.C College, the Director of K.C College of Management Studies and the former Secretary of the Hyderabad & (Sind) National Collegiate Board, H(S) NC Board. Besides the above official designations, at heart, she is forever an educationist and a feminist. Under her able leadership, K C College has been thrice re-accredited 'A' grade by NAAC. Another fine feather in her cap was KC College being awarded the Best College by University of Mumbai for the year 2103-14.

Under Dr. Nichani's dynamic vision, KC College also celebrated its Diamond Jubilee in 2014 with the festivities being Inaugurated by none other than the then President of India. A pragmatic, perfectionist yet empathetic leader, a lady with an iron hand and gentle heart, standing tall as a lighthouse in the field of academics - that is our dear Manju Madam or Dr. Manju Nichani. Being a visionary, she sensed the importance of training young minds in research at undergraduate levels and started the Science Honors Program – SHP which is identified now as 'Jigyaasa', during the Golden Jubilee Year of K.C. College, 2004-2005.

With a new batch of energetic and curious minds trained year after year, she supported and encouraged every single activity of SHP for twelve glorious years. SHP entrants would

eagerly await and enjoy her talks on 'Time Management', 'Effective Leadership Skills, 'Seven Habits of Successful People' and many more. Most of the participants were awe struck by the fluency and spontaneity of her talks and looked up to her as their role model in communication skills and public speaking. She strongly believed that a good scientist should harbor a combination of scientific temper and humane, empathetic approach with a keen sense of social responsibility. She created a pool of dedicated teachers and resources that shaped and nurtured the young researchers and entrepreneurs that college is proud of.

The recognition of K.C. College in the field of research today, especially at the undergraduate research achievements, is all due to Dr. Nichani's constant encouragement and support for 'Jigyaasa' program at K.C. She took pride in all achievements and laurels of Shpians, from the prizes won across the conferences and seminars to poster competitions to the 32 publications in the International peer reviewed journals. Batch XII of SHP is extra special because it is the last batch to graduate under the Super Mentorship of Dr. Nichani at the time of her superannuation. They witnessed the smooth transition of their super mentor, Manju ma'am from one role into other with the same fervor and enthusiasm after more than forty years in the field of education. We, the members of Team Jigyaasa- SHP, wish her many beautiful, fruitful years of inspiring work that would enlighten hundreds of young minds and help them to reach their goals.

Dr. Manjula J. Nichani

Ex-Principal

From the Conveners' Desk

'My mind's garden is my most beautiful creation, for whatever I sow now will reap in multiples. It teaches me patience in the beginning and allows me to enjoy nature's bounty at the harvest'.

We, at K.C. College, believe in not just learning from syllabus but also widening our student's knowledge boundaries by innovative and experimental learning. The faculty members of Science departments at K.C., have been involved in this unique activity, though an Undergraduate Research program, called 'Jigyaasa-SHP' with a tagline of 'Nurturing Young Minds from Lab to Life'. The activity that started under the leadership of our pragmatic Ex-Principal, Dr. Manjula Nichani during the Golden Jubilee year (2004-05) of K.C., completes twelve years and enters into its thirteenth year. This current volume XII of our research magazine 'Jigyaasa', includes not only the research papers of our young budding scientists, but also their well penned module reports that offer the readers, glimpses of the multifarious activities organized under SHP program, that help our 'Jigyaasus' to develop into confident human being with a good mix of scientific temper and values and ethics, that would guide them onto the right path.

This research magazine 'Jigyaasa' is one of the highlights and a true testimony of the hard work put in by the teachers and students throughout the eighteen long months of 'SHP-Jigyaasa' program. It is also a magazine created by our students under the guidance of teachers, with a special mention of Ms. Geeta Brijwani and her team, who have worked meticulously and tirelessly to bring out this issue of Jigyaasa-Volume XII.

I take this opportunity to express my heartfelt gratitude towards our Principal Dr. Hemlata K. Bagla, who has ignited a thirst for achieving excellence in all our research activities. I also thank the Vice Principals, Prof. Smarajit Padhi and Dr. Shalini Sinha, for extending their support, leading to the successful completion of yet another batch of Jigyaasa- SHP. I express my sincere thanks towards

the coordinator of batch XII, Dr. Anupma Harshal and all the mentor teachers for supporting their students throughout the SHP program. I appreciate the extra efforts put in by various teachers of SHP committees, Prof. Prabha Padmanabha, Prof. Sheela Valecha, Dr. Tejashree Shanbhag and Dr. Archana Thite, for contributing towards effective and time-bound evaluation process and technical support. I take this opportunity to congratulate students of Batch XII, for successful completion of SHP program. We are proud of their achievements of research publications in International journals and representing K.C. at various research platforms, winning prizes. On behalf of the entire Team 'Jigyaasa-SHP', I wish them good luck for a bright future.

Dr. Sagarika Damle

Convener, Jigyaasa, SHPKCC

Message from the Co-ordinator

A Decade of exuberance of innovative ideas by young

Kishinchand Chellaram College at the University of Mumbai offers an excellent environment for the pursuit of graduate education. The college is home to an exciting Undergraduate Research Program called 'Jigyaasa' Science Honors Program initiated in the year 2004. Under the able leadership of Dr. Hemalata K. Bagla and the Star DBT Scheme at the college, JSHP has gained a new momentum in its 14th year. It aims to providing students with essential, research-based professional development.

The 18 month program journey began with the star gazing event at Tandulwadi, nature trail at Sanjay Gandhi National Park, Laughter yoga, wellness for self and included coffee with the Vice Chancellor. The program focuses on studying the diversity of experiences that include, theoretical perspectives of research, its methodology, Resume writing, Gender sensitization, Communication skills, Culture, Film appreciation to name a few. The students enjoyed the food extravaganza, role play, and Interactive sessions and developed a sense of giving quotient with the visit to the Saphale Baal wadi. The other modules include awareness about infectious diseases, financial transactions in the stock market and body language etiquette. They horned their technical skills by learning how to make an app, use Photoshop, word press and a firsthand experience of ethical hacking

The batch harnessed experience by presentations at various prestigious University level competitions and also at the National Indian Youth Science Congress, wherein many won accolades and received appreciation for their research work. Advanced communication skills, enhanced student's expression power and they won the heart of the renowned judges from prestigious academic institution during the 9th Intercollegiate Research Meet, organized by K.C. College.

The journey culminates in the much awaited Felicitation Ceremony, the highlight of the ceremony being, release of Research volume 'Jigyaasa'.

We release the XIIth volume of the Undergraduate student research journal that is a publication of original research undertaken by the students. The publication enables undergraduate students to actively contribute to an international knowledge base. The students are heavily involved in the editorial process making corrections based on the reviewers' suggestions, as well as grammatical and structural changes. This enables them to gain editorial and leadership expertise. A special thanks is due to all the mentors, student volunteers and teachers of Computer Science department for this published outcome.

'Team Jigyaasa', is grateful for the constant motivation and guidance given to us by our pragmatic ex Principal, Ms. Manju Nichani, in all our endeavors. We are also thankful to the Vice principals, Dr. Shalini Sinha and Mr. Smarajit Padhi for their support and ensuing this journey together. Special thanks are due to our mentor and former Vice Principal, Dr. Vijay Dabholkar for troubleshooting and making this journey as smooth as possible.

The success of the JSHP has only been possible due to the commitment and passion of our faculty members, Industry mentors, Resource persons leading to continuous quality improvement. I would like say a big heartfelt 'Thank You' to all the teacher members of 'Team Jigyaasa' for their active participation. I value the contribution and assistance of the non-teaching staff and office staff for their technical assistance. At K.C. College, Jigyaasa SHP we believe that a holistic grooming of undergraduates nurtures the scientific community and in turn prepares them as communicators of science to both the scientific community and the public. Here's wishing Batch XII good Luck.

Dr. Anupma Harshal
Coordinator

SHPKCC Batch XII

Jigyaasa Volume XII is the compilation of adventures explored by the SHP Batch XII students, and their research papers that showcase their hard work driven by passion, curiosity and guidance of their skilled mentors. We have enrolled the 14th Batch and it is a moment of pride to say that the program successfully engages in "Nurturing Young Minds from Lab to Life". 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.

The Cover page reflects willingness to learn, Upcoming with innovative ideas, unlearn the principles that have a two-edged sword and dynamics the elements of life. Also, Cover Page speculates about researchers, Researchers are always hungry for satisfying their curiosities and being an innovator as well as zeal for research existing knowledge that can resolve to the existing problem in our society and help to immensely exploring our knowledge.

Jigyaasa XII is an attempt to raise the standard of our manuscripts at par with other academic journals. It covers the journey of the SHP Batch XII 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 n 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 ex-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 Happy Reading…

Jigyaasa 2015-2016

Jigyaasa 2015-2016

Research Publications

Department	Nature/ Year of publication Title of publication	List of author/s	Book/Journal Issue no., Page nos.	ISSN /ISBN number, Impact Factor
Life Science	Antimicrobial property of Capsaicin 12th July 2017	Pranay Agarwal, Chandan Das, Oliva Dias and Dr. Tejashree Shanbhag	International Research Journal of Biological Sciences	ISSN: 2278-3202 Impact Factor:3.67
Chemistry	Qualitative Analysis of Various Adulterants and Microbes in Milk Samples Collected from Different Areas of Mumbai 15th July 2017	Krisha Desai, Jayshree Sonagra and Dr. Sheela Valecha	International Journal of Innovative Research in Science, Engineering and Technology Vol.6, Issue 7.	ISSN: 2319-8753 (online) 2347-6710 (print) Impact Factor: 6.209
Biotechnology	Study of Total phenol, flavonoid contents and phytochemical screening of methanolic crude extracts of two weed plants. 2nd July 2017	Shreshtha Shah, and Dr. Anupma Harshal and Mrs. Anushi Divan	Annals of Science Vol. 6	ISSN: 2287-688X
Biotechnology	Prevalence of Polycystic Ovary Syndrome among Women in Mumbai and Association of its Symptoms with Work Hours	Madhu Kumari, Rutuja Walavakar, Madha Shaikh , Dr. Anupma Harshal and Ms. Nandini Desai	International Journal of Innovative Research in Science, Engineering and Technology Vol.6, Issue 7.	ISSN: 2319-8759 (online) 2347-6710 (print) Impact Factor : 6.209

Department	Nature/ Year of publication Title of publication	List of author/s	Book/Journal Issue no., Page nos.	ISSN /ISBN number, Impact Factor
Chemistry	Characterization of commercial detergents and natural cleansing agents with comparison of their potential for biodegradability December 2017	Anjali Mer, Rajesh Samant, Prabha Padmanabha	International Journal of Advanced Chemistry, Vol. 6, Pages 1-7	ISSN: 2310-2977

Gene Sequence

Dr. Anupma Harshal, Adarsh Chalke, Jareena Joseph, Sharma, A Nucleotide Sequences of novel Pseudomonas strain from oral swabs of smokeless tobacco consumers. Accession Bank SUB1391942 Sequence ID1 KU937106 Released on 4th Apr 2016.

Achievements

Department	Student/s	Mentor/s	Name of Competition
Biotechnology	Jareena Joseph, Adarsh Chalke	Dr. Anupma Harshal	1. 3rd District Qualifiers at Avishkar conducted by university of Mumbai 19th Dec 2016 2. Awarded the 3rd Prize in the National Research Meet conducted by Saijan Gupta Konark Group of companies. 18th Jan 2017. 3. 3rd prize in the 8th Indian Youth Science Congress (Science & tech) organized by University of Mumbai, M S Swaminathan Research Foundation, SRM University and Rajiv Gandhi National Institute of Youth Development 16th Feb 2017
Biotechnology	Shreshtha Shah	Dr. Anupma Harshal, Anushi Divan	1.2nd Prize- TantraAvishkar 2k17 a National Level Engineering Symposium at I.E(I)-TSEC[Thadomal Shahani Engineering College] 2.Best Paper Presentation Award (1st Prize)- National Seminar on Plant Biology-New Frontiers at Bhavans College (Andheri)

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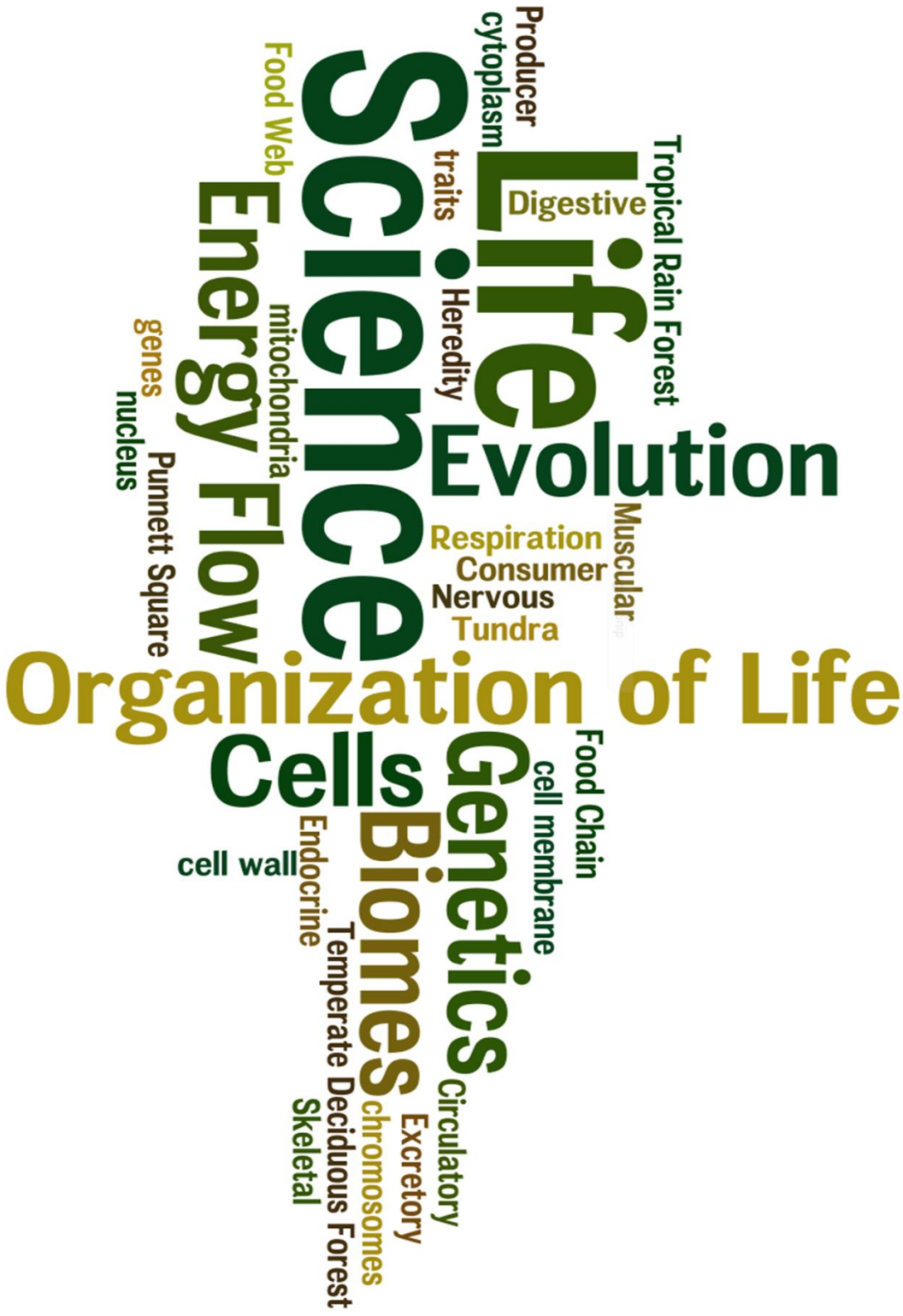
LIFE SCIENCES

What's different here is that we have now technologies that allow these life science companies to bypass classical breeding. That's what makes it both powerful and exciting.

JEREMY RIFKIN


Producer
cytoplasm
Food Web
Science
Life
traits
Digestive
Tropical Rain Forest
Energy Flow
Heredity
mitochondria
genes
nucleus
Punnett Square
Evolution
Respiration
Consumer
Nervous
Tundra
Muscular
Organization of Life
Cells
Genetics
Biomes
Food Chain
cell membrane
cell wall
Endocrine
Temperate Deciduous Forest
Circulatory
Excretory
chromosomes
Skeletal

DEVELOPMENT, FORMULATION AND EVALUATION OF HERBAL TEETH WHITENING TOOTHPASTE CONTAINING *CINNAMOMUM TAMALA*

Urmi Dhamnaskar

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*Author for correspondence **Dr. Tejashree Shanbhag**

ABSTRACT

Objective: White teeth are what a lot of people desire for. The use of a toothpaste formulated by using a herbal constituent as the Active Pharmaceutical Ingredient (API) is much safer option rather than opting for chemical procedures like bleaching which may leave the teeth with sensitivity.

Methods: In this study, a teeth whitening herbal toothpaste with API as *Cinnamomum tamala* was developed, formulated and evaluated on the basis of its pH, foaming capacity, antimicrobial activity and whitening effect on teeth. The whitening effect was checked by distributing the toothpaste to volunteers, and checking their tooth shade before and after by using VITA Toothguide 3D- Master.

Results: The formulated toothpaste showed the pH of 7.70, foaming capacity of 142 ml and a clear zone of inhibition of 1.7 cm against C. albicans. Out of the total number of volunteers, 71% of the volunteers showed increase in teeth whiteness by 1 shade in 20 days.

Conclusion: The formulated toothpaste showed about a neutral pH and good foaming capacity which was appropriate according to Indian Standards. It showed significant antimicrobial activity, and whitening of teeth was observed as expected.

Keywords: Toothpaste, formulation, whitening effect, *Cinnamomum tamala*.

INTRODUCTION

Aesthetics of the teeth is of great importance to many patients. Public demand for aesthetic dentistry, including tooth whitening, has increased in recent years [12]. The anatomic part of the tooth consists of the crown, root, enamel, dentine, pulp, and root, etc. [5]. Tooth discoloration is a common dental complaint in most populations [2]. It is imperative to determine if the discoloration is extrinsic, which is associated with the absorption of such materials as tea, red wine, some medications, iron salts, tobacco, and foods, onto the surface of the enamel and, in particular, the pellicle coating, or intrinsic, where the tooth color is associated with the light-scattering and absorption properties of the enamel and dentine [12]. Whitening of teeth is provided by dentists or other dental professionals but is more costly than whitening carried out by patients. As a result, there is interest in the development of methods for stain removal and tooth whitening that may be applied at home. Today, in developed countries, most people brush their teeth and many appear to do so in order to have 'aesthetically beautiful teeth' when they smile [2]. The clinical procedures of teeth whitening include subjecting teeth to bleaching agents like 10 – 20 % solutions of carbamide peroxide or sodium perborate (a hydrogen peroxide releasing agent). But bleaching of teeth has its own disadvantages like a possibility of soft tissue damage due to the caustic nature of the high concentrations of peroxide or a greater risk of getting post-operative sensitivity [7], so a more safer option is preferred. Toothpaste is a dentifrice which improves the aesthetic appearance and the health of the teeth [3]. The effectiveness of toothpastes at reducing or removing extrinsic dental stain has improved with the introduction of more whitening toothpastes onto the market [2]. Certain plants used in folk medicine serve as a source of therapeutic agents having multi-potential effects in addition to their antimicrobial activity. Herbal formulations can provide an option for a safe and long term- use [6]. Though various studies have been done on the effects of these indigenous products on oral health promotion and interventions, very little literature is available on the effects of these herbal products as a bleaching or tooth whitening agent [1]. *Cinnamomum tamala* has good antimicrobial effect [8]. Therefore in the present study *or tejpat* is checked for whitening effect on teeth by using it in a toothpaste formulation.

MATERIALS AND METHODS

2.1 Selection and Purchase of Materials:

The Tejpat powder was purchased from an Ayurvedic shop in Thane. The ingredients of the toothpaste i.e. Calcium carbonate, Glycerin, Sodium lauryl sulphate, Sodium Saccharine, were purchased from Vikash Pharma, Goregaon, Mumbai. Sodium Carboxy Methyl cellulose was purchased from Vikash Drugs, Princess Street Mumbai. All the ingredients mentioned above

were of I.P. Grade. 70% Sorbitol Solution of L.R. Grade was purchased from S D Fine Chemicals and Sodium benzoate was issued from the Life Sciences Laboratory of the college. For antimicrobial assay, 24 hour cultures of E. coli and C. albicans were used. Nutrient agar plates were used for the antimicrobial assay. An edge runner was used while making the toothpaste so as to bring all the ingredients together and for thorough mixing. Two commercially available toothpastes – Himalaya Sparkling White and Pepsodent Whitening were used for comparative study.

2.2 Methods:

An antimicrobial herbal toothpaste formulated by Dave et al which was evaluated as the best by them was developed further using Tejpat powder. First the herbal extracts were replaced by Tejpat powder and the toothpaste was made according to that formula. The toothpaste formula was developed by altering the amounts of different ingredients so as to get good consistency, good foaming, good taste and after feel i.e. on the basis of sensory tests. Five different formulations were formulated. The best formulation was evaluated on the basis of pH, foaming capacity and antimicrobial activity. Whitening effect was evaluated by distributing it to volunteers and checking their tooth shade before and after.

General procedure for preparation of thetoothpaste:

All the ingredients used were measured accurately. The liquid ingredients i.e. water , 70% Sorbitol Solution, Glycerin were mixed under stirrer and then transferred to the edge runner. After that the powder ingredients in the following sequence – Calcium carbonate, Tejpat powder, Sodium saccharine, Sodium benzoate, Sodium Carboxy methyl cellulose were added to the edge runner containing the liquids and all the ingredients were mixed until a paste without any lumps was formed. Then the mint oil was added as per requirement. The toothpaste was mixed for another 1-2 minutes. The Sodium laryl sulphate was added at last and the toothpaste was mixed at slow speed to avoid formation of air bubbles.

Procedure for evaluation of Foaming Capacity:

About 5 gm of the toothpaste was accurately weighed in a 100ml glass beaker, and 10ml of water was added to it. The beaker was covered with a watch glass and allowed to stand for 30 minutes to disperse the toothpaste. The contents of the beaker were transferred to a 250ml graduated measuring cylinder after mixing it properly with a glass rod. It was made sure that no lumps of the toothpaste and no foam more 2 ml went into the measuring cylinder. The residue left in the beaker was transferred with the help of further portions of 5-6 ml of

water, ensuring that the entire residue was transferred to the cylinder. The contents in the cylinder were adjusted to 50 ml by adding sufficient water. A thermometer was used to mix the contents to ensure a uniform suspension. As soon as the temperature reached 30ºC the cylinder was stoppered and given 12 complete shakes. The cylinder was allowed to stand for 15 minutes and volumes of the following were noted down:

1. foam plus water (V_1 ml) and
2. water only (V_2ml)

Calculate Foaming power ml: V_1 - V_2

Procedure for evaluation of the pH of thetoothpaste:

10gms of the toothpaste was taken into a 50 ml beaker and 10 ml of freshly boiled and cooled water at 27ºC was added to make a 50 % aqueous suspension. The pH of the suspension was measured within 5 minutes using a pH meter.

Procedure for evaluation of antimicrobial activity:

Nutrient Agar plates were used for the antimicrobial study. 48 hours culture of Escherichia coli and Candida albicans were used. Anti microbial study was done using disk diffusion technique, maintaining aseptic conditions. For comparative study, Pepsodent Whitening and Himalaya Sparkling White were used. The plates were incubated at 37 ºC for 24 hours. The antimicrobial effect was evaluated by measuring the zones of inhibition [5].

Procedure for evaluation of teeth whitening:

The volunteers were given the sample toothpaste formulation no. 5. Their tooth shade was determined and noted down using VITA toothguide 3D- Master. Volunteers were told to use the sample toothpaste once a day for 20 days. Their toothshade after 20 days was checked and noted down again. Selection of tooth shade: VITA Toothguide 3D – Master was used for checking the tooth shade. The shade Guide was held close to the teeth in natural lighting near a window. The tooth shades were checked in the same surroundings to maintain the constancy of lighting.

Table no. 2: Formulation per 100 gms of Toothpaste

Ingredients	F1	F2	F3	F4	F5
70% Sorbitol	45.0 ml	45.0 ml	45.0 ml	45.0 ml	45.0 ml
Glycerin	5.0 ml	5.0 ml	5.0 ml	5.0 ml	5.0 ml
Distilled water	15.0 ml	14.25 ml	14.0 ml	14.25 ml	13.85 ml
Calcium Carbonate	20.0 gm	20.0 gm	20.0 gm	20.0 gm	20.0 gm

Tejpat Powder (A.P.I.)	10.0 gm	10.0 gm	10.0 gm	10.0 gm	10.0 gm
Sodium Saccharine	0.25 gm	0.25 gm	0.25 gm	0.25 gm	0.15 gm
Benzyl Alcohol (BA) Sodium benzoate (SB)	0.5 ml (BA)	0.5 ml (BA)	0.5 ml (BA)	0.5 gm (SB)	0.25 gm (SB)
Sodium CMC	3.00 gm	3.00 gm	3.00 gm	2.75 gm	1.75 gm
Sodium Lauryl Sulphate	1.25 gm	2.00 gm	2.25 gm	2.25 gm	4 gm
Mint oil (Flavour)	Q. S.	Q. S.	Q. S.	Q. S.	Q. S.

Feedback Survey:

An online survey was conducted using Google Forms. The survey questionnaire asked the volunteers questions about the taste, flavor, foaming capacity, teeth whitening, freshness and afterfeel based on their experience of using the toothpaste. The results were analyzed using pie charts.
formulations were developed by trial and error, and evaluated on the basis of taste, foaming and consistency i.e. on sensory properties, by using it, for developing the formula.

OBSERVATIONS AND RESULTS

The optimized formulation was Toothpaste Formulation 5. The

Foaming capacity, pH, anti microbial activity

The Foaming Capacity of the toothpaste Formulation 5 was 142 ml. The results of the pH of the toothpastes were recorded as follows:

Table no. 3: pH of the toothpastes

Toothpaste	Toothpaste Formulation 5	Pepsodent Whitening	Himalaya Sparkling White
pH of the toothpaste:	7.70	8.34	5.76

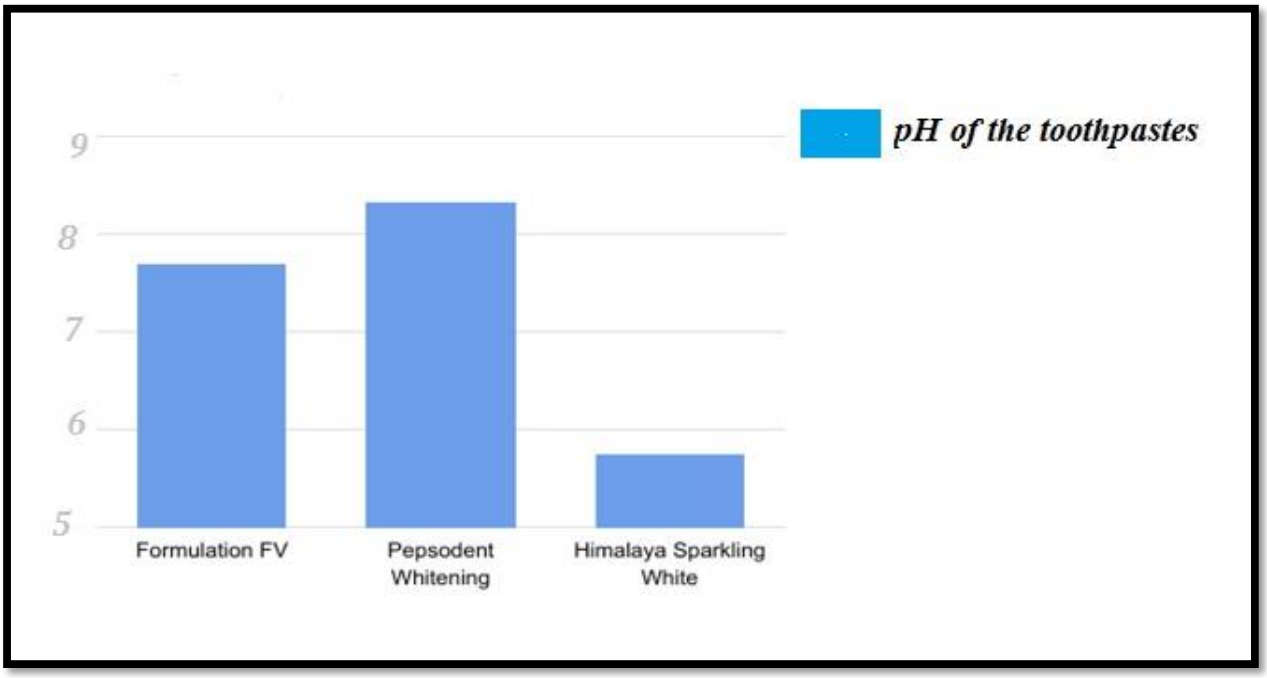

Fig. No. 4: pH of the toothpaste

None of the toothpastes showed antimicrobial activity against Escherichia coli. The Anti-microbial activity results against Candida albicans were as follows:

Table No. 4: Zones of inhibiton of toothpastes against C. albicans

Toothpaste	Toothpaste Formulation 5	Pepsodent Whitening	Himalaya Sparkling White
Zone of Inhibition (in mm)	17 mm	16 mm	15 mm

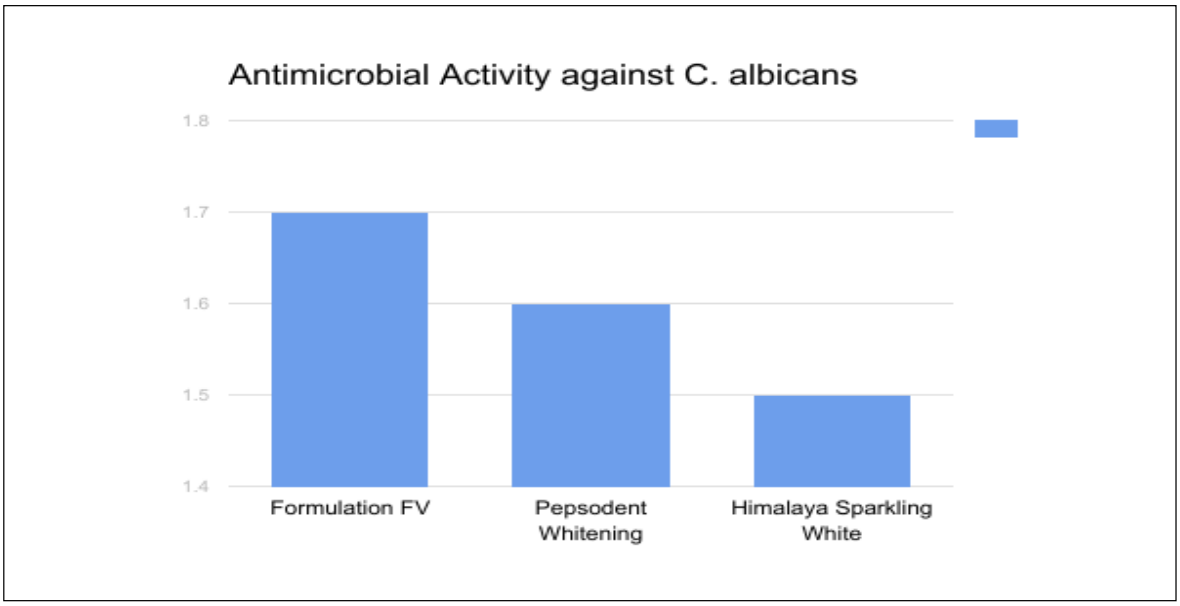

Fig. no. 5: Antimicrobial activity against C. albicans

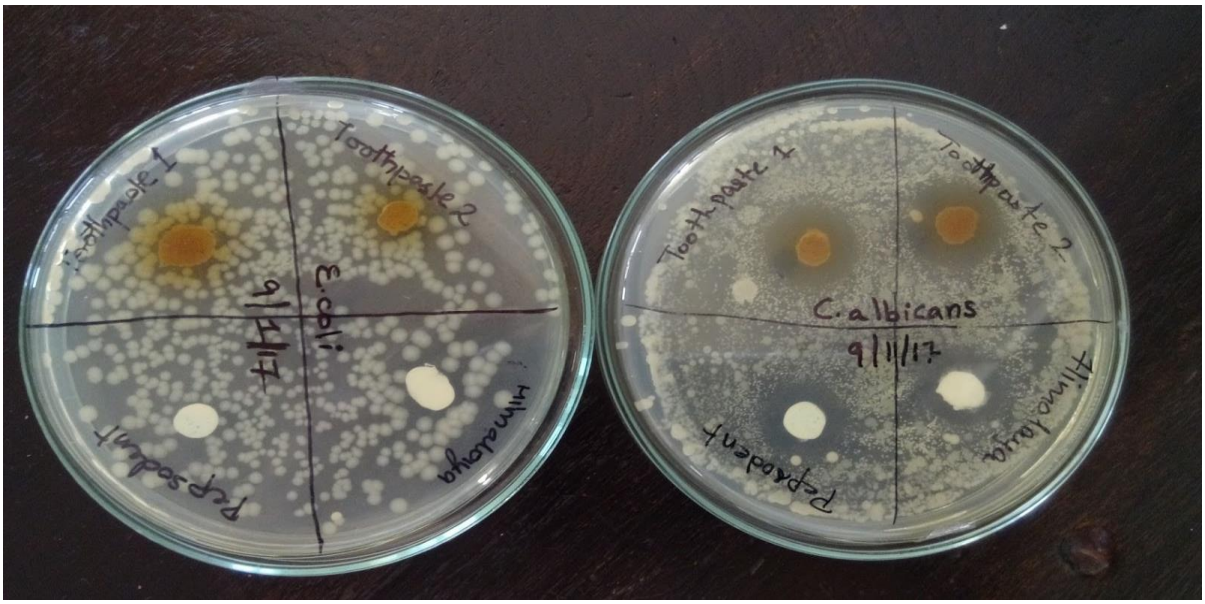

Fig no. 6: Antimicrobial activity of toothpaste against E. coli (left) and C. albicans (right)

Change in toothshade:

Out of the total volunteers 70% of the volunteers showed increase in tooth whiteness by one shade, in 20 days.

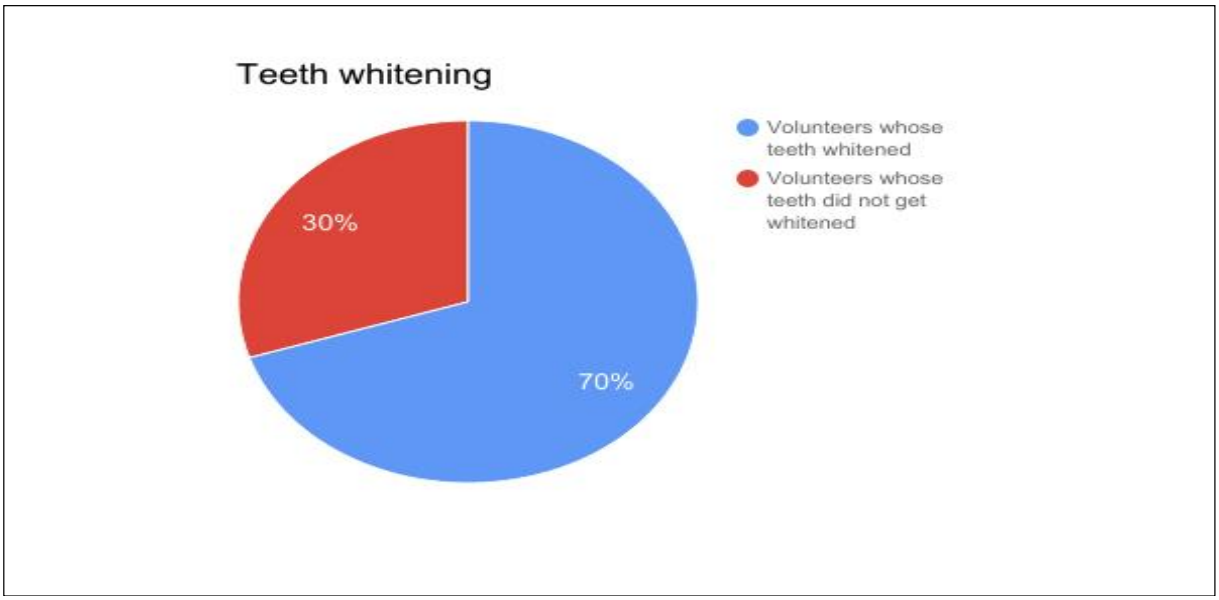

Fig no. 7: Teeth Whitening

Survey Observations and Results:

The results of the online survey are depicted in the form of pie charts:

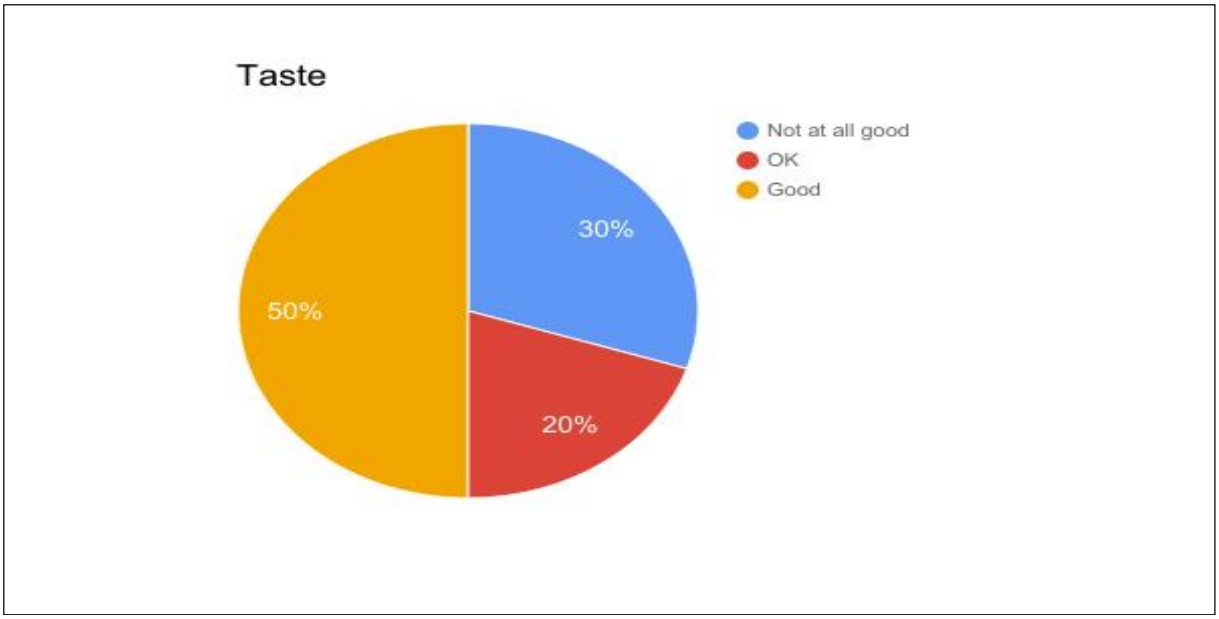

Fig. no. 8: Taste

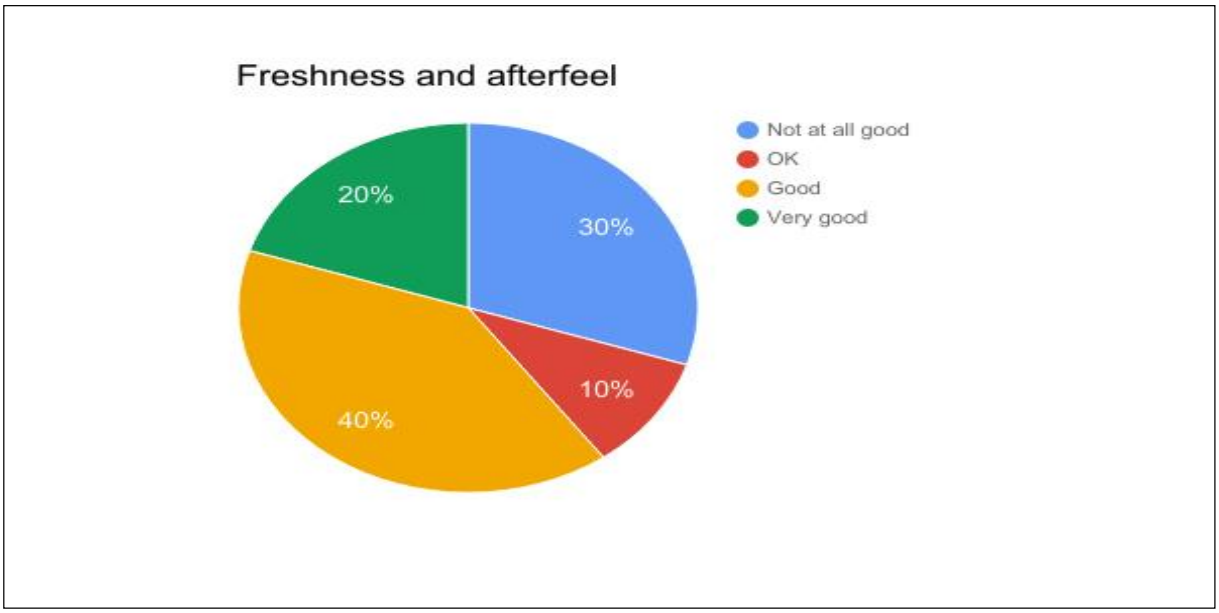

Fig. no. 9: Freshness and after feel

About 60% of the volunteers were satisfied and 10% were very satisfied using the toothpaste. 80% of the volunteers would like to use the toothpaste again.

Two of the volunteers having gum problems, also stated that they felt their teeth being strengthened after using the toothpaste. One of the volunteers applied the toothpaste to the ulcer on the inside of the lips and observed that it was healed a little.

DISCUSSION AND CONCLUSION

4.1 Discussion:

A key feature of whitening toothpastes is that they include proteolytic enzymes that remove extrinsic stains from teeth. It is claimed that some whitening toothpastes also remove pellicle (external membrane) from a tooth surface [14]. Commercially available toothpaste have the properties like anti plaque, antimicrobial activity, tooth whitening property but there are some demerits, such as most of the companies are using fluoride as an ingredient of tooth paste which may produce many associated adverse effect such as dental fluorosis [9].

Nowadays the use of herbal personal care products is on the rise. People tend to use herbal products more instead of chemical based products. The toothpaste formulation had the Active Pharmaceutical Ingredient as the Tejpat powder. An herbal alternative to get white teeth is always better as the chemical bleaching may result in damages to the teeth. In the present study the toothpaste formulation 5 showed anti microbial activity against C.

albicans. C. albicans was selected as it is a common oral microbe.

It has been previously reported that enamel erosion can occur at a pH lower than 5.2 to 5.8 [16]. And the toothpaste developed in this study has a pH of 7.70.

As the packaging was in a simple plastic jar with a lid, the flavouring added that is mint oil was getting evaporated as it is volatile.

4.2 Conclusion:

The optimized toothpaste formulation 5 had the pH near neutrality - 7.70, Foaming capacity of 142 ml, was proper according to Indian Standards toothpaste specifications, and showed antimicrobial activity against C. albicans of 17 mm. It showed significant antimicrobial activity, and whitening of teeth was observed as expected.

4.3 Future Scope:

Developing the toothpaste in a finer way can be done, like packing the toothpaste in Aluminium collapsible tubes which in turn maintain the flavor of the toothpaste, and also help it to stay disinfected. In the present study, the powder of the plant *C. tamala* was used directly in the formulation. Instead, water extracts evaporated to dryness can be used to make the toothpaste a little attractive. Further studies must be done in order to find out which is the chemical constituent of the plant that carries out teeth whitening. A mouthwash can be formulated and developed which gives a fresh breath and anti microbial effects too.

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ISOLATION AND CHARACTERIZATION OF L-ASPARAGINASE PRODUCING MICRO-ORGANISMS

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ABSTRACT

L-Asparaginase, also known as Crisantaspase, is an enzyme has a significant role in medical and bakery industry. Asparagine is an important amino acid required by cancerous cells, in absence of enzyme Asparaginase, it promotes cancer. Asparginase breaks the amino acid Asparagine into Aspartic acid and ammonia thus preventing tumors of the tissues. There is always an inflow for requirement of the enzyme due to increase in hypersensitivity reactions and development of neutralizing anti-asparaginase antibodies in patients suffering from cancer. There is a continuous need for new sources for the enzyme and extremophiles are a good alternative to explore.Salt water from salt pans were used to isolate the microbial species for the source of asparginase. Isolation and identification of the species producing the enzyme was done. L-asparaginase activity was determined by nesslerization assay, where the rate of hydrolysis of asparagine was determined by measuring ammonia released.Three strains were identified by various biochemical tests. Amongst the species identified, *micrococcus luteus* was found to produce maximum amount of said enzyme.This could provide a preliminary idea for considering the species as an alternative source to combat the problems associated with drug resistance in cancer patients specifically with Acute Lymphoblastic Leukemia (ALL). Further, purification of enzyme and media optimization for maximum production of enzyme is required.

Keywords: L-Asparaginase, Extremophiles, Acute Lymphoblastic Leukemia (ALL), *micrococcus luteus.*

INTRODUCTION

L-Asparaginase (L-asparagine amino hydrolases (EC 3.5.1.1)) is a potent antineoplastic agent, widely exploited in the treatment of certain cancers, especially acute lymphoblastic leukemia [1]. L-Asparaginase catalyzes the hydrolysis of the amino acid asparagine into aspartic acid and ammonia. Lymphocytic leukemia cells are not capable of synthesizing L-asparaginase and rely on the exogenous pool of this amino acid for their growth and survival. L-asparaginase exploits this dependency of the leukemia cells on L-asparaginase to kill them. L-asparaginase hydrolyses L-asparagine to aspartic acid and ammonia, thus; depleting the amino acid supply to leukemia cells and starves them to death [2]. On the contrary, normal cells are protected from L-asparaginase starvation due to their ability to produce this essential amino acid [3]. Since the observation that L-asparaginase from *E.coli* has an antitumor activity similar to that of the guinea pig serum, there has been considerable interest in asparaginase from various sources specially microorganisms [4]. Although various microorganisms like *E.coli* [5,6], *Erwinia corotovora* [7], *Pseudomonas acidovoras*, *Pseudomonas aerginosa* [8] *Erwinia om extreme chrysanthemi* [9], *Enterobacter aerogenes* [10], *Thermus thermophiles* [11], *Nocardia levis* [12], *Bacillus circulans* [13], *Bacillus cereus* [14] and *Bacillus brevis* [15] have a potential for the enzyme production, just the purified enzyme from *E.coli* and *Erwinia sp*. are currently in medical use as efficient as drugs in the lymphocytic leukemia, because of high substrate affinity [16].

The therapeutic use of L-asparaginase from above two sources was limited due to immunological responses. L-asparaginase from bacterial origin has several issues like hypersensitivity due to long-term use leading to allergic reactions and anaphylaxis [17], asparaginase resistance [18], leukemoginicity [19], and glutaminase activity [20]. So, due to all these factors, there is a need for newer asparaginases with new immunological properties.

Microorganisms from extreme environments like marine water and hypersaline lakes are expected to have modified structure and different immunological properties [1]. The L-asparaginase from these halophilic organisms may be used in the treatment for hypersensitive patients. In the current study, an L-asparaginase producing organism was isolated from salt pans of Bhayander region, Maharashtra, India.

MATERIALS AND METHODS

Isolation of microorganisms from salt water

The water sample was collected from Bhayander region, located in Maharashtra, India. The water was enriched in Glucose Asparagine broth, Nutrient broth appropriately diluted and inoculated on respective agar plates. The plates were incubated at room temperature for 24 hours. A total of 32 isolates were obtained from the plates, which were further screened for L-asparaginase production.

Screening of isolates for L-asparaginase production

The isolates obtained were primarily screened for L-asparaginase production using a rapid plate assay as described by Gulati et al. [21]. The isolates showing the L-asparaginase production were further screened using submerged fermentation process. The isolates were inoculated in Glucose Asparagine broth and L-asparaginase production was measured every 24 hours using L-asparaginase assay. The isolate with highest amount of enzyme production was selected for further studies.

L-asparaginase assay

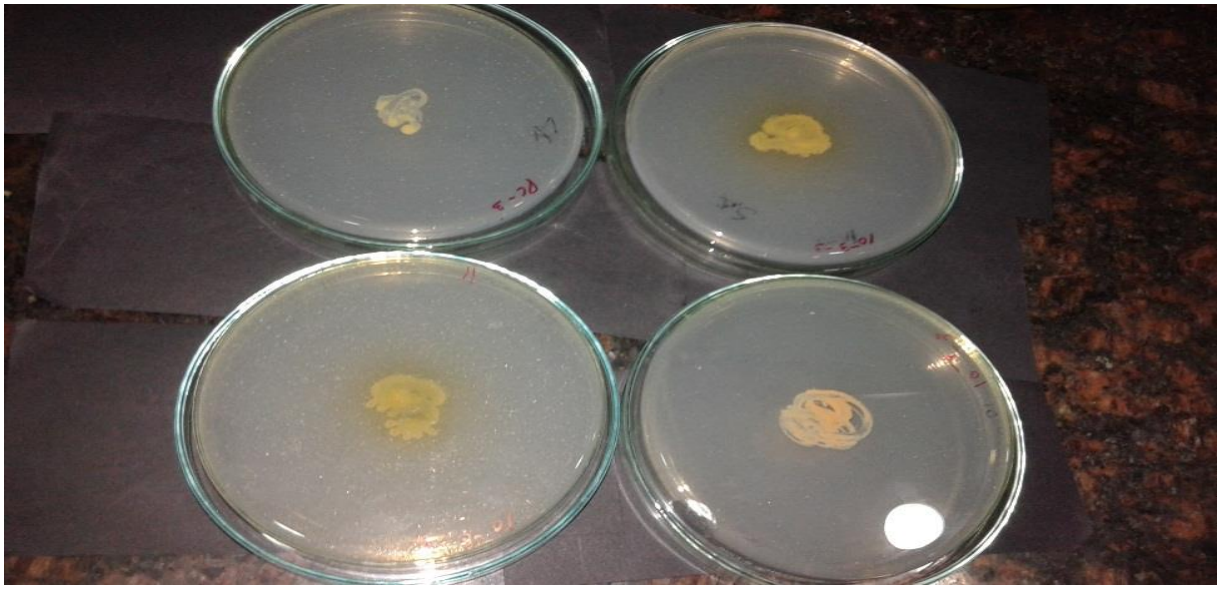
L-asparaginase activity was measured by Nessler's reaction. The assay procedure is based on direct Nesslerization of ammonia. The assay was performed according to the procedure described by Alapati and Muvva [12], with certain modifications. Cell free extract was obtained by centrifuging the culture broth at 5,000 rpm for 20 min. This cell free extract (0.5 ml) was mixed with 0.8 ml of 50mM Tris-HCL buffer pH 8.6 and 0.2 ml of 40mM L-asparagine. The reaction mixture was incubated for 30 min at 37 degree Celsius and was terminated by addition of 15% trichloroacetic acid. Precipitated proteins were removed by centrifugation and the liberated ammonia was measured colorimetrically at 500 nm by nessleriaztion. Enzyme activity was determined with reference to ammonia liberated using ammonium sulphate standard graph.

Identification of Hypersaline isolates

Different morphological, cultural and physiological characteristics of the isolates were studied for identification purpose and compared with the standard description of Bergey's Manual of Deteminative Bacteriology and also performed various biochemical tests.

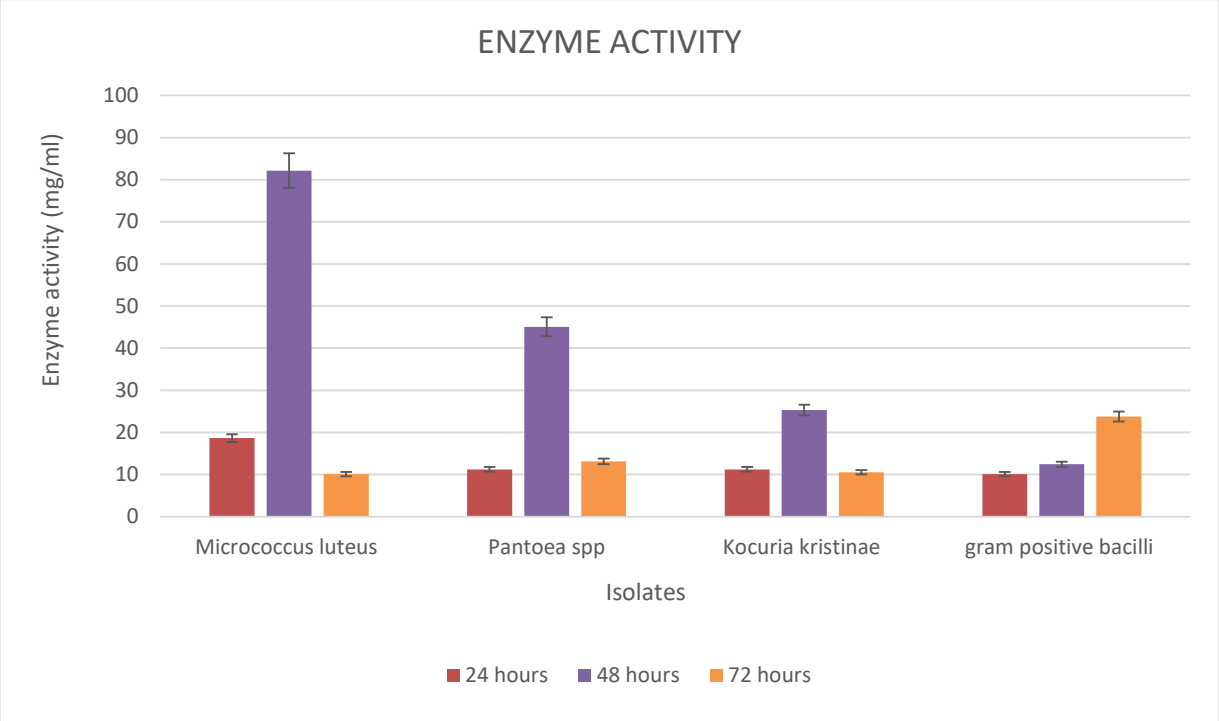
RESULTS

A total of 32 isolates were obtained from a marine soil sample of which 4 isolates (MI 1-MI 4) showed growth on Glucose asparagine agar were found to be positive in primary Screening and also checked further using secondary screening.
The figure shows 4 positive obtained strains producing L-asparaginase



With the help of secondary screening, 4 isolates were screened for enzyme activity using submerged fermentation technique. Most of the isolates showed highest enzyme activity after 48 hours of incubation. The isolates were ranked on the basis of enzyme production and the isolate MI 1 showed the maximum enzyme activity (82.15 IU/ml). The graphical representation of the enzyme production by various isolates is given below:

Rank	Isolates	L-asparaginase activity (IU/ml)
1	MI 1	82.15
2	MI 2	45.06
3	MI 3	25.34
4	MI 4	12.44



ENZYME PRODUCTION BY DIFFERENT ISOLATES AT DIFFERENT TIME INTERVALS

Identification of the isolate MI 1:

The isolate MI 1 was identified on the basis of colony characteristics and by various biochemical tests.The isolate was found to be aerobic, Gram-negative,Circular and endospore forming cocii. The colonies when examined on solid mediawere white in color, circular, medium sized, glossy and flat in appearance. The results ofthe biochemical tests are given below:

SR. NO.	BIOCHEMICAL TEST	OBSERVATION
1	Gram staining	Gram negative with paracental to sub-terminal endospore
2	Catalase test	+
3	Oxidase test	+

4	Sugar fermentation tests	
	Glucose	+
	Sucrose	+
	Galactose	+
	Arabinose	–
	Lactose	–
	Maltose	–
	Mannitol	–
	Xylose	–
5	Methyl red test	+
6	Voges Proskauer test	–
7	Citrate utilization test	–
8	Nitrate reduction test	–
9	Gelatin liquification test	–
10	Starch hydrolysis test	–
11	Growth in medium with 10% NaCl	+

Key: + positive, -negative

DISCUSSION

L-asparaginase has been well documented to possess antilymphomic, anti-leukemic and anti neoplastic activities. Organisms like *E.coli*, *Serratia marcescens*, *Erwinia corotovora*, *Psedomonas acidivoras* and *P. geniculata* have been reported to produce L-asparaginase. The most important application of the enzyme l-asparaginase is in the treatment of acute lymphoblastic leukemia, Hodgkin disease, chronic lymphoblastic leukemia and melanosarcoma [16]. Hence, in the present study organism isolation from hypersaline environment, having L-asparaginase activity was identified for maximum enzyme production by secondary screening. The isolate was identified as *Micrococcus luteus.*

CONCLUSION

The enzymes from microbial sources have gained interest due to their applications in various fields like industries, therapeutics, etc. Microorganisms inhabiting the marine and hypersaline environments are expected to have enzymes with different features compared to that of non-saline environment organisms. In the present study, isolate obtained from the marine soil sediment displayed a potential for L-asparaginase production. The isolate showed an appreciable amount of enzyme production (82.15 IU/ml) by when supplemented with glucose asparagine broth. By understanding the regulatory concepts for the synthesis of the enzyme, large scale production of asparaginase can be enhanced. The isolate was identified as *micrococcus luteus* by biochemical tests.

FUTURE PROPECTS

L-asparaginase has been a major research topic for many researchers worldwide. Its chemotherapeutic potential in treating acute lymphoblastic leukaemia has been one of the eminent discovery of the modern times. But the use of this enzyme is limited due to the glutaminase side activity leading to numerous side effects in patients. So, the emphasis was on the exploration of different sources for the enzyme production. Microorganisms from marine environment were the prime focus as they are expected to produce Lasparaginase having different immunological properties that can be used in hypersensitive patients. In the present study, media optimization for L-asparaginase from marine soil isolate *micrococcus luteus* was investigated. The molecular weight and glutaminase activity of the enzyme were also determined. The enzyme should be further characterized for its kinetic properties. The stability of the enzyme at higher temperatures should be investigated as the enzyme has an application in the food industry, especially in the bakery industry for production of acrylamide free food. The molecular studies of the enzyme including its structure determination by crystallization, amino acid sequencing, and determination of the active sites will enhance the knowledge about its mechanism of action in treatment of ALL, its immunogenicity and resistance. The enzyme can be modified genetically by site-directed mutagenesis or chemically by conjugating it with compounds which help reduce its immunogenicity and also increase its half –life. The enzyme can be a potential candidate for the biodrug concept, with the approach of drug production directly in the digestive environment by ingesting living recombinant microorganisms. Thus the enzyme and the source organism *micrococcus luteus* have a great potential

which needs to be worked upon and nourished.

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A STUDY OF ISOLATION OF MYB GENE FROM *Aloe vera*

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ABSTRACT

Objective: The Myeloblastosis (MYB) superfamily is the largest class of transcription factors. It plays a role in resistance against abiotic stress, especially water and phosphate. It regulates the growth and development of the plant. *Aloe vera* is a succulent plant growing well under water stress. It shows drought resistance to some extent. Hence, *Aloe vera* was selected to study the genes belonging to this family.

Methods: The genomic DNA of *Aloe vera* was extracted and purified. The primers from a sequenced MYB gene from *A. thaliana* were used. A PCR product of a MYB gene in *Aloe vera* was amplified. AGE was performed to identify the presence of PCR product.

Result: An approximate 200 bp part of MYB gene is expected to be sequenced which is involved in stress tolerance mechanism.

Conclusion: The technique is yet to be optimized to get a MYB gene amplified and cloned. From this study, *Aloe vera* DNA extraction and purification was optimally done. The concentration and quality of DNA as well as primer quality play an important role in the PCR amplification of a gene.

Keywords: Aloe vera, MYB gene, PCR

INTRODUCTION

Aloe vera is a succulent perennial xerophyte. It is a monocot and belongs to the grass family. It stores water in its leaves, in the form of a gel, hence it can survive well under water stress. It doesn't have any special nutritional requirements for its growth. It propagates vegetatively, no seeds are borne by this plant [1].

The medicinal properties of Aloe have been extensively explored for centuries. The gel, especially, has been used to treat injuries, minor burns and rashes. It is commonly used as a soothing and cooling agent. Aloe gel is a part of many cosmetic formulations.

Aloe, as a plant can withstand various stresses, like water stress and phosphate stress. There are many genes involved in this stress tolerance mechanism [2]. The interplay of these genes gives the plant the plant its stress tolerance ability [3]. These genes, which have similar functions or are associated with the same property are clubbed under one gene family. Most of the time, these genes are inherited together.

The Myeloblastosis (MYB) gene family is the largest class of transcription factors [4]. These genes are responsible for the overall growth and development of the plant. They regulate the circadian cycle of the plant . They help in the defence mechanism against certain viruses [5]. Some of these genes are involved in the stress tolerance mechanism.

In many plants, these genes have been isolated and sequenced. In *Arabidopsis thaliana,* about 190 genes belonging to the MYB family have been identified and sequenced [6]. However in Aloe, only some of them are sequenced.

Plants have different susceptibility to stress, depending on their ability to tolerate stress. There is an intricate mechanism, which has its roots in the genes. The expression of the right type of genes helps the plant to survive under stress. Hence, the identification and sequencing of these genes becomes essential to study this mechanism in detail. Their expression in different plants in relation to the stress tolerance property can be explained.

The aim of this study is to identify a particular MYB gene in *Aloe vera,* isolate it and further sequence it. Its expression can further be researched, to illustrate the stress tolerance mechanism. The same findings can be extrapolated to other plant models.

Objective:

1. To extract genomic DNA from Aloe vera.
2. To purify the gDNA using Purification kit.
3. To PCR amplify a clone of MYB gene from *Aloe vera.*
4. To repurify and sequence the PCR product.

MATERIALS AND METHODS

1. Extraction of genomic DNA from Aloe vera

The genomic DNA extracted from *Aloe vera* using CTAB method. The original protocol was standardized for *Aloe vera*, since it has a lot of gel content [7].

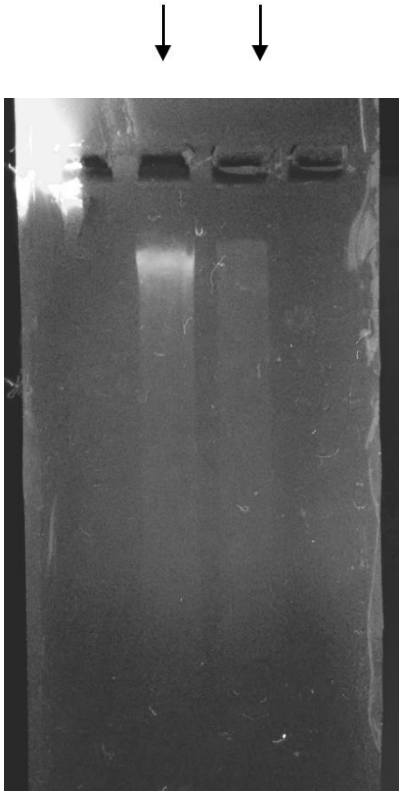
2. Agarose Gel Electrophoresis

The extracted gDNA was run on an AGE gel to visualize the DNA band. The DNA was checked for any signs of degradation [8].

3.Purification using Invitrogen`s Purelink Purification Kit

The proteins and other molecules associated with DNA were removed in the purification process. An AGE gel was run and the purified DNA was used in the PCR.

gDNA Purified gDNA



The concentration of the gDNA product decreases after purification. Since degradation and contamination are not seen, the purified product can be used for running PCR reaction, as template DNA.

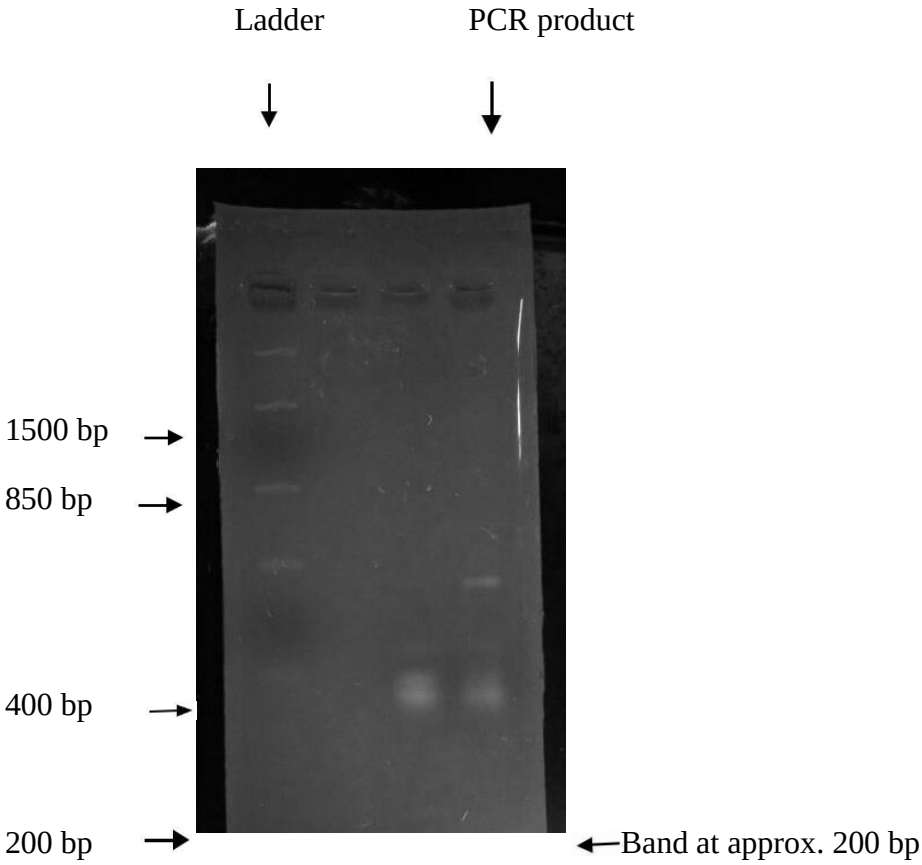
4. Polymerase Chain Reaction

The primers were synthesized from a Homolog of this gene in *A.thalaina*. The PCR was standardized at annealing temperature 54°C [9].

RESULTS

The genomic DNA of Aloe vera was extracted using the above mentioned protocol. The gDNA was purified and run on the gel. The following bands were obtained.

The PCR reaction was run several times under different temperature and concentrations of DNA. The PCR product was run on a gel along with a molecular ladder. A band was seen at approximately 200 bp.



DISCUSSION

Aloe, being a succulent plant has large amounts of gel content. During the extraction process, it is essential to slowly get rid of the aqueous gel content. The extraction of DNA, thus becomes a tedious process. Many variations were tried with different reagents and finally the above method was standardized.

The extraction protocol plays a role in the quality of DNA obtained in the final step.

There are various factors involved in the amplification of a gene in a PCR reaction. They are as follows.

1. Concentration of template DNA
2. Specificity and concentration of the primers
3. Annealing temperature

Every batch of extraction yields a different concentration of DNA. There is very less manual control on the amount of DNA that will be present in the eluted tube. After purification, the concentration further reduces. It is difficult to standardize the concentration of DNA in the template DNA used in the PCR.

The PCR needs to be standardized to the concentration of DNA in that particular batch. Since, very little amount of DNA is obtained in one extraction, the gDNA often gets used up before the PCR is standardized. The whole process has to be repeated for the next batch. The crucial

part is extracting DNA with more or less similar concentration and very little contamination.

The primers were designed by using NCBI Primer BLAST software. The gene of interest has been sequenced in *A.thaliana*. The length of this gene is 2837 bp. (Accession ID: X90381.2) The primer designing was outsourced by Sigma-Aldrich. The expected size of the fragment of gene that it can bind to 200 to 400 bp. The forward and reverse primers used very 20-22 bp and highly specific.The primer concentration needs to be optimum or excess. It should be sufficient enough to bind for 35 cyles. [10].

The annealing temperature is very important for primer specificity. Low temperature may result in unspecific binding and spurious product amplification. However, the annealing temperature used was 54°C, which is sufficiently high for binding.

The storage conditions of the PCR reagents such as RedTaq , dNTPs and primers should be maintained at -20°C. Aliquots of these reagents should be made to reduce the freeze and thaw cycles. The sterile conditions while setting up the PCR reaction , especially, DNase activity needs to be inhibited. The primers and DNA template need to be routinely checked for degradation.

Considering all the facts the PCR band of 200 bp obtaind could be that of MYB homolog of Arabidopsis from *Aloe vera.* The product obtained was not sufficient for sequencing. Also further PCR did not yield reproducicble PCR product due to variation in the quality of gDNA of Aloe.

CONCLUSION

A single band was seen at 200 bp in the PCR product. It was obtained multiple times with different concentrations of DNA. It was subjected to reamplification and only one band was seen. A PCR product isolated from *Aloe vera* which may be a part of MYB gene.

FUTURE PROSPECTS

The isolated gene, which might be MYB gene, can further be sequenced and matched with MYB genes from other plants using bioinformatics. A conserved domain of MYB should be identified.

The expression of this gene can be studied using recombinant DNA technology. This gene can be induced in a callus culture and its effect can be monitored. The stress tolerance mechanism can be illustrated by understanding the relationship between various genes.

The MYB genes are expressed in animals and humans too [11]. Some of these genes are established as oncogenes [12]. The homology between this gene and these oncogenes can be identified. The relationship between a stress tolerance gene and an oncogene can help in understanding both these mechanisms better.

This is a major area of study and there are various options to explore more about these genes, in Aloe as well as other plants.

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COFFEE CONSUMPTION IN URBAN POPULATION AND ESTIMATION OF CAFFEINE FROM DIFFERENT COFFEE BRANDS

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ABSTRACT

Objectives: Caffeine is the most common drug in the world. Its consumption from low to moderate doses is generally safe. However, if taken in excess, it causes health problems, such as insomnia, gastric intestinal disturbance etc. causing risk to human health. Coffee consumption is more in teenagers and working population. Therefore, it would be important to know the caffeine contents of some popular coffee brands and spread the awareness regarding ill effects of excess coffee consumptions.

Methodology:A survey was conducted amongst urban population of various age groups to know the coffee brand preferences who consumed coffee on daily basis. They were analysed on the criteria of coffee brands used, male /female percentage and awareness regarding the advantages and disadvantages of coffee consumption.

Caffeine was extracted from the coffee powder and coffee beans belonging to different popular brands. Method of liquid-liquid extraction using chloroform was employed for the same. Caffeine was obtained in the powder form by following steps liquid-liquid extraction, evaporation and re-crystallisation.

Results: According to the survey the most preferred coffee brand is Nestle and coffee consumption is highest among the female population of age group 10-20. Amongst the coffee brands under study, Bru was found to contain highest amount of caffeine followed by Nestle and Filter coffee.

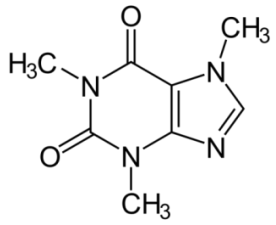
Conclusion: As Filter coffee shows less amount of caffeine than the other two instant coffee brands used in the experiment, awareness should be spread that people who consume, more that 2-3 cups of instant coffee per day, should switch to filter coffee to avoid health hazards.

Keywords: Caffeine, liquid –liquid extraction, recrystallization.

INTRODUCTION

Coffee is not only one of the most important commodities in international trade but also the world's second most popular drink, after water[1]. Coffee drinking has become an everyday affair with the proliferation of cafes, fast food outlets, takeaway coffee, home coffee makers, and a society that seems unable to function without its daily coffee fix[2].Caffeine is the most common drug in the world. Its consumption from low to moderate is generally safe[3].Caffeine is found in tea, coffee, chocolate, many soft drinks, and pain relievers and other over-the-counter medications. In its natural form, caffeine tastes very bitter[4]. But its excess consumption causes health hazards, therefore, excess consumption of coffee can also be imposing health risks amongst children, adolescents and young adults who are drinking more coffee[5].Therefore, the current research paper attempts to estimate caffeine content of some popular instant coffee brands, filter coffee and coffee beans that would help the consumers to make an informed choice about brand selection.

1.2 Structure of caffeine



NAME : Caffeine
CHEMICAL NAME : 3,7-Dihydro-1,3,7-trimethyl-1H-purine-2,6-dione
CHEMICAL NAMES :1,3,7-trimethylxanthine; 1,3,7-trimethyl-2,6-dioxopurine; caffeine
ALTERNATE CHEMICAL NAMES: thein; uaranine; methyltheobromine; No-Doz
CHEMICAL FORMULA: C8H10N4O2
MOLECULAR WEIGHT: 194.19[6].

Source and Neuro-chemical properties: Caffeine is a psychoactive CNS stimulant drug discovered by German chemist Friedrich Ferdinand Runge in 1819. He coined the term 'Kaffein' which became Caffeine[7]. Caffeine is defined as a drug because it stimulates the central nervous system, causing increased alertness. Functionally, caffeine produces a range of effects opposite those of adenosine, including the behavioural stimulant effects associated with the drug[3]. It gives most people a temporary energy boost and elevates mood and is used to reduce physical fatigue and to prevent or treat drowsiness. It produces increased wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination Caffeine is a chemical compound which is naturally found in plant sources including coffee, cocoa, tea, cola nuts, guarana etc[8]. It acts as an antagonist at adenosine receptors, thereby blocking endogenous adenosine. However, sensitive adults and children in particular, may suffer from various side effects due to caffeine[9].Hence if caffeine taken in excess, causes health problems, such as insomnia, increases the formation of urine, gastric intestinal disturbance and stimulates acid production in the stomach that increases occurrence of gastric ulcer [10]. Therefore excess consumption of coffee would be a risk to human health [5].

Fig1. Plant of *Coffea arabica*(L.)

Fig. 2 Coffee Beans

MATERIALSANDMETHODS

A] Survey Method

1) Collection of 500 forms from target population using random sampling method.

2) Analysis and representation of survey results.

B] Extraction Method

Materials: Coffee Powder from different market brands, coffee beans, filter coffee powder

Apparatus: 250ml beakers,

50ml Burette, Stirrer, Funnel.

Chemicals:Chloroform,Sodium carbonate,Calcium sulphate

(A) Extraction of caffeine from raw coffee powder:

Preparation of raw coffee solution (Ho Chun Lok, et al) [11]

1) 4.58g of fine grinded raw coffee powder is weighed, 100 cm^3 of distilled water is added along with 3.0g of sodium carbonate to increase solubility of caffeine in hot water (Acidic compounds in coffee are converted to water soluble salts by sodium carbonate and remain in aqueous layer during extraction). Solution is boiled and brewed.
2) The raw coffee solution is cooled to room temperature.

(B) Solvent Extraction
1. All apparatus is rinsed with chloroform.
2. Raw coffee solution is filled in separating funnel.
3. 10 cm^3 of chloroform is added to it and swirled vigorously. The mixture is allowed to stand and chloroform layer separates out at the bottom which is collected in a beaker. This step is repeated for 5 times.
4. Calcium sulphate is added to the separated chloroform layer to remove water. Shake well and stop until fluffy, cloudy effect is observed.
5. The beaker holding the filtrate is weighed.
6. Excess calcium sulphate is filtered out.
7. The beaker with filtrate is placed into hot water bath to evaporate chloroform (Boiling Point : 61.2 degree Celsius).

C) Re-crystallisation:
1. The extracted powder in taken a beaker and 25ml hot water is added to dissolve it.
2. The solution is boiled till the solution gets clear.
3. It is filtered while hot, using cotton plug.
4. The solution is further boiled to reduce the volume to 5ml.

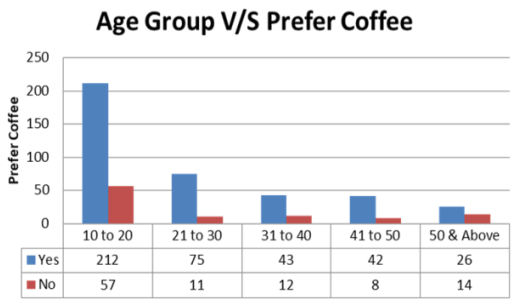
5. The solution is cooled un till the formation of crystals is observed.

8. The crystals are powdered, weighed to calculate the amount of powder extracted.

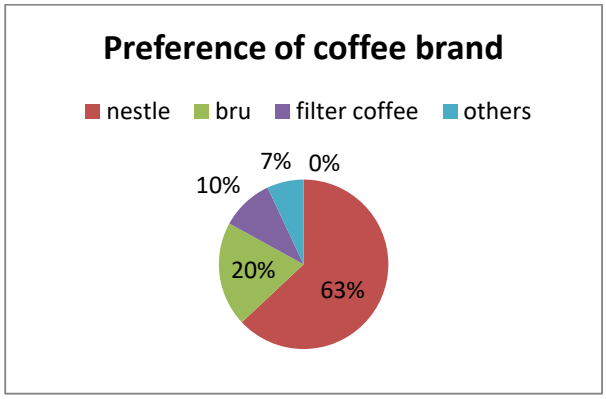
Observations and Results:

A] Survey Method: According to analysis, age group of 10-20 are more coffee drinkers and female population prefer more coffee as compared to males. It was found that most of the respondent prefered Nestle as compared to other brands.

Coffee brands	Coffee in grams (Approx 1 teaspoon)	Caffeine in grams	Percentage of caffeine
Nestle	1.208 grams	0.0279 grams	2.309%
Bru	1.208 grams	0.0443 grams	3.667%
Filter Coffee	1.208 grams	0.0105 grams	0.869%
Coffee beans	1.208 grams	0.0110 grams	0.910%



It was followed by Bru, then filter coffee and followed by other brands.



B] Estimation of Caffeine content: Amongst the coffee brands analysed coffee brand Bru was found to contain highest amount of caffeine followed by Nestle followed by local coffee beans from HP and least was found in Filter coffee.

The WHO standards of caffeine are as follows:

•0.400 grams of caffeine per day appears to be safe for Healthy Adults.

•Teenagers should limit themselves to less than 0.100 grams of caffeine.

•Heavy coffee drinkers, intake more than 0.500-0.600 grams a day, which cause side effects like insomnia, nervousness, restlessness, stomach upset, irritability, fast heartbeat ,muscle tremor etc. in sensitive individuals[6].

DISCUSSION

A survey conducted with 500 individual as target population from urban city, with an assumption that people of age group 21-30 and the working class are the highest coffee drinkers. It was proved wrong after the data obtained from the survey was analysed, as it showed that children and adolescent of age group between 10-20, preferred drinking coffee more than any other age groups under

study. This may be due to Though Nestlé was the most preferred brand as expected before the survey, it did not contain the highest caffeine content but Bru contained the maximum caffeine content amongst the brands studied.

Since coffee beans collected from a local market in Himachal Pradesh contained less caffeine than the two most popular instant coffee brands, it did have more caffeine than the filter coffee.

CONCLUSION

As Filter coffee shows least amount of caffeine from the other brands used in the experiment, awareness should be spread amongst the teenage and working population, that people who consume more than 2-3 cups of coffee per day, should prefer filter coffee to avoid ill effects of excess coffee consumptions.

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ANTIMICROBIAL PROPERTY OF CAPSAICIN

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ABSTRACT

Objective: Antimicrobial agents have always been of clinical significance. The effectiveness of antibiotics in the near future is unpredictable due to increasing antibiotic resistance among common pathogenic microbial strains. Capsaicin, the active ingredient in chillies has numerous biological properties which are yet to be explored. Due to its characteristic pungent nature, it has attracted interest in the field of antimicrobial studies, especially in the past two decades.

Methods: In the current study, pure capsaicin at different concentrations and the Soxhlet extract of Bhut jolokia (India's 'hottest' chilli) were screened for their antimicrobial effects on the common pathogenic bacterial strains Salmonella paratyphi A, Salmonella paratyphi B, Proteus mirabilis and Micrococcus luteus as an extensive literature study revealed no screening performed on these strains.

Results: Antimicrobial activity was observed in Salmonella paratyphi A, Salmonella paratyphi B and M. luteus with M. luteus showing the maximum susceptibility.

Conclusion: In this age of antibiotic resistance and emergence of pathogenic microbial mutants, it is of significant importance to have knowledge about secondary antimicrobial compounds apart from the currently known antibiotics. In a different aspect, a genetic relationship can be established between eukaryotes and prokaryotes which respond to capsaicin due a common receptor gene.

Keywords: Antimicrobial activity, Antibiotic resistance, Capsaicin, Pathogenic

INTRODUCTION

Antimicrobial agents have always carried major clinical significance, especially in this age of growing antibiotic resistance amongst common disease causing pathogens. In a country like India where no clinical prescription is required to purchase

antibiotics, resistance to antibiotics is promoted significantly. The growing trend of antibiotic resistance is becoming increasingly difficult to counter with emergence of

pathogenic, antibiotic-resistant bacterial strains like MRSA (Methicillin resistant Staphylococcus aureus).

Among human population, nutritional agents are preferred over conventional therapeutics by the former's virtue of being safe, cost-effective and regularly consumed in the given geographic area. Edibles like Manuka honey are a known traditional remedy against skin infections caused by Staphylococus aureus[1].

Capsaicin (8-methyl-N-vanillyl-6-nonenamide)[2] is an active component present in Capsicum plants (chili peppers) and is responsible for 'burning' sensation caused by chillies. It is a part of the secondary metabolite family 'Capsaicinoids' that has been known to evolve as a defence mechanism in plants against mammals, whose characteristic grinding teeth repress seed dispersion in several plants. It is noted that members of class Aves lack the TRPV1 receptor and facilitate effective seed dispersal in plants[3].

Capsaicin has been mainly employed in the past for its analgesic purposes[4]. Due to its characteristic pungent nature, it has attracted interest in the field of antimicrobial studies in the recent times. A bactericidal effect has been identified against food borne pathogens Escherichia coli, Helicobacter pylori, Pseudomonas aeruginosa and Salmonella typhi[5, 6].

India is reported to be the world's biggest producer, consumer and exporter of chilli peppers[7]. Bhut jolokia is the 'hottest' known chilli in India and the third hottest in the world according to the Guinness world records[8, 9].It is commonly cultivated in Assam and is known to have high capsaicinoid content along with other plant metabolites.

Table-1: Bacterial strains assayed for antimicrobial property

Pathogen	Basics	Sources	Symptoms	Incub-ation	Antibiotic resistance
Salmonella paratyphi A[10]	Pathogen responsible for causing paratyphoid fever.	Food and water contaminated with faeces of an infected individual.	Diarrhoea, high fever, vomiting, headache, nausea and stomach cramps.	6-30 Days	Multidrug-resistant strains (resistant to chloramphenicol, trimethoprim-sulfamethoxazole and fluoroquinolone) have been commonly observed[11].

Salmonella paratyphi B[10]	Another pathogen which causes the enteric fever - paratyphoid.	Food and water contaminated with faeces of an infected individual.	Diarrhoea, high fever, vomiting, headache, nausea and stomach cramps.	6-30 Days	Multidrug-resistant strains (resistant to chloramphenicol, trimethoprim-sulfamethoxazole and fluoroquinolone) have been commonly observed[11].
Proteus mirabilis[12]	A gram-negative, facultatively anaerobic bacterium that commonly causes urinary tract infections. It increases the alkalinity of urine due to its high level of urease production which hydrolyses urea to ammonia.	Soil, sewage, birds, reptiles and as normal flora of human intestines.	Kidney stones, painful urination, obstruction in the urinary tract.	16-24 Hours	Insusceptible to tetracycline and nitrofurantoin. Strains resistant to first generation cephalosporins and ampicillin have also been observed[13].
Micrococcus luteus[14]	A spherical, gram-positive bacterium that contributes to body odour.	Found in water bodies, soil and in the skin microflora.	Skin infections in immunocompromised patients.	16-18 Hours	Resistant to Penicillin. Inhibition of 24mm with tetracycline[14].

MATERIALS AND METHODS

Test microorganisms

Non-pathogenic bacterial strains of Salmonella paratyphi A (SpA), Salmonella paratyphi B (SpB), Proteus mirabilis(P. mirabilis) and Micrococcus luteus(M. Luteus) were obtained (Dept. of Microbiology, K.C. College) and were cultured in Nutrient Agar slants at 37^{o}C for 24 hrs.

Capsaicin
Pure Capsaicin (HiMedia Labs, Mumbai, India) was dissolved in dimethyl sulphoxide (DMSO) and dilution was performed to obtain the following concentrations: 5µgml^{-1}, 10µgml^{-1}, 50µgml^{-1}, 100µgml^{-1} and 500µgml^{-1}.

Chilli extract
Chilli extract (C.E.) was obtained by the Soxhlet extraction of 10g Bhut jolokiapeppers with methanol as solvent[15]. Methanol was completely evaporated in a hot air oven and the extract obtained (0.477g) was dissolved in 10 ml of DMSO.

Susceptibility Tests
Susceptibility was studied by the methods of agar disc diffusion (Kirby-Bauer method) and agar well diffusion on bacteria cultured by spread plate and bulk seed methods on solid nutrient media. Zones of inhibition were measured for each concentration. Wells were created immediately after culturing with a sterile cork borer and 100 µl of test sample was poured in each well by means of a micropipette.
Concentration of bacterial suspension was adjusted by the use of colorimeter to obtain a mat growth on spread plate culture and 10^8 cfu ml^{-1}for bulk seed culture.

- All the data is representative of minimumthree replicates for each test.
- All tests were performed in a Laminar Air Flow to avoid contamination.
- Negative controls were prepared using plain DMSO in the same method. Microbial growth was found to be unaffected.

RESULTS AND DISCUSSION

Kirby-Bauer method

Table-2: Results of Kirby-Bauer method

Test organism	Zone of inhibition (mm)
SpA	Neg
SpB	Neg
P. mirabilis	Neg
M. luteus	Neg

No zone of inhibition was observed in any of the organismsin Kirby Bauer method. Lower volume of test sample provided by disc was postulatedto be the reason for negative result

Agar well diffusion method

1. Spread plate culture

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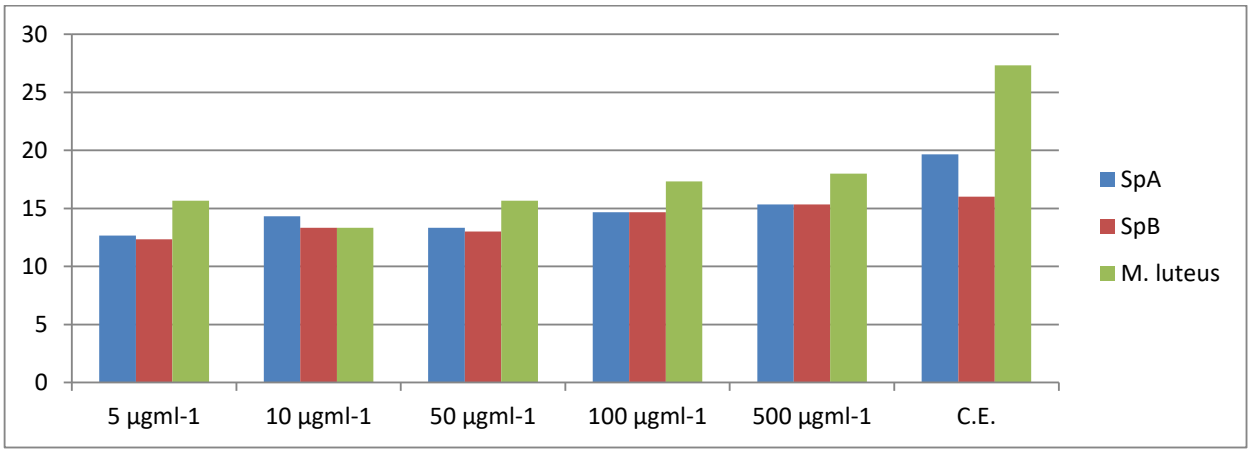
Table-3: Zones of inhibition in spread plate culture

Test organism	Mean zone of inhibition (mm)	Concentration of Capsaicin (µgml^{-1})					
		5	10	50	100	500	C.E.
SpA		12.66±1.15	14.33±0.57	13.33±1.10	14.66±0.57	15.33±0.57	19.66±0.57
SpB		12.33±0.57	13.33±0.57	13.00±1.73	14.66±0.57	15.33±0.57	16.00±0.00
P. mirabilis		Neg	Neg	Neg	Neg	Neg	Neg
M. luteus		15.66±2.08	13.33±2.88	15.66±0.57	17.33±1.15	18.00±3.00	27.33±2.51

Fig.-1: Comparative analysis of zones of inhibition in spread plate culture

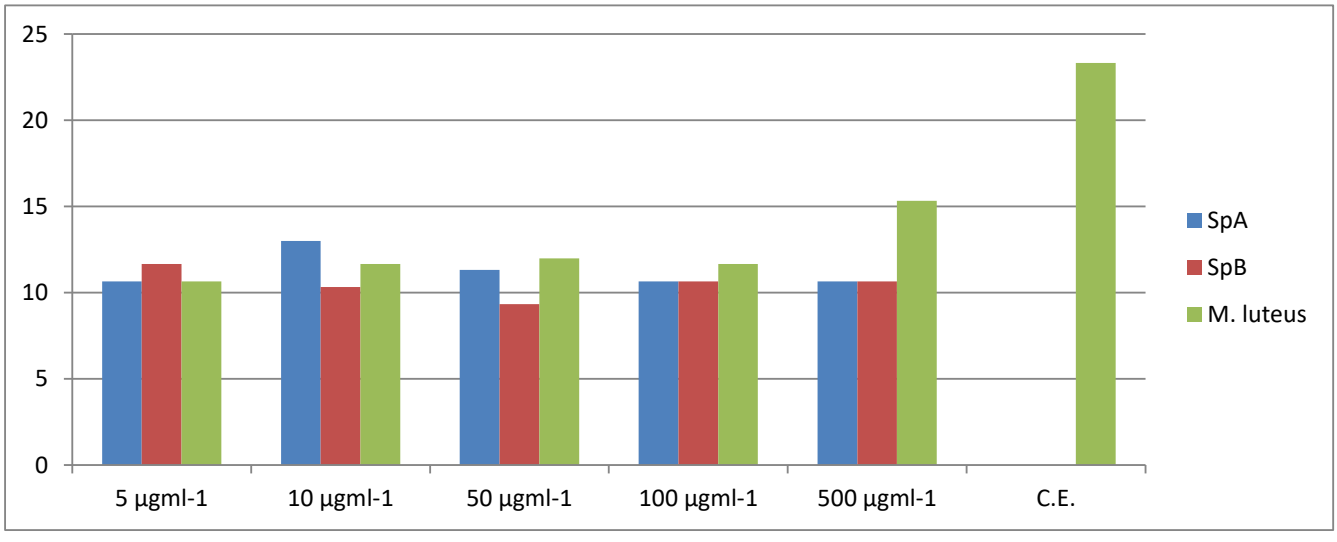


1. Bulk seed culture

Table-4: Zones of inhibition in bulk seed culture

Test organism	Mean zone of inhibition (mm)	Concentration of Capsaicin (µgml^{-1})					
		5	10	50	100	500	C.E.
SpA		10.66±1.15	13.00±1.73	11.33±1.15	10.66±1.15	10.66±1.15	neg
SpB		11.66±2.08	10.33±0.57	9.33±1.15	10.66±1.15	10.66±2.30	neg
P. mirabilis		Neg	Neg	Neg	Neg	Neg	Neg
M. luteus		10.66±1.15	11.66±1.52	12.00±1.73	11.66±1.52	15.33±0.57	23.33±2.88

Fig.-2: Comparative analysis of zones of inhibition in bulk seed culture



Salmonella paratyphi A and Salmonella paratyphi B

The causative agent of paratyphoid fever did not present with any strikingly significant susceptibility to Capsaicin in both, pure and chilli extract forms as compared to the currently available antibiotics employed for treatment.

The field of inhibition observed only displayed a reduction in number of colonies

and not a completely clear zone. The result is consistent with a similar analysis previously performed on Salmonella typhi[6] where no significant inhibition was observed with pure Capsaicin and chilli extract.
It was noted that a lower zone of inhibition was observed when culturing was done with bulk seed method. This reason suggested for this effect has been described that the colonies present in the bulk of the medium may have affected the diffusion of the sample in the medium; however, further analysis has to be performed to get any conclusive explanation.

Micrococcus luteus
M. luteus is resistant to penicillin. Mean zone of inhibition obtained with C.E. (Spread plate method) was 27.33 mm which is greater than 24 mm (zone of inhibition with tetracycline) and strikingly close to 34 mm, the zone of inhibition with chloramphenicol. A significant zone of inhibition was observed with both, standard capsaicin and C.E.; however, a striking observation was made - susceptibility towards C.E. was higher than that towards standard capsaicin even at its highest concentration. The other components in chilli namely carotenoids, chlorophyll and other plant metabolites may have contributed to the inhibitory effect; however, further detailed analysis is required for a specific conclusion.

Proteus mirabilis
A negative result was obtained i.e. P. mirabilis grew normally in the presence of Capsaicin and was hence concluded to be resistant to Capsaicin.

CONCLUSION

The organisms of genus Salmonella have been majorly discussed in the subject of antibiotic resistance. Significant evidence is available to suggest the antibiotic resistance among Salmonella species [18]. The lookout for alternative antimicrobial agents must continue to prepare for worst case scenarios relating to typhoid and paratyphoid fever, especially in developing countries like India or underdeveloped countries of Africa where hygiene standards are low as compared to developed countries.

M. luteus is abacterial isolate from the normal micro flora of the skin; this organism is known for causing body odour due to its products of metabolism. It is also known for causing skin infections in immunocompromised/immunodeficient patients. M. luteus is resistant to penicillin. Further testing can be performed to amplify the data in order to meet the requirements for commercialization of an idea. Tests like bactericidal versus bacteriostatic effect, analysis with different species of chillies coupled with HPLC, testing with more

Mean zone of inhibition obtained with C.E. (Spread plate method) was 27.33 mm which is greater than 24 mm (zone of inhibition with tetracycline) and strikingly close to 34 mm, the zone of inhibition with chloramphenicol. These findings suggest promising prospect of chilli as a natural antimicrobial agent. Topical ointments containing capsaicin are currently employed for analgesic purposes. Modified ointments can be formulated to tackle skin infections caused by M. luteus to replace the use of antibiotics.

This study can be of significant importance in the field of ayurveda. Topical ointments can be prepared to counter skin infections instead of administering antibiotics which are known to have side effects. Capsaicin ointments are currently used as analgesics.

microorganisms are beneficial to obtain the required data.

It has been observed that even eukaryotes are susceptible to high doses of capsaicin. High dose of Capsaicin is toxic to humans and low

doses cause symptoms like irritation, sneezing and a burning effect in the superficial layers of the body. This suggests that a common gene may be present in eukaryotes and susceptible prokaryotes responsible for generating a similar response. Thus, with further investigation a genetic relationship can be established and the theory of common ancestry can gain further solidity.

There is a possibility of existence of genetic orthologs between eukaryotes and prokaryotes which are responsible for generating susceptibility towards Capsaicin (which evolved as a defense mechanism in plants).

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SCREENING OF FLOWERS OF *PELTOPHORUM PTEROCARPUM* FOR DIFFERENT BIOLOGICAL ACTIVITIES

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ABSTRACT

Objective: To investigate the preliminary phytochemistry, antioxidant, antibacterial activity and natural pigment content of flower extracts of *Peltophorum pterocarpum*, a beautiful flowering plant of Family Caesalpiniaceae, widely seen in the different pockets of Mumbai.

Methods: Preliminary phytochemical analysis was conducted by using the standard methods. The isolation of different pigments was carried using TLC. Antioxidant activity was done using DPPH method. The antibacterial activity of the aqueous and methanolic extracts was checked against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Salmonella typhi* by disc diffusion and agar well methods. The aqueous and Methanolic extract were subjected to UV spectrophotometer analysis for silver nanoparticles synthesis. Larvicidal activity of the extracts were also checked on *Aedes aegyptii*.

Results: The preliminary phytochemical screening of the flower extract showed presence of alkaloids, phenolic compounds, saponins, flavonoids, proteins and amino acids. In TLC, carotenoids were observed. The methanolic flower extract showed potent antioxidant activity as well as antimicrobial activity against all four bacteria at low and high concentrations. The concentration at which there is maximum synthesis of silver nanoparticles for methanolic and aqueous extract is 25 micrograms. The extracts did not show any larvicidal activity.

Conclusion: The study revealed that the flower extract shows antioxidant and antibacterial activities in all concentrations taken into consideration for this experiment and can be used in the future to make cosmetic formulations.

Keywords: Peltophorum, Phytochemistry, Antibacterial, TLC, Carotenoids, Nanoparticles.

INTRODUCTION

Plants produce secondary metabolites which is said to be the origin of all natural and herbal products. They have been extensively studied and are being used as an alternative for treatment of diseases such as cancer and diabetes. [16]. For survival and reproduction purposes, flowers have been evolved to have pigments on petals. It is these pigments that produce colour. Ornamental trees have a tremendous ecological importance. Colour produced by the petals is usually based on the season as well as their need. Flowers that bloom in daylight are pretty with bright coloured petals to attract nectarivores while those that bloom in the evening have pollinators that are nocturnal and hence are dark or white. Yellow coloured flowers are common throughout the year and is seen to be used the most as avenue trees. These pigmented bodies are a source of secondary metabolites which possess a wide array of bioactive components used to treat health problems. [2]. Micro-organisms have increasingly developed resistance due to uncontrolled and unrestrained use of anti-microorganisms by the general population irrespective of whether they have been prescribed by a doctor. Hence, a need to formulate alternatives to it has become imminent.

Peltophorum pterocarpum (DC.) Baker ex Heyne, belonging to Family Caesalpiniaceae, is a common avenue tree planted for its bright yellow colored flowers which bloom twice a year. The common name is Copper Pod, Golden Flamboyant and Yellow Poicinia. It is a huge umbrella shaped crown of many branches consisting of feathery mimosa like leaves and abundance of bright yellow flowers. It is a fast-growing evergreen tree with dense spreading crown. Flowering season lasts for few weeks. Different parts of this tree have been used in the treatment of insomnia, skin diseases, constipation, etc. [1] Its bark is used for dysentery, eye lotion and leaves for skin irritations. There has been reports of other applications of various parts of *P. pterocarpum*. Since, the flowers were not really used for any concrete purposes, this study was undertaken. The flower extracts were tested for Phytochemical analysis, Thin Layer Chromatography, Antimicrobial, Larvicidal and Nanoparticle synthesis [1].

MATERIALS AND METHODS

Fully developed flowers of *P. pterocarpum* were collected from area around Churchgate station and flora fountain, Mumbai, India. The collected plant material was air dried for two weeks and pulverized using an electric blender to increase the surface area of extraction.

2.2. Preparation of Plant extract

2.1. Collection and Preparation of plant sample

The air dried and finely grounded plant material (20 g of each) were extracted with 250 ml of methanol by using a Soxhlet apparatus at a temperature not exceeding the boiling point of solvent. The extract was concentrated to semisolid mass using water bath and stored in a refrigerator at 4°C till further use. The extract was dissolved in 0.1% Dimethylsulfoxide

(DMSO) for antibacterial studies. Aqueous extract was prepared with 10 g of powdered plant material and 100 ml distilled water.

2.3. Preliminary Phytochemical Analysis

Phytochemical screening of plant extract was carried out qualitatively for the presence of alkaloids, phenols, terpenes, saponins, amino acids, carbohydrates, xanthoproteins, quinones, steroids and tannins by using standard techniques.

2.4. Thin Layer Chromatography

TLC plates were prepared using silicon, calcium carbonate and chloroform. A baseline was drawn on the TLC plate. A spot of the plant extract was placed on the baseline with use of the capillary tube and allowed to dry. The plates were placed in the developing chamber with solvent. Solvents (acetic acid and acetone) with different proportions (1:9, 3:7, 4:6, 6:4) were used. The Rf values were determined.

2.5. Antimicrobial assay

Microorganisms *Escherichia coli, Salmonella typhi, Streptococcus pyogenes, streptococcus aureus* was used for the present study. These were obtained from the microbiology laboratory, Kishinchand Chellaram College. Stock culture were maintained at 4°C on slants of nutrient agar. Agile microorganisms for the experiments were prepared by transferring a loopful of colonies to 9ml saline from the stock culture. Antimicrobial activity was determined by agar cup and disc diffusion method. In disc diffusion method, the suspension of bacteria was inoculated on the agar plates. Sterile paper discs dipped in different concentrations were placed on the surface of the labelled agar plates and incubated at 37ºC for 24 hours. For agar cup method, the well was made by using a 6mm cork borer. The microorganisms were then swabbed on the nutrient agar plates with the help of sterile cotton swabs. The plates were divided into quadrants and wells were made using 6mm cork borer that was sterilized with alcohol and flame. At the end of incubation, inhibition zones were formed.

2.6. Nano particle Synthesis

A quality of 0.008 grams of AgNO3 was weighed and made up to 100 ml with distilled water. Total amount of AgNO3 solution prepared was 400ml. For making 5% concentration, 0.005 grams of extract was dissolved in 10 ml DMSO and then added to 90 ml of AgNO3 solution. Similarly, the concentrations 10%, 25%, 50%, 75% and 100% were prepared.

2.7. Larvicidal Activity

The larvicidal Activity of the Methanolic and Aqueous extract of the plant and plant extract with nanoparticles synthesized by it, against the larvae of the dengue vector, *Aedes aegypti* mosquito was determined through mosquito larval bioassay. The mortality of the mosquito larvae was noted in the 100µg/ml ,250µg/ml ,500µg/ml ,750µg/ml concentration of the plant extract after 24hour exposure.

RESULTS

3.1. Phytochemical Analysis

The methanolic extracts were prepared using Soxhlet apparatus and aqueous extract was made using Buchner funnel. In

this study, the methanolic extract showed presence of alkaloids, saponins, phenols, diterpenes, proteins and amino acids while it showed negative results for Phyto sterols, carbohydrates and flavonoids. Alkaloids, phenols, saponins, flavonoids, proteins and amino acids were present in the aqueous extract and showed absence of carbohydrates and phyto sterols. Based on these results, antimicrobial and larvicidal activity as well as nanoparticle synthesis was performed on this plant sample.

3.2. TLC Assay

The solvents used were acetic acid and acetone in varying concentrations i.e. 1:9, 3:7, 4:6 and 6:4. The component observed was carotenoids with relative factor for the same were 0.631, 0.523, 0.532 and 0.639 respectively. The solvent ratio which showed the best result was 6:4.

3.3. Antimicrobial activity

For agar well and disc diffusion method, the concentrations used took both high and low range into consideration. The concentrations used were 25µg/ml, 50µg/ml, 75µg/ml, 200µg/ml, and 500µg/ml. The positive control was Gentamicin discs and the negative control was DMSO. All the concentrations of methanolic and aqueous extracts showed the zone of inhibition. But the maximum diameter was observed to be 25µg. Both the extracts were subjected to antibacterial activity against two gram positive and gram negative bacteria i.e. *Escherichia coli*, *Salmonella typhi*, *Streptococcus pyogenes* and *Streptococcus aureus*.

3.4. NP Synthesis

The extracts prepared by adding silver nitrate solution to the extract, was subjected to UV spectrophotometer analysis. The concentrations prepared were 5%, 10%, 25%, 50%, 75% and 100%. The positive control was Dimethylsulfoxide. At wavelength of 430 nm, the highest peak was observed to be of 25 percent. (Fig. 1).

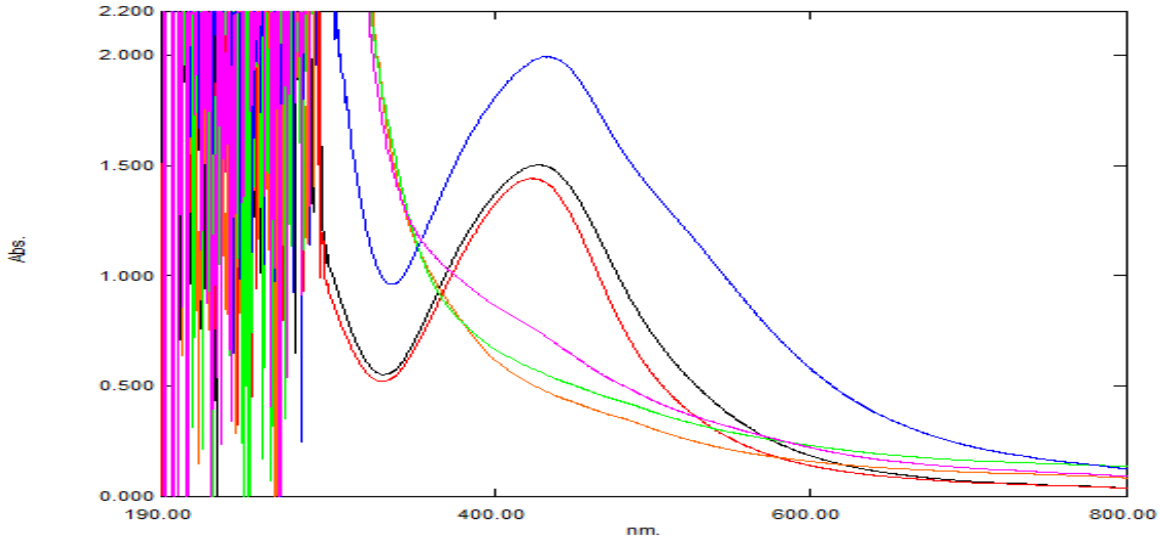
3.5. Larvicidal Assay

The extract was tested for larvicidal activity using the larvae of *Aedes aegyptii*. The concentrations prepared were 100µg/ml, 250µg/ml, 500µg/ml and 750µg/ml. Positive control was the larvae without any addition of external material while the negative control was Dimethyl Sulfoxide. Since only 100µl of the extracts prepared was infused in the beakers containing the larvae, no activity was observed. Each treatment was done in triplicates.

DISCUSSION

When natural products are formulated to drugs, they play a vital role in therapeutics and other treatments of human ailments. Secondary metabolites of plants serve as a defense mechanism against several microorganisms, insects and other herbivores. The metabolites found in this study were alkaloids, saponins, phenols, diterpenes, proteins, flavonoids and amino acids which are also termed as phytochemical constituents[1]. Alkaloids are nitrogen containing heterocyclic organic bases, biosynthesized and derived from amino acids. They are toxic to man and animals in high doses but have great medical uses in lower doses. Phenols have antioxidant, anticarcinogenic, antimutagenic, antitumoral, antibacterial, antiviral and inflammatory properties.

Other constituents such as tannins serve as defense mechanisms[2].



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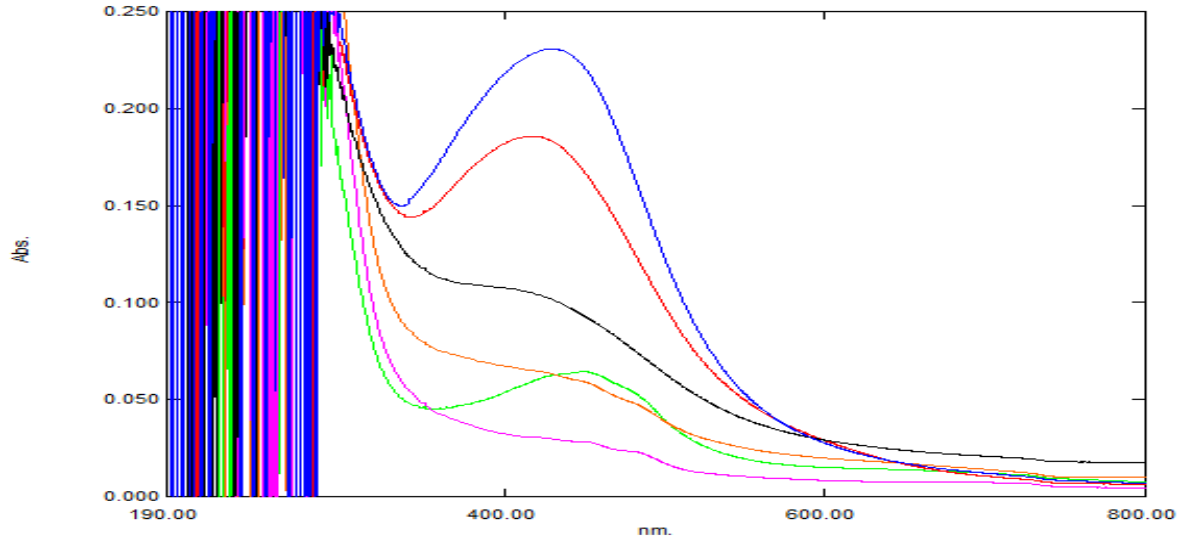
Blue 25%. Green 50%

Pink 75% Orange 100%

Fig.1(a).: UV Spec. Graph showing peaks at different concentrations of aqueous extract.



Black 5% Red 10%.

Blue 25%. Green 50%

Pink 75% Orange 100%

Fig. 1(b).:UV Spec. Graph showing peaks at different concentrations of methanolic extract.

Hence, phytochemical analysis was the first step done in this study. Based on previous reports, Methanol was found to be the most effective solvent for extraction and so methanolic extracts were prepared[3]. For comparison with it, aqueous extract was also made.

Plant compounds that show presence of colour are referred to as pigments. Carotenoids and anthocyanins are the reason for presence of red and yellow pigments in petals of flowers of family Caesalpiniaceae. The carotenoids class of compounds comprises yellow, orange and red pigments. They are found in plasmids which are specialized protoplasmic bodies present in the higher plants. The production of Carotenoids increase when the leaves approach senescence. This is due to the breakdown of photosynthetic apparatus[2].

The positive results of antimicrobial activity against both Gram positive and Gram negative bacteria may be indicative of presence of broad spectrum antibiotic compounds. This is confirmed by the presence of secondary metabolites in the flower extracts[1]. For production of nanoparticles, various methods have been used out of which, most of them are still in the developmental stages. The stability and aggregation of nanoparticles, control of crystal growth, morphology and size are the problems faced by the scientists. Separation of the produced nanoparticles for further applications is a bigger issue. It was observed that the nanoparticles produced by plants are more stable compared to those produced synthetically or using other organisms. Plant extracts are reported to reduce metal ions faster than fungi or bacteria. Hence, due to easy availability of the flowers, they were subjected to synthesis of nanoparticles in this study.

CONCLUSION

From the current studies, we can conclude that the *Peltophorum pterocarpum* is rich in carotenoid and its aqueous and methanolic extracts showed potent antibacterial activity which can be further explored for antifungal activity as well. This may find its application in formulation of antimicrobial lotion/cream. The plant also shows potential for synthesis of silver nanoparticles which finds a wide range of applications from biomedical to consumer goods as well as it can be used as a drug delivery device. Larvicidal activity was negligent at the lowest concentration and this can be studied further by increasing the concentrations of the plant extracts.

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HEALTH PROBLEMS OF TRAFFIC POLICE OFFICERS IN MUMBAI

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ABSTRACT

Air quality crisis in cities is predominantly due to vehicular emissions. Traffic policemen are a group of population most exposed to constant vehicular emission, dust particles, and harmful gases. Therefore, in this study we aimed to assess the health status of traffic policemen stationed at various traffic junctions in Mumbai (city & suburb). The impact of air pollution on the respiratory system of the traffic police officers using Peak Flow Meter, along with their Blood Pressure levels & Body Mass Index was recorded in this study. Vegetation cover at every junction was recorded. The purpose of our research was to shed light on the health risks, to which traffic police officers are vulnerable to & suffering from, and to check whether vegetation cover has any positive influence on the traffic personnel's health. So, further appropriate preventive measures could be taken to safeguard their health. For the lung capacity test,— 45.86% traffic police officers were found to be in the Green Zone (indicates that lung function is good), 52.68% in the Yellow Zone (indicates caution i.e. they are susceptible to asthma & should get a checkup done)& 1.46% were in the Red Zone (indicates medical emergency).

Keywords: Vehicular emissions, Atmospheric pollution, Traffic personnel, Health issue

INTRODUCTION

Occupational environment plays a major role on the health of the exposed population. One such example would be the group of Traffic Police Officers who are exposed to the increasing air pollution at traffic junctions.

They are constantly exposed to the impure atmosphere which is of poor quality particularly in mega cities like Mumbai. The health hazards get more severe when the duration of the exposure increases. This fact is more important in situation where the personnel are engaged in traffic duty. The factors that affect the health of Traffic Police Personnel are many including atmospheric gases such as Sulphur dioxide, Oxides of nitrogen, Carbon Monoxide & Particulate matters. Other factors include unhealthy lifestyle (smoking & drinking), family history of respiratory diseases, etc. Therefore, our research aimed to determine the extent of health retrogression of Traffic Police Officers due to the ever deteriorating air quality.

Also, greenery in the megacities is diminishing day by day. There are several reasons for poor vegetation in the cities. It is said that green plants are the lungs of cities as they play a major role in reducing carbon dioxide burden on the atmosphere. Municipal Corporation has been increasing traffic islands at the traffic junctions, but not all the junctions have decorative vegetation. Therefore, we have also tried to check whether presence of plants at or near junctions can have some reduction in the damage to health of traffic personnel.

METHODOLOGY

Research Approach: Our research was a Cross-sectional Survey study conducted from May to July 2016 with prior permission from superintendent of Mumbai Police.

Study Design: The data for this study was collected by performing 3 tests namely -

1. Wright's Peak Flow Meter.
2. Blood Pressure Test.
3. BMI Test.

   Along with, a direct interview method with the help of a relevant questionnaire was done.

Study setting: Traffic Junctions & Traffic police HQ of Mumbai & Suburbs. The traffic junctions selected for this study were Tardeo, Worli, Byculla, Mulund, Chembur, Ghatkopar, Nagpada, Colaba, Bandra, Airport, Borivali, Goregaon.

Population of study: Traffic Police Officers of Mumbai & Suburbs.

Sample size: 205

Inclusion criteria: Traffic Police Officers working in Traffic Junctions & Administrative Offices.

The Wright's Peak Flow Meter test was used to check the lung capacity of the traffic police personnel. The test signifies whether their lungs function normally or have been affected by the pollution. The blood pressure test shows whether their work conditions and occupational stress have affected their health.The BMI test determines the fitness criteria.

The questionnaire contained the following information- the traffic junction areas, the duration of their service (administrative office & traffic junction), whether they

work at day/night & the average hours they work for.

The other part of the questionnaire dealt with the problems they face during their duties such as :

-Experiencing shortness of breath or any other respiratory disorder (Asthma, Bronchitis etc.)

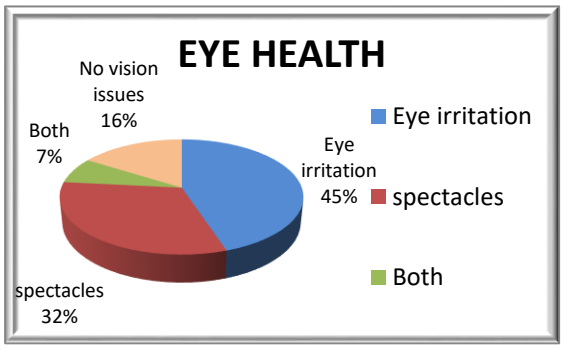
-Whether they are on any medication for it.Any hypersensitivity to dust particles or specific gases.

-Irritation of eye or redness after the end of the day.

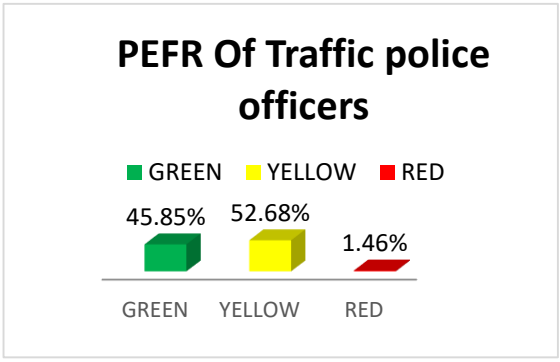
- One of the important questions included was whether they were provided with any protection masks and if they used it on their duty hours.

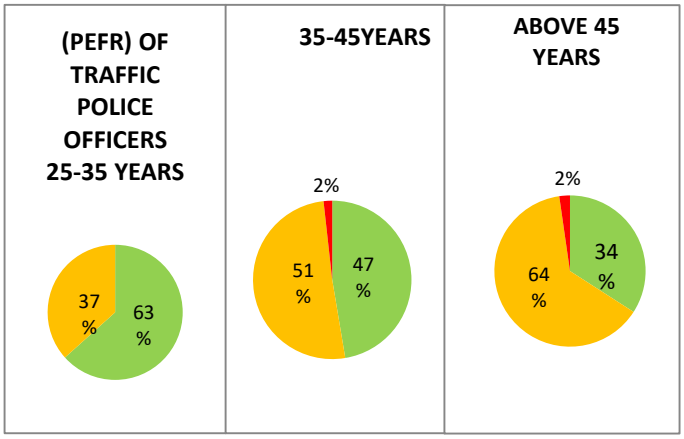
RESULTS

Our finding showed that, out of 205 traffic police personnel 16.58% reported family history of respiratory disorders amongst which 50% were suffering from hypersensitivity to dust or pollen. Overall 32.68% had hypersensitivity problem amongst which (55.22%) were in yellow zone i.e. susceptible to asthma and (44.77%) in green zone i.e. healthy lung function. About 42.87% personnel were suffering from eye irritation problem this was followed by spectacles issues as 31.70%.

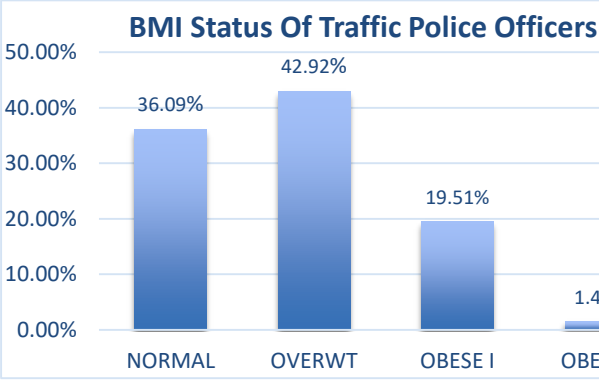


The data revealed that percentage of Yellow zone (52.68%) is greater than that of Green zone (45.85%) thus; there Lung capacity of traffic police personnel is at a high risk of various respiratory disorders (COPD). Some traffic police personnel come under Red zone (1.46%) this shows they are at immediate danger and need medical attention to prevent the further exposure and risk factors.

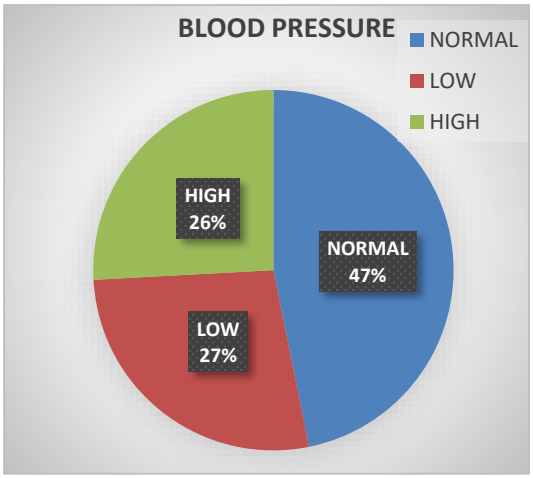




It was observed that 42.92% of people were overweight and 19.51% were obese class I (According to their BMI). Whereas, 1.46% people were obese class II and the rest 36.09% were normal.



Most of the traffic personnel were suffering from high and low blood pressure problems about (53%) whereas, (47%) individuals were in the normal blood pressure range.



Pulmonary lung function of normal people and traffic police personnel were compared using chi-square test. Statistically found significant difference between the pulmonary function of both of the sample set. Chi-square value was found to be (5.18) where tabulated value (1.48). (80.48%) of the traffic police personnel did not wear protection masks and about (19.51%) wore protection masks. Statistically no significant difference was found in the peak expiratory flow rate of traffic police personnel wearing mask and not wearing [(Tcal = 0.87) & (Ttab = 1.97)]. From t test distribution it was found to be statistically that there was no significance difference found in peak expiratory flow rate (PEFR) of traffic police officers working in administrative offices and stationed at traffic junction [(Tcal = 0.83)] & (Ttab = 1.97)]. It was revealed that there was negative correlation between the age of the traffic police personnel and PEFR (correlation value – 0.43).

CONCLUSIONS

The traffic police personnel who had family history of respiratory disorders were less prone to the allergic reactions thus; it does not depend upon the family lineage. In current study sample protection masks has no significant impact on PEFR thus it is not efficient measure. Dust and Particulate matter (PM) present in air causes eye irritation. Overall no. of personnel in yellow zone were more than the no. of personnel under green zone this suggests that most traffic police officers are at greater risk of developing pulmonary dysfunctions. With age the lung function found to be declining. The PEFR value of personnel working in office and junction were statistically almost similar due to rotating duties which neutralized or minimized impact on the lung function capacity. Vegetation cover may have positive impact on overall health status.

SUGGESTIONS

- After certain age traffic police officers should must be given duties in administrative offices rather than junctions.

- Trees that help in minimizing pollution should be planted along the road sides and traffic islands.

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CHEMISTRY

Chemistry is necessarily an experimental science: its conclusions are drawn from data, and its principles supported by evidence from facts.

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science
Environmental
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SOIL TESTING AND DETERMINATION OF HEAVY METAL CONCENTRATION IN SOIL

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ABSTRACT

Objective: The basic aim of this project is to test the soil for its fertility level and to determine the heavy metal contamination of the soil.

Methodology: The methodology included identification of the area, soil sample collection, determination of physical parameters of soil by instrumentation, determination of chemical parameters of soil- carbon, nitrogen and micronutrient content, etc. by various techniques like volumetric method, Kjeldahl method; determination of heavy metal concentration in soil by Atomic Absorption Spectrophotometry, etc.

Results: The soil samples were alkaline to slightly acidic with low percentage of moisture and high electrical conductivity. The samples were highly fertile in context with organic matter and other parameters whereas the samples contained low amounts of potassium. The samples were deficient in manganese and iron and moderate for zinc concentration and were highly contaminated with nickel.

Conclusion: The soil samples were fertile for plant growth of specific types that can survive in high nickel and essential nutrient concentrations along with low manganese and iron concentration. The high concentrations of essential nutrients can be attributed to dumping of wastes.

INTRODUCTION:

[1]Soil may be defined as a thin layer of earth's crust which serves as a natural medium for the growth of plants. It is the unconsolidated mineral matter that has been subjected to, and influenced by genetic and environmental factors- parent material, climate, organisms and topography all acting over period of time. Soil differs from the parent material in the morphological, physical, chemical and biological properties. A study of soil profile supplemented by physical, chemical and biological properties of the soil will give full picture of soil fertility and productivity. Physical properties of the soil include water holding capacity, aeration, plasticity, texture, structure, density and colour etc. Chemical properties refer to the mineralogical composition and the content of the type of mineral such as Kaolinite, illite and montmorillonite, base saturation, humus and organic matter content. The biological property refers to a content of extent and

types of microbes in the soil which include bacteria, fungi, worms and insects. The soils are studied and classified according to their use which is termed as land capability classification.

Site location:

The selected site was a dumping ground located in Bhayander east. The location is at Navghar road at a distance of about 600 metres from Bhayander station. An unofficial dumping ground was created by the nearby residents of a ground in Bhayander (east) .The site is surrounded by stainless steel utensils manufacturing units on two of its sides. The dumping ground is covered with household wastes on the side facing the residential buildings on a larger extent which decreases towards the ends of the ground. The side facing the playground have piles of dirt and soil recovered from the whole ground in order to make the ground flat and the pile of dirt contains human sanitary wastes as well. The sides facing the factory are covered with effluents from the factory.

There are a few plant species growing in the dumping ground near the household wastes site and near the soil piles site.

Literature review:

[1]Soil is the major factor to be considered when agriculture comes in mind. India being an agricultural country is highly influenced by soil quality. Soil testing is necessary to keep a regular check upon the quality and potential of a particular soil type or farms in a particular locality. Soil testing is also carried out to prescribe appropriate treatment for soils that are losing their potency. Another aspect of latest scientific input in the soil testing programme would be to analyse these extracted amounts of nutrients by modern, heavy duty and fast analysing equipment so that the capacity and accuracy of the soil testing laboratories could be enhanced by equipment like auto analysers, atomic absorption spectrophotometer and inductively coupled plasma-atomic emission spectrometer. [1]

Ruqia Nazir et al. conducted a study to estimate levels of heavy metal in plant, water and soil in Tanda Dam Kohat. Results showed that concentration of heavy metal in soil was above permissible limit. [3] It is observed that as the heavy metal content in soil increases, the uptake of heavy metals from soil by plants also increase.

The most important sources of heavy metals in environment are anthropogenic activities such as mining, smelting, steel and iron industry, dumping of waste etc. The heavy metals accumulate in the soil and are a potential threat to public and eco-system integrity. [4] The level of heavy metals in dumping sites and in the plants grown in that area was determined and was found that a considerable amount of metals accumulate in the plants.

Metals like mercury, lead, cadmium, silver are non-degradable and highly toxic at low concentrations. [5]

Environmental pollution by heavy metals, even if it is at low concentrations and the long- term cumulative health effects that go with it, is of major health concerns all over the world. For instance bioaccumulation of lead (Pb) in the human body interferes with proper functioning of the mitochondria thereby impairing respiration as well as causing constipation, swelling of the brain, paralysis and could eventually lead to death.[6]

Sutterland (2000) stated that the problem worsens as daily traffic increases. Cement dust has been shown to adversely affect

the soil and exhibit elevated pH levels. Cement industry also plays a vital role in the imbalances of the environment and produces air pollution hazards. It was well documented that work done on the composition of soil around cement factories has shown that there were very high levels of chromium, silica, iron and calcium with contamination levels decreasing dramatically with distance from the factories. [8]

MATERIALS AND METHODS

The area under study was identified and soil samples were collected from three different location within the site namely; soil from the rhizosphere of plant 1 (sample 1), soil from the rhizosphere of plant 2 (sample 2) and soil under effluent discharge (sample 3). The soil samples were collected by the method of random sampling as the distribution of plants and effluent in the site was not uniform. The soil samples were then sieved in order to remove unwanted pebbles, stones and undecayed material such as whole grass, polythene cut-outs, glass pieces, etc. the soil was weighed and oven- dried at 105°c and cooled in desiccator. The cooled soil was again weighed and the loss in weight was determined to be the amount of moisture present in the samples. the oven-dried soil was then subjected to various tests to determine its physical and chemical properties. The physical properties such as pH and soil conductivity was determined using pH meter and potentiometer respectively. The chemical parameter of gypsum requirement was determined by volumetric method by titrating 1ml of aliquot of soil in gypsum solution against versenate solution (0.01N). organic matter was determined by volumetric method (Walkley and Black, 1934). Total nitrogen was estimated by Kjeldahl method which gave an estimate of all forms of nitrogen present in the soil. The amount of potassium available in soil was estimated by flame photometric method (Toth and Prince, 1949). The calcium concentration in soil was estimated by volumetric method by titrating the aliquot against versenate solution (0.01N). the concentrations of zinc, manganese, hexavalent chromium and cadmium was determined using Atomic Absorption Spectrophotometer by reading the extracts made from soil in concentrated HNO_3 at 213.9nm, 279.5 nm, 357.9 nm and 228.8 nm for zinc, manganese, hexavalent chromium and cadmium respectively. The amount of iron was determined using colorimeter by using 0.25% 1,10- phenanthroline as the colour developing agent and was read at 520 nm. The nickel concentration was estimated by volumetric method by titrating the aliquot prepared in aquaregia against 0.01M EDTA solution using muroxide indicator.

CALCULATED RESULTS

Table 1- Physical parameters of soil

Samples	Soil moisture (gram)	Moisture percentage	pH	Conductivity (mS/cm) 0.1N KCl solution- 12.88
1	5.219g	24.94%	7.46	0.73
2	2.873g	8.52%	7.62	0.76
3	0.210g	0.74%	6.74	1.90

Table 2- Chemical parameters of soil

Samples	Gypsum requirement (tonnes/ha)	Organic matter (%)	Total nitrogen (kg/ha) Soil samples= 10g made up to 100ml volume	Exchangeable calcium (me/100g soil)	Potassium estimation (kg/ha)
1	42.02	3.77	22029	3.2	28
2	3.82	6.50	25582	5.6	32
3	22.92	9.06	14923	5.8	99

Table 3- Heavy metal concentration in soil

Samples	Zinc concentration (ppm)	Manganese concentration (ppm)	Iron concentration (ppm)	Nickel concentration (mg/ 50 g soil)	Cadmium concentration (ppm)	Chromium concentration (ppm)
1	0.315	0.320	0.650	476.5	0.2911	0.0435
2	0.412	0.388	0.650	476.5	0.1552	0.0172
3	0.661	0.315	1.275	317.7	2.3386	0.0256

RESULTS

The pH of sample 1 and sample 2 ranged from 7.1- 8.5 and hence the soil is moderately alkaline thus supporting plant growth. The pH of sample 3 ranges from 6.6- 6.9 .The values indicate a slightly acidic nature of the soil. Literature suggests that this type of soil may not be very suitable for plant growth. The pH values of the soil samples are slightly acidic to alkaline in the range of 6.5-8.5.This suggests that lime treatment may not be necessary for the selected soil sample.

The electrical conductivity of soil samples range from 1-2 mS/cm. The electrical conductivity measure is a direct indication of salt content .The values of electrical conductivity are in agreement with a salt content less than 0.15%. Among the selected samples the conductivity of sample 3 is higher compared to the other two samples. This indicates the sample 3 may have a higher concentration of ions. The organic matter in soils nearby plants was found to be lower than in the sample where effluents were present. Hence either the plants utilised the organic matter from the soil for their metabolic requirements or the microbes present in the soil have utilised the organic matter for their growth. Since the organic matter in all the samples are more than 1%, the soils are highly fertile to support plant growth. All the samples are very low in available potassium for plants i.e. below 140kg/ha. Thus, the soils are very low in fertility levels as far as potassium concentration is considered. The maximum concentration of nitrogen in soil should be 700kg/ha. All the samples contained more than 700kg/ha of nitrogen and hence are highly fertile as far as nitrogen concentration is considered. Critical limit of calcium in soil is estimated to be less than 1.5 me Ca/100g standardised by Government of India under All India Coordinated Research Project. All the samples had calcium levels above the critical limit. Hence the soils are having high concentrations of calcium and are highly fertile. The upper limit for metals such as zinc, manganese and iron as specified by the Government of India suggests that the soils are rich in all the metals indicated above.[1] The nickel concentration in the area was found to be 954 times more than the upper limit specified by World Health Organization. [2] The concentration of cadmium was above the critical limit specified by WHO whereas the concentration of hexavalent chromium was under the limit specified by WHO. [2] The samples on qualitative analysis showed absence of the two metals. Thus, the two metals could be present either as co-ordination complexes or they could be absent. If they are present as co-ordination complexes, methods to detect these complexes need to be devised.

CONCLUSIONS

The soil samples were low in total moisture content but had high amounts of ions or salts. Thus, the deposition of effluents in the area may have resulted in high salt concentration and they are motile in soil by virtue of strong adherence to water molecules may be due to hydrogen bonds or other weak bonds. The amount of moisture is too low in soils for the soil to be classified under garden soils. Since the soils are free of salts and hence no effect of salts that can hinder in the growth of plants except for very sensitive crops. The

soils were slightly acidic to alkaline (pH range from 6.74- 7.46) in nature which makes them susceptible for supporting plant growth. The soil under effluent area was slightly acidic in nature and hence may not be able to support growth of pH sensitive plants.

Since the soils were slightly acidic to moderately alkaline, they need not be treated with lime or gypsum. Gypsum is required to treat alkaline soils that have excess of sodium. Sodium is not an essential nutrient for plant growth. Plants do not absorb sodium from soil. Excess of sodium in soil makes it alkaline and hence interferes in its ability to support plant growth.

Organic matter usually contains 58% organic carbon and a constant amount of nitrogen that is to be supplied to plants. Only 77% of total organic carbon can be recovered, the method has some limitations. The organic matter in soils nearby plants was lower than in the samples where effluents were present. This can be attributed to either the plants took up the organic matter from soil for their metabolic requirements or the microbes present in the soil have utilised the organic matter for their growth. Since the organic matter in all samples is more than 1% (the average amount of organic matter to be found in soils of Maharashtra deciphered under All India Co-ordinated Research Project), the soils were highly fertile to support growth of plants or micro-organisms.

Potassium is an essential primary nutrient for plant growth. All the samples are very low in available potassium to plants i.e. below 140 kg/ha. All the samples were above the critical limit specified for calcium by the government of India. This may be attributed to the deposition of left-over of building material and domestic waste disposal. The samples were having high concentrations of manganese and iron. The soil samples were having very high concentrations of zinc. The soils had high concentrations of nickel which can be attributed to deposition of effluent waste from industries that may contain high concentrations of nickel. Since nickel is an allergen; it can cause serious problems for workers working in the industry as well as for people residing in the nearby residential colonies. The samples also had high concentrations of cadmium which can also be due to effluent deposition. The samples were low in hexavalent chromium concentration which can be due to partial treatment of the effluents released in the soil or chromium utilisation in steel manufacturing was done efficiently.

[2]The concentration of zinc ions in soil has to be less than or equal to 150mg/kg according to World Health Organization. The soil samples had high concentrations of zinc in them. Hence, the soils were not deficient in zinc concentration as per WHO guidelines. The upper limit of manganese in soil has to be 1800 mg/kg and the soil samples had manganese in lower concentration as compared to WHO guidelines. The absence of copper pose a threat to plant growth as copper is an essential component of an enzyme responsible for oxidation-reduction potential in leaves that will ultimately affect photosynthesis. [1]

The absence of lead in the samples indicates that the effluents from the factories which are partially treated and are able to remediate lead.

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CHARACTERIZATION OF COMMERCIAL DETERGENTS AND NATURAL CLEANSING AGENTS WITH COMPARISON OF THEIR POTENTIAL FOR BIODEGRADABILITY

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ABSTRACT

Commercial detergents are chemical formulations designed to dissolve or disperse grease, grime, and dirt by making them water soluble or suspending it in water. They are best known for its wide use in laundry industries and household cleaning. A critical analytical study was conducted on the quality of some of the popular detergent powders sold in Indian market viz. Ariel, Surf Excel, Rin and Tide with respect to their moisture content, active and total alkalinity, active detergent matter, water-insoluble matter, oxygen releasing capacity and pH. Two natural cleansing agents viz. *Areetha* and *Shikakai* were tested with the same parameters and were found effective as detergents but with certain limitations.

After use, the residual detergents are discharged into sewage system and are carried to water bodies which result in damaging the biodiversity of aquatic environment due to non-degradable nature of the active detergent matter present in detergents. Bacterial cultures were isolated from detergent-rich soil in *Dhobighat*, Mumbai and used to study detergent degradation over a period of time. Methylene Blue Photometric Assay was used to estimate the reduction in active detergent matter. Degradation was seen in the commercial detergents over a period of time.

Keywords: Detergents, Biodegradation, Methylene Blue Photometric Assay

INTRODUCTION

Detergents, by definition are anything that cleans; which include soaps, synthetic detergent powders and liquids, alkaline materials, solvents etc. But popularly, the term 'detergent' is used for packaged cleansing products added to water for household laundry.[1]Laundry detergent or washing powder, is a type of detergent (cleaning agent) that is added for cleaning laundry. Commonly, the mixtures of chemical compounds that includealkylbenzenesulfonates, which are similar to soap but are less affected by hard water, are used for the manufacturing of detergents.

Utilization of detergents:

The synthetic detergent industry is a profitable industry due to the need for laundry and cleaning by every individual considering the increasing world population.[2]The commercial sector is enlarging its market share rapidly by increasing the influence on consumers with the help of mass media. The detergent market has grown into a highly competitive one,where various vivid brands grapple to get consumers' recognition. Each brand claims to clean

spotlessly and brighter, bragging of the technologically questionable phrases like fighting granules, dirt gravitator, etc. Humongous quantities of surfactants are being used in household and industries daily, and most of it ends up disseminating in soil, water, etc.[3]Surfactants are usually the largest contributor of artificial organic carbon to the aquatic ecosystem. The sewage treatments in countries like India are extremely poor, and the use of detergents is increasing exponentially. In India, per capita consumption of detergents in 1994 was 2.8 kg per annum. This was predicted to rise to over 4 kg/capita by 2005. In rural areas the use of detergent bars was expected to grow by 7-8 percent annually.[4]

Adverse effects of detergents:

Detergents being used on large scale and their poor sewage treatment are responsible for its toxic effects on aquatic animals such as fishes, microbes like yeasts and bacteria.[5][6] Eutrophication, commonly known as Nutrient pollution, is also a pressing issue caused byexcessive deposition of surfactants in freshwater bodies. The aquatic plants like algae use the readily available nutrients like Phosphorous, Nitrogen and Carbon from the detergents, rapidly to cause algal blooms which in turn lead to a decrease in the concentration of dissolved oxygen in water. This creates hypoxic and anoxic condition killing fishes, producing foul odour and increasing the anaerobic pathogenic flora.[7]

Constant exposure to detergents is also dangerous to mammals including humans.[8]A study done to perceive the Indian consumers' conditions due to the adverse effects of detergents on health, showed that 77.6 % of the subjects had experienced some kinds of skin irritation due to the detergent use and the major population among them, comprised of *dhobis* and rural women. The primary reason for health problems arisesas the conventional laundry detergents leave chemical residues on the clothes which enter human body via the skin or through the lungs. The usual result of a steady and intemperate exposure of the skin to detergents is drying, fissuring and dotting of the keratin layer leading to increased permeability that causes sensitization, which may develop into dermatitis.

Elderly people are more susceptible to infections that may lead to developing eczema. Other health problems could be allergies, skin infections and in rare cases, cancer. The fragrances used in laundry detergents can prove allergic and be highly irritating to lungs, causing serious health effects to people with asthma or chronic heart problems.[7] Detergents are also partly responsible for the decline of coastal plants subjected to polluted spray; the cloudy water phenomenon i.e. formation of large foam ponds due to excessive foaming in rivers and for the contamination of groundwater by contributing nutrients and mineral salts such as phosphates, nitrates, ammonium, boron, etc.[9][10][11] Due to the growing complications, it was felt that bioremediation of detergents was a necessity.

Biodegradability of detergents:

Biodegradability is simply defined as the breakdown of an organic substance to simpler substances (carbon dioxide, water, methane or other simple organic molecules) by micro-organisms (bacteria,

fungi) or other biologicalmeans. The rate of biodegradation depends on pH, temperature, oxygen, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of compound, cellular transport properties,and chemical portioningin growth medium.[11]

A number of bacterial species are known to degrade surfactants and most of them are isolated from contaminated soil, water or sediments. Many aerobic bacterial species have the potential to degrade surfactants in peptone medium gratuitously but very few species grow in defined medium with surfactant as sole carbon/energy source.[12]

MATERIALS AND METHODS

Four brands of marketed detergent powders were procured from the open market in India, and taken up for the study of commercial detergents.

Brand name	Manufactured by	Barcode No.	Date of Packing
Ariel	Procter & Gamble Company, Mumbai	4 902430651783	Packed on 9/15
Surf Excel (Quick wash)	Hindustan Unilever Ltd. (HUL), Mumbai	8 901030515927	Packed on 7/15
Rin (Whites like new)	Hindustan Unilever Ltd. (HUL), Mumbai	8 901030454301	Packed on 3/15
Tide Plus (Jasmine and Rose)	Procter & Gamble Company, Mumbai	4 902430653176	Packed on 7/15

The powdered seeds of Areetha(*Sapindus trifoliatus*) and Shikakai (*Acacia concinna*)were
taken up for the purpose of studying natural detergents.

1. Moisture Content:

Weigh accurately about 1 g of the material into a dry tarred dish, and dry to constant mass in an air-oven at a temperature of 105 ± 1ºC. Cool in a desiccator and weigh.

Moisture content = (M – m) * 100/M

M = mass in g of the material taken for the test and m = mass in g of the material after drying

2. Alkalinity:

Pipette out 25 ml of stock sample solution and titrate against 0.1 N HCl till it becomes colourless (Phenolphthalein as Indicator), let the burette reading be **'A ml'** for determining Active Alkalinity. For the same solution, continue to titrate with 0.1 N HCl, till the colour changes from yellow to orangish red (Methyl Orange as indicator), let the burette reading be **'B ml'** for determining Total Alkalinity.

Active alkalinity (x) = A * 0.1 * 31 / 1000 g of Na_2O

Total alkalinity (y) = B * 0.1 * 31 / 1000 g of Na_2O

3. Active Detergent Matter:

Pipette 10 ml of sample. Add 10 ml of chloroform and 5 ml of 0.005% methylene blue solution to it. Shake well. The chloroform layer (lower) will be coloured blue. The mixture is then titrated with standardized cetrimide solution. After each

addition of 0.5ml, shake well and allow the phases to separate. Initially the chloroform phase will be coloured blue. Towards the end, the colour would start migrating to the aqueous layer. Note the reading at which the colour intensity in both the phases is the same.

x ml of 0.002 M Cetrimide = x* 0.002 * 348 / 1000 g of SLS

4. Water Insoluble Impurity:

Weigh a G_4 grade crucible and fit to a Buchner flask, to which suction is applied. Pour 1% sample solution sample. Dry the sintered glass filter funnel with the residue in an air-oven at a temperature of 105 ± 2°C. Cool in a desiccator and weigh till constant mass is obtained.

Matter insoluble impurity= 100 (m / M)

m = mass in g of the matter insoluble in water, and M = mass in g of the sample taken for the test.

5. Oxygen Releasing capacity

50 ml of 1% solution was pipette out in conical flask. To this, 1 test tube of HCl was added and titrated against 0.1 N $KMnO_4$ till faint pink colour persists.

x ml of O.1 N $KMnO_4$ =8 * 0.1 * x / 1000 of O_2 in 50ml

6. pH:

Weigh 1 ± 0.001 g of the material and transfer to a 100 ml volumetric flask. Partially fill the flask with distilled water and agitate until the sample is completely dissolved. Filter the solution through Whatman filter paper: 41, in case of residual solids. Adjust the temperature of the solution and the distilled water to 30 ± 0.5, and make up the volume to the calibration mark with distilled water. Stopper the flask, mix thoroughly, and allow the solution to stand at a temperature of 30 for two hours prior to measuring the pH. Measure the PH of the solution using a glass electrode.

Biodegradation of Synthetic Detergents:

i. Source of bacterial sample and collection:

Soil from the outlet of laundry washing water from *Dhobighat*, a commercial laundry cleaning area in Mahalaxmi, Mumbai, Maharashtra, India was collected in sterile containers, stored at 4˚C till processed and serially diluted. This served as the source of detergent-degrading bacteria. The bacteria were isolated on Nutrient Agar plates supplemented with a synthetic detergent. Gram nature and colony characteristics of two bacterial isolates were studied.

ii. Cultivation of isolated bacterial cultures;

Isolated bacterial was subcultured on sterile Nutrient Agar slant and M9 media supplemented with a synthetic detergent and incubated at 37° C for 48 hours to screen its degradative capacity. The 48-72 hour cultures were suspended in sterile saline and set to 0.1 O.D.at 540 nm.

iii. Sterilization of Detergent sample:

1g of Detergent samples viz. Ariel, surf excel, Rin and Tide were weighed, packed in packets and put in a dry hot air oven for sterilization.

iv. Inoculation of Bacterial Sample:

a. Sterile M9 media (incomplete mineral medium) was used as growth media to check for the 2 bacterial cultures' capability to use detergent as a primary source of carbon.
b. Sterile 250 ml conical flask labelled as 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 were filled with 100 ml sterile saline. Flask 1, 3, 4, 5 and 6 was inoculated with 1 ml mix culture of the two types of organisms.

Flasks 3, 4, 5, 6, 7, 8, 9 and 10 contained 1g of surfactant.

c. Flask 1 served as positive control and flask 2 served as media control. Flask 3 and 7 contained Ariel, Flask 4 and 8 contained Surf Excel, Flask 5 and 9 contained Rin; Flask 6 and 10 contained Tide.
d. These flasks were incubated at 37° C for 1 month. 10 ml aliquots were pipette out in a sterile test-tube maintaining aseptic conditions at the end of 0 days, 15 day and 30 days and were analysed using Methylene Blue Photometric Assay[13] at 620 nm.
e. From the calibration curve, read the micrograms of apparent SLS, corresponding to the measured absorbance.

mg MBAS/L = / mL original sample.

RESULTS AND DISCUSSION

1. Moisture Content:

Table 1: Moisture content of various detergents

Sr. No.	Sample	Moisture content (in %)
1.	Ariel	2.639
2.	Surf Excel	6.312
3.	Rin	2.320
4.	Tide	2.881
5.	Areetha	6.390
6.	Shikakai	6.395

As shown in Table 1, only Surf Excel has 6.312% of moisture which is equal to 6.39% of moisture in both the natural products (*Areetha* and *Shikakai*). One can understand the presence of moisture in natural products, but if commercial criteria are adopted, it becomes difficult to justify the moisture content in Surf Excel.

2. Alkalinity:

Alkaline nature of the detergents is very important with respect to its effectiveness

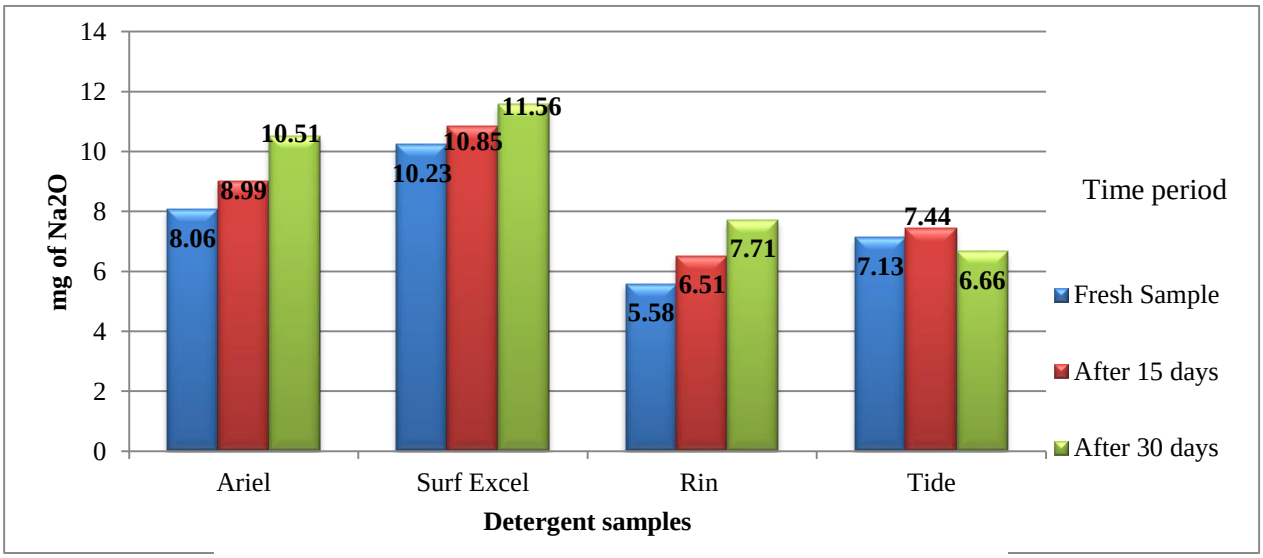


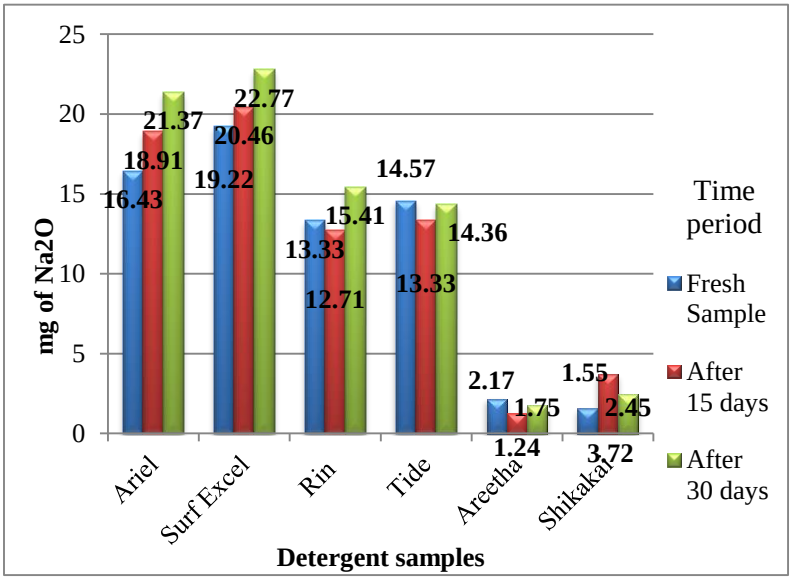
Figure 2. a. Active Alkalinity of Detergent Samples

of its cleaning ability. Oil or grease can be removed from the cloth only in alkaline conditions; also mild alkaline conditions make the hard water soft and improve the detergency action. Effectiveness in hard

water is the advantage of detergents, over soap. The alkalinity of a detergent is measured in terms of active alkalinity (or bicarbonate alkalinity) and total alkalinity (or carbonate alkalinity). Active alkalinity helps in water softening and total alkalinity helps in the removal of oil and grease.

As the detergents are non-biodegradable, it is expected to show the same alkalinity after a long span of time, when they are disposed in water. Hence, the known concentrations of detergent solutions were preserved in anopencontainer (beaker) for about a month.The same procedure was followed and readings were taken after 15 days and 30 days from the date of preparation. The results obtained for the Active and Total alkalinities of the samples are reported in Figure 2. a. and Figure 2. b.

Figure 2. b. Total Alkalinity of Detergent Samples

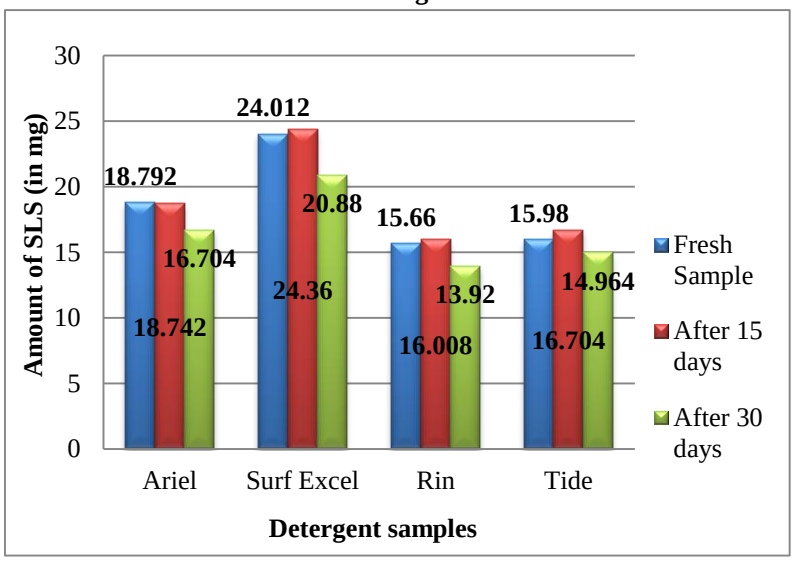


3. Active Detergent Matter:

The activity of a detergent varies with its active detergent matter content like Sodium Lauryl Sulphate. The effectiveness of a detergent can be easily correlated with the active detergent matter. As it is known that the detergents are majorly non-biodegradable, this indicates that Sodium Lauryl Sulphate remains unaffected in the solution for a longer time.

The solutions once prepared were kept for one month in an open atmosphere and the measurements were carried out for the active detergent matter on the first, 15th and the 30th day. The results obtained are shown in Figure 3. As the natural detergents had no Sodium Lauryl Sulphate, it could not be detected in any case of sample and period.

Figure 3: Active detergent matter of the samples of various detergents



4. Water Insoluble Impurity:

As the detergents are non-degradable, the insoluble matter in the detergents remains in water body for a long time. This has made determination of the insoluble matter of detergents a point of interest. The amount of insoluble matter in the various detergent samples is shown in Table 4.

Table 4: Insoluble matter in various detergents

Sr. No.	Sample	Insoluble matter (in %)
1.	Ariel	14.24
2.	Surf Excel	16.80
3.	Rin	1.72
4.	Tide	0.56
5.	Areetha	47.80
6.	Shikakai	88.78

5. Oxygen Releasing capacity

Nascent oxygen generally used for bleaching/cleaning of the cloth. The property of releasing oxygen has given a tremendousadvantage to the detergent over soaps. The detergent which releases more oxygen is considered to be better detergent. The results of oxygen releasing capacity of all the samples are reported in the Table 5.

Table 5: Oxygen releasing capacity of various detergents.

Sr. No.	Sample	Oxygen releasing capacity (in ppm)
1.	Ariel	14.40
2.	Surf Excel	8.00
3.	Rin	4.80
4.	Tide	3.20
5.	Areetha	128.00
6.	Shikakai	176.00

6. pH:

We have already discussed about the alkalinity of the detergent solutions. The pH of the solutions doesn't matter much but to have data for correlation, pH parameterof the 1% solution of the sample is taken. The results of pH of all the samples are reported in the Table 6.

Table 6: pH of fresh 1 % solutions of various detergents

Sr. No.	Sample	pH
1.	Ariel	10.26
2.	Surf Excel	10.27
3.	Rin	10.16
4.	Tide	10.12
5.	Areetha	6.59
6.	Shikakai	6.51

7. Biodegradation of Synthetic Detergents

i. Isolation and Cultivation of Detergent degrading Bacteria:

Two bacterial strains of detergent degrading bacteria were isolated from detergent rich soil in *Dhobighat*, Mahalaxmi, Mumbai, Maharashtra, India. This place was selected because it is a commercial area for the washer men for washing of clothes. Using enrichment technique, the isolates were inoculated in Nutrient Media supplemented with Detergent. The plates were incubated in at 37°C. After 2-3 days, when prominent growth was observed, the colony characteristics of the isolates were studied. These isolates recovered from *Dhobighat* were designated as C1 and C2 respectively.

Characteristics	C 1	C 2
Size	large	medium
Shape	ovoid	circular
Colour	colourless	cream
Elevation	flat	low convex
Margin	entire	entire
Opacity	translucent	translucent
Gram nature	Gram negative	Gram negative
Morphology	coccobacilli	coccobacilli

ii. Degradation of active detergent matter by bacterial culture isolated from soil in *Dhobighat*and quantifying the result using Methylene Blue Photometric Assay:

The determination of the biodegradation for synthetic detergent samples is crucial to the outcome of the investigation and this was done using the Methylene Blue Photometric Method. The Methylene Blue Active substance (MBAS) analysis was used to determine the concentration of active detergent matter in each of the commercial detergent products. The mixture of 2 isolated Gram negative bacterial cultures were used to study detergent degradation over a period of 30 days. Reduction of absorbanceof MBAS was used to estimate the reduction in active detergent matter in the solutions. Degradation was followed over a period of time.

A significant reduction was observed in the absorbance and concentration of MBAS (shown in Table 7. a. and Table 7. b.) in the 1% sample detergent solutions viz. Ariel, Surf Excel, Rin and Tide. This indicates that the active detergent matter present in them can be degraded slowly by the bacterial cultures isolated from the detergent rich soil in *Dhobighat*.

The Figure 7. c. gives the percentage degradation of the active detergent matter of the sample detergents, over a span of 15 days, 15 – 30 days and overall degradation in 30 days. Ariel, surf Excel, Rin and Tide are degraded to 86.21%, 90.14%, 77.88% and 66.42 % respectively over 30 days.

Bureau of Indian Standards (BIS) does not enlist the criteria of biodegradability of detergents in I.S.: 4955 – 2001. Whereas, under Environment Protection Authority (EPA) of Australia, the Australian Standard for biodegradability (AS1792 – Methods to determine the Biodegradability of Surfactants) requires 80% of the mixture to be degraded within 21 days, to carry the 'biodegradable' label. So according to EPA, except for Ariel, the other sample detergents are non-biodegradable.[14]

Table 7. a.: The Absorbance of MBAS (mg MBAS/L) on Day 1, Day 15 and Day 30 for various detergent samples

Sample (1% Detergent solution)	O. D. at 620 nm for Day 1	O. D. at 620 nm for Day 15	O. D. at 620 nm for Day 30
Ariel	0.41	0.06	0.06
Surf Excel	0.09	0.06	0.01
Rin	0.17	0.10	0.04
Tide	0.17	0.08	0.06

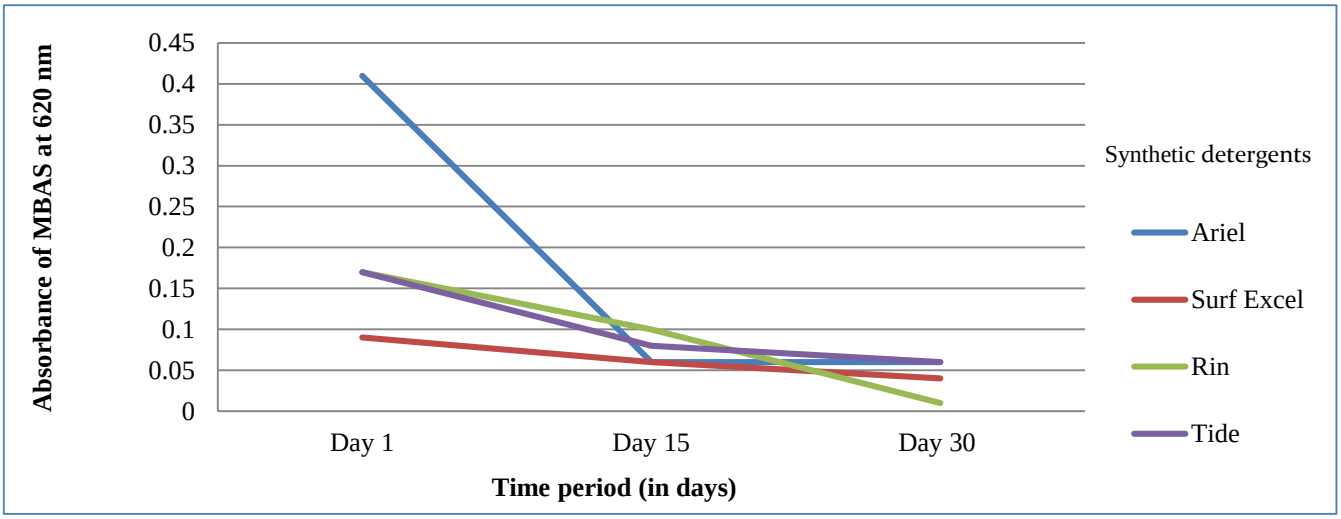


Table 7. b.: The concentration of MBAS (mg MBAS/L) on Day 1, Day 15 and Day 30 for various detergent samples.

Sample	Concentration of MBAS on Day 1	Concentration of MBAS on Day 15	Concentration of MBAS on Day 30
Ariel	0.682	0.094	0.094
Surf Excel	0.142	0.094	0.014
Rin	0.280	0.160	0.062
Tide	0.280	0.126	0.094

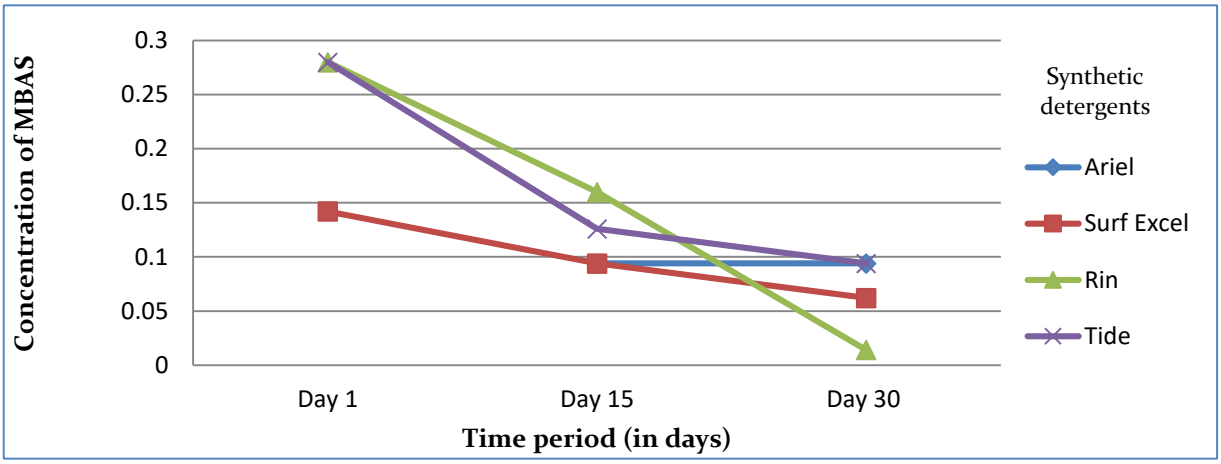
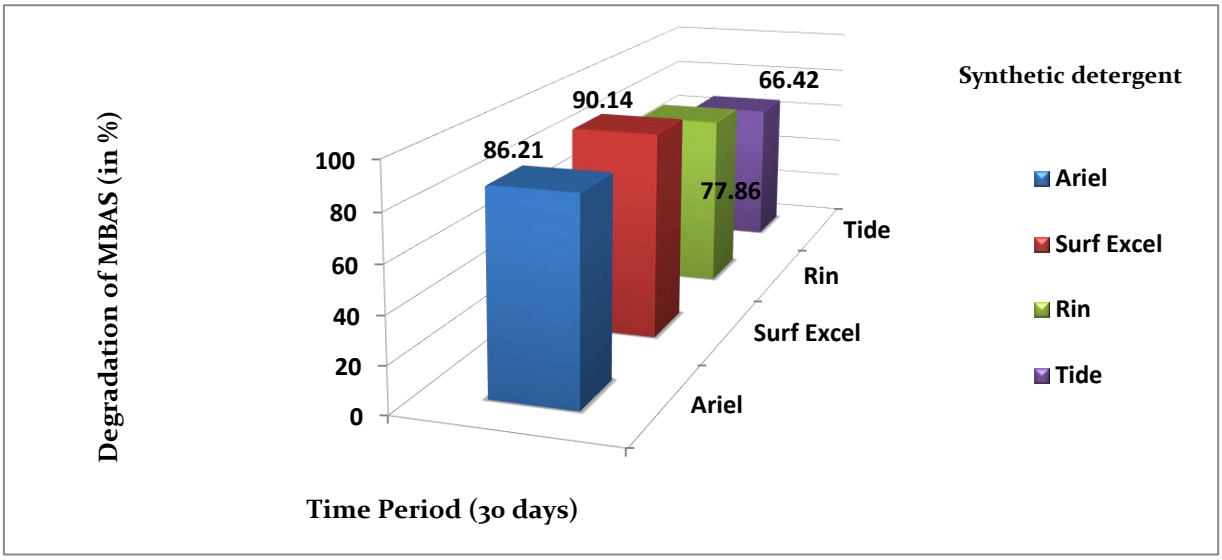


Figure 7. c.: The percent total degradation of the sample synthetic detergents in 30 days



CONCLUSION

The purpose of this work was to compare the properties of commercially available detergents viz. Ariel, Surf Excel, Rin, Tide and the natural cleansing agents viz. *Areetha* and *Shikakai*. After reviewing the results that were obtained from chemical and microbial analysis, it is observed that the natural cleansing agents are almost at par with the commercial samples. It is an accepted fact that the natural cleansing agents do not perfectly match with the commercial samples in some tests, but can be made equivalent to the properties of the commercial samples by using minimal quantity of chemical aid.

The moisture in natural cleansing agents is comparatively high, but this could be justified by the process of obtaining the natural samples. The natural samples were obtained by crushing of the seeds directly, but heat drying was not performed in oven before use. Thus it can be stated that, after heat drying, the moisture content can be lowered and the other results would have been further improved.

The natural cleansing agents are mildly alkaline; rather, almost neutral. Thishas an advantage in washing of the delicate fibres like wool and other synthetic fibres. Simultaneously, the colours of the threads are also secured in the moderately low alkaline detergent solutions and here the natural cleansing agents have tremendous advantage over commercial detergents. At the same time, it will be of further interest to compare the oil / grease removal capacity of the natural cleansing agents with the specialized commercial detergents.

Pollution control was one of the otheragenda behind this study. If the results of solubility are reviewed, it is observed that both the naturalcleansing agents are not sufficiently soluble in water.If the effluent containing natural cleansing agentsis filtered after use, then the insoluble matter can be easily removed. This will help the reduction in load of effluent treatment for removal of Chemical Oxygen Demand (C. O. D.) / Biological Oxygen Demand (B. O. D.)

The functioning of the natural cleansingagents is mainly based on its capacity to release the nascentoxygen. Huge amount of oxygen is released by these products; that will indirectly help in reduction of C. O. D. in the effluent.

The natural cleansing agents are biodegradable and hence can be removed from the effluent with minimal processing. Whereas, the Environment Protection Authority (EPA) requires 80% of the detergent mixture to be degraded within 21 days. This signifies that the advantage of natural cleansing agents is limited to 21 days, over the commercial detergents.

If some water soluble softeners and active-detergent matter are added, then greater performance from the natural products can be achieved. The systematic drying and proper packing will improve the performance of the natural materials and a great impact on the environment can be achieved.

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DETERMINATION OF HEAVY METALS IN VARIOUS BRANDS OF LIPSTICKS

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ABSTRACT

Objective: Heavy metals toxicity to humans is the result of long term low or high level exposure topollutants common in our environment. Metals like lead, cadmium, chromium and nickel were determined in different lipstick brands that targets the unbranded lipsticks and comparing them with international brands and with standards prescribed by USFDA.

Methods: Heavy metals like Pb and Ni were analysed volumetrically whereas Cr and Cd weredetermined by Atomic Adsorption Spectrophotometrically.

Results: Lead and Nickel concentration was found to be much higher than the standards prescribed byUSFDA in unbranded lipsticks as well as in comparison with branded lipsticks. Also the chromium and cadmium concentration was higher n the unbranded ones.

Conclusion: Unbranded lipsticks are sold at low cost for use and target the wider population in India.These lipsticks had high heavy metals concentration indicating the practice of use of cheap raw materials. Many of these heavy metals like nickel, lead, cadmium show wide spread chronic effects on the user.

Keywords: volumetrically, Atomic Adsorption Spectrophotometrically, chronic effects.

INTRODUCTION

1. Cosmetics

Cosmetic is defined as "Any article intended to be rubbed, poured, sprinkled or sprayed on, or introduced into, or otherwise applied to, the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and includes any article intended for use as a component of cosmetic". Cosmetic products are regulated for health and safety. There are concerns regarding the presence of harmful chemicals, including heavy metals, in these products. There have not been many studies on presence of heavy metals in cosmetics in India.

A large number of heavy metals are present in lipsticks. Some of these are used as colouring agent. Some of these metals are added as intentionally ingredients while some added accidently from environment. Some cosmetics products are directly applied to the skin such as lipsticks. These enter into the body and cause the hazardous effect to the internal body organs. Cosmetics are the important source of releasing heavy metals in the environment. Cosmetics represent an important source of sensitization, since they are used every day and are applied to the thinnest areas of facial skin, such as the pre-ocular areas and lips, where absorption is very high.

1.1 Lipstick as a Cosmetic

Lipstick -a cosmetic product containing pigments, oils, waxes, and emollients that applycolour, texture, and protection to the lips.

Many colours and types of lipstick exist. As with most other types of makeup, lipstick is typically, but not exclusively, worn by women . Some lipsticks are also lip balms, to add colour and hydration. Lipstick contains wax, oils, antioxidants and emollients.[31]Wax provides the structure to the solid lipstick. Lipsticks may be made from several waxes such as beeswax, ozokerite and candelillawax. Because of its high melting point, Carnauba wax is a keyingredient in terms of strengthening the lipstick. Various oils and fats are used in lipsticks, such as olive oil, mineral oil, cocoa butter, lanolin,and petrolatum. Lead and other trace metals are also found in many lipsticks. It is impossible to know whether these metals are in the lipstick by looking at the ingredient list because they Hydrogenated cotton seed oil, sodium hexametaphosphate on the ingredients list of lipsticks. These chemicals contain trace amounts of naturally occurring metals, such as lead and many more.[33]Matte lipsticks contain more filling agents like silica but do not have many emollients. Crème lipsticks contain more waxes than oils. Sheer and long lasting lipsticks contain more oil, while long lasting lipsticks also contain silicone oil, which seals the colours to the wearer's lips. Glossy lipstick contains more oil to give a shiny finish to the lips.

1.2 Constituents of Lipstick

It is a solidified fatty base containing dyes and suspended pigments .the base is usually composed of waxes, oils and fatty materials like bee wax, hydrocarbon, castor oil, oleic alcohol, are not an intentional ingredient added, but rather, an unintentional contaminant. These trace metals are naturally occurring and accidentally get taken up with other chemicals that are used in lipstick production. Lead acetate, chromium, thimerosal,butyl stearate, propylene glycol, polyethylene glycol, cocoa butter and lanoline.

The gloss and hardness of the product are determined by the characteristics of waxes. The oils may account for more than half of the total weight of the lipstick the fatty material gives lipstick more body, softens skin of lips and promotes dispersion of insoluble pigments.

Dyes including fluorescein are used predominantly to stain the lips. Lakes and other pigments are used for their brightening and covering effects. A typical product may contain 10% lakes and only 2-3% pure dyes.

Aims and objectives

Heavy metals like lead, cadmium, chromium and nickel were determined in different lipstick brands that targets the

'B' grade market and comparing them with the standards prescribed by US FDA as well as with international lipstick brands.

Unbranded lipsticks are sold at low cost for use and target the wider population in India. These may be toxic in the long run. Many of these toxic metals like nickel, lead, cadmium show widespread chronic effects.

MATERIAL AND METHODS

The heavy metal concentration was determined using atomic absorption spectrophotometry and volumetrically also (for reference).

The lipsticks samples of different brands were collected from different cosmetics shops from the local market of Mumbai. The samples were of different qualities and popular brands as well a 'B' grade market brands with different price ranges. For each brands same colours were taken. Those colours were taken that were mostly used. Collections of samples were made according to their use by the different societies of peoples. Some brands were used by the lower and middle class peoples and some were used by the upper class peoples.

AAS

Sample Preparation

Lipsticks are present in the semi-solid form, so these required pre-treatment before analysis. Lipsticks are organic in nature. For this purpose **1 g** of lipstick samples were exactly weighted with electrical analytical balance and put into digestion flask. Lipsticks samples were digested by using wet digestion method by repeatedly addition of HNO_3 and $HCLO_4$(acid mixture in ratio 3:1) at

atmospheric pressure under open system at hot plate under 90-100 ºC for 3-4 hours and heated until the white fumes started evolving, which showed the completion of digestion process and samples were cooled. The resulting digestate was mixed with 5ml distilled water and the digestate was filtered with Whatmanfilter paper(Number 41). The volumes of sample were made up to 10 ml by the addition of distilled deionised water. Samples were stored in clean labelled sample bottles until analysis and metal quantification.

Sample analysis forLead, Cadmium, Chromium and Nickel determination

Lead, Cadmium, Chromium and Nickel determination using flame atomic absorption spectrometry. Air acetylene flame was used for Lead, Cadmium and Nickel while air-acetylene-nitrous oxide flames to be used for Chromium determination. Standard solutions were prepared in five different concentrations for each metal separately to obtain calibration curve for quantitative analysis. Deuterium lamp was used for background correction.

Volumetrically

The concentration of lead was determined by complex metric titration with EDTA whereas nickel was determined volumetrically by Nickel-DMG method. (FOR REFERENCE)

RESULTS

The distributions of heavy metals in 6brands were studied. Table 1 showed values of metals for all determined metals. Lead detected by AAS in all tested brands of lipsticks in the range of 1.10-4.80ppm. The USFDA (US Food and Drug Administration) limit for lead as colour additive in cosmetics is 20 ppm. So in all samples the lead concentration was below the FDA limit. The campaign for safe cosmetics has given the 0.1 ppm lead level in candy. Safe level of lead according to EPA lead safe level is 0.5 ppm. Using these values it was concluded that in all brands lead level was higher than CSC (Campaign for Safe Cosmetics) and EPA (Environmental Protection Agency) safe limit values. In the results of this study the lead level was lower in expensive (branded) samples. In all brands the cadmium was present in the range of 1.49-3.72 ppm. The oral limited value for the cadmium is 0.9 to 3ppm. When cadmium is directly subjected it lowers the blood pressure. So its trace amount is also not safe. In all lipsticks brand samples chromium was present in the range of 2.15-3.40 ppm. It is added as colorant in lipsticks. Its amount should be less than 5 ppm. According to EPA the safe level of chromium is 1 ppm. So in cheap brand and chromium concentration was maximum while in expensive brand it was lowest, Nickel was present in the range of 2.02-3.64 ppm. Nickel concentration above 1 ppm can cause the contact dermatitis [13]. So in cheap brands nickel concentration was highest and lowest was in branded lipstick.

Sample 1 was the cheapest brand of the lipsticks and mostly used by the lower class peoples. The lead and cadmium

concentrations were higher in all the tested samples when mean value of all metals were calculated followed by the nickel in this brand. The mean value for lead was 4.53 ppm in all 3 samples. In Brand 2 which was also all metals were present in greater concentration, but the level of cadmium and lead were greater as compared to other metals.

When mean value of metals was determined for cadmium its concentration was 3.59 ppm. In Brand 3 cadmium concentration was higher. The mean values for Cd, Cr, and Ni were 3.04 ppm, 2.92 ppm and 2.83 ppm respectively (Figure 1). It was mostly used by the middle class societies. In this all the metals were present in lower limited vales. Only the nickel was present in greater concentration followed by the lead and chromium. Brand 5 was mostly used by the upper class society. In this brand cadmium contents were found higher. In Brand 6 all metals contents were lower. Hence it was a branded company lipstick. In our results of all tested samples in all 6 brands lead (4.80ppm), cadmium (3.72 ppm) and nickel (3.64 ppm) levels were higher, followed by the chromium (3.40 ppm)

Table 1: Concentration (ppm) of Metals in Different Brands

Brands	***Lead***	***Cadmium***	***Chromium***	***Nickel***
1	4.53	3.72	2.54	3.04
2	3.30	3.59	3.40	3.34
3	2.22	3.04	2.92	2.83
4	4.80	1.49	2.15	3.64
5	1.10	3.56	2.87	2.02
6	2.53	2.02	2.21	2.38

NOTE: The values are the Average of Triplicate Samples (n = 3)

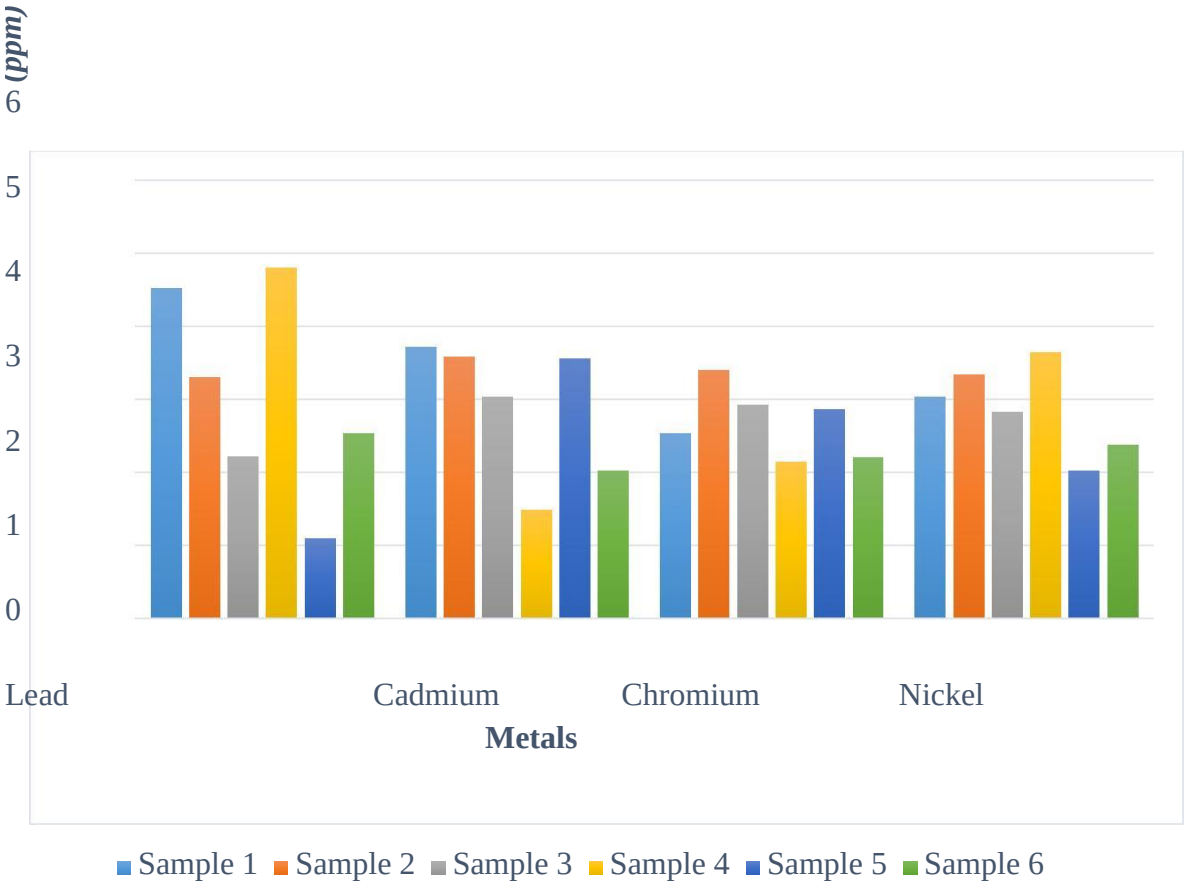

Figure 1 : Concentration of Metals in Different brands of lipsticks

CONCLUSION

Atomic absorption spectrophotometer was used for the determination of heavy metals (Lead, Cadmium, Chromium and Nickel) Samples of six different brands of Lipsticks sold at local markets in Mumbai.

- These unbranded lipsticks had high heavy metal concentration indicating the practice of use of cheap raw materials.
- The appearance of unbranded sample was flaky whereas the branded variety had a smooth uniform appearance.
- The unbranded variety tended to leave stubborn stains after use.
- On application on a smooth surface, the unbranded variety showed an uneven appearance.
- On keeping the samples for a duration of about 8 months the unbranded sample showed colour variation.
- During digestion, the pigment particles of the branded sample started dispersing whilst the unbranded sample got clumped.
- The branded sample showed heavy metal concentration within the permissible limit prescribed by the United States of America Food and Drug Administration.
- The unbranded sample had high concentrations of all the metals in which lead had the maximum concentration.

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COMPARATIVE STUDY OF MATTING AGENTS IN NAIL LACQUERS USING TITANIUM DIOXIDE AND FUMED SILICA

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ABSTRACT

Different combinations of matting agents in nail lacquers have been tried to get the perfect suiting formula for the desired matte product. Also with regular and timely lab work we came up with new formulas that can be incorporated to get any desired effect for e.g.: ice matte, sand paper matte, milky matte etc, without increasing the cost of overall product. Through this project we have increased the life of nail polish that earlier sustained for a few months to 10 months of applicability. Another breakthrough in the project is the matting effect has been enhanced and the compatibility of the pigment with solvent system has been increased.

The comparison of matting properties is required to acquaint us with the enormous possibilities on how we can bring about maximum potential out of the limited resources. Through comparison we have developed an ideal formula that one can replicate to enhance their product's life span and matting effect without increasing the cost of the product, for some formulators, this modification may even reduce the cost of formulating matte nail polish and still give out the desired results.

Keywords: Nail lacquers, Matting,surface coating, pigment solvent system.

INTRODUCTION

Chemistry of nail lacquers

There is no single formula for nail polish. However, the main ingredients include film forming polymers like nitrocellulose, film modifiers – plasticizers like N-substituted toluene sulphonamides, solvents, suspending agents- colouring agents such as inorganic and organic pigments and resins for adhesion.

In traditional nail polish formulations, the even surface of the coating leads the light to be reflected directly off the surface into the eyes. This direct reflection leads the eye to perceive the surface as glossy. When a matting agent is added to a system, the surface of the coating becomes rough, resulting in the amount of light that is directly reflected reduced. This alteration of the surface causes the eye to perceive the finish to be matted as opposed to glossy. Like colour, both matting and gloss are subjective impressions that are identified by our senses.

The major problem faced by the industry today is that when titanium dioxide is dispersed in paste form as a whitener, matting agent and opacifier, the molecule of tio2 does not fully bind with the lacquer

system, causing settling of the pigment and the polish can no longer be used for its purpose.

Aim of this paper

The goal of this research is to come up with sustainable model/formulations that can be replicated and studied. The aim of this paper is to achieve matting in nail lacquers.

Conventionally for achieving matting in nail polish titanium dioxide has been used in itsilmenite form (dissolved in solvent, white paste). Titanium dioxide still remains in usage because of its outstanding whiteness, Opacifying agent and matting properties. It does not react with the solvent system and hence remains neutral in the lacquer. In this project, the whiteness of nail polish was disregarded for matting studies and titanium dioxide in Rutile form (off white in colour or buff coloured) was used as compared to titanium dioxide white ilmenite (99.2% pure) .The lacquer is generally manufactured in bulk and the pigment is added later on to smaller systems.

When titanium dioxide is added to the lacquer system and shear mixing is done, it makes this system into a white suspension mixture of pigment bound with the lacquer system. But due to slightly larger size of titanium dioxide molecule, it does not bind with solvent system even after shear mixing of the suspension. After the addition titanium dioxide, other pigments for example: blue pigment or red lakes are added to get the desired colour of the matte finish. These secondary pigments in turn bind with titanium dioxide-solvent system bounded suspension. If the pigment starts to settle then soon the product will be of no use. To stop this settling of pigment we have come up with formula through this experiment with the desired matte finish without affecting the chemistry of the suspension and the cost remains close to same.

MATERIALS

Nail lacquer , Titanium dioxide (cosmetic grade) , titanium dioxide rutile (20 microns ltd), fumed silica (8 – 20 micron size) , any FDA certified pigment for later use , 250 ml beakers , glass slides , glass rod , lab mixer , accurate weight balance up to 500 grams.

BATCH 1	TITANIUM DIOXIDE	SILICON DIOXIDE
A	1%	1%
	1.7%	1.7%
	2%	2%
B	1.7%	2%
	1.7%	2.5%
	1.7%	3%
C	2%	1.7%
	2.5%	1.7%
	3%	1.7%
D	-	1%
	-	1.5%
	-	2%
E	1%	-
	1.5%	-
	2%	-

METHODOLOGY

Comparison of matting agents in combination is a problem in itself. Conventionally what one would do is try to separate out all the agents and make batches from different concentrations of the agents to give loads of samples to test. In this project i have made my own protocols to go about it . I have used

titanium dioxide in different concentrations so as to maintain the whiteness of the product with varying concentrations of fumed silica per batch. Each batch has four sub batches that have three formulations in each. By using these kinds of protocols 36 new formulas have been formed and each one is unique in its own way. Conventionally titanium dioxide white is used. But in this project we have used not only white but also Rutile/buff form of titanium that is slightly off white and cream in colour. Titanium dioxide in rutile form has been used because it's molecule size is way smaller than that of conventional titanium dioxide, this will help in dissolving the off white pigment better in the solvent system. Also it can bind with the coloured pigments easily as it has lower molecular size.

THE EXPERIMENT

Take 200g. Nail lacquer base and add titanium and silica in the range of the given formulae. Shear mixing needs to be done for at least 10 minutes. Only then other tests can be performed on the lacquer or nail polish.

In the first batch Titanium dioxide thick white paste has been used with ACE-MATT TS 100, a registered brand of EVONIK is untreated thermal fumed silica specially manufactured to give matte effect in paints and solvent systems. It also increases the storage stability of the lacquer.

For certain formulae where the concentration of fumed silica increases the mixing time should be increased to 15- 20 minutes.

BATCH 2	TITANIUM DIOXIDE GL	SILICON DIOXIDE
A	1%	0%
	1.5%	1%
	2%	1.5%
B	1.7%	2%
	1.7%	2.5%
	1.7%	3%
C	2%	1.7%
	2.5%	1.7%
	3%	1.7%
D	1%	1%
	1.7%	1.7%
	2%	2%

In second and third batches titanium dioxide in two varied forms has been used naming GLOWTOX-D & GLOWTOX-90 both registered brands of 20 MICRONS ltd. Fumed silica that has brand name FMSIL412+ also from the same company.

In batch number 2 GLOWTOX-D titanium dioxide has been used and in batch number 3 GLOWTOX-90 has been used.

BATCH 3	TITANIUM DIOXIDE	SILICON DIOXIDE
A	1%	0%
	1.5%	1%
	2%	1.5%
B	2%	1.7%
	2.5%	1.7%
	3%	1.7%
C	1.7%	2%
	1.7%	2.5%
	1.7%	3%
D	1%	1%
	1.7%	1.7%
	2%	2%

Addition of these components to the nail lacquer base may vary from one formulation of nail lacquers to another but similar results can be achieved if some combinations are changed to 0.5% of dosage. Note that dosage of titanium dioxide in white paste form should not exceed more than 3% and of ACEMATT TS100 should not exceed more than 4%.

Also the dosage of GLOWTOX – D & GLOWTOX- 90 should not exceed 4.5 % - 5% and of FMSIL412+ should not exceed more than 4%. If the dosage values of the above pre requisite conditions is exceeded the product may not be fit for usage. Generally with high silica content the product becomes more viscous and application is affected due to this.

RESULTS & DISCUSSION

The basic constituents of nail lacquers are discussed below

1. Film forming polymers – Nitrocellulose, film modifiers – di butyl phthalates. Nitrocellulose provides an unusual combination of properties of toughness, durability, solubility and solvent release. Nitrocellulose films have a high gloss transition point, tendency to shrink and have to be used with plasticizers. It is the most common film former used today. Secondary plasticizers have been used to give better flexibility of film and better adhesion.
2. Resins – toluenesulfonamides. These are added to nitrocellulose based lacquers to increase the solid content without increasing viscosity of lacquer. Resins provide excellent gloss, depth, flow and adhesion. Other resins that are often used are acrylates and poly amides.
3. Suspending agents – Organo clays. Rheological modifiers and thixotropic agents prevent the colorants from settling down.

4. Solvents – toluene, butyl acetate, ethyl acetate etc. Solvent blend in nail lacquer influences viscosity, application, drying time, gloss, film hardness, and long term stability of product.
5. Colorants - colorants for nail enamels are usually confined to non-bleeding types. The colours selected for nail enamels need to be relatively light fast. Organic colours are responsible for producing light coloured shades and inorganic for a little dull shade. The degree of dispersion of pigment has a major influence on gloss of the product. Colorants with high specific gravity like titanium dioxide tend to settle.

In the above results it has been observed that some formulations stand out and others do not. This only shows that particular concentration of combination of fumed silica and titanium dioxide can bring about superior matting in nail lacquers. To confirm the safety of products that were created in the lab we did a heavy metal test and various tests according to Indian Standards IS9245:1994. The products passed all the tests and are now suitable to be sold in the market. The lab reports of the batch C-3 and C-2 have been attached in the project report.

CONCLUSIONS

Matting in nail lacquers has been achieved using fumed silica and titanium dioxide. The perfect suiting formulae have been analysed for making a superior matte product. The products that have come out as a result have a better matting property, they have a better shelf life and this addition of new matting agents does not increase the overall cost of the product. Over 39 formulae have been made in combinations, out of which few are mentioned below.

Some of the matting agents such as white paste titanium dioxide with fumed silica (ACEMATT TS 100) give astounding matting effect even at low dosage, such worth mentioning formulas are A-2 from Batch 1 that has 1.7% titanium dioxide white paste and 1.7% of fumed silica. This particular product gave sand paper matte effect even at such low concentrations of the matting agents. The drying time of this product is approximately 30 seconds. Another astounding formula is B-1 from Batch -1. This formula has 1.7% of titanium dioxide and 2% of fumed silica. The average drying time is around 20 seconds. The product has a shelf life of over 1 year and 4 months. There are no major changes in it's viscosity and applications. The product gives superior ice matte finish. The formula named D-1 form batch 1 is an example of matte products that we get in the market today. They have high gloss and low matting.

The formula C-2 and C-3 of Batch -1 are very good matte products. Other commendable formulae in Batch -1 are B-3 that has superior sand paper matte effect and B-2 that has a classical matte finish.

Matting agents such as titanium dioxide rutile/buff and fumed silica of a different cosmetics grade gave altogether astonishing results. Although the colour of the product changed, it did bring about better matting at low and medium concentrations. One such example is B-1 from Batch 2 that uses dark buff coloured titanium dioxide and fumed silica to achieve sand paper matte with medium gloss. The concentrations in this formula

are 1.7% of titanium dioxide and 2% of fumed silica. The overall colour of this formula is dull but the matting effect is commendable As the molecule size of titanium dioxide is less than that of titanium dioxide white paste the titanium dioxide buff/rutile suspends better in the suspension. The shelf life of this product is almost 1 year and 8 months – 2 years. There is not much change in viscosity and application of the product. the average drying time of this product is around 50 seconds. Another formula in the same batch is A3 having concentration of only 2% buff/rutile titanium dioxide and 2.5% fumed silica. Surprisingly this product is not dull. It has a good matting effect and drying time is little over 1 minute. This is a promising formula.

Matting agents such as light buff / rutile titanium dioxide and fumed silica also gave astounding but fewer results. The formula C-3 which has 1.7% titanium dioxide and 3% fumed silica gave an ice matte effect. The gloss levels were normal. the shelf life is also over 2 years. Another formula is B-3 of same Batch – 3 . it has the concentrations of 3% titanium dioxide and 1.7 % fumed silica. It gives a rich creamy matte effect.

The overall quality of these products is superior. The shelf life of the above mentioned products may vary according to nail enamel formulations and atmospheric conditions.

These products are safe to use on a regular basis as they passed all the laboratory tests. The analysis that was carried out is blush test, heavy metal tests, Arsenic test, Adhesion test, drying time and non volatile matter. The products passed the Indian Standards IS9245:1994. This proves that the products are safe to use and can be marketed.

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ESTIMATION OF METALLIC ELEMENTS PRESENT IN BANANA INFLORESCENCE ESSENTIAL FOR PROGESTERONE REGULATION

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ABSTRACT

Banana inflorescence is hardly used as edible food in India. However, study based on metallic elements present in Banana flowers has never been studied for Indian species of banana. The Musa acuminata flower was collected from vegetable markets of Mumbai. The inflorescence was separated from bracts and was then subjected to sun drying for 48 hours. Dry sample was then subjected to solvent extraction using methanol; to get rid of organic matter. The residue was dried in oven at 250C for 10 min; and then it was converted to ash- by incinerating sample in muffle furnace for 3.50 mins at 5000C. The ash was studied for the determination of metallic elements by different techniques. The experiment was arranged in a completely randomised design and was performed in replicate. The metallic content analysis revealed presence of key metallic elements like Zn and Cu. These elements play a key role in progesterone secretion regulation in females. Hence use of Musa acuminate flower, in the form of vegetable, in diet proves to be valuable for females.

Keywords: Banana inflorescence, Musa acuminate, metallic content analysis, Elemental analysis

INTRODUCTION

India is the world's largest producer of bananas, with 28% of world production.[1]Fruits, leaves, root and stalks from banana plants have been used to treat fevers, burns, diarrhoea, inflammation, pains and snakebite in folkloric medicine.[2]Recently, more attention has been focused on the utilization of agricultural by-products.[3] The banana blossom is a large, dark purple-red blossom that grows from the end of a bunch of bananas. Its sizable bracts, or leaves, snugly enclose delicate, sweetly scented male blossoms [7]. The banana blossom grows at the end of a bunch of bananas. It is a leafy maroon coloured cone with cream coloured florets layered inside.[8] These florets need to be cleaned well before they are cooked as a vegetable. The banana blossom is rich in vitamins, flavonoids and proteins.[9] The flavour is a little starchy and bitter. The banana is a typical climacteric fruit and mainly grows in tropical and subtropical regions.[10] Banana flower has tremendous nutritional value and is being consumed as food additive, in many Asian countries such as Sri Lanka, Indonesia and Thailand. In Sri Lanka, it is consumed as a curry as well as a boiled or deep fried salad with rice and wheat bread.[4] Besides being consumed fresh, banana flower can also be made into various

products such as dehydrated vegetable, pickle and canned food.[5] In commercial situation after collecting the single bunch of bananas, lots of banana flowers are produced which is only been used as organic material and fertilizer in plantations (in China) until today (Yang et al., 2003).[6]

Zinc is involved in numerous aspects of cellular metabolism.[22]Zinc supports normal growth and development during pregnancy, childhood, and adolescence.[23-26] Zn serves a purely structural role in zinc fingers [27] Zinc fingers form parts of some transcription factors, which are proteins that recognize DNA base sequences during the replication and transcription of DNA. Each of the nine or ten Zn^{2+} ions in a zinc finger helps maintain the finger's structure by co-ordinately binding to four amino acids in the transcription factor.[28] The transcription factor wraps around the DNA helix and uses its fingers to accurately bind to the DNA sequence.[29,30] Zn ions are coordinated to the amino acid side chains of aspartic acid, glutamic acid, cysteine and histidine.[31] The metal also has a flexible coordination geometry, which allows proteins using it to rapidly shift conformations to perform biological reactions.[32]Copper and zinc play an important role in regulating progesterone production by luteal cells via involvement of superoxide dismutase.[11]The ratio of copper to zinc is clinically more important than the concentration of either of these trace metals.[33]Zinc is involved in the reorganization of ovarian follicles which are the source of progesterone. This occurs through the involvement of metalloproteinase-2 (MMP-2) enzyme, which is a member of zinc endopeptidase family.[12] Zinc is also involved in the secretion and function of male hormone testosterone through the enzymes that control the arachidonic acid cascade.[13,14] Involvement of manganese in the synthesis and production of oestrogen and progesterone may be due to the fact that it acts as a cofactor in the synthesis of cholesterol, a precursor for steroids, including estrogen and progesterone.[15] Iron also plays an important role in ovarian activity.[16] Trace elements are important for reproduction [17,18] also via contributing to the normal health of reproductive organs and reproductive cycles.

Nearly two billion people in the developing world are deficient in zinc.[19] Inadequate zinc levels have been associated with decreased fertility, abnormal oestrus, and abortions.[20,21]Zinc deficiency is characterized by growth retardation, loss of appetite, and impaired immune function.[34-36] In more severe cases, zinc deficiency causes hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin lesions. Weight loss and impaired appetite, delayed healing of wounds, taste abnormalities, and altered cognition can also occur.[34,37-41] Pregnant women, particularly those starting their pregnancy with marginal zinc status, are at increased risk of becoming zinc insufficient due, in part, to high fetal requirements for zinc.[42]Lactation can also deplete maternal zinc stores.[43] Zinc deficiency in breast-fed infants is a rare disease caused by a low level of zinc in their mother's milk.[44] Testosterone deficiency is associated with late-onset hypogonadism. Micronutrients including

copper and zinc have influence on testosterone synthesis. The association between micronutrient concentrations in hair tissue and serum testosterone was studied in Korean men. Subjects with normal testosterone group had a significantly higher Zn level compared to low testosterone group (P=0.003). Significant negative correlations were evident between total testosterone and Cu level (P=0.022), and the Cu/Zn ratio (P=0.008). Normal testosterone is associated with a higher Zn level. Decreased serum testosterone is significantly associated with a high level of Cu and elevated Cu/Zn ratio in hair tissue.[45]

The World Health Organization (WHO) estimates that 80% of the people in developing countries depend on traditional medicine for their primary health care, and about 85% of traditional medicines involve the use of plant extracts. [46]Women take progesterone by mouth for inducing menstrual periods; and treating abnormal uterine bleeding associated with hormonal imbalance, and severe symptoms of premenstrual syndrome (PMS). Progesterone is also used in combination with the hormone estrogen to "oppose estrogen" as part of hormone

replacement therapy. If estrogen is given without progesterone, estrogen increases the risk of uterine cancer.

American Family Physicians 2000;62:1339-46.- "Due to the side effects of synthetic progestins, natural progesterone is preferred. Progesterone has proven bio-availability and no side effects making it the preferred hormone for menopause.[48]

Journal of Women's Health Gender-based Medicine: 2000; 9 (4): 381-7. – "The side effects of the synthetics include bloating, nausea, and depression were eliminated by using natural progesterone. In fact, unexpected improvement in the feeling of well-being was observed when natural progesterone was used." [49]New research shows that progesterone supplements in the first trimester of pregnancy do not improve outcomes in women with a history of unexplained recurrent miscarriages [50]

It is not suggested to use synthetic progesterone in pregnancy, which can be found in some hormone replacement therapy and birth control pills. [47]

MATERIALS AND METHODS

MATERIALS: Banana flower, Blender, Beakers, Flasks, Pipettes (2ml to 25ml capacity), Burette, Muffle furnace, Oven, Acid solutions of Concentrated HCl, Concentrated HNO3. All the chemicals used are AR grade.

Sample preparation

Banana is one of the most popular fruits in Mumbai (Maharashtra). The blossoms of Musa acuminate were collected from Mumbai (Vegetable market), Maharashtra State, India, in 2016. The inflorescence were separated from bracts i.e. cleaned manually and cut into small pieces. It was then sun dried for 48 hours. Dry sample was then subjected to solvent extraction using methanol; to get rid of organic matter. The residue was then dried in oven at 40 oC. The dried residue was then blended into powder and converted to ash by subjecting it to incineration in a muffle furnace maintained at 500 oC for 3:30 hours.

Samples digestions

Ash of 1.0g of powdered flower sample was digested in concentrated H2SO4 for

two hours. The solution obtained was filtered and diluted to 100ml with distilled water.

Determination of Metallic Elements Present in Sample:

The digested samples were analyzed to determine presence of different essential and toxic metallic elements; using different Analysis techniques like Voltammetry, Flame-Photometry, Atomic Absorption Spectroscopy. The statistical analysis was conducted to determine the descriptive and inferential statistics such as Average Deviation, R.A.D, Standard Deviation and Variance.

A. Determination of Zinc by Voltammetry:

Reagents: Concentrated Nitric acid, Concentrated Perchloric acid, Distilled water, Acetate Buffer (pH 4.6).

Sample Preparation: Banana flower was Sun Dried for 48 hours. The dried flower was then blended in a mixer and a powder was obtained. The dried powder was then subjected to ashing in a muffle furnace at 350 C for 3.50 hours. Ash of 1 gram of powder was digested with Nitric Acid and Perchloric acid. The residue was reconstituted with 10ml distilled water and 1ml acetate buffer (pH 4.6).

Analysis: Zinc was estimated using Voltmeter (797 va computrance).

Result:

8.51 mg/100g

B. Quantitative analysis for Sodium and Potassium by Flame-Photometry:

Lab: Analytical Chemistry Lab, Kinshinchand Chellaram College. Vidyasagar K.M. Kundnani Chowk, D.W. Road, Churchgate, Mumbai - 20

Sample Solution Preparation: Banana flower was sun Dried for 48 hours. The dried flower was then blended in a mixer and a powder was obtained. 1 gram of powder was digested in distilled water for 1:30 hrs. The solution was then filtered and diluted to 100 cm3 with distilled water.

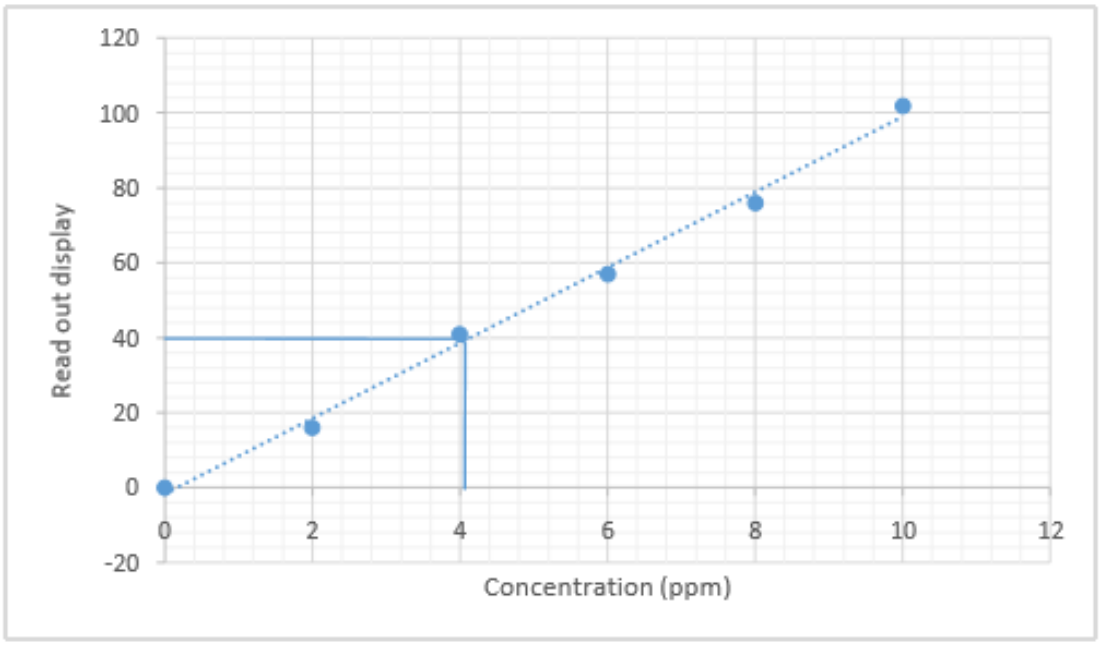
Instrument: Flame photometer Equiptronics Model EG 850 A

A) Estimation of Sodium

Concentration of Solution (Season1) (ppm)	Read-out Display		Concentration of Solution (Season2) (ppm)	Read-out Display
2	16		2	15
4	41		4	40
6	57		6	58
8	76		8	75
10	102		10	104
U.K. A1	40		U.K. A1	44
U.K. A2	38		U.K. A2	40

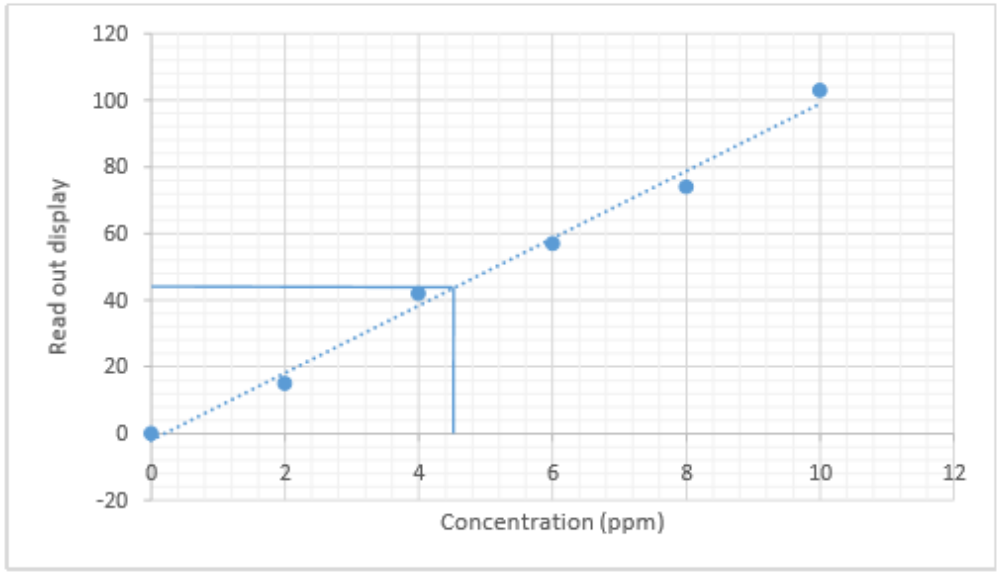
Graph Plot:

Season 1



From graph, 1.0 g powdered sample in 100 ml contains 4.100 ppm Sodium. Therefore, Sodium content in flower sample is, 4.100 mg/100g.

Season 2

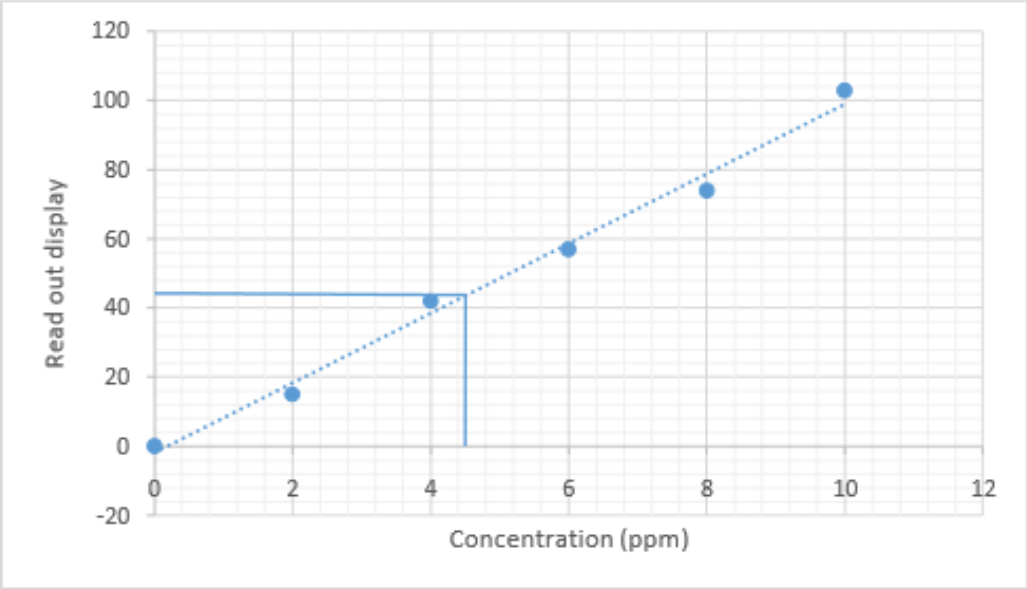


B) Estimation of Potassium

Concentration of Solution (Season1) (ppm)	Read-out Display		Concentration of Solution (Season2) (ppm)	Read-out Display
2	15		2	18

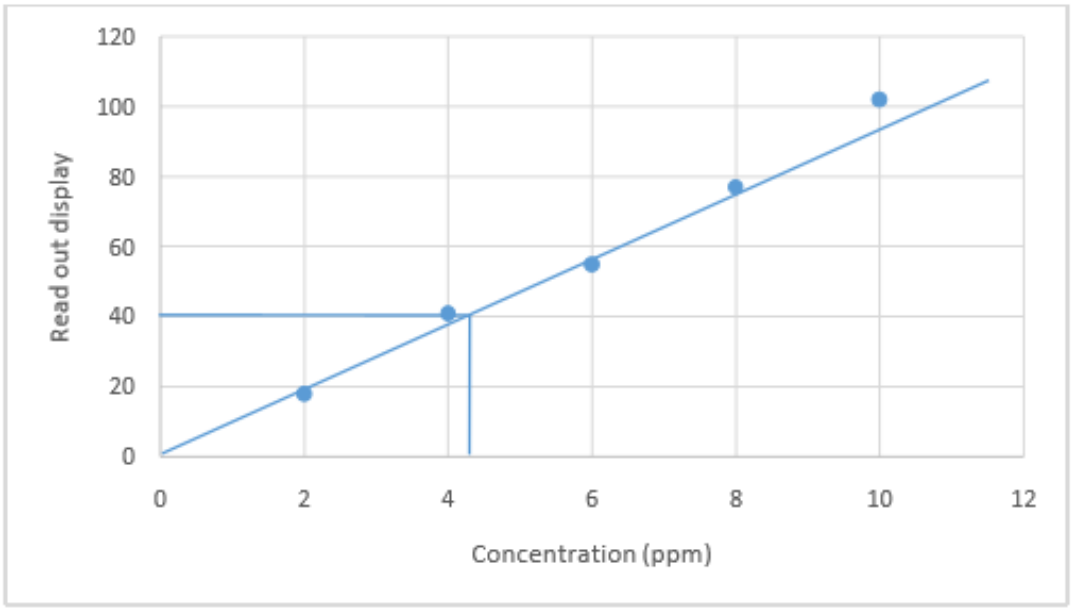
4	42		4	41
6	57		6	55
8	74		8	77
10	103		10	102
U.K. A1	44		U.K. A1	37
U.K. A2	42		U.K. A2	44

Graph Plot:
Season 1



From graph, 1.0 g powdered sample in 100 ml contains 4.400 ppm Potassium. Therefore, Potassium content in flower sample is, 4.400 mg/100g.

Season 2



RESULTS AND DISCUSSION

In this study different samples were investigated for their metallic elements content in different labs, during different seasons, using different methods of analysis.

Voltammetry and Flame Photometry:

	Average content (mg/100g)	Average deviation	R.A.D	Standard deviation	Variance	A.I for pregnant/ lactating women (mg/d)
Potassium (Flame Photometry)	134.32	4.1975	0.03125	5.5329	30.6130	3225
Sodium (Flame Photometry)	4.400	--	--	--	--	1902

A.I Adequate Intake of Adult (Pregnant/Lactating) Indian women.[51]

Atomic Absorption Spectroscopy:

	Average content (mg/100g)	Average deviation	R.A.D	Standard deviation	Variance	A.I for pregnant/ lactating women (mg/d)
Ca(mg/100g)	65.694	1.1773	0.0179	1.3604	1.8506	1200
Zn(mg/100g)	9.125	0.0826	0.00905	0.1162	0.0135	12
Mg(mg/100g)	11.692	0.0191	0.001633	0.0236	0.00056	310
Cu(mg/100g)	3.259	0.5504	0.16889	0.0787	0.0062	1.35
Mn(mg/100g)	15.429	0.0547	0.00354	0.0857	0.0073	2-5
Fe(mg/100g)	32.203	0.7187	0.02232	1.0625	1.1290	2.80/1.27
Na(mg/100g)	4.065	2.1805	0.5364	2.7337	7.4730	1902

Heavy metals (AAS)

	Average content (mg/100g)	Average deviation	R.A.D	Standard deviation	Variance
Cd (mg/100g)	0.244	0.046	0.1885	0.03253	0.001058
Pb (mg/100g)	0.3365	0.3365	1.000	0.2379	0.056596
As (mg/100g)	0.000	--	--	--	--

CONCLUSIONS

The purpose of this work was to identify and detect Qualitative and Quantitative presence of certain metallic elements, present in Banana inflorescence, which help in regulation of progesterone secretion in Females. Even though inflorescence discarded at the time of harvest do not present any environmental problem, their nutritional value is lost. The analysis of M. acuminata inflorescence revealed considerable metal content necessary for Progesterone regulation in females of all age group, including the requirement during pregnancy. In a view of its high metallic content of required metals, Banana inflorescence can be introduced in diet as a vegetable or its dehydrated dried form as flour. Along with Zinc, Banana inflorescence also contains certain other metallic elements like Sodium, Calcium, Magnesium, Potassium,

Manganese, Copper and Iron, in considerable mount, which are very essential for physiological functioning, normal growth and development of human beings. Also Banana Inflorescence were found not to contain toxic heavy metals like Cadmium, Lead and Arsenic (Cd being in permissible limits) which could possess a threat to health of pregnant women as well as the baby; thus making it fit for consumption.Hence, based on this research it was found that introducing Banana Inflorescence into Human (Female) diet can become a good source of metallic elements like Zinc, Sodium, Calcium, Magnesium, Potassium, Manganese, Copper and Iron; thus helping in normal growth and development along with proper Progesterone secretion regulation.

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QUALITATIVE ANALYSIS OF VARIOUS ADULTERANTS AND MICROBES IN MILK SAMPLES COLLECTED FROM DIFFERENT AREAS OF MUMBAI

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ABSTRACT

The study was carried out keeping the view of recently emerging concern of adulteration of natural milk with various illegal substances to increase its marketability. Milk forms a significant part of diet for all age groups and important source of nutrient required for growth in infants and children for maintenance of health. But the pure nutritious milk is not reached to the people because of milk adulteration. Since milk provides essential nutrients for excellent growth of many microbes, contamination of milk is very frequent which causes various diseases to human health. Qualitative analysis was carried out on 10 milk samples; standardmilk adulteration methods followed by FSSAI (Food Safety and Standard Authority of India)2012 Manual .Hence checking for adulterants and microbial load present in milk was the main objective of this study. Chemical analysis was carried out for detection of adulterants and microbial load was determined by microbiological tests such as SPC, Coliforms count followed by quality check including Methylene blue and Resazurin dye Reductase test (MBRT and RRT). Formalin, Urea, Detergents and Benzoic acid were detected as adulterants in milk samples. Water was also detected majorly in some of the milk sample. The microbiological analysis was significantly high in case of loose milk samples than brand milk. Varying results were observed for dye reduction tests. This concludes that the milk analysis and testing should meet all FSSAI standards to be safe for consumption and pasteurization is necessary to avoid bacterial contamination of milk.

Keywords: Adulterants, SPC, MBRT, RRT, FSSAI, Pasteurization

INTRODUCTION

Milk is the nature's most nearly perfect food supplying wide range of nutrients and has a very high nutrient density in relation to calorie content of the food. The human body is in need of milk throughout the life as it is useful for all ages and groups. It has all the substances needed by the organisms in its easiest assimilable form. Hence milk is a perfect food, readily digested and absorbed. It is chiefly a valuable source of good quality protein, fat, carbohydrates, vitamins and minerals. Milk is high in nutrition and majority of Indian population rely on milk for their protein supplement.

When it leaves the udder of the animal, all the interplay's of adulteration begin.The term adulteration in general may be defined as the addition of some of the legally prohibited substances into a more valuable genuine product. According to PFA act 1954 "adulterant" means any material, which is or could be employed for the purpose of adulteration.Quality of milk is deteriorated due to its adulteration in different marketing channels. Adulteration of milk is usually done by adding inferior cheaper materials/elements like pond water, cane sugar and powdered milk and there were reports of adulteration in dairy products also in many parts of India. (Ramya P, Feb 2015). The nature of adulterants generally encountered in milk and milk products are water, removal of fat, addition of skim milk powder, reconstituted milk, thickening agents such as starch, flour, glucose, urea, salt, chlorine. Preservatives such as neutralizers which usually consists of sodium bicarbonate, sodium carbonate, sodium hydroxide and calcium hydroxide. (MK, 2014). Fertilizers or additives such as Urea, Ammonium sulphate, glucose, Water and Detergents are also added to the milk. To increase the shelf life of milk preservatives such as Benzoic acid, Salicylic acid, Formalinand hydrogen peroxide are added. As a result of these malpractices, the ultimate victim is a consumer, who innocently takes adulterated milk. These all adulterants and Preservatives in excess amounts have very serious health effects on human body causing various types of diseases and other complications. Being an essential food for human beings, milk also acts as a good medium for the growth of many microorganisms. Microbial contamination of raw milk can occur from various sources like air, milking equipment, feed, soil, grass and feces. In appropriate conditions milk can act as a carrier of disease from milking animals to human via microorganisms. (Muhammad Naseer Abbas, July-Aug 2013). Despite food legislation, adulteration remains uncontrolled, furthermore legal steps laid down in the PFA Act are extremely difficult to maintain due to inadequate and untrained man power and laboratory facilities. Such is the state in the country where we are one of the largest nations of milk producers. In the year 2010-2011, India was ranked among the top 5 countries in the world producing 121.8 million tons of milk. (MK, 2014). The aims and objectives of the study was to carry out Qualitative analysis of various adulterants and determination of microbiological quality of milk of 5 loose and 5 brand milk samples collected from different areas of Mumbai.

MATERIALS AND METHODS

I. Chemical Analysis:-

1. Test For Detection Of Neutralizers:-

Rosolic acid test (Soda Test)-

•Take 5 ml of milk in a test tube and add 5 ml alcohol followed by 2-3 drops of rosolic acid. If thecolor of milk changes to pinkish red, it is inferred that the milk is adulterated with sodiumcarbonate /sodium bicarbonate and so unfit for human consumption.

2. Test For Detection Of Hydrogen Peroxide:-

• Take 5 ml milk in a test tube. Add 3 drops of paraphenylene diamine and shake

well. Change in color of the milk to blue confirms that the milk is adulterated with hydrogen peroxide.

• To 10 ml of milk sample in a test tube add 10-15 drops of Vanadium Pentoxide reagent and mix.

The development of pink or red color indicates presence of hydrogen peroxide.

3. Test For Detection Of Formalin:-

Formalin (40%) although poisonous, can preserve milk for a long time.

•Take 10 ml of milk in a test tube. Add 5 ml conc. sulphuric acid through the sides of the test tube without shaking. If a violet or blue ring appears at the intersection of the two layers, it shows the presence of formalin. Note violet coloration usually does not appear when relatively large quantities of formaldehyde are present.

4. Test For Detection Of Cane Sugar:-

Generally cane sugar is mixed in milk to increase the percentage solids content of milk i.e., to increase the lactometer reading of milk, that was already diluted with water.

• Take 10 ml of milk in a test tube. Add 5 ml of hydrochloric acid along with 0.1 g of resorcinol. Shake the test tube well and place it in a boiling water bath for 5 min. Appearance of red color indicates the presence of added cane sugar in milk.

5. Test For Detection Of Starch:-

Addition of starch increases the SNF content of milk. Wheat flour, arrowroot, rice flour, etc., can also be added for increasing the SNF content.

• Take 3 ml milk in a test tube and boil it thoroughly. Cool the milk to room temperature. Add 2 to3 drops of 1% iodine solution. Change of color to blue indicates that the milk is adulterated with starch.

6. Test For Detection Of Glucose:-

Poor quality glucose is sometimes added to milk to increase the lactometer reading.

• Take 3 ml of milk in a test tube. Add 3 ml Barford's reagent and mix it thoroughly. Keep the test tube in a boiling water bath for 3 min and then cool it for 2 min by immersing it in tap water without disturbance. Add 1 ml of phosphomolybdic acid and shake. If blue color is visible, then glucose is present in the milk sample.

7. Test For Detection Of Urea:-

Urea is generally added in the preparation of synthetic milk to raise the SNF value.

• 5 ml of milk is mixed well with 5 ml paradimethyl amino benzaldehyde reagent. If the solution turns distinct yellow in color, then the given sample of milk contains urea. Control, normal milk may show a faint yellow color due to presence of natural urea.

8. Test For Detection Of Ammonium Sulphate:-

The presence of sulphate in milk increases the lactometer reading.

• Take 5 ml of milk add 2.5 ml of 2% sodium hydroxide, 2.5 ml of 2% sodium hypochlorite and 2.5ml of 5% phenol solution then heating for 20 seconds in boiling water bath. If bluish color turns to deep blue it indicates the presence of

ammonium sulphate, however in case it turns to pink it shows that the sample is free from Ammonium sulphate.

9. Test For Detection Of Salt:-

Addition of salt in milk is mainly resorted to with the aim of increasing the corrected lactometer reading.

• 5 ml of silver nitrate reagent is taken in a test tube. Add 2-3 drops of potassium dichromate reagent. Add 1 ml of milk in the above test tube and mix thoroughly. If the contents of the test tube turn yellow in color, then milk contains salt. If it turns to chocolate or reddish brown in color, the milk sample is free from salt.

10. Detection Of Detergents:-

• Take 5 ml of milk in a test tube and add 1-2 drops of bromocresol purple solution. Mix well. Appearance of violet color indicates the presence of detergent in milk. Unadulterated milk samples will show a very faint violet coloration.

11. Test For Water :-

Lactometer reading detects adulteration of milk with water.

• Take raw milk in a long stemmed wide mouth bottle or a measuring cylinder. Place the lactometer in it taking care to see that the lactometer does not touch the sides of the bottle or the measuring cylinder. Note down the reading at the surface of milk sample taken.

12. Detection Of Benzoic And Salicylic Acid:-

• Take 5 ml of milk in a test tube. Add 3-4 drops of concentrated sulphuric acid. Add 0.5% ferric chloride solution drop by drop and mix well. Development of buff color indicates presence of benzoic acid and violet color indicates presence of salicylic acid.

II. MICROBIAL ANLAYSIS:-

1. STANDARD PLATE COUNT (SPC):-

Standard Plate Count is a method used for determining the total number of viable bacteria in milk samples. Milk is serially diluted 10-fold using saline to reduce the load of microbial growth and to obtain well isolated colonies followed by spread plate technique. After making dilutions, plate out 0.1ml on to 10^{-4}, 10^{-5} and 10^{-6}on Nutrient Agar which supports the growth of all organisms. Incubation is done at ambient temperature for 24-48hr. Observe and counts the colonies from all plates and interprets by comparing to standard result table.

INTERPRETATION TABLE:-

Raw Milk per ml (organisms)	Grade
Not exceeding 2,00,000	Very Good
Between $2x10^5$ - $1x10^6$	Good
Between $1x10^6$ - $5x10^7$	Fair
Over $5x10^7$	Poor

2. COLIFORM COUNT:-

It is important in quality control of milk as it is indicative of possible fecal contamination and presence of pathogens. For pasteurized milk it indicates post pasteurization contamination. Absence of coliforms in 1:100 dilution of raw milk or

1:10 dilution of pasteurized milk is considered satisfactory. Dilutions are made and pated out 0.1ml on 10^{-1}, 10^{-2} plates. Coliforms count is performed using Violet Red Bile Agar incubated at 37°C for 24 hr. Violet colonies are indicative of coliforms.

3. METHYLENE BLUE REDUCTASE TEST:-

The organism present in the milk reduces Methylene blue to a colorless compound. The time taken to reduce the Methylene blue by a given sample of milk indicates the quality of milk. The sample which shows decolourization of blue dye in 30 mins at 37°C is not acceptable as per standards. Incubate all tubes at 37°C. Note the time for decolourisation in the **TEST** tube.

INTERPRETATION TABLE:-

Time for Decolourisation	Interpretation
8 hrs. and above	Excellent
6-8 hrs.	Good
2-6 hrs.	Fair
Less than 2 hrs.	Poor

4. RESAZURIN REDUCTASE TEST:-

This is a redox dye which changes color as the pH of the milk changes due to growth of organisms. At the same time the dye is also reduced in 2 stages. In the first stage an irreversible change from the blue resorufin and then finally to the colorless dihydroresorufin takes place. The test is intended as a platform test for detecting milk of poor quality.Incubate all tubes at 37°C for 30mins for color change.

INTERPRETATION TABLE:-

Color	Quality
Blue/Lilac	Good
Mauve/Pink	Fair
Pink/Colorless	Poor

RESULTS AND DISCUSSION

The loose milk samples were collected from Goregoan, Bhayander, Kandivali and Church gate and brand milk samples such as Amul, Gokul, Mother Dairy, Gowardhan, and Mahananda were collected from different dairies.

I. CHEMICAL ANALYSIS:-

SR. NO	ADULTERANTS	LM-1	LM-2	LM-3	LM-4	LM-5	AMUL	GOKUL	MOTHER DAIRY	GOWAR-DHAN	MAHA-NANDA
	PRESERVATIVES										
1)	Formaldehyde	_	+	_	_	_	_	_	_	_	_
2)	Benzoic Acid	+	+	+	+	+	+	+	+	+	+

3)	Salicylic Acid	_	_	_	_	_	_	_	_	_	_
4)	Hydrogen Peroxide	_	_	_	_	_	_	_	_	_	_
	NEUTRALIZERS-										
1)	Sodium Carbonate/ Bicarbonate	_	_	_	_	_	_	_	_	_	_
	THICKENING AGENTS-										
1)	Starch	_	_	_	_	_	_	_	_	_	_
2)	Cane Sugar	_	_	_	_	_	_	+	_	_	_
	FERTILIZERS/ ADDITIVES-										
1)	Urea	_	_	_	_	_	_	_	_	_	_
2)	Ammonium Sulphate	+	_	_	_	+	_	_	+	+	+
3)	Glucose	_	+	+	+	_	+	+	_	_	+
4)	Salts	_	_	_	_	_	_	_	_	_	_
5)	Water	30	29	30	28	20	30	30	32	30	31
6)	Detergents	_	_	_	_	_	_	_	_	_	+

KEY: - '+' –POSITIVE, '–' - NEGATIVE

II. MICROBIOLOGICAL ANALYSIS:-

1) STANDARD PLATE COUNT-

Sr. No	Samples	SPC	Interpretation
1)	LM-1	$2.0X10^{6}$	Fair
2)	LM-2	$2.6X10^{8}$	Poor
3)	LM-3	$5.9X10^{7}$	Poor
4)	LM-4	$2.9X10^{9}$	Poor
5)	LM-5	$9.2X10^{7}$	Poor
6)	Amul	$3.9X10^{7}$	Fair
7)	Gokul	$1.0X10^{9}$	Poor
8)	Mother Dairy	$2.7X10^{8}$	Poor
9)	Gowardhan	$9.2X10^{7}$	Poor
10)	Mahananda	$1.1X10^{8}$	Poor

2) COLIFORM COUNT-

SR.NO	SAMPLES	COLIFORM COUNT
1)	LM-1	$2.7X10^{5}$
2)	LM-2	TNTC
3)	LM-3	$1.08X10^{6}$
4)	LM-4	TNTC
5)	LM-5	$9.8X10^{4}$
6)	Amul	$2.9X10^{5}$
7)	Gokul	TNTC
8)	Mother Dairy	$4.6X10^{4}$
9)	Gowardhan	$1.8X10^{5}$
10)	Mahananda	$4.8X10^{5}$

KEY:-TNTC- TOO NUMERABLE TO COUNT

According to standard table, **absence of coliforms in 1:100 dilution of raw milk or 1: dilution of pasteurized milk is considered satisfactory.**

3) METHYLENE BLUE REDUCTASE TEST-

Sr. No	Samples	Test	C-1	C-2	Interpretation
1)	LM-1	C	B	C	Good

2)	**LM-2**	**C**	**B**	**C**	**Fair**
3)	**LM-3**	**C**	**B**	**C**	**Fair**
4)	**LM-4**	**C**	**B**	**C**	**Good**
5)	**LM-5**	**B**	**B**	**C**	**Excellent**
6)	**Amul**	**C**	**B**	**C**	**Good**
7)	**Gokul**	**B**	**B**	**C**	**Excellent**
8)	**Mother Dairy**	**B**	**B**	**C**	**Excellent**
9)	**Gowardhan**	**B**	**B**	**C**	**Excellent**
10)	**Mahananda**	**C**	**B**	**C**	**Fair**

KEY: - C –COLORLESS, B- BLUE

4)RESAZURIN REDUCTASE TEST-

Sr. No	Samples	Test	C-1	C-2	Interpretation
1)	**LM-1**	**Pi**	**Pi**	**C**	**Fair**
2)	**LM-2**	**Pi**	**C**	**C**	**Fair**
3)	**LM-3**	**Pi**	**Pi**	**C**	**Fair**
4)	**LM-4**	**C**	**Pi**	**C**	**Poor**
5)	**LM-5**	**Li**	**Pi**	**C**	**Good**
6)	**Amul**	**Li**	**Pi**	**C**	**Good**
7)	**Gokul**	**Pi**	**Pi**	**C**	**Fair**
8)	**Mother Dairy**	**Li**	**Pi**	**C**	**Good**
9)	**Gowardhan**	**Li**	**Pi**	**C**	**Good**
10)	Mahananda	Pi	C	C	Fair

KEY: - Li-GOOD, Pi-PINK, C-COLORLESS

CONCLUSION

Qualitative Analysis was carried out check various adulterants and their microbiological quality of loose and brand milk collected from different areas of Mumbai. On the basis of data obtained in the present study, conclusion may be drawn that milk quality is not completely as per standards and adulteration in milk is still in practice and has not been checked completely.

In a country such as India where milk and milk products play an important role in different foodstuffs, this analysis carried out will bring about more

awareness to the general public about the malpractices and negligence during milk production and transportation.

In adulterants, **Formaldehyde and Benzoic Acid** were detected in all samples of milk. They serve us preservatives in the milk for increasing shelf life of milk and preventing it from sour or bitter taste. Natural preservatives such as honey can also be used to reduce chemical preservatives during milk storage. Further quantitative analysis is required to find out the quantity of preservatives present in milk samples which should be preferably in limited amounts. Excess amount of these preservatives causes renal failure, respiratory disease, vomiting, metabolic acidosis etc. **Ammonium sulphate** was detected in brand milk sample of Mother Dairy, Gowardhan and Mahananda. It serves as Additives which is added to increase the lactometer reading by maintaining density of milk. On higher amounts it can cause severe irritation and inflammation of respiratory tract. **Glucose** was detected in three loose milk and brand milk (Amul, Gokul, and Mahananda) samples. The poor quality glucose is added to increase carbohydrate content of the milk and thus density is also increased. Due to this the milk can now be adulterated with water and it will not be detected by lactometer test. **Detergent** was detected in Mahananda sample which indicates negligence of improper washing of utensils, boilers, storage tanks during production and transportation of milk.**Loose milk sample -5 showed significant results as lactometer reading was below 30. This indicates milk is adulterated with water.** The source of water also is of great matter. Contaminated water used for adulteration can lead to serious health related infections and diseases.

The **microbiological nature of loose and brand milk was poor.** Hence hygienic conditions need to be maintained right from the collection centre to the consumers. The **coliforms count** was very high as compared to standard table which indicates high level of risk. The presence of coliforms indicates faecal contamination which can take place easily due to unhygienic maintenance of milk in the cattle.

The **Dye Reduction Tests** also gave intermediate results which were not too good or too bad. Handling of milk from cattle, production stage where it passes through various steel pipes, boilers etc. till storage and transportation has to be maintained properly. **Pasteurisation** is very much important before consumption.

To ensure that **good quality milk** is reached to the consumers, there should be **proper quality control system**. This will improve the quality and reputation of milk entities which increases the profits as well as protects health of animals and consumers of milk.

FUTURE PROSPECTS

Milk and milk products need to be analyzed for variety of reasons such as assessment of milk quality, determination of nutritive value, detection of adulteration and in research and development. Government bodies regulate the permitted levels of certain preservatives and contaminant compounds; much of this advancement has been driven by increased sensitivity and specificity of determination e.g. using analytical instruments. But sometimes it becomes difficult and

impossible to accurately analyses one component in the presence of others using classical method of analysis. Due to lack of specificity and sensitivity of classical method of analysis it may lead to inaccurate, unreliable results, erroneous and false results. Therefore in order to achieve the reliability of results, nowadays instrumental analytical techniques have become mandatory in quality control, safety and meeting the regulatory norms. Selecting appropriate instrumental technique is also very important because criteria such as Precision, Reproducibility, Accuracy, Speed, Sensitivity, Specificity needs to be taken into consideration.

Analytical Balance, Electric Ovens, Muffle Furnace, pH Meter, Infra-Red Moisture Balance, Laminar Air Flow Chamber, Freeze Dryer (Lyophilizer), Centrifuge and Cream Separator are the common laboratory instruments used during analysis of milk products in any laboratory. Some other used instruments that are frequently used in quality control lab are as follows: Milko-tester, Infra-red Milk analyzer (Milk scan), FTIR-Milko-scan, Lacto star Automatic Milk Analyzer, Pro-Milk MK II, Polari meter, Butyro Refractometer, Automated Kjeldahl for Protein Estimation (Kjeltec). Besides common equipment's certain Sophisticated instruments are also available that help in detection of adulterants and quantification of bio-actives. These sophisticated instruments are follows: Thin layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPLTC), Gas Liquid Chromatography (GLC), High Performance Liquid Chromatography (HPLC), Mass Spectrometry, LC-MS, GC-MS, Texture profile analyzer, Spectrophotometer, Flame Photometer, Atomic Absorption Spectrophotometer, Rancimat. By using such instruments not only adulterants can be detected but also the protein value, fat value and other nutritive information can be determined.

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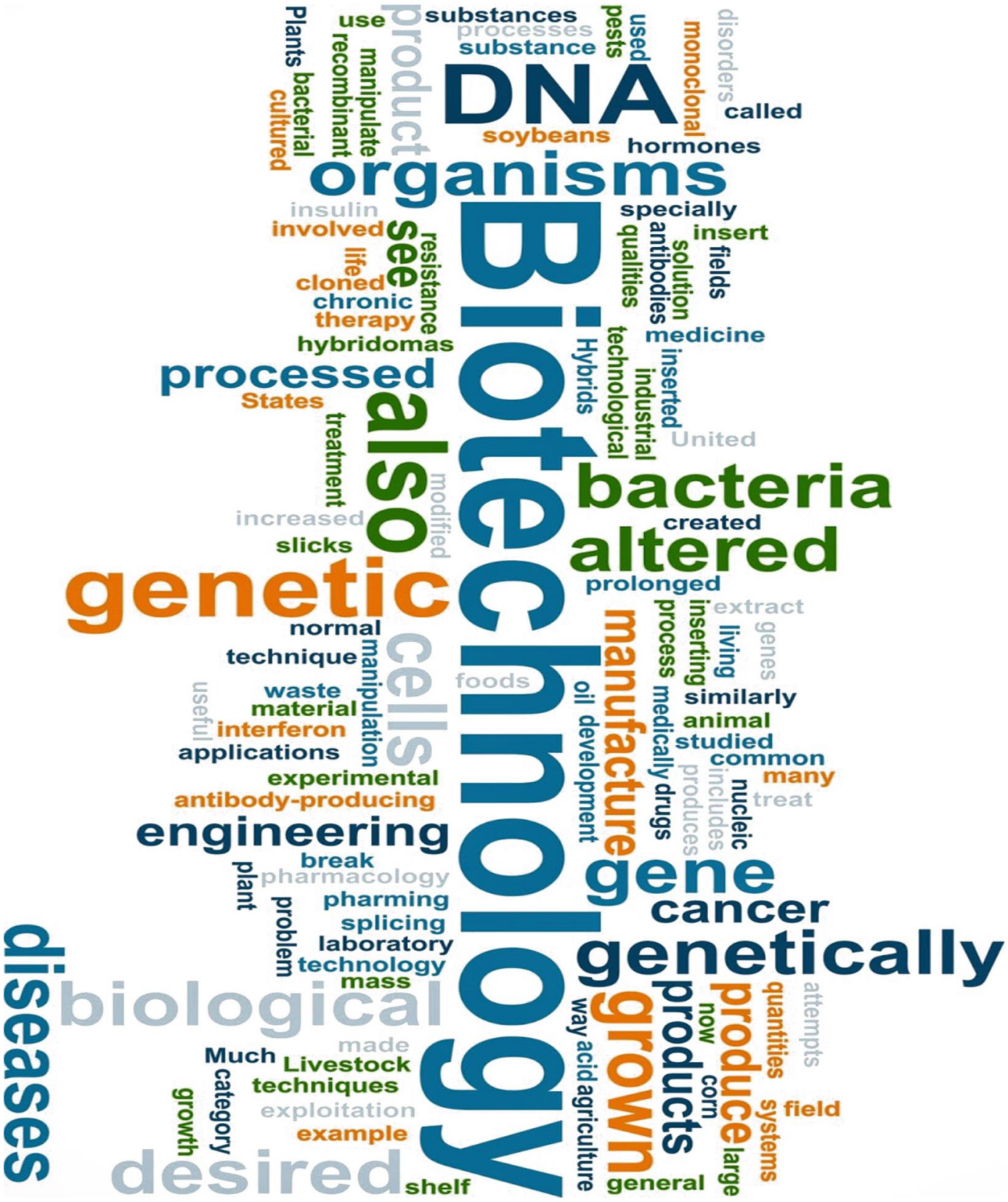
BIOTECHNOLOGY

Almost everybody is enthusiastic about the promise of biotechnology to cure disease and to relieve suffering.

LEON KASS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COMPREHENSIVE STUDY OF ALTERED ORAL MICROBIOME, SALIVARY BIOMARKERS IN SMOKELESS TOBACCO (SLT) CONSUMERS

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ABSTRACT

Tobacco chewing is attributed to oral cancer and a major challenge is to identify tobacco users who are at risk. The present study aimed to analyse biomarkers and monitor the altered oral biome in the SLT consumers. Healthy tobacco chewers and control were enrolled for the study. Unstimulated saliva samples were collected and screened for the presence of microbes. Biochemical markers such as sialic acid and thiocyanate were estimated from the normal oral microflora & any variants.

We report the presence of *Pseudomonas aeruginosa, Klebsiella pneumoniae, Rhizopus oryzae* in many of the samples. The strain of P. aeruginosa was characterized and the sequence was submitted to NCBI (Accession no. KU937106). The Antibiotic Susceptibility Test of different strains isolated showed that few of the strains isolated exhibited drug resistance which needs further investigation.

We propose that the antibiotic resistance to the different antibiotics may have implication in the manifestation of other diseases, low immunity and subjects may exhibit deteriorating oral health. The altered oral microbiome in the individual may put them at a higher risk of oral cancer development & other diseases.

INTRODUCTION

Smokeless tobacco users in India and Pakistan together have been estimated to number 100 million. Habitual betel quid chewing is commonly practised by men and women in Bangladesh, India, Pakistan and Sri Lanka, while tobacco smoking is much more common among men in these countries compared to women, except for certain small geographic areas. Countries in South Asia are major producers of tobacco and the region is a net exporter.[1]The magnitude of the health risk associated with smokeless products appears to be associated with the type of tobacco and method of cultivation used. Greater potential for harm is associated with fire-curing (resulting in deposits of polycyclic aromatic hydrocarbons on the leaf), bacterial contamination, fermentation during production (which may favour the activity of micro-organisms that reduce nitrates to nitrites,

leading to formation of nitrosamines), inclusion of certain additives in Asian products (e.g., areca nuts) and particular methods of product storage (some of which may promote continued bacterial formation of nitrosamines). Behavioural influences on health risks include amount of smokeless tobacco consumed and frequency of use, length of application, surface of application, oral hygiene, and rates of salivating, swallowing, and spitting. Risk associated with use may be modified by other exposures such as diet, alcohol consumption, and genetics[2]. The contents of tobacco are – nitrosamines (nicotine), polycyclic aromatic hydrocarbons, polonium, nitrosodietheinal amine and nitrosoproline. They also chew pan or betel nut to which a variety of spices are added. These products of pan and tobacco have psychotropic and anthelminthic activity due to the presence of areca alkaloids, predominantly arecoline and lauric acid. These alkaloids have powerful parasympathetic action, produce euphoria, and counteract fatigue. The products of betel nut are antiseptic, bactericidal and antioxidant as reported by few investigators.[3]

Assessment of nicotine metabolism and disposition has become an integral part of nicotine dependency treatment programs. Serum nicotine concentrations or urine cotinine concentrations can be used to guide nicotine patch dose to achieve biological concentrations adequate to provide the patient with immediate relief from nicotine withdrawal symptoms, an important factor in nicotine withdrawal success. Absence of nicotine metabolites can be used to document abstinence from tobacco products, an indicator of treatment success[4-5]

MATERIALS AND METHODS

Study design:

This study was a community based cross-sectional study.

Subjects

A convenient sampling method was used to recruit smokeless tobacco consumers from a mixed group consisting of taxi drivers, dabbawalas, local vendors and support staff at K.C. College.

Sample collection

1.5 to 2 ml of unstimulated whole saliva also was collected under resting condition during the hours 10am-12am, 2 hours after the subject's usual breakfast time, according to the method of [6]Navazesh. Saliva samples were collected in sterile centrifuge tubes. The sample was centrifuged to remove unwanted particulates. The supernatant was separated and biochemical analysis was done.

Microbial analysis of the sample

All the chemicals and reagents used for testing and/or analysis were of AR/ GR grade.(Hi media) The samples were screened on different media: Nutrient Agar, Mac Conkey's Agar, Sabouraud's Agar, and Veillonella Agar (containing Vancomycin 60µg %). Vancomycin is a

selective agent which suppresses the growth of other organism without hampering the growth of Veillonella.

The plates were incubated as follows:
Nutrient Agar, Mac Conkeys and Veillonella Agar - 37° C for 24 - 48 hours
Sabouraud's Agar – Room Temperature for 48 hours.
Morphological and cultural characteristics of the growth on the respective media were studied.

Isolation and Characterisation of the organisms:
The colonies observed on the plate were characterized by Hans Christian Gram's method of Gram Staining [7] and observed under the light microscope (100X magnification-Oil immersion lens). Capsule staining was also carried out by [8]Maneval'smethod.Fungal spores were observed after [9]Lactophenol Cotton Blue Staining. The results and observations were noted down. The fluorescing colonies were further tested for their susceptibility against various antibiotics on the Nutrient Agar Medium.

Antibiotic Sensitivity Test:
[10]The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial sensitivity profiles of the isolates using different antibiotics. The antibiotics used were as follows: Tetracycline (30 µg), Ciprofloxacin (5 µg), Amikacin (30 µg) and Vancomycin (30 µg), Neomycin (30µg), Gentamycin (10µg), Methicillin (5 µg), Tetracycline (30 µg), Penicillin (10 units), Ticarcillin(75 µg)
A lawn growth of the microbial culture on sterile nutrient agar plate was used for the antibiotic sensitivity test. The AST plates were incubated at 37°C for 24h. The zone of inhibition (in millimeters) was measured and reported. The sensitivity pattern was scored as either sensitive or resistant.

Inhibition zone diameters were measured and the strains were categorized as resistant or susceptible to the antimicrobial agents based on the inhibition zone size given in the standard chart (Performance Standards for Antimicrobial Disk Susceptibility Tests, CLSI Vol.28 No.1, Jan 2008).

Estimation of Total Protein:
The amount of proteins present in the sample was estimated by using Folin Lowry's method of estimation of protein. [11]

Sr. No	Vol. of BSA (ml)	Conc. of BSA (mg/ml)	Vol. of D/W (ml)	Vol. of Reagent 1 (ml)		Vol. of Reagent 2 (ml)	
Blank	0	0	1	3	**Mix the contents of the**	0.5	**Vortex each tube immediately.**
1	0.1	10	0.9	3		0.5	
2	0.2	20	0.8	3		0.5	

3	0.4	40	0.6	3	**tube. Incubate for 10 minutes at RT**	0.5	**Incubate at RT in the dark for 30 minutes.**
4	0.6	60	0.4	3		0.5	
5	0.8	80	0.2	3		0.5	
6	1.0	100	0	3		0.5	
Sample	250 µl	-	750 µl	3			

Estimation of Thiocyanate

Thiocyanate is known to be the biomarker of exposure. Biomarkers of tobacco exposure are used to confirm the absorption of specific smoke constituents in a quantitative manner. (447nm) [12-13]

Sr. No	Volume of Fe(NO3)3 0.20M (ml)	Volume of KSCN (conc- 2.00 ´ 10^{-4}) (ml)	Volume of Distilled water. (ml)
Blank	5	0	5
1	5	1	4
2	5	2	3
3	5	3	2
4	5	4	1
5	5	5	0
Sample	5	500ul	500ul

Estimation of Sialic Acid:

Estimation of sialic acid was estimated by the method of Yao et al.[14]

Sr. No.	Conc of NANA	Vol. of Standard	Vol. of water	Vol. of glacial acetic acid	Vol. of Ninhydrin Reagent	**Separate the supernatant from the precipitate, Add the contents and keep them in a boiling water bath for 10 minutes.**
Blank	0	0	3	1	1	
1	20	0.6	2.4	1	1	
2	40	1.2	1.8	1	1	
3	60	1.8	1.2	1	1	
4	80	2.4	0.6	1	1	
5	100	3	0	1	1	
Sample	Unknown	500ul	500ul Saline + 4ml Ethanol **Centrifuge the sample tubes for 30 min**	1	1	

RESULTS AND DISCUSSIONS

1.) Characterization of altered oral microbiome from the oral cavity

Our analysis provides evidence that the oral flora of smokeless tobacco consumers varies significantly from those of non-consumers. The present study found the presence of *Pseudomonas aeruginosa, K. pneumoniae, R. oryzae* in many of the samples.

30 male respondents (21 consumers, 7 control) ranging between 20-60 years of age, participated in the study. The presence of Gram negative cocco-bacilli was found out to be in 15 out of 21 samples.

One of the respondent's saliva samples showed the presence of Gram negative short rods that displayed the presence of a capsule. These unusual green- blue fluorescent colonies were confirmed to be *Pseudomonas aeruginosa*, this was characterized using phenotypic and genotypic approaches. Sequence comparison of 16S rRNA gene and phylogenetic analysis confirmed the *Pseudomonas spp* strain.

[IMViC Scheme: -,-,-,+]

The strain of *P. aeruginosa* that exhibited a blue – green fluorescence was further characterized and the novel sequence was submitted to NCBI. (Fig1)

Chalke,A., Joseph, J., Sharma, A. and Harshal, A.

A Nucleotide Sequences of novel fluorescent *Pseudomonas aeruginosa* from the oral cavity of smokeless tobacco consumers under the Accession No: KU937106 to Gene Bank.

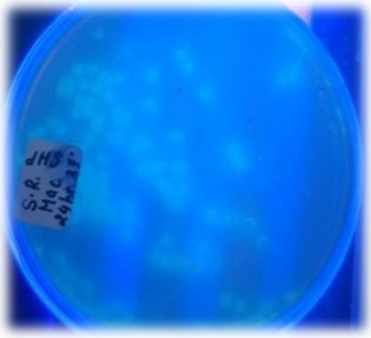

Fig 1. Colonies of *Pseudomonas aeruginosa* under UV transilluminator

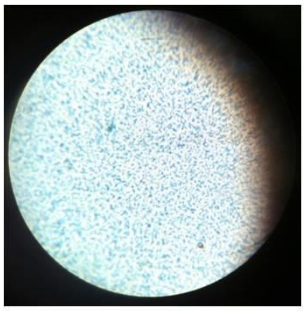

Fig2. Maneval's Capsule Staining

Sequence comparison of 16S rRNA gene and phylogenetic analysis of another strain isolated confirmed *Klebsiella pneumoniae*(Fig 2)

[IMViC Scheme: -,-,+,+]

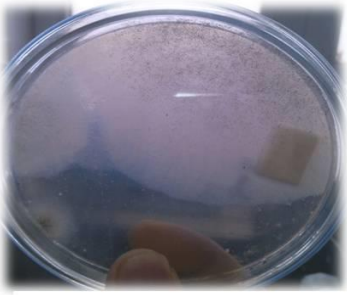

Fig 3.Organism name: *Rhizopus oryzae* strain WM 06.833

The presence *Rhizopus oryzae* in the saliva sample of the subject can be co-related to its property to degrade tobacco waste &

enhance xylose utilisation to produce lactic acid. Therefore, excess of lactic acid is a major cause of tooth decay, mouth ulcers, demineralisation, formation of dental caries, etc.[15]

Oral health is influenced by oral microbial flora. Consumption of tobacco increases the growth of bacteria in the oral cavity. Oral bacteria cause oral diseases, such as gingivitis, which later lead to periodontitis if left untreated. If treatment is administered too late, precancer and cancer may develop.[16] We report the presence of Gram-negative short rods, *Pseudomonas aeruginosa Klebsiella pneumoniae*, in the oral flora of smokeless tobacco consumers. These micro-organisms must have gained entry into the oral cavity through the crude and mediocre quality tobacco products.[17]

As in case of - tobacco wildfire disease caused by *Pseudomonas syringae* pv. tabaci is a kind of bacterial leaf disease.[18] When such infected leaves are used to manufacture the product, they end up in the oral cavity of the respondent leading to alternation of the oral microbiome.[19]

2.) Antibiotic sensitivity Profile

The sudden acquisition of resistance to antibiotics poses difficulties in treating infections. Resistance to several different antibiotics at the same time is even more significant problem. It is because of the acquired resistance that bacterial isolates must be subjected to antibiotic susceptibility testing. Bacteria showing reduced susceptibility or resistance to an antibiotic imply that it should not be used on the patient.[20]

Out of 21 subjects selected 15 subject samples showed the presence of the Gram negative coccobacilli strains, with unusual morphological and genetic characteristics. The culture was obtained from Veillonella Agar that contained 60µg % of Vancomycin. AST was performed for these fluorescent samples using the Kirby Bauer method.

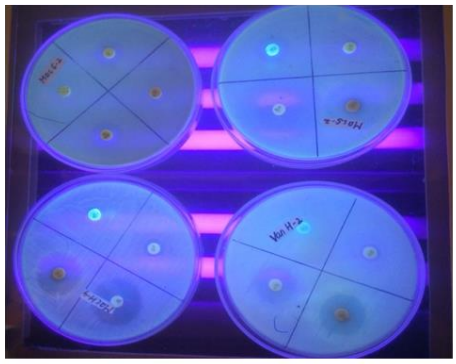

Fig 4 Antibiotic sensitivity profile of the isolates

The role of microbial communities in human health is emerging as one of the most important and fascinating biomedical challenges of our times.[21]The study of Zarco, M et al states that periodontitis is extremely difficult to treat because of the nature of the disease & its complications of antimicrobial therapy. Furthermore, periodontal pathogens develop virulent factors, like encapsulation, that make them resistant to antibiotics. [22]Pathogens hiding within plaque are one thousand times more resistant to antimicrobials than those which are more exposed.[23]

Table 1: Batch

Antibiotic	Concentration	B1(mm)	B2(mm)	B3(mm)	B4(mm)
Ticarcillin	75	7	10	14	11
		R	**R**	**R**	**R**
Ciprofloxin	5	25	20	23	25
		S	I	S	S
Gentamycin	10	15	17	17	18
		S	S	S	S
Neomycin	10	15	13	13	15
		I	I	I	I
Amikacin	10	16	18	20	20
		S	S	S	S
Vancomycin	30	-	-	-	-
		R	**R**	**R**	**R**

Table 2: Batch

Antibiotic	Concentration(mg)	A1(mm)	A2(mm)	A3(mm)	A4(mm)	A5(mm)
Tetracycline	30	20	-	19	31	-
		S	**R**	S	S	**R**
Ciprofloxin	5	19	24	26	24	23
		I	S	S	S	S
Gentamycin	10	26	19	18	28	-
		S	S	S	S	R
Neomycin	10	17	13	16	22	-
		S	I	I	S	**R**
Amikacin	10	30	-	14	15	-

		S	**R**	I	**R**	**R**
Vancomycin	30	10	-	-	25	23
		I	**R**	**R**	S	S
Penicillin	10	12	-	9	19	-
		R	**R**	**R**	I	**R**

Effective use of antibiotics in the future requires genomic analysis of the patient's oral microbiome to recognize the microbes that are present and to determine whether they will respond to specific treatments. Therefore, the oral microbiome will likely play a central role in the development and advancement of personalized medicine.

3.) Quantitative estimation of salivary biomarkers

Early detection of oral cancer is the most effective way to improve survival. The treatment planning of oral cancer is mainly based on the tumor, node and metastasis (TNM) classification and histopathological diagnosis. There is an urgent need to develop tumour markers to identify high risk individuals, improve cancer detection in early stages, predict disease outcome and response to therapy.[24]

Aberrant glycosylation is the universal feature of cancer and components of various glycoconjugates such as Sialic acid was estimated using Yao et al method. N-acetyl neuraminic acid (referred to as sialic acids) is a negatively charged nine carbon monosaccharide.[25]These glycoconjugates are released into the circulation through increased turnover, secretion, and/or shedding from malignant cells. [26]

Fig 5:N-Acetyl

Neuraminic acid (Sialic acid) Estimation

Other exposure marker such as Salivary thiocyanate (SCN) was estimated. SCN a metabolic product of cyanide, is an anion found in organic and inorganic compounds. SCN has a property to induce cancerous changes in epithelium. SCN is secreted in saliva and has a long half-life of 10-14 days in normal adults and is in continuous contact with epithelium through blood and saliva.[27-28]Thiselevated level of SCN in the saliva of smokers may be responsible for excessive cancer risk of smokers through the nitrosylation process, which is a process of converting organic compounds into nitros derivatives which are potent carcinogens and teratogens.[29-31] Elevation of SCN content was estimated in the SLT consuming mass, (5.16±4.54)mM, when

compared to the control group, (2.48±1. 25)mM

Table 3: Estimation Mean of Biomarkers

Biomarkers	Test Group	Mean	Standard Deviation
Protein	Sample	93.89	76.13
	Control	61.62	50.80
Thiocyanate	Sample	5.16	4.43
	Control	2.48	1.18
Bound Sialic acid	Sample	5.33	3.03
	Control	3.95	2.29
Free Sialic acid	Sample	10.80	10.60
	Control	8.44	9.88

Total sialic acid is a major constituent of glycoproteins and has been studied by several investigators. Elevation of total sialic acid level in the SLT consuming population was observed; protein bound sialic acid (5.33±3.11) mg% and free sialic acid (10.80±10.8) mg% as compared to the control group; protein bound sialic acid (3.95±2.43) mg% and free sialic acid (8.44±10.8) mg%. The protein estimate, when compared to the protein bound sialic acid was found to be statistically significant (r= 0.48, P= 0.027; P< 0.05)

Elevated levels of salivary sialic acid and thiocyanate in the respondents indicate its importance as a biomarker and may have a potential for using for early diagnosis. As these biomarkers were present in detectable amount in saliva, saliva can be used as a reliable marker.[32] Further studies are required to identify the value of this biomarker in predicting disease outcome and response to therapy in oral cancer patients. Further advancement in technologies is also required to develop portable, low cost, automated devices for rapid estimation of salivary sialic acid.[33]

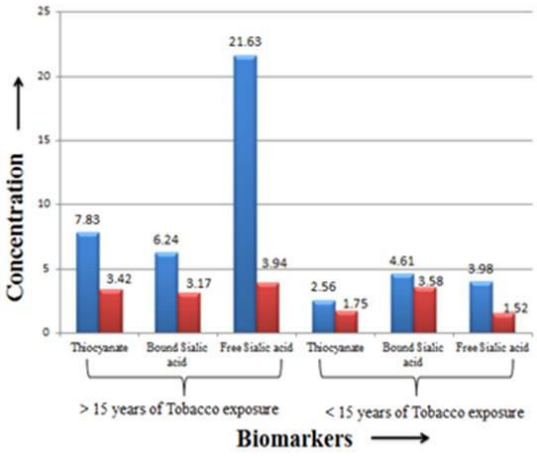

Fig 6: Biomarkers Estimate

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STUDY OF THE PHYSIOCHEMICAL CHARACTERISTICS OF COMMERCIALLY AVAILABLE SMOKELESS TOBACCO PRODUCTS

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ABSTRACT

Smokeless tobacco products have been identified as group 1 carcinogen and owing to considerable difference in patterns of its usage there has been rapid proliferation with wide range of chemical constituents. While studies have revealed that Smokeless Tobacco products manufacturing process involve extra fermentation and high temperature aging practices which encourages bacterial growth that could be detrimental to human health. So to better understand the potential microbiological and health risks associated with smokeless tobacco products use there is a need in assessing the presence of microbial contamination and profiling existing chemical constituents present in it.

Popular brands of gutka, pan masala, masheri, and tobacco products available in the market were used for the study. Through GC-MS profiling the presence of carcinogenic compounds such as Ethane,1,1,2-trichloro, Isoprothiolane, Diazoxon and 1,4-Dioxane were confirmed and on microscopic examination of colonies the occurrence of gram negative rods were reported. Further by Sequence comparison of 16S rRNA gene and phylogenetic analysis it was confirmed to be *Klebsiella* and *beta proteobacterium* species which were subjected to plasmid isolation while 50% of the analysed tobacco samples were in the alkaline range, which results to unprotonated nicotine absorption into the mucosal membrane as reported earlier. Therefore the present study demonstrates that the tobacco products are characterized by bacterial population which may influence not only the chemical constituents of the tobacco products but also the health of the consumers.

Keywords: Smokeless Tobacco, pH, 16S rRNA, *Klebsiella*, *beta protobacterium*, plasmid, Chemical constituents, GC-MS.

INTRODUCTION

Smokeless tobacco (ST) is a major public health problem in Indian subcontinent and India is considered as the global capital of ST use. As per the Global Adult Tobacco Survey (GATS - 2010), more than one-third (35%) of adults in India are tobacco users. Of them,21%are addicted only to Smokeless

Tobacco products where as 9% are addicted to smoking alone. Rest 5% are addicted to both forms; i.e. smoking as

well as ST. Contrary to this, in the United States, 20.6% of adults are smokers, whereas only 4.4% of men and 0.7% of women are ST users. This data clearly shows that unlike western countries, ST use is far more common than smoking in India. As per the World Health Organization report, the most significant risk factor for cancer is tobacco use, which alone is responsible for 22% of cancer deaths world over. India is the second most populous country in the world. It is a secular country but the Hindus form the majority. Hinduism traditionally advocates abstinence from all intoxicants. Even then, India is the third largest producer and consumer of tobacco in the world. The country has a long history of tobacco use. Tobacco is used in a variety of ways in India. Considering the enormous health complications associated with tobacco use, it is of utmost importance to understand the factors leading to its use and to plan strategies to reduce its intake. This is especially relevant for the developing countries like India, where tobacco use continues to be common notwithstanding the recognition of harmful consequences of its usage [1-5].There is sufficient evidence in humans for the carcinogenicity of smokeless tobacco. Several studies have established a causal association between use of smokeless tobacco and cancers of oral cavity, esophagus and pancreas. Smokeless tobacco causes acute increases in blood pressure and heart rate, and has been associated with a small increase of cardiovascular disease risk. Effects on insulin sensitivity, glucose tolerance and the risk for diabetes from smokeless tobacco use are plausible [6]. The use of smokeless tobacco causes reproductive and developmental toxicity, and its use during pregnancy increases the risks for preeclampsia and premature birth, causes increased placental weight and reduces mean birth weight. Smokeless tobacco use by men causes reduced semen volume, reduced sperm count, reduced sperm motility and an increased frequency of abnormal spermatozoa [6].

MATERIALS AND METHODS

This project was undertaken in Mumbai City. We collaborated as a team of undergraduate researchers. The purpose of the study was to examine and analyse smokeless tobacco products in Southern Mumbai and to ascertain about the risk associated with consumption of smokeless tobacco in various forms and its effect on the health.

I} Study design: 15 different brands of Smokeless tobacco products were collected from the local retail markets of Mumbai from the month of November 2015 to April 2016. The samples were selected on the basis of popularity among the people, representing a large and uniform sample pool. They were personally collected from the shops due to the absence of a national level manufacturer. These were then labelled with unique identification codes and stored. For ease of use, the samples have been identified with numbering as sample T1 , T2 , T3 , T4 , T5 , T6 , T7 , T8 , PM1 , PM2 , PM3 , PM4 , M1 , G1 , G2. The preparation

procedures adopted to obtain the extract of different Smokeless Tobacco product was as follow: 0.5 grams of theaccurately weighed smokeless tobacco samples were crushed with the help of Mortar and pestle and 5ml of saline was added to it under sterile condition to get a liquid mixture. The liquid mixture was then collected into sterile centrifuge tubes and kept for incubation in incubator for 30 minutes at 37degree Celsius. After incubation period the centrifuge tubes with the mixture was centrifuged for 10 minutes at 5000 rpm using centrifuger. After centrifuging supernatant was collected into another sterile centrifuge tube and stored for further analysis.

II} pH Analysis: The obtained extract was analysed using a laboratory grade LI 613 (ELICO) pH meter and pH paper.

III} Microbial Analysis of the samples:

Different extract samples were screened on different media that included Sabouraud's Agar, Nutrient Agar, MacConkey's Agar and Veillonella Agar (containing Vancomycin 60µg %). Vancomycin is a selective agent which supresses the growth of other organism without hampering the growth of Veillonella. A nicromeloop was used for the streaking on the respective media plates.

The plates were incubated at their respective incubation conditions:

Nutrient Agar, MacConkey's Agar and Veillonella Agar - 37^{o} C for 24-48 hours, Sabouraud's Agar - RT for 48 hours.

Morphological and cultural characteristics of the growth on the respective media were studied.

IV} Isolation and Characterization of the organisms:

The colonies observed on the plate were characterised by Hans Christian Gram's method of Gram Staining [7] and observed under the light microscope (100X magnification-Oil immersion lens).

V} Preparation of Plasmid DNA [8] and Protocol for extraction and determination of Chemical constituents: By Alkaline Lysis method using SDS (Minipreparation)

1) A powdered plant material (Smokeless Tobacco sample) -50 g was treated with 15 ml of NH_4OH (25%) at room temperature.

And solvent extraction was performed with 300ml of ethyl acetate for 72 h.

2) The extract was filtered and air dried at 40 degree C .the residue was then dissolved in H2O and acidified with H_2SO_4 to pH 3.

3) Then it was extracted with petroleum ether and diethyl ether and adjusted to pH of aqueous phase to 9-10 with NH4OH (25%).after this process it was extracted with chloroform and washed with distilled water.

4) Crude constituents was concentrated to dryness under reduced pressure and dried over sodium sulphate.

5) The residue obtained were then dissolved in methanol and subjected to GC-MS analysis (Gas Chromatography-Mass Spectroscopy)

RESULT AND DISCUSSION

From the 15 different brands of Smokeless Tobacco Products only Two products extract T6 and T8 showed growth of bacterial colonies after isolating on Nutrient Agar, MacConkey's Agar and Veillonella Agar which showed the presence of Gram negative rod shaped bacterial characteristic on microscopic observation and confirmed to be klebsiella and beta proteobacterium by sequence comparison of 16S rRNA gene and phylogenic analysis.

FIG -1 Bacterial Colonies onNutrient Agar, MacConkey's Agar and Veillonella Agar**.**

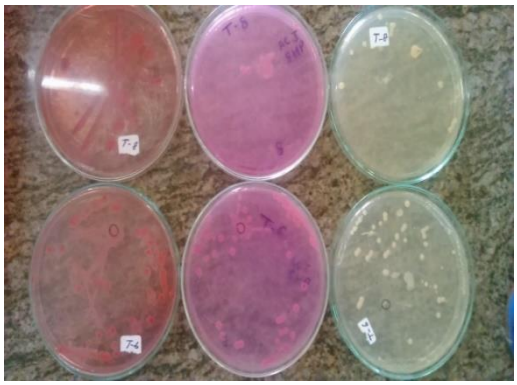


FIG-2 Gram staining of***Klebsiella sp***

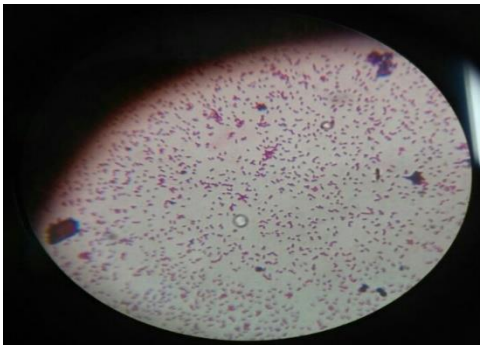
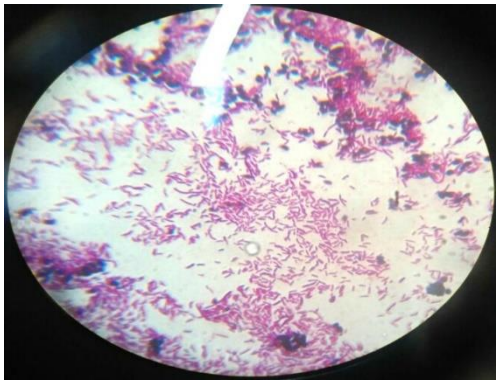


FIG-3 Gram staining ***beta proteobacterium***



Biochemical test-

Table-1*Klebsiella sp.*

Indole	Methyl red	Voges proskauer	Citrate test
-	-	+	-

Sugar Tests	**Inference**
Xylose	+
lactose	+
Glucose	+
Maltose	+
Mannitol	+
Urease Test	Urease utilized

Table-2 *beta proteobacterium sp.*

Indole	**Methyl red**	**Voges proskauer**	**Citrate test**
-	-	-	-

Sugar Tests	**Inference**
Sucrose	-
lactose	-
Glucose	+
Maltose	-

Mannitol	-
Urease Test	-

Table-3

Isolate name	Genus	Strain
A	*klebsiella*	Clone HLB-47
B	*beta proteobacterium*	Clone B11

Table-4 pH Analysis using pH meter

Smokeless Tobacco Product with following code	pH Reading
T1	8.6
T2	9.06
T3	5.77
T4	9.8
T5	5.5
T6	5.5
T7	5.5
T8	6
PM1	9.2
PM2	9
PM3	9.3
PM4	8.5
M1	6.5
G1	6.04
G2	8.0

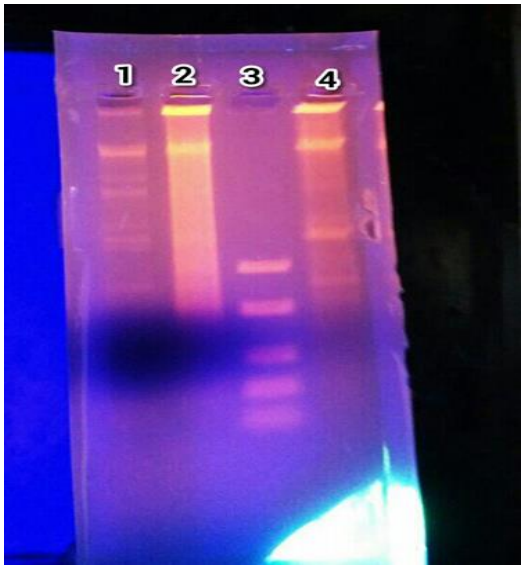
Table-5

SLT Products	Mean ± SD
PM	9 ± 0.36
G	7.02 ± 1.39
T	6.96 ± 1.85

From The above readings obtained Pan Masala showed high pH reading followed by gutka and other tobacco products due to its Tobacco content , Nicotine, Heavy Metals ,Benzo[a]pyrene and Tobacco specific Nitrosamines**.**

FIG – 4 Preparation of plasmid DNA by Alkaline Lysis method with SDS and then

Isolation of plasmid using Gel Electrophoresis method from the bacterial colonies obtained.



Lane 1 = 2-3 plasmid DNA
Lane 2 = 2 plasmid DNA
Lane 3 = Ladder DNA
Lane 4 = 4 Plasmid DNA

Several species of bacteria found in smokeless tobacco products have been associated with opportunistic infections. Part of the concern over microbial risk from smokeless Tobacco products stems from the fact that consumers typically hold the product in their mouth in close contact with mucus membrane for extended period of time in order to allow nicotine to pass into the blood stream. So doing provides an opportunity for the consumer to be exposed to bacteria present in the product. Microbial community on tobacco leaf changes during the air curing process especially during high

heat and high humidity under these conditions microbes that converts nitrate to nitrite appear to increase. Nitrate to Nitrite conversion is key in forming carcinogenic tobacco specific nitrosamine[9-11].

High pH is associated with high nicotine absorption. Nicotine absorption from smokeless tobacco is importantly influenced by the pH at the buccal-product interface. In an alkaline (high) pH, environment nicotine is unionized and rapidly absorbed into bloodstream and then reaches brain where there is a kick caused in part by the drugs stimulation of the adrenal glands and resulting discharge of epinephrine (adrenaline). The rush of adrenaline stimulates the body and causes and increase in blood pressure, respiration and heart rate whereas in acidic (low) pH nicotine is ionized and does not cross biological membranes also nicotine induced DNA strand breakage is pH dependent thus pH appears to be the primary means by which the speed of nicotine absorption is determined[12,13].

Gram-negative rods are unusual pharyngeal isolates in normal man, suggesting the presence of effective oral defence mechanisms against these organisms. The findings from this study have provided evidence for the presence of Gram-negative short rods of *Klebsiella* and *beta proteobacterium* in the smokeless tobacco product. *Klebsiella* is a gram negative microorganism of the *Enterobacteriaceae* family present in the digestive tract, classified as coliform.*Klebsiella* is also considered as an ubiquitous opportunistic pathogen that colonizes at the mucosal surfaces in humans and causes severe diseases, such as septicaemia, pneumonia, urinary tract infections, and soft tissue infections while the *beta proteobacterium* consist of several groups of aerobic or facultative bacteria that are often highly versatile in their degradation capacities. They are highly metabolically diverse and containchemolithoautotrophs,photoautotro p-hs and heterotrophs. This rare bacteria oxidizes ammonia into nitrite as a metabolic process which help in forming carcinogenic TSNAs[14-16].

While plasmid isolated indicates antibiotic resistant genes conferred to the microbe.

TABLE- 6

Chemical constituent	Retent -ion time	Area	Height
Ethane,1,1 ,2- trichloro	12.021	54675	22060
1,4- Dioxane	10.374	321516	130481
Isoprothiol ane	26.786	58354	15678
Diazoxon	34.877	90967	18020

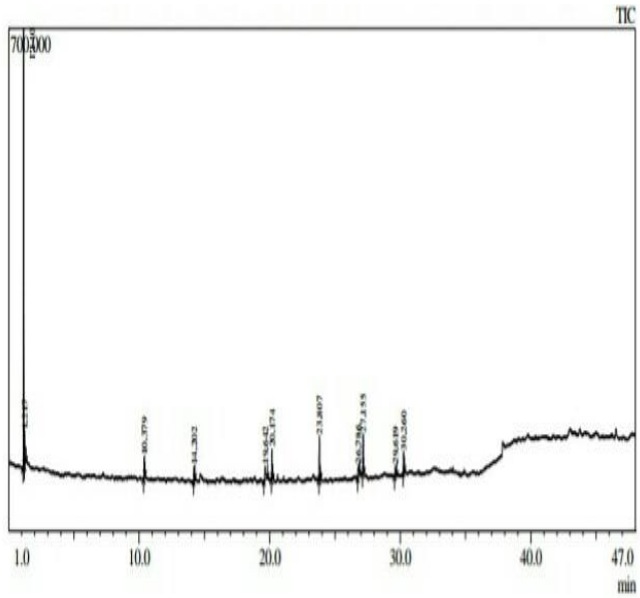
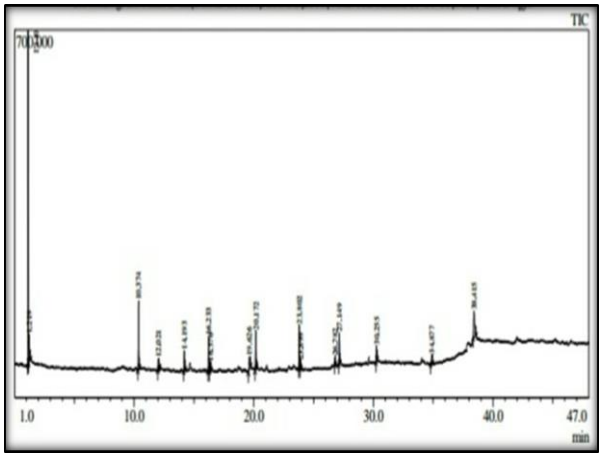


FIG-5 (GC-MS) Chromatogram 1

FIG- 6 (GC-MS) Chromatogram 2



There has been continuing debate about the toxicology and possible impacts on public health associated with the use of Smokeless Tobacco products. 28 chemical agents are listed including nitrosamines, carbonyls, benzo(a)pyrene, angelica lactones, coumarin, ethyl carbonate and a series of metallic and radioactive species. There is significant data in the literature concerning the levels of nitrosamines in smokeless tobaccos, but there is little information available for the majority of the other species. Moreover, most of the existing data has been generated on a small number of brands in each study, with little comprehensive comparative information available on the contents of different product style.

Recent advances in Gas Chromatography tandem Mass Spectroscopy allows for greater compound specificity by eliminating matrix ions arising from other compound that share the same parent mass but lack the correct transition ion drastically decreasing background interferences and detection limits.[17,18]

From this study the presence of carcinogenic insecticidal and pesticidal residues in Smokeless Tobacco such as Ethane,1,1,2-trichloro, Isoprothiolane, Diazoxon and 1,4-Dioxane were confirmed.

Ethane,1,1,2-trichloro is a derivative of an high persistent organochlorine compound DDT,

fatty tissues which results in intermediate syndrome after 24-96 hours of DDT is used as insecticide for protection purpose but due to its carcinogenic effect it has been totally banned. According to the statistical studies carried out in Mumbai 64% of women use only one type of smokeless tobacco product while 34% women use more than one type of smokeless tobacco products and no difference was noted between pregnant and non-pregnant women in smokeless tobacco consumption. Pregnant women consuming Tobacco products with such toxic residues present in it are more likely to give birth prematurely or low birth babies while studies in mice have found that DDT blocks the binding of hormone progesterone to it receptors and in theory this could cause both prematurity and low birth weight. High level oral exposure may lead to endocrine disruptance and abnormalities of liver function and nervous system[19,20]

Diazoxon is a contact organophosphorous insecticide which kills insects by altering normal neurotransmission within the nervous system of insect. Symptoms of acute diazoxon on exposure are nausea, dizziness, salivation, headache, sweating, lacrimation and further can

progress to vomiting, weakness, tremor, respiratory depression,tightness in chest, wheezing, productive cough. Furthermore some studies have even reported memory loss, confusion and depression while it is fat soluble it may led to delayed toxicity if significant amount of diazoxon get stored in exposure[21]

Exposure of 1,4-Dioxane in humans may occur through ingestion of contaminated tobacco products which at high level result in drowsiness ,irritation to throat and chronic exposure can even lead to buccal cavity disruption so it is likely classified to be carcenogenic to humans[22] while Isoprothiolane is a kind of systemic fungicide which act as an respiratory irritant and known for its degradation dynamics[23]. Thus the result obtained from this study indicates the variable nature and chronic effects of using such insecticidal/fungicidal contaminated Smokeless Tobacco products.

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HPLC ANALYSIS & COMPREHENSIVE EVALUATION OF ANTIMICROBIAL PROPERTIES OF TWO WEEDS & FORMULATION OF HERBAL LOTION.

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ABSTRACT

Weeds have been neglected and their use for medicinal purpose has not been considered on a large scale. Our current research was based on the identification of phytochemicals & determination of antimicrobial properties of two weeds. The weeds used were *Ipomoea carnea* and *Alternanthera sessilis.* Qualitative and Quantitative analysis of Phenols and Flavonoids was done by the Folin Ciocalteau and Aluminium chloride method respectively. Antimicrobial analysis was done by Agar well Diffusion method and the synergistic action of weeds was also studied on 4 bacteria, 2 fungi and bacillus. Both weeds showed positive response for presence of secondary metabolites like flavonoids, phenols, tannins, terpenoids, etc. which indicates that they can be used as herbal components. The weeds also exhibited positive antimicrobial activity against the bacteria. The quantitative phytochemicals analysis stated the TPC and TFC value were found out. HPLC analysis confirmed the presence of phenols and terpenoids. The research work suggests a herbal antibacterial formulation and its ingredients were studied to be consistent in quality and can be easily used as skin cream for treatment of various skin infections. Weeds can be further used to study various properties like anti-carcinogenic, anti-diabetic, allelopathy, etc

Keywords: Weeds, Phytochemicals, Antimicrobial, HPLC, Herbal Cream.

INTRODUCTION

Weeds, commonly defined as plants that grow out of place and are competitive, persistent and pernicious [1] have been a part of civilization and many ancient documents mention about humans battling weeds in the crop fields. Weeds are the important and unused components of the agricultural ecosystem. Weeds are a component of plant genetic resources that when well protected in agro-ecosystems, can become useful plants or become crops themselves [2]The role of weeds, commonly found in disturbed areas, in traditional medicine floras has been overlooked. [3]However, weeds are useful to human beings as food, erosion control, medicines, aesthetic value, shelter, supply of organic matter and mineral nutrients to the soil. Consumption of agricultural weeds is a world-wide phenomenon as some of the plants are characterized by high nutritional value and medicinal properties. [4]Traditional healers recognized their medicinal potential and have utilized them for the treatment of human ailments. Weeds are also found to be resistant to most of the microbial diseases when compared to the cultivated crops. [5] The resistance of weeds towards the microbial diseases provoked many workers to explore the reasons for such potency.

Weed plants selected for study: *Ipomoea spp.* and *Alternanthera spp. Ipomoea spp is a* common weed popularly known as Besharam, Behaya in India. It is a medicinal plant used in different traditional medical systems including Ayurveda, Siddha, and Unani to treat Leucoderma. *Alternanthera spp is a* common weed with several medicinal and dietary uses. Itis used internally against intestinal inflammation, externally to treat wounds, to hepatitis, tight chest, bronchitis, asthma and lung troubles.

MATERIALS AND METHODS

Collection and preparation of plant materials

Fresh plants were collected randomly from different areas of Mumbai region, India.

Preparation of powdered extracts of the plants:

The part of plant selected for this study was leaf. These leaves were surface sterilized to remove any dust and dirt present on the surface. The leaves were dried in an oven and later transferred in a homogenizer where they were crushed into a coarse powder. [6]

Extraction of phytochemicals using Soxhlet extraction:

The choice of solvent for the extraction process was methanol. The extracts obtained after the completion of the cycle, were filtered and concentrated to remove methanol. The filtered extracts were labelled and kept at 4^{o}C till further use.[6]

3.01QUALITATIVE PHYTOCHEMICAL ANALYSIS.[7] [8][9]

Test for Proteins, Carbohydrates, Flavonoids, Glycosides, Terpenoids, Saponins, Alkaloids, Reducing Sugar, Phenols, Tannins, Quinones, Coumarin, Emodins, Steroids, Anthocyanins, Leucoanthocyanins was performed by

biochemical methods as given by **Savithramma, N (2011), Wadood, A (2013) and Soni, A (2013)[7][8] [9]**

3.02 QUANTITTIVE PHYTOCHEMICAL ANALYSIS:

3.02.01 Determination of total phenolic content (TPC)

The TPC of plants was evaluated quantitatively by Folin-Ciocalteau method.TPC was expressed as Gallic acid equivalent (mg of Gallic acid/mg of dry weight of extract) based on the calibration curve. [10]

3.02.02 Determination of total flavonoid content (TFC)

The TFC of plants was determined by Aluminium chloride colorimetric method. The TFC was expressed as mg of Quercetin equivalent (QE)/g dry weight of weeds.[10]

3.02.03 High Performance Liquid Chromatography (HPLC)

Linalool and Gallic Acid were used as the standards. The HPLC column used is Kromasil- C18 OctadecyeSilane. The mobile phase was1:1(methanol: water) for both standards as well as sample. The peaks for the same were obtained at 254 nm. [11]

3.02 ANTIMIROBIAL ANLAYSIS:

Plant extracts obtained from soxhlet, were dissolved in 10% Dimethyl sulphoxide (DMSO) for antimicrobial study. The mixture was sonicated using a sonicator before use. The Microorganisms used are *E.coli, S.typhi, S. aureus, C.diphtheriae, B.subtilis, B. cereus, S.cerevisiae and C.albicans*. Positive and negative controls were also set up for the study.

Agar well diffusion assay:

Mueller-Hinton agar and Sabourauds media was the choice of medium for growth of bacteria and fungi respectively. The agar well diffusion medium was prepared and approximately 100µl of the test organism was surface spread on the media using a sterile cotton swab. Holes punched into the agar using cork-borer. Positive and negative control was also added. The plates were kept for Pre-diffusion at 4°C for 30 minutes. After the Pre-diffusion period was over, the plates for bacteria were incubated at 37°C for 24 hours to observe the results. The plates with fungal cultures were incubated at room temperature for 48 hours to observe the results. The entire set was run in triplicates and the average result for the three readings was taken into consideration. [12]

3.03 SYNERGISTIC EFFECT

Mueller-Hinton agar and Sabourauds media was the choice of medium for growth of bacteria and fungi respectively. 0.1 ml of 18-24 hour old culture were swabbed under aseptic conditions. Strips of adsorbent sterile whatmann filter paper (6cm × 1cm) were saturated with extract solution and then placed on the agar plate such that the strips touch each other at 90 degrees. The plates were then incubated at 37°C for 24 hours for bacteria and 28°C for 48 hours for fungus. [12]

3.05 FORMULATION OF A

HERBAL LOTION

Formulation Preparation:

1:1 ratio of coconut oil and almond oil was taken in a beaker and heated in a double boiler. As soon as the oil becomes warm, bees wax, aloe Vera gel, vitamin E, Nutmeg, Aromatic oil, plant extracts were added with continuous blending till the mixture is smooth. [13]

3.05.01 Evaluation of properties of herbal lotion

The herbal lotion was tested for its Physical Properties, Thermal Stability, pH, Patch Test, Homogeneity and Microbial growth.

RESULTS AND DISCUSSION

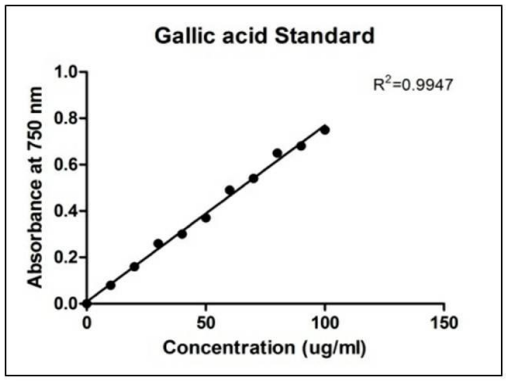
1. **Qualitative Phytochemical Analysis**

   Out of the 16 phytochemicals that were being analysed, 8 phytochemicals like Proteins, Carbohydrates, Phenols, Flavonoids, Terpenoids, Glycosides, tannins and Reducing sugars were found to be positive for both *Ipomoea carnea* and *Alternanthera sessilis.*

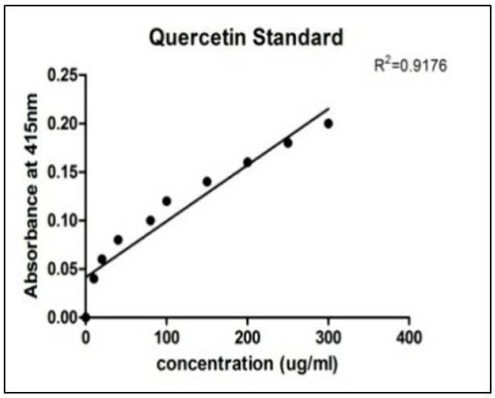
2. **Quantitative Phytochemical analysis**

Total Flavonoid Content (TFC)		
Concentration(mg Quercetin equivalent/g)	**Extract**	**Sr. No.**
0.09	*Ipomoea carnea*	1
0.2	*Alternanthera sessilis*	2

Total Phenolic Content		
Concentration(mg Gallic acid equivalent/g)	**Extract**	**Sr. No.**
0.0526	*Ipomoea carnea*	1
0.0657	*Alternanthera sessilis*	2

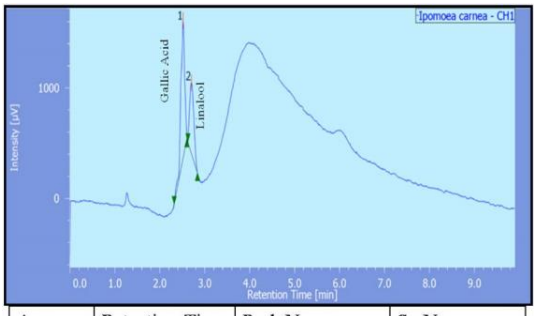


Graph 1: Standard calibration plot of Gallic acid

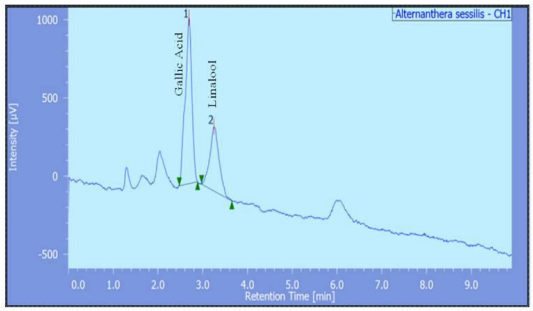


Graph 2: Standard calibration plot of Quercetin

3. High Performance Liquid Chromatography (HPLC) Analysis



Area	Retention Time	Peak Name	Sr. No
7324	2.517	Gallic Acid	1
4227	2.700	Linalool	2



Area	Retention Time	Peak Name	Sr. No
10929	2.692	Gallic Acid	1
5937	3.250	Linalool	2

4. Antimicrobial Activity:

Zone of inhibition (mm)						
Alternanthera sessilis	*Ipomoea carnea*	Positive control	Negative control			
35	36	36	0	*E.coli*	Gram Negative	Organism
36	35	39	0	*S.typhi*		
0	0	52	0	*S.aureus*	Gram Positive	
11	12	47	0	*C.diptheriae*		
13.5	12.5	27.5	0	*B.subtilis*	Bacillus	
14	13	28	0	*B. Cereus*		
0	0	22	0	*C.albicans*	Fungus	
0	0	25	0	*S.cerevisiae*		

5. Synergistic Effect

The synergistic effect was however not very evident for both the weeds. When both the weeds were used in combination they did not enhance or suppress each other's individual potential. Therefore, these weed cannot be used as a potential combined drug therapy treatment.

6. Herbal lotion.

The physical parameters of stability like colour, odour, appearance, consistency was tested on 10 people. The Physical Properties of the lotion were found to be stable with respect to color, odour and appearance during the period of study. The Test for Thermal Stability revealed that the Lotion

was found to be stable at 28°C, 37°C and 55°C.The pH of the lotion with the extract was found to be around 6 which is suitable for topical application as pH of the skin is between5-6. No residue of colored patch of Lotion was observed during the study period. Homogeneity of the lotion was ranked as +++ = excellent. The formulated Lotion showed no microbial growth.

CONCLUSION

By determining the antimicrobial potential of the two weeds, they can be used as potential natural antibiotics against a host of microorganisms.This in vitro study demonstrated that both weed plants showed positive response for presence of phytochemicals like flavonoids, terpenoids, saponins, phenols, etc. Phenols and flavonoids were found in good concentration in both the weeds by TPC and TFC. The present study provides evidence that these weeds act as antibiotics to *S.typhi* and *E.coli*. The activity of weeds may be due to presence of phenols and flavonoids which were quantified by TPC and TFC and Terpenoids and phenols which were quantified using HPLC. Phytochemicals like terpenoids and phenols were estimated by HPLC using Linalool and Gallic Acid as the standard & 1:1 methanol:water as the mobile phase at 254 nm. Inhibitory effect showed that Gram negative bacteria were more sensitive to *Ipomoea* and *Alternanthera* leaf extracts. The herbal lotion formulated was pure and stable in consistency, color, fragrance, appearance .The prepared formulations showed no evidence of irritation and phase separation. Stability parameters like stable consistency, homogeneity, nature and fragrance of the formulations showed that there was no significant variation during the study period. No effect was observed on fungus in the entire study. Also no combined effect of both weeds was observed which concludes that both weeds cannot be used in combination with each other.

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FACTORS LEADING TO RISE IN CONSUMPTION OF ALCOHOL AND HOOKAH SMOKING AMONG THE YOUTH, A COHORT STUDY

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ABSTRACT

Objective: To find the factors affecting consumption of alcohol and hookah smoking among the youth (15-30 years of age) of Mumbai.

Methodology: The study was conducted by circulating questionnaire among random subjects in the town and suburban areas on Mumbai. Then 330 correctly filled forms were analyzed through chi square test, Z test and coefficient of colligation. The data was filtered and presented.

Results: The factors affecting consumption levels among youth of Mumbai are sex of the individual, educational status of the individual, and their occupation. Besides this, parental acceptance also plays a crucial role in the consumption of alcohol and hookah. Other factors such as age at which the consumption begins and the locality of an individual was also studied along with individual's perception on hookah smoking .

Conclusion: Through our study we concluded that there are differences in the preference of hookah and alcohol amongst youth of Mumbai and it is majorly influenced by the sex of the individual. There are several factors which lead to smoking and alcohol consumption in the younger population of Mumbai, including educational status of the individual.

Keywords: Alcohol, hookah, youth, Mumbai.

INTRODUCTION

Alcohol or Ethyl alcohol, or ethanol, is an intoxicating ingredient found in beer, wine and liquor. Alcohol is produced by the fermentation of yeast, sugars and starch. Globally, alcohol is a major cause of mortality and morbidity [1]. Since 60% of India's population comprises of the younger generation, therefore the rise in alcohol consumption among the younger generation has led to an alarming situation and thus it has become very essential to investigate the root cause behind this rise and tackle the factors leading to the rise. It is imperative to know the patterns of alcohol consumption among different types of consumers to launch a well-planned program for prevention and control of this social pathology [1]. This is a complex issue and the necessity to pay attention to the adolescent and young adults' population is very high. Thus, in order to curtail early alcohol use and its consequences support from family, school and society plays a key role.

Hookah: Tobacco is a green, leafy plant that is grown in warm climates. After it is picked, it is dried, ground up, and used in different ways. If one form of tobacco use is curbed, the tobacco companies find an alternative, the latest being the use of hookah. While youngsters may see it as the new cool, the health impacts of hookah smoking is severe as it has both immediate and long term implications. It is commonly thought that smoking shisha and hookah can be less harmful than cigarettes, but the truth is that one shisha is equivalent to 50 to 60 cigarettes, and a two hour to three hour session of smoking a shisha is equivalent to smoking 25 cigarettes [2].

According to a report from the World Health Organization (WHO), a hookah smoking session may expose the smoker to more smoke over a longer period of time than occurs when smoking a cigarette. Also, due to the method of smoking—including frequency of puffing, depth of inhalation, and length of the smoking session—hookah smokers may absorb higher concentrations of the same toxins found in cigarette smoke [3].

MATERIALS AND METHODS

A cross-sectional study was carried out involving 330 male and female adolescents between the age group of 15 to 30 years of age in the city of Mumbai. Participants from both the Suburban as well as from South Mumbai were taken into consideration for this broad spectrum study. The participants selected for the study were students, working class individuals and housewives.

The questionnaire comprised of two sections- one for details regarding alcohol consumption and the second section for details regarding hookah smoking.

The results obtained were analyzed using chi square test, coefficient of colligation and Z test

RESULTS AND DISCUSSION

The research analyzed 330 subjects within the age group of 15 to 30 years with the average age being 19.21 years with standard deviation of 3.48 years.

i) Out of these, 196 (59.39%, 95% confidence interval = 59.39 $\pm$ 0.96) subjects self-reported consumption of either alcohol or hookah or both. With 5% error, it can be concluded that around 60% of the population in this age group consumes either alcohol or hookah or both. Consumption of either alcohol or hookah or both among the 330 subjects is shown in the pie chart below. From the pie chart it can be concluded that both alcohol and hookah are equally preferred in this age group.

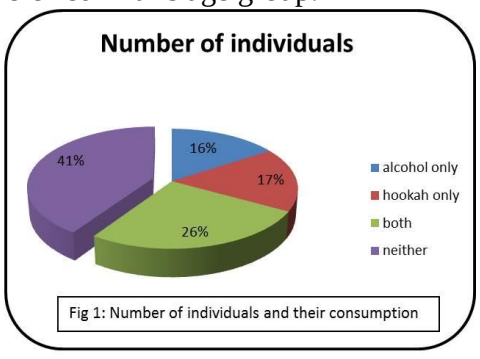

Fig 1: Number of individuals and their consumption

ii) The 330 subjects consisted of 163(49.39%) males and 167 (50.61%) female subjects. There were 121 males as compared to 75 females who consumed alcohol and hookah and 75 men. 43 men and 91 women consumed neither.Chi square test for this data showed that there is association between sex of an individual and consumption of alcohol and hookah. (chi^2_{stat} =26.8056, P_{value} = <0.0001).

Ƶ test was performed to check if there is more consumption of alcohol and in males compared to number of females (Ƶ $_{value}$=5.3, P_{value}= <0.0001, Ho: $P_1=P_2$, H_1: $P_1 > P_2$. P1= Proportion of males consuming alcohol, hookah or both. P_2= Proportion of female consuming either alcohol, hookah or both)

SEX	NUMBER OF INDIVIDUALS		
	ALCOHOL	HOOKAH	BOTH
Male	24	35	62
Female	28	22	25

Table 1: Type of consumption and gender of individuls

Of these 164 males, 121(73.78%) males have consumed either alcohol or hookah or both. Among these 121 males, 24(19.83%) consume alcohol only, 34(28.1%) smoke hookah only and 62(51.24%) consume both alcohol and hookah. In the class of 166 females, 75(45.18%) females have consumed either alcohol or hookah or both. The 75 females consisted of 28(37.33%) females consuming alcohol only, 22(29.33%) females smoking hookah only and 25(33.33%) females using both alcohol and hookah.

From the above data, it is evident that males prefer smoking hookah over consumption of alcohol, and females prefer alcohol consumption over hookah smoking.

The age at which consumption of alcohol and hookah starts is represented in the following table:

AGE AT WHICH CONSUMPTION STARTS	NUMBER OF INDIVIDUALS	
	ALCOHOL CONSUMPTION	SMOKING HOOKAH
<15	11	23
15-20	113	100
>21	15	11

Table 2: Age at which consumption starts

The data in table 2 shows the type of consumption (alcohol or hookah) and the age at which the consumption begins. Chi square test reveals that there is no association between type of consumption (alcohol or hookah) and age at which consumption starts i.e. when people start consumption of alcohol and hookah they are not partial to either and consume both without a preference.(chi^2_{stat} =5.5544, P_{value}= 0.0622, Ho: P_1= 0.5, H_1 = P_1>0.5 where P_1= proportion of youth(15-20 years) consuming either alcohol or hookah).

iii) The subjects can be divided into different classes in accordance with their educational status.

The consumption habits of individuals in these groups are as follow:

EDUCATIONAL STATUS	NUMBER OF INDIVIDUALS	
	CONSUMPTION OF ALCOHOL OR HOOKAH OR BOTH	DO NOT CONSUME
Below 12th grade	34	16
Undergraduate	23	28
Graduate	122	85
Post graduate	15	5

Table 3: Type of consumption and educational qualification of individuals

From Chi^2 test for the data recorded in table 3 it was concluded that there is association between educational status of an individual and their susceptibility to consumption ($chi^2_{(cal)}$= 7.8715, P_{value}= 0.0487)

Ƶ test for the above data shows that more number of individuals belonging to the below 12th grade community consume alcohol or hookah or both and as more compared to the individuals in the undergraduate community. ($Ƶ_{cal}$= 2.3, P_{value}=0.0101, Ho: P_1=P_2, H_1 : P_1>P_2, where P_1= Proportion of people below 12th grade and consume alcohol or hookah or both, P_2= Proportion of undergraduates consuming alcohol or hookah or both). Also, more undergraduates consume alcohol or hookah or both and as more compared to the individuals in the graduate community. ($Ƶ_{cal}$= 1.8, P-value=0.0372, Ho: P_1=P_2, H_1 : P_1>P_2, where, P_1= Proportion of undergraduates consuming alcohol or hookah or bothP_2= Proportion of graduates

consuming alcohol or hookah or both). Ƶtest also revealed that number of graduates that consume alcohol or hookah or both is same as the number individuals in the post graduate community. ($Ƶ_{cal}$= 1.4, P-value=0.0803, Ho: $P_1=P_2$, $H_1 : P_1>P_2$, where, P_1= Proportion of graduates consuming alcohol or hookah or bothP_2= Proportion of post graduates consuming alcohol or hookah or both)

i) Our subjects are greatly diverse in terms of their occupation. The three major communities that they belong to are student community, working community and housewives (home maker). This diversification is demonstrated in the table given below:

OCCUPATION	CONSUMPTION OF ALCOHOL OR HOOKAH OR BOTH	NO CONSUMPTION	TOTAL
STUDETS	154	115	269
WORKING	37	12	47
HOUSEWIVES	7	7	14

Table 4: Consumption and occupation status of individuals.

The student community makes up the largest part of the subject group with 269 (81.51%) individuals followed by the working section with 47(14.24%) individuals and the housewives community is represented by 14(4.24%) individuals.

The results of chi square test performed on the data from table 4 showed that there is indeed association between occupational background of an individual and susceptibility of consumption ($chi^2_{(cal)}$ =6.3064, P_{value}= 0.0427).

Ƶ test revealed that the proportion of people consuming alcohol and hookah is in working community is more as compared to student community ($Ƶ_{value}$= 2.8 P-value= 0.0027) Ho: $P_1 = P_2$, H1 = $P_1>P_2$, where P_1= Proportion of individuals in the working community that consume either alcohol or hookah or both, P_2 = Proportion of individuals in the student community that consume either alcohol or hookah or both).

ii) Out of the students 154 students 42(27.27%) students consume alcohol only, 51(33.15%) students only smoke hookah and 61(39.61%) students consume both alcohol and hookah.

Through Z test, it was found that students prefer both hookah and alcohol equally ($Ƶ_{(cal)}$ =1.2, P_{value}= 0.1098)

Amongst the 35 working subjects, 9(25.71%) individuals consume alcohol ONLY, 3(8.6%) only smoke hookah and the rest i.e. 23(65.71%) consume both alcohol and hookah.

Results from Z tests showed that working individuals prefer alcohol consumption over hookah smoking ($Ƶ_{(cal)}$ =2.4, P_{value}= 0.0072)

The sample for this research was collected from town and suburbs of Mumbai. Of the 330 participants, 120(36.36%) participants were from town and 210(63.64%) participants belonged to the suburbs of Mumbai.

Of the total 120 participants from town, 16(13.33%) consumed alcohol only, 16(13.33%) smoked hookah only and 41(31.17%) consumed both alcohol and hookah.

Amongst the 210 participants from suburbs, 36(17.14%) consumed alcohol only, 41(19.52%) smoke hookah only and 47(22.38%) consumed both alcohol and hookah.

Chi square test was performed to check whether there is association between locality

and consumption of alcohol or hookah. The result of the test showed that there is no association between the two (chi^2_{stat} =0.0818, P_{value}= 0.7749)

Parental Acceptance

The individuals that consume alcohol and hookah were asked to state whether or not their parents approve of their consumption habits, and if they did approve of it till what were the conditions in which they would allow their children to consume alcohol and hookah.

(One Subject Has Not Responded To This Question In The Hookah Section.)

Parental Acceptance To Alcohol Consumption Only

The data shows that 47% of the parents, of individuals who consume alcohol, would never permit their children to consume alcohol. This shows that there is awareness in the parent community about the ill effects of alcohol consumption. However, the data also depicts that parents do allow their children alcohol consumption under their supervision (31%) and on special occasions (11%). From this one can conclude that alcohol consumption has started to become a common social norm. There is also some percentage (11%) of parents that permit their children to consume alcohol whenever they wish.

Parental Acceptance To Smoking Hookah Only

Some parents permit the use of hookah but only under their supervision (17%), this could mean that parents are not completely aware of the harmful effects of hookah and so are permitting the use of it. Percentage of parents that never permit smoking hookah (32%) is around the same as the percentage of parents that allow hookah smoking only on special occasions (31%). However, the percentage of parents (20%) permitting hookah smoking anytime their child wants to smoke is more than the parents.The above data depicts that there is more acceptance of parents towards hookah smoking when compared to alcohol consumption

Number Of Parents That Have Identical Opinions Towards Consumption Of, Both, Alcohol And Hookah

Parental acceptance	Consumption of alcohol and hookah smoking.
Never	32
Under parental supervision	4
On special occasions	9
Anytime I want to	7

Table 5: Number of parents that have identical opinions towards consumption of both, alcohol and hookah.

When we combine the data, we can see that the number of parents that say no for consuming alcohol also say no to hookah smoking.

PREFERENCE

58 subjects have not responded to the question and 2 participants have no preference.

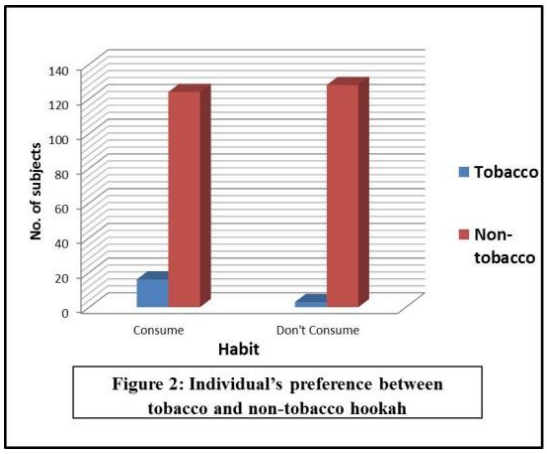

Figure 2: Individual's preference between tobacco and non-tobacco hookah

Traditionally hookah contains tobacco, however, nowadays there are non- tobacco hookahs also available. When given the choice, subjects would choose non-tobacco hookah over hookah containing tobacco.

From this we can infer that, largely, individuals are aware of the ill effects of usage of tobacco irrespective of whether or not they smoke hookah.

iii) **PERCEPTION OF HOOKAH: (degree of awareness)**

Out of the subjects that do smoke hookah 106 (73.61%) do so even when they are aware that it is harmful and 38 (26.39%) hookah smokers are under the false assumption that hookah isn't harmful.

CONCLUSION

This study was conducted in order to find the factors that affect the consumption of alcohol and hookah among individuals that are in the age group of 15-30 years (with the average age being 19.21 years with standard deviation of 3.48 years) and are living in Mumbai.

Previous reports have stated that, as of 2010, about 30 percent of India's population consumed alcohol regularly. Some 11 percent are moderate to heavy drinkers. The average Indian consumes about 4.3 litres of alcohol per annum, says the report. The rural average is much higher at about 11.4 liters a year [4].

Testing Of Association Of Factors Mentioned In The Table With Consumption Of Alcohol And Hookah:

FACTOR	CHI^2 VALUE	P value
SEX	26.8056	<0.0001
TYPE OF CONSUMPTION AND ONSET AGE	5.5544	0.0622
OCCUPATION	6.3064	0.0427
EDUCATIONAL STATUS	7.8715	0.0487
LOCALITY	**0.0818**	**0.7749**

For the above table, P_{value} = <0.05 shows association between the mentioned factor and consumption of alcohol and hookah.

Z Test For Proportion For Factors Given Below:

FACTOR	Z VALUE	P value
SEX	4.8	<0.0001
OCCUPATION	2.8	0.0027
EDUCATIONAL STATUS*	$Z_{value(below12 and ug)}$=2.3 $Z_{value(ug and grads)}$=1.8 $Z_{value(grads and pg)}$=1.4	0.0101 0.0372 0.0803

*Key: UG = undergraduate
Grads = graduate
PG = post graduate

For the above table, if the $P_{value}$is less than 0.05, then reject null hypothesis.

Alcohol consumption has been identified as a component cause for more than 200 diseases, injuries and other health conditions with ICD-10 codes (see section 1.6.1; WHO, 1992; Rehm et al., 2009a). In spite of the negative health effects of waterpipe tobacco smoking, its use is becoming more common [5].

It has, therefore, become imperative to study the factors affecting alcohol consumption and hookah smoking.

1. The first element that plays a role in consumption of alcohol and hookah smoking is the sex of an individual.

   According to the WHO report of 2014, in Indiathe total alcohol per capita (15+) consumption, drinkers only (in litres of pure alcohol) in the year 2010:-

   Males (15+) =32.1 Females (15+) =10.6

   90% of women are lifetime abstainers of alcohol, and only 59% of males are lifetime abstainers.

   Through our study we concluded that, males consume more alcohol, hookah or both as compared to females.

   However, sex is not a highly associated factor(Yule's coefficient=0.5469). This may be due to the fact that girls are catching up with the guys in terms of consumption of alcohol [6].

   Male gender is significantly associated with hookah smoking [7].

   Our study showed that more females prefer alcohol compared to smoking hookah, and more males prefer hookah over drinking alcohol.

2. This study also showed us that majority of people start consumption of alcohol and hookah when they are in the age group of 15-20 years.

   Also, when individuals try consumption for the first time, they do not have a preference i.e., they will either consume alcohol or hookah.

3. Occupation of an individual is also an important factor. WHO report (2014) states that primary working individuals are ten times more likely to report alcohol abuse in India [4].

   This survey of youth staying in Mumbai also showed that there is more consumption in the working community as compared to the student community.

   It was also found out that students consume both alcohol and hookah equally whereas working individuals prefer alcohol consumption over hookah smoking.

4. Educational status of an individual also influences the consumption of alcohol and hookah.
   The P value for chi square test, for checking association between consumption of alcohol and hookah, is slightly less than 0.05. This shows that the association might be weak. To check association Ztest was performed.
   From our study it can be seen that higher the educational status, lower the consumption of alcohol and hookah. Consumption of alcohol, hookah or both is highest in individuals who are below 12^{th} grade as compared to undergraduates, graduates and post graduate individuals. This means that when an individual gets proper education they tend to stay away from social evils like drinking and smoking.
5. **The locality of the individual (suburb or town) does not affect the consumption.** This goes to show that the problem of alcohol consumption and hookah smoking is wide spread in Mumbai.
6. **Another reason for rise in hookah smoking could be higher parental acceptance. The parental acceptance is more for hookah smoking compared to alcohol consumption. This goes to show that parents must be unaware of the harmful side effects of hookah smoking.**
   The above discussed factors are responsible for rise in alcohol consumption and hookah smoking among youths of Mumbai.

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ASSOCIATION OF POLYCYSTIC OVARIAN SYNDROME SYMPTOMS WITH WORK HOURS OF MUMBAI WOMEN

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in women of reproductive age and despite decades of research, the etiology of the disorder is unknown. The objective of this study was to determine the effect of working hours on its symptoms and occurrence in a small cohort of women of reproductive age in Mumbai.

Keywords: Polycystic Ovarian Syndrome, PCOS, Work hours, Symptoms.

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is an endocrine disorder, which occurs due to inappropriate secretory levels of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Imbalance in the hormonal levels leads to conditions like hirsutism, infertility issues and polycystic ovaries. The Syndrome is the most common endocrine irregularity that affects 5%-20% women of reproductive age.[1] The symptoms of PCOS also include Diabetes Mellitus, Irregular menstrual cycles, acne and abnormal BMI, which in turn can cause psychosocial disruptions such as anxiety and depression. The cause of Polycystic Ovarian Syndrome is unknown but it is most commonly considered to have genetic roots. The presence of polycystic ovaries can increase the levels of testosterone in the body, which results in establishment of masculine characteristics like hyperandrogenism (excess secretion of androgens)[2]. Regular exercise, balanced diet and hormonal pills in combination are the most widely prescribed treatment for PCOS[3]. Acupuncture or ovarian drilling may be required in cases where a large number of cysts are present in the ovaries[4]. This

involves surgical drilling of the ovaries to enable removal of the cysts.

This study was designed to assess the effect of work and work related stresses upon the manifestation of PCOS Symptoms. The study

suggests that greater physical and psychological involvement in work may prove beneficial to the treatment of this disorder, since there is no definitive cure for PCOS. The findings were aimed to ascertain whether the nature of work influenced to benefit or aggravate the symptoms.

MATERIALS AND METHODS

The questionnaire included mandatory questions regarding personal details of the respondent, such as the age, BMI and marital status. It also enquired about the respondent's work-related details, dietary habits and level of physical activity.

The next set of questions included the symptoms of Polycystic Ovaries Syndrome (PCOS), the responses of which were collected in terms of Yes/No responses.

The questionnaire enquired about medical diagnosis or tests, which the respondents may have undergone. The respondents were given a choice of filling in the details of the tests or leaving the space blank.

The survey was conducted among women of reproductive age group, ranging from 12 to 48 years, residing in the Suburbs of Mumbai, South Mumbai and Navi Mumbai. The sample included women (married/unmarried) working in corporate institutes, professional and business fields as well as students and homemakers. The total number of forms received was 353, of which 19 forms were incomplete, making the sample size 334.

The collected data was subjected to statistical analysis for validating various hypotheses was carried out and the tools used were Chi-squared test, Yule's coefficient of association and Z test.

Chi-squared test:

A chi square test is a statistical test applied when two categorical attributes from a single population are available. It is used to determine whether there is a significant association between the two attributes. A hypothesis is formulated which can then be accepted or rejected based on the observed data values and expected values. The chi squared analysis was carried out with a significance level of 0.05 and degree of freedom was 1. P-values for all associations between attributes being tested were calculated and further interpretation of the result was done.

Z- test:

A z-test is a statistical tool used to test for a single population proportion or to compare two population proportions when the sample size is large. The test statistic is assumed to have a normal distribution.

It explains the statistical significance that helps decide whether or not the null hypothesis should be rejected. The p-value is the probability that you have falsely rejected the null hypothesis. Z scores are measures of standard deviation.

The Z- test is usually conducted for specific reasons, i.e. if the result obtained from the

chi squared test does not confer with the logical reasoning of the researcher.

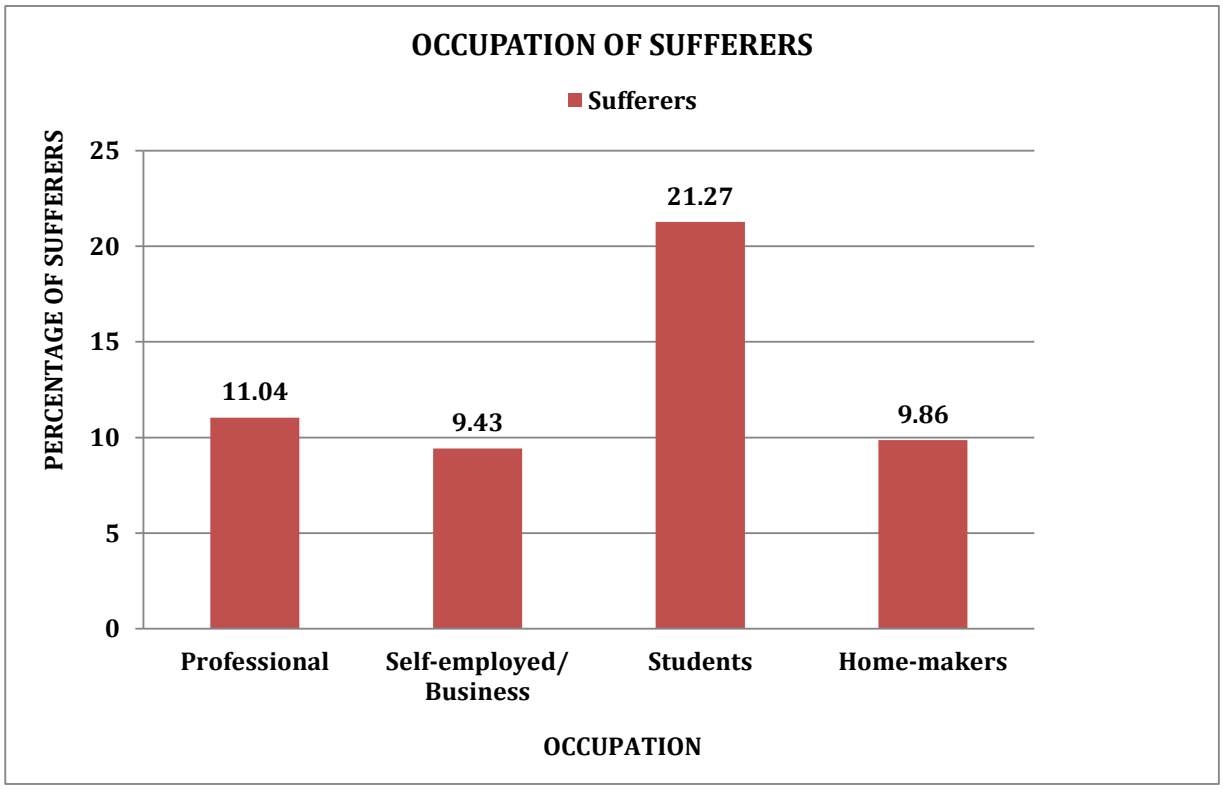
Yule's Coefficient of Association:

This test provides insight into the correlation between two attributes, their association, disassociation or complete independence. Yule's coefficient of association has the advantage of simplicity. If the attributes are independent of each other, the coefficient of association will be zero. If the attributes are perfectly or positively associated, the coefficient will be +1. If they are completely negatively associated or disassociated, the coefficient will be -1. Thus the value of coefficient of association ranges from -1 to +1.

This test gives a measure of the extent of correlation between two independent attributes, and is more reliable than the chi-squared test results.

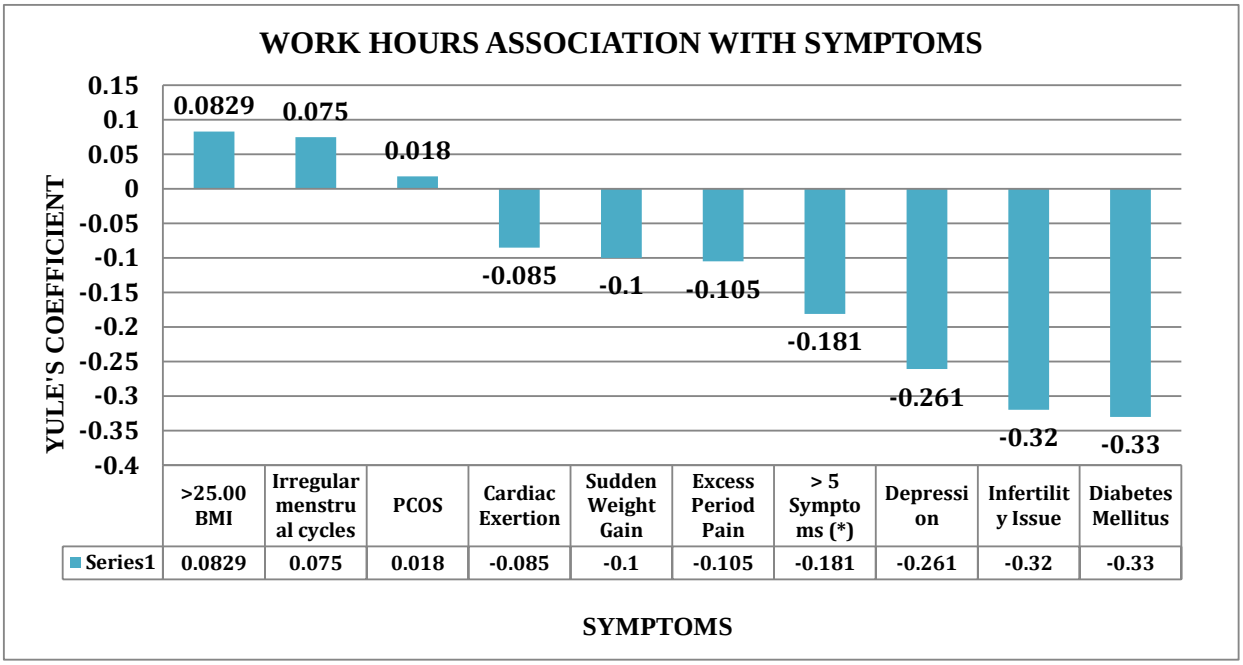
RESULT

Of the total 334 analyzed cases, 47 women reported no symptoms of PCOS included in the questionnaire and thus these cases were concluded to be the non-probable ones (14.07%). 287 women reported suffering from at least 1 symptom of PCOS and were thus concluded to be the probable cases (85.93%), of which the 40 self-reported sufferers were a part.



The above bar graph represents the occupation of PCOS sufferers, among the total sample. It can be observed that maximum percentage of sufferers is seen among the students (47). This may be attributed to hormonal imbalances because of stress or depression seen among this category. Lower prevalence of the disorder was observed in the other categories, with women from the self-employed/business field showing least prevalence

WORK HOURS



(*) Symptoms considered: Irregular menstrual cycles, Excess Period pain, Infertility Issues, Greater than normal BMI, Facial Hair, Acne, Problems in weight loss, sudden weight gain, Diabetes Mellitus.

The above bar graph represents the extent of association of PCOS symptoms with working hours of the respondents using Yule's Coefficient.

It can be observed that majority of the considered PCOS Symptoms, including greater than 5(*) show a trending negative correlation with an increase in the hours of work. This indicates that secondary diseases like depression, infertility and Diabetes Mellitus may show a decreased manifestation among

women having long working hours, which needs further investigation. Thus an active lifestyle may prove beneficial for women suffering from PCOS, as it may possibly prevent or decrease the manifestation of secondary diseases caused due to PCOD.

Table 3**: X^2statistic with respective P-values of relation between presence of symptoms of PCOS and greater than 30 hours of work per week among Mumbai women .**

H_0: There is no association between the considered symptoms of PCOS and Working hours of Mumbai women.

H_1: There is an association between the considered symptoms of PCOS and Working hours of Mumbai women.

Symptoms of PCOS	X^2statistic	P-value	Result	Yules
PCOS	0.011	0.91639	No Association	0.018
Infertility Issue	4.7165	0.0229875	Association	-0.32
Irregular menstrual cycles	0.3819	0.5365	No Association	0.075
Excess Period Pain	0.8082	0.3686	No Association	-0.105
Diabetes Mellitus	3.2385	0.7192	No Association	-0.33
Depression	5.267	0.02173	Association	-0.261
Cardiac Exertion	0.4218	0.5160	No Association	-0.085
Sudden Weight Gain	0.5632	0.4529	No Association	-0.10
>25.00 BMI	0.5887	0.4429	No Association	0.0829
>5 Symptoms of PCOS(*)	10.899	0.00096	Association	-0.181

(*) Symptoms considered: Irregular menstrual cycles, Excess Period pain, Infertility Issues, Greater than normal BMI, Facial Hair, Acne, Problems in weight loss, Sudden weight gain, Diabetes mellitus.

The Chi-square test showed an association can be observed between more than 30 hours of work per week and presence of infertility, depression and occurrence of greater than 5 of mentioned symptoms with P-value lesser than 0.05 at 5% level of significance.

As the rest of the symptoms show P-value > 0.05, no association can be found between them and working for greater than 30 hours per week.

By Yule's Coefficient of Association, more than 30 hours of work per week shows a very weak positive association with occurrence of PCOS. Thus, more the hours of work, more may the occurrence of PCOS. As for the symptoms Irregular Menstrual Cycles and Greater than 25.00 BMI, a very weak positive correlation

with more than 30 hours of work per week can be observed, may indicate that more the hours of work, more the presence of these symptoms.

Very weak negative correlation detected by Yule's Coefficient test can be observed for symptoms including Infertility Issue, Excess Period Pain, Diabetes Mellitus, Depression, Cardiac Stress, Sudden Weight Gain and Greater than 5 tabulated symptoms. This indicates that with more than 30 hours per week, the manifestation of these symptoms may decrease.

Table 4:**Z statistic with respective P-values for testing the mentioned hypotheses between symptoms of PCOS and greater than 30 hours of work per week among Mumbai women.**

H_0: The proportion of women showing symptoms of PCOS and working for more than 30 hours per week is the same as the proportion of women showing the symptoms and working for less than 30 hours per week.

H_1: The proportion of women showing symptoms of PCOS and working for more than 30 hours per week is lesser than the proportion of women showing the symptoms and working for less than 30 hours per week.

Symptoms of PCOS	Z statistic	|Z statistic|	P-value	Result
PCOS	0.105	0.105	0.46017	H_0 not rejected
Infertility Issue	-2.1717	2.1717	0.015	H_0 rejected
Irregular menstrual cycles	0.618	0.618	0.26763	H_0 not rejected
Excess Period Pain	-0.899	0.899	0.18406	H_0 not rejected
Diabetes Mellitus	-1.7996	1.7996	0.03593	H_0 rejected
Depression	-2.295	2.295	0.01101	H_0 rejected
Cardiac Exertion	-0.6495	0.6495	0.25785	H_0 not rejected
Sudden Weight Gain	-0.7505	0.7505	0.22663	H_0 not rejected
>25.00 BMI	0.6888	0.6888	0.2451	H_0 not rejected
>5 Symptoms of PCOS(*)	-3.3015	3.3015	0.00048	H_0 rejected

(*) Symptoms considered: Irregular menstrual cycles, Excess Period pain, Infertility Issues, Greater than normal BMI, Facial Hair, Acne, Problems in weight loss, Sudden weight gain, Diabetes Mellitus.

In the cases of infertility, Diabetes Mellitus, depression and presence of greater than 5 tabulated symptoms, the null hypothesis (H_0) can be rejected (P-value<0.05) at 5% Level of Significance. This indicates that the manifestation of these symptoms can be correlated with the number of working hours.

The rest of the symptoms do not show such a relation as the P-value>0.05. Thus the null hypothesis (H_0) cannot be rejected for symptoms like Irregular Menstrual Cycles, Excess Menstrual pain, Cardiac Exertion, Sudden Weight gain and Greater than normal BMI. This indicates that the manifestation of these symptoms of PCOS is completely independent of their working hours.

DISCUSSION

The chi-squared test and the Ƶ test were used to estimate the presence of an association between the tabulated symptoms of PCOS and working hours of Mumbai women. Both these tests detected that symptoms like Depression, Infertility and presence of Greater than 5 tabulated symptoms show a distinct Association with more than 30 hours per week.

The chi-squared test detected the symptom Diabetes Mellitus as not correlating with the hours of work. The Ƶ test also showed this parameter as having no correlation with the number of working hours, when a two-tailed result was considered (as P-value>0.05 for two-tailed hypothesis).However, no association could be detected between the rest of the symptoms considered and the hours of work.

The Yule's test detected a low positive correlation for symptoms like Irregular menstrual cycles, greater than normal BMI as well as PCOS itself. This indicates that these parameters may show slight increase in manifestation with increased Work hours. A negative correlation was observed between the rest of the tabulated symptoms and greater than 30 work hours per week. Thus the cohort shows a decreased manifestation of majority of the PCOS symptoms when they have longer working hours.

CONCLUSION

The respondent population belonged to the reproductive age group of 12-48 years. The maximum respondents belonged to the professional field. The category which included highest number of sufferers was the student's category, with 21.27% of them suffering from the disorder. Women belonging to the business or self-employed category included the lowest number of sufferers. This indicates that younger women are more prone to hormonal imbalances, thus may show greater manifestation of the symptoms.

Our results indicate that greater involvement in work, either physical or mental, can help reduce the severity of the various symptoms of PCOS. According to the Yule's test, depression shows a negative correlation with greater hours of work, thus indicating that work involvement can develop a

positive mindset among the concerned population.

The progress made in the study of Polycystic Ovarian Syndrome and Polycystic Ovarian Disorder can be majorly credited to the survey data made available from different countries and due to paucity in the data available in the Indian subcontinent, it is necessary to put forth more such data for comparative analysis. This study was a small attempt in this direction.

More precise results could possibly be obtained if a greater sample size is used for analysis.

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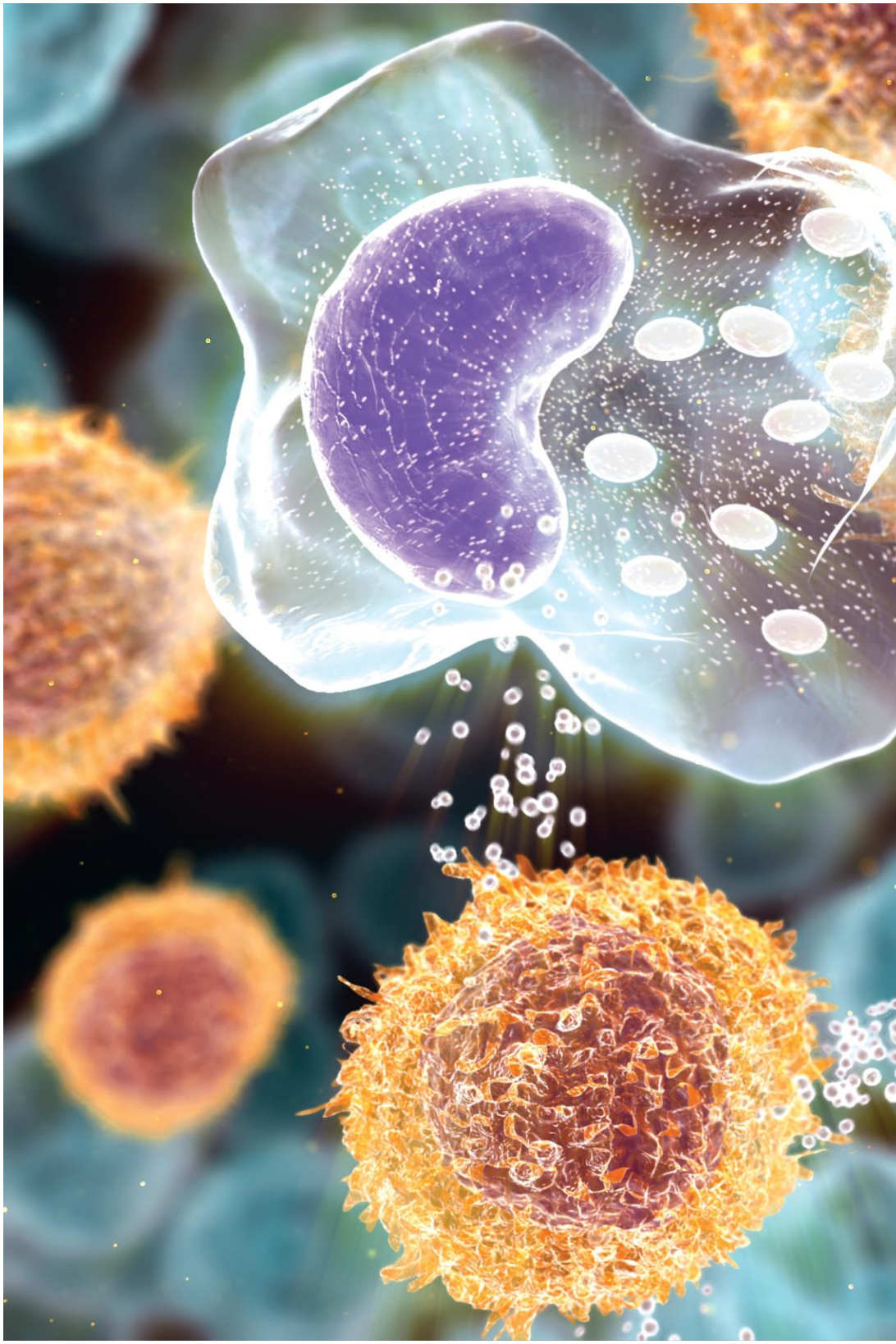
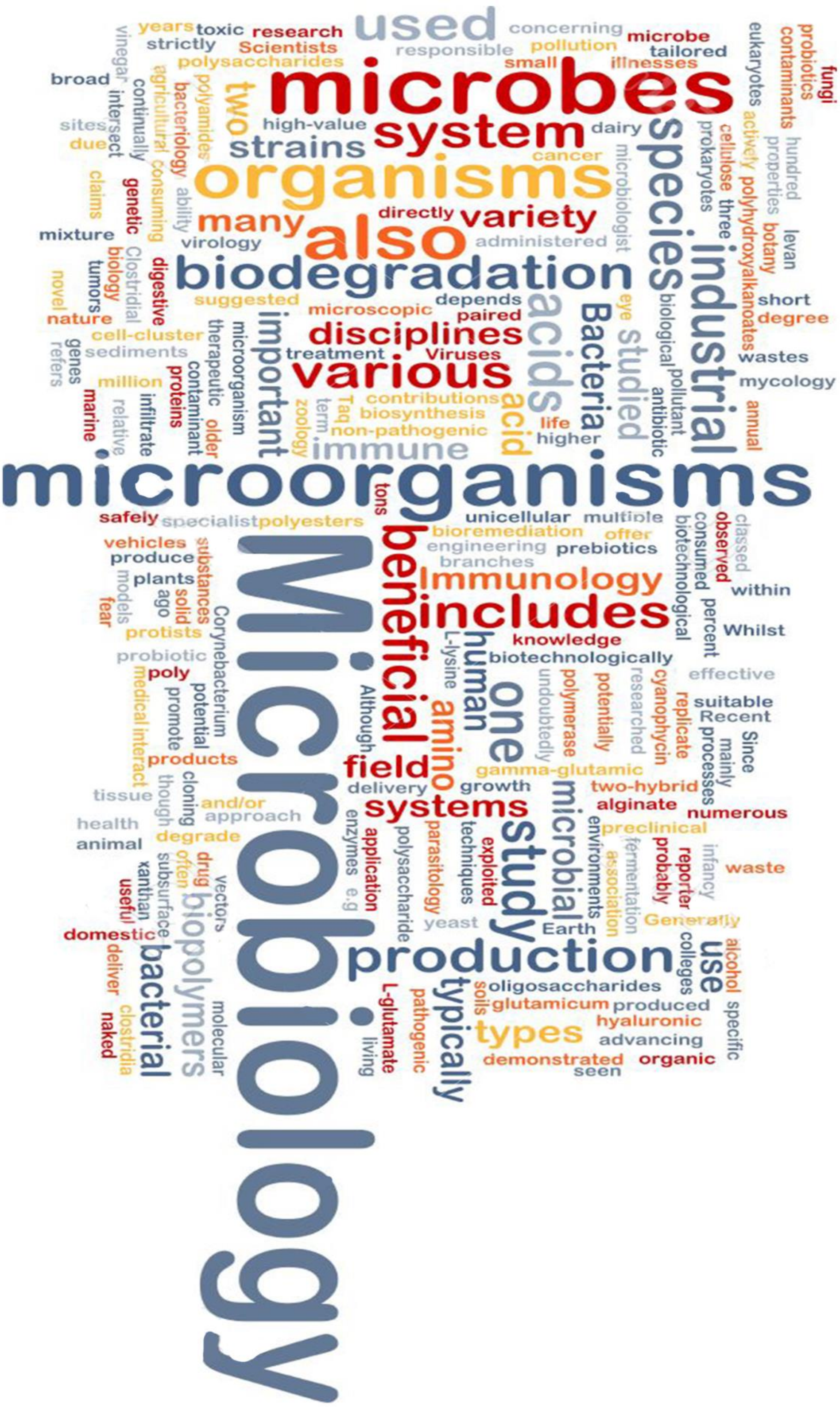
MICROBIOLOGY

In microbiology the roles of mutation and selection in evolution are coming to be better understood through the use of bacterial cultures of mutant strains.

EDWARD LAWRIE TATUM

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DETECTION OF DRUG RESISTANT ORGANISMS FROM NATURAL WATER BODIES.

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ABSTRACT

Antibiotic resistance of microorganisms 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. Antibiotic Resistant organisms were isolated and identified from natural water bodies. A total of 13 isolates were identified using conventional biochemical and Vitek method. Antibiotic susceptibility testing of all the isolates was performed using disc diffusion method. Most of the isolated organisms were found to be resistant to multiple antibiotics.

Keywords: Antibiotic Resistant organisms, natural water bodies, Plasmid coded resistance.

INTRODUCTION

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. [1]

Water bodies harbor various organisms such *Escherichia coli, Enterobacter spp., Klebsiella spp., Salmonella spp., Pseudomonas aeruginosa, Vibrio parahemolyticus,Aeromonashydrophila*[2]. Natural water bodies get contaminated due to the discharge of sewage containing human and animal faecal matter. This faecal matter contains a mixture of pathogenic and non-pathogenic organisms. Antibiotic resistant organisms have been found in natural water bodies which is a major health concern. Antibiotic resistance occurs when bacteria undergo a genetic change that reduces or eliminates the effectiveness of the drugs or other agents designed to cure or prevent infection. Resistant bacterial infections have inevitably followed the widespread use of every new antibiotic introduced. [2] Non-judicious use of antibiotics in animal feeds to boost growth,

improper dosages of antibiotics are the two most common reasons for the emergence of antibiotic resistant bacteria and these bacteria often follow the route of sewage into the natural water resources. Antibioticsresiduesand antibiotic resistant organisms as environmental pollutants have largely been overlooked. Studies have demonstrated that hospital waste water is highly selective environments and that they contribute to high rates of resistant bacteria that are being discharged in the natural environment.

Many of the genes coding for antibiotic resistance have been found on extrachromosomal DNA like plasmids which could serve as vectors for horizontal transfer of these genes.Higher numbers of resistant bacteria occur in polluted habitats, indicating that humans have contributed substantially to the increased proportion of resistant bacteria occurring in the environment. [3]

MATERIALS AND METHODS

***Collection Of Water Samples*:** Clean plastic bottles were used forwatersample 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 4^0C.

***Enumeration Of Bacterial Population By Standard Plate Count Method*:** Bacterial population was determined using standard plate count or viable count method. 0.1ml of 10^{-3} to 10^{-10}dilution series was plated on Sterile Nutrient Agar plate using an alcohol-sterilized glass spreader and was incubated at 37^0c for 24-48 hrsin triplicate.

Identification Of Bacterial Isolates–Cultural, Morphological Characters:Sub-culturing 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. [5]

***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. 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^{10}), Chloramphenicol(C^{30}), Rifampicin (Rif^{5}), Nitrofurantoin(Nit^{100}), Tetracycline (Te^{30}), Ticarcillin(Ti^{75}), Oxacillin(Ox^{1}), Erythromycin (E^{15}), Vancomycin (Va^{30}). Imipenem (Imp^{10}), Penicillin G (P^{10}), Piperacillin (Pi^{100}). Carbenicillin (Cb^{100}), Amoxyclav (Amc^{30}), Ticarcillin (Ti^{35}), Amoxycillin (Amx^{30})., Cefotaxime (Ctx^{30}), Aztreonam ($At30$) Ceftriaxone (Cf^{30}), Amoxyclav (Amc^{30}), Tobramycin (Tob^{10}), Gentamycin (Gen^{10})Clindamycin (Cd^{20}). Methicillin (Met^{5}).

RESULTS AND DISCUSSION

Viable count of all the water samples was carried out. Table 1 shows the results of the count.

Table 1- Viable Count:

SAMPLE NO.	SOURCE	VIABLE COUNT (Cfu/ml)
1.	RAMWADI (VIRAR)	1.29 x 10^6cfu/ml
2.	PIMPLEWADI (VIRAR)	8.70 x 10^3cfu/ml
3.	MUMBAI CENTRAL	1.75 x 10^9cfu/ml
4.	MARINE LINES	2.45 x 10^6cfu/ml
5.	VILE PARLE	3.45 x 10^6cfu/ml
6.	VAITARNA RIVER	2.10 x 10^6cfu/ml
7.	TANSA RIVER	3.09 x10^5cfu/ml

As observed in the table 1, all the water samples showed a very high bacterial contamination. All these water sources were used as potable though the water is highly unfit for consumption.

Water sample was also streaked on selective media to isolate and then to carry out the identification of the organisms. Thirteen isolates showing varied cultural characteristics were selected to be processed further. Table 2 shows the results of identification of the organisms.

Table 2 - Identification of isolates by Vitek 2 compact

SAMPLE	NO. OF ISOLATES OBTAINED	ISOLATED ORGANISMS BY VITEK 2 COMPACT
1.	2 (ISOLATE-1,2)	1: Klebsiella pneumonia *2: Acinetobacter* lwoffi
2.	2 (ISOLATE 3,4)	*3 & 4 :* Strains *Of Aeromonassobria*
3.	4 (ISOLATE5,6,7 ,8)	5,6,7 & 8: Strains Of *Ochrobactrumanthropic*
4.	1 (ISOLATE 9)	9: *Stenotrophomonasmaltophilia*
5.	1 (ISOLATE 10)	10: *Stenotrophomonasmaltophilia*
6.	2 (ISOLATE 11,12)	11: *Brevundimonasdiminuta/vesicularis* 12: *Enterobacter cloacae complex*
7.	1 (ISOLATE 13)	13: *Pseudomonas putida*

The characteristics of each organisms are as follows-

- *Klebsiella pnemoniae* is a gram negative, non-motile, facultatively anaerobic organism, causative agent of nosocomial and community acquired infections. It causes pneumonia, UTI, septicemia and

rarely diarrhea. *Klebsiella pneumoniae* are resistant to a wide range of antibiotics. Of particular concern is the Extended Spectrum Beta Lactamase (ESBL) producing strains *of Klebsiellapnemoniae.*[10]

- *Acinetobacter lwoffi* –. It is a gram negative, non-fermentative, bacillus. It can cause infections in human hosts, particularly catheter-associated infections in immuno-compromised patients [11].

- *Aeromonassobria* – They are water borne organisms that have been implicated repeatedly as the causative agents of clinical illnesses, often serious, ranging from gastrointestinal and wound infections to septicemia[13].Sometimes it is a lactose non-fermenter and when grown on MacConkey's Agar can ferment lactose and gives a negative oxidase test.

- *Ochrobactrumanthropi* – It is an oxidase-positive, lactose non-fermenting, gram negative bacillus that is an emerging pathogen in immunocompromised patients, particularly in those with dwelling central venous catheters.[14]

- *Stenotrophomonasmaltophilia*– It is an aerobic, lactose non-fermenting, motile, Gram-negative bacteria.It is becoming a relevant opportunistic pathogen causing bacteremia, pneumonia and intra-abdominal and muco-cutaneous infections. The treatment of infections can become cumbersome because the pathogen harbours an intrinsic resistance to several classes of antibiotics, through beta-lactamase production, drug efflux pumps and decreased permeability. [15]

- *Enterobacter cloacae complex*- It is a rod shaped, gram-negative, oxidase negative but catalase positivefacultatively anaerobic bacteria from the Enterobacteriaceae family. *Enterobacter cloacae* are nosocomial pathogens that can cause a range of infections such as bacteremia, lower respiratory tract infections, skin and soft tissue infections, urinary tract infections, endocarditis, intra-abdominal infection, septic arthritis, osteomyelitis and ophthalmic infections. These bacteria contain beta-lactamase, which is undetectable in-vitro and is highly resistant to antibiotics such as third generation cephalosporins. [16,17]

- *Brevundimonasdiminuta* – It is a lactose non-fermenting, gram negative environmental bacilli. *Brevundimonasdiminuta* and *Brevundimonasvesicularis* were previously assigned to genus *Pseudomonas*. *B.diminuta* is commonly used as a test organism for validation of sterilizing grade

membrane filters due to small size of the bacterium.[18,19]

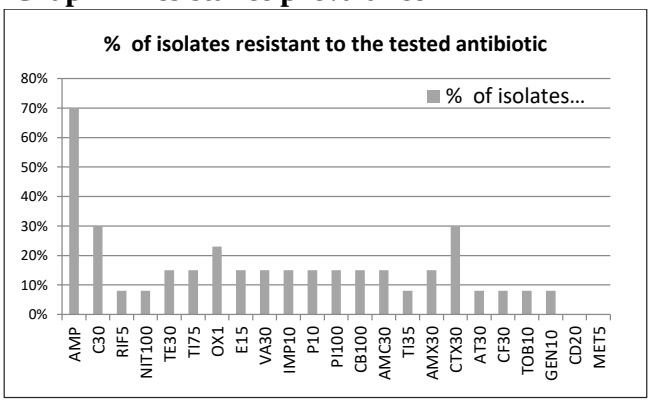
- *Pseudomonas putida* – It was considered a low virulence pathogen and was recognized as a rare cause of bacteremia. Recently, however multi-drug resistant and carbapenem resistant *P.putida* isolates have emerged, causing difficulty in treating nosocomial infections in seriously ill patients. Nosocomial *P.putida* bacteremia showed high resistant rates to most potent beta-lactams and carbapenems and was associated with high mortality rates.

The antibiotic resistance pattern was studied using disc diffusion method . Table 3 shows the antibiogram of the thirteen isolates.

Table 3 - Antibiotic resistance pattern of bacterial isolates from water samples.

Sr. No.	Organism	Resistance pattern
1.	*Klebsiella pneumoniae*	AMP^{10}, TE^{30}, TI^{75}, OX^{1}, E^{15}, VA^{30}.
2.	*Acinetobacter lwoffii*	AMP^{10},CTX^{30}, OX^{1}, VA^{30}.
3.	*Aeromonassobria(STRAIN 1)*	CB^{100}.
4.	*Aeromonassobria(STRAIN 2)*	IMP^{10},AMP^{10}, P^{10}, PI^{100}.
5.	*Ochrobactru manthropi(STRAIN 1)*	CB^{100}, AMP^{10}, PI^{100}.
6.	*Ochrobactru manthropi (STRAIN 2)*	(AMC^{30}), (TI^{35}), (AMX^{30}).
7.	*Ochrobactru manthropi (STRAIN 3)*	(AMP^{10}), (C^{30}), (CTX^{30}), (AT^{30}), (CF^{30}),(AMC^{30}), (AMX^{30}), (TOB^{10}), (P^{10}).
8.	*Ochrobactru manthropi (STRAIN 4)*	(TE^{30}), (AMP^{10}), (CTX^{30}), (GEN^{10}).
9.	*Stenotrophomonasmaltophilia(STRAIN 1)*	(AMP^{10}), (TI^{75}), (OX^{1}), (E^{15}).
10.	*Stenotrophomonasmaltophilia(STRAIN 2)*	–
11.	*Brevundimonasdiminuta/vesicularis*	(IMP^{10}), (CTX^{30}), (PI^{100}), (CD^{20}).
12.	*Enterobacter cloacae complex*	(AMP^{10}), (C^{30}), (MET^{5}), (CD^{20}).
13.	*Pseudomonas putida*	(AMP^{10}), (C^{30}), (RIF^{5}), (NIT^{100})

Graph 1- resistance prevalance



The observed high frequency of bacterial resistance as seen in graph 1, may not only result in the therapeutic failure, but also endanger the health of the people who are at

risk of infection with these pathogens. There is also a possibility of plasmid transfer of antibiotic resistance to human pathogenic bacteria.

The number of viable organisms found varied from sample to sample owing to the diverse conditions of the locations where the water bodies are situated.

The organisms studied were generally gram negative bacteria. The organisms were found to be resistant to beta lactam antibiotics like ampicillin and other widely used antibiotics. Some were also found to be resistant to multiple drugs. 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. Some strains also showed resistance towards third and fourth generation antibiotics. Such 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 threat is highly magnified by the fact that these organisms are found in natural water bodies that are being continuously used by people for various activities and purposes, many-a-times without any further effective antimicrobial treatment.

Further studies were done on the resistant and intermediate resistant strains of organisms to check whether the resistance conferred upon the organisms was plasmid borne or chromosomal. Many of them showed plasmid borne antibiotic resistance. This can further compound the problem of antibiotic resistance due to the horizontal intra- and inter-genus transfer of plasmids.

CONCLUSION

MultipleAntibiotic resistant bacteria were found in all the water samples. It must also be made sure that usage of waters from water bodies suspected of harboring such organisms is avoided as much as possible and if used, is properly treated to destroy the potential antibiotic resistant pathogens present.

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ANTIBACTERIAL ACTIVITY OF *TINOSPORA CORDIFOLIA&CINNAMON*

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ABSTRACT

The stem of *Tinospora cordifolia* has been used in ayurvedic preparations to treat general debility, fever and urinary diseases. Cinnamon is a spice that has been used for treatment of conditions like arthritis, coughing, and sore throat. This study aims to study antimicrobial activity of *Tinospora cordifolia* and Cinnamon. The screening of antibacterial activity of chloroform, methanolic and ethanolic extracts of T.cordifolia and Cinnamon was performed by agar well diffusion method against *E.coli, S pyogenes, S.typhi, P.aeruginosa, Shigella, Salmonella para typhi b* and *B. Subtilis*.

Amongst the three extracts, the methanolic extract of *T.cordifolia* and Cinnamon was found to be most effective against the selected strains. The average zone of inhibition was recorded as 15.5$\pm$ 3.3 mm and 12.3 $\pm$2.4 mm, for methanolic extract of Cinnamon and *T.cordifolia*, respectively, using 100% concentration of the extracts. The MIC was found to be in the range of 25-50 mg/ml for both the herbal extracts and synergistic association was established against the selected organisms. Gram positive and Gram negative organisms were inhibited by both the herbs, indicating a broad spectrum activity of the extracts. The synergism between *T.cordifolia* and Cinnamon indicated that they can be used together for various therapeutic purposes, subject to in-vivo studies.

Keywords: *Tinospora cordifolia*, Cinnamon, MIC, Synergistic activity.

INTRODUCTION

Tinosporacordifolia, commonly known as Guduchi, is the species of Menispermaceaefamily. It is a common climbing shrub found in tropical deciduous forest of south Indian peninsular plains. It is a large, climbing shrub with several elongated twining branches.
The seeds are curved and it is considered to be a unique character. Fruits are fleshy and single
seeded.
It is also propagated by stem nodal cuttings. The leaves are membranous and chordate. The flowers are small and yellow or greenish yellow. The bark is creamy white to grey, deeply left spirally, the space in between being spotted with large rosette-like lenticels. The stem is soft wooded, dry, cylindrical and 5 mm to 25 mm in diameter.
Guduchi is used in Ayurvedic rasayans to improve the immune system, memory and mental intelligence. (1).
T. cordifolia is mentioned in Ayurvedic literature as a constituent of several compound formulations used in general debility, dyspepsia, fever and urinary diseases. It is considered as a bitter tonic, astringent, diuretic and potent aphrodisiac and curative against skin infections, jaundice, diabetes and chronic diarrhoea and dysentery (2)
Stem is a bitter stomachic; stimulates bile secretion; causes constipation; tonic; allays thirst, fever, burning sensation, prevents vomiting; diuretic; enriches the blood; cures jaundice; useful in skin diseases; the juice is useful in diabetes, vaginal and urethral discharges, low fevers, and enlarged spleen (Ayurveda). The roots and stems are prescribed in combination with other drugs as an antidote to snake bite and scorpion sting.

Cinnamon is a spice obtained from the inner bark of several tree species from the genus Cinnamomum. Cinnamon is used in both sweet and savoury foods. The term "cinnamon" also refers to its mid-brown colour. Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products (2). The most important constituents of cinnamon are cinnamaldehyde and trans-cinnamaldehyde (Cin), which are present in the essential oil, thus contributing to the fragrance and to the various biological activities observed with cinnamon (3).
Cinnamon bark contains procyanidins and catechins. The components of procyanidins include both procyanidin A-type and B-type linkages. These procyanidins extracted from cinnamon and berries also possess antioxidant activities. (4)
Cinnamon is used as a spice and aromatic. Traditionally, the bark or oil has been used to combat microorganisms, diarrheal, and other Gastrointestinal disorders, and dysmenorrhoea, although there is limited data to support these uses. Research has focused on anti-inflammatory, antioxidant, and antimicrobial activity. Cinnamon extracts have been shown to exert in-vitro activity against some common human pathogens, as well as fungicidal activity against plant pathogens. (6)

The aim of the study was to study the antibacterial activity of Guduchi(Tinospora cordifolia) and Cinnamon against laboratory strains, individually and combined.

MATERIALS & METHODOLOGY

Bacterial cultures used:

Cultures used were lab isolates namely *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Shigella*

spp, Bacillus spp, Salmonella para B. Suspensions of these bacterial isolates were made in sterile saline &density was adjusted to 0.5 McFarland.(8)

Preparation of Cinnamon extract:

Dried, fresh sticks of cinnamon spice were purchase from the market. The cinnamon sticks were then washed with distilled water & dried in shade. They were then homogenized in a mixer.

15g of this dried powder was extracted in 3 solvents namely ethanol, methanol and chloroform using Soxhlet method.

The solvent was evaporated and remaining residue was then weighed and mixed in 20% DMSO (Dimethyl Sulfoxide) to get a concentration of 250mg/ml. This extract was considered as 100% concentration. Desired concentrations were prepared (v/v %) by diluting the concentrated extract with appropriate volume of 20% DMSO.

Preparation of Guduchi extract:

Fresh Guduchi stem was collected from a rural area of Satara, Maharashtra. The stems were washed with distilled water and dried in shade.

They were further homogenized in a grinder and extracted by the aforementioned process. (7)A concentration of 200mg/ml was prepared.

Sterility of both the extracts was checked by their spot inoculation on sterile nutrient agar plate and no growth was observed after 24 hrs of incubation.

DETERMINATION OF ANTIBACTERIAL ACTIVITY OF CINNAMON AND GUDUCHI

Agar Well Diffusion Method:

20ml of molten Nutrient Agar media was bulk seeded with 0.3ml of the bacterial culture of 0.5 McFarland standard, and aseptically poured in a sterile plate. The plates were then allowed to set. Wells were punched into the cooled agar plate with the help of surface sterilized cork borer 6 mm in diameter and extracts were added to the wells accordingly. The plates were then incubated at 37 ^{0}C for 24 hours. (1) 20% DMSO was kept as control zone of inhibitions were measured accordingly.

Further the 100% extract (200mg/ml) was diluted to different concentrations as 50mg/ml, 100mg/ml, and 150mg/ml i.e. 25%, 50%, 75% & 100% and were checked for antibacterial activity against the selected strains.

Determination of Minimum Inhibitory Concentration.

The minimum inhibitory concentration (MIC) of cinnamon and Guduchi was determined for organisms by agar dilution method. (8)

Standard stocks of 100% concentration of cinnamon and Guduchi were prepared. Range (6.25mg/ml-50.5mg/ml) for cinnamon and Guduchi were prepared from standard stock using sterile molten nutrient agar as diluents.

This was then poured in sterile plate and allowed to set for 24 hrs old cultures of (organisms) of 0.5 McFarland's were spot inoculated on the plate. Nutrient agar lacking extract was kept as positive control. The plates were incubated at 37^{0}C for 24 hrs. The lowest concentration of the extract that produced no visible bacterial growth on the plate was the MIC. The results were recorded.

Synergistic activity of Cinnamon and Guduchi:

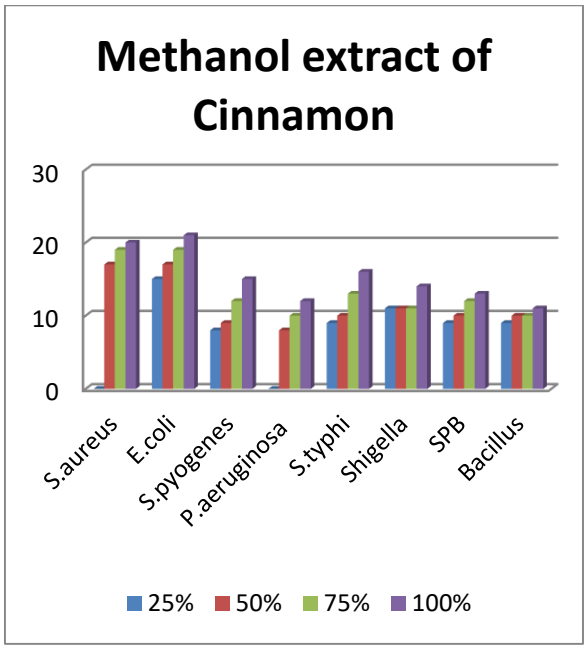
For synergism, agar well diffusion performed.All the 4 concentrations (i.e. 25%, 50%, 75% and 100%) of the extract of Guduchi and cinnamon were mixed and the extracts were added to the agar wells and the activity was checked against individual test organisms and the results were recorded.

The culture density was 0.5 McFarland's standard. The plates were incubated at 37^0C for 24hrs and all the 3 extracts namely Guduchi in and cinnamon in ethanol, methanol & chloroform were checked.

RESULTS AND DISCUSSION

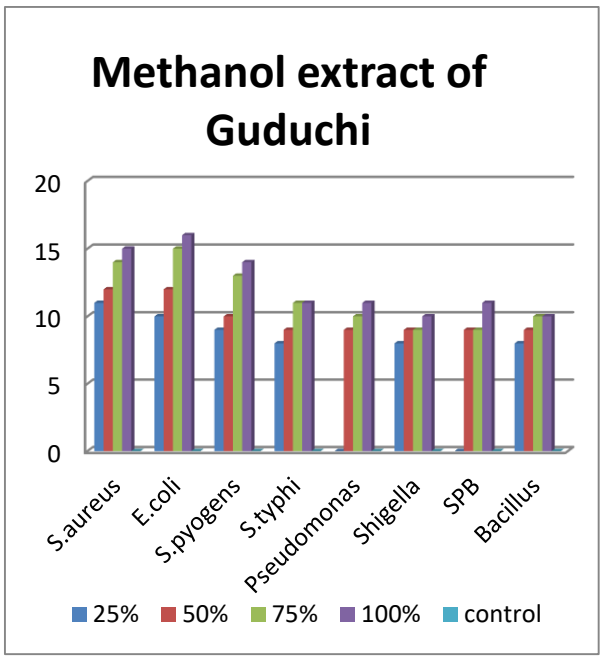
From the test organisms, methanol extract of Cinnamon at 100% has showed best activity against *E. coli* (21mm) as compared to *Bacillus* (11 mm) in agar well diffusion method. All organisms except *S. aureus* showed inhibition at 25% concentration. The activity of 100% Cinnamon extract in methanol was maximum for *E.coli* (21mm) followed by *S. aureus* (20mm), *S. typhi* (16mm), *S. pyogenes* (15mm), *Shigella* (14mm), *Salmonella para B* (13mm), *Pseudomonas aeruginosa* (12mm) and *Bacillus* (11mm). Methanol and ethanol extracts of Cinnamon were more effective as compared to chloroform extract for all test organisms.

Figure 1 – Antibacterial activity of cinnamon by Agar well diffusion method.



For Guduchi, the methanol extract at 100% has showed best activity against E. coli (16mm) as compared to *Bacillus* and*Shigella* (10 mm) in agar well diffusion method. All organisms except *P. aeruginosa* and *Salmonella para B* showed inhibition at 25% concentration. The activity of 100% Guduchi extract in ethanol was maximum for *E.coli* (16mm) followed by *S.aureus (*15mm), *S. pyogenes* (14mm), and *S. typhi*, *P. aeruginosa* and *Salmonella para B* showed 11 mm zone size. Methanol extracts of Guduchiwas more effective as compared to chloroform and ethanol extract for all test organisms and similar results were obtained by Mukhtar and Ghori et al.

Figure 2 - Antibacterial activity of Guduchi by Agar well diffusion method.



Concentration (mg/ml)	*E. coli*	*S.aureus*	*S.typhi*	*P.aeruginosa*	*SPB*	*Shigella*
6.25	+	+	+	+	+	+
12.5	+	+	+	+	+	+
25.5	+	+	+	+	+	-
50.5	-	-	-	-	-	-

MIC of both the extract was 50 mg/ml for *E. coli*, *S.aureus. S. typhi*, *Salmonella para B*, *S. pyogenes* and *P. aeruginosa* and for Shigella it was 25 mg/ml.

Table.1- MIC of Cinnamon by Agar well diffusion method.

Table.2- MIC of Guduchi by Agar well diffusion method.

Concentration (mg/ml)	*E. coli*	*S.aureus*	*S.typhi*	*P.aeruginosa*	*SPB*	*Shigella*
6.25	+	+	+	+	+	+
12.5	+	+	+	+	+	+
25.5	+	+	+	+	+	-
50.5	-	-	-	-	-	-

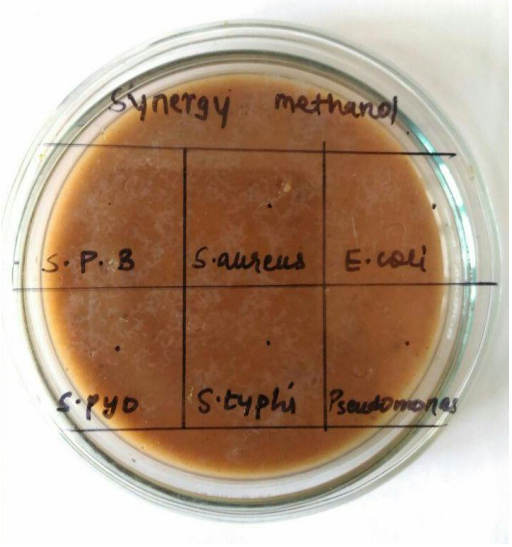
CONCLUSION

Both gram positive and gram negative organisms were inhibited by Cinnamon and Guduchi extracts indicating the broad-spectrum activity of the extracts hence, proving the potency of Cinnamon and Guduchi as good antibacterial agents against bacteria. Significant synergistic activity of Guduchi and Cinnamon against

the lab isolates was recorded in this study. As nowadays bacteria are becoming resistance to antibiotics, Cinnamon and Guduchi in lower concentration can be used as alternative sources of medicines. They can serve as cheap and alternative source of medicines.

Synergistic activity of Cinnamon and Guduchi was checked by agar dilution method and Agar-well diffusion method by combining sub inhibitory concentrations of each extracts (i.e. ¼th concentration of methanol extract of Cinnamon and Guduchi). No growth of all test organisms at sub inhibitory concentration of both extracts proved the synergism.

Figure.3 Synergy by agar dilution method



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COMPUTER SCIENCE

Software is a great combination between artistry and engineering

BILL GATES


Process
Programming
Control
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Information
Stochastic
Machine
Network
Constraint
Networks
Modelling
Methods
Spaces
Learning
Resource
Complexity
Problems
Computational
Techniques
Ontology
New
Automated
Analysis
Software
Algorithms
Engineering
Optimisation
Model
Flow
Foundations
Applications
Systems
Logic
Embedded
Constraints
Efficient
Checking
Models
Support
Reasoning
Design
Theory
Data
Inference
Verification
Multicore
Refinement
Games
Search
Neural

AN APPROACH FOR DESIGN AND DEVELOPMENT OF A MOBILE APPLICATION FOR PROJECTING IMAGES USING HOLOGRAM

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ABSTRACT

Objective: A holographic image can be generated by digitally computing a holographic pattern and displaying onto a transparent acrylic sheet film for illumination by suitable coherent light source. Virtual image is projected using by Hologram reflection through handheld device and the suitable method is selected for reflecting the image. Holography records the scene through an area of perspective. The viewing window of a hologram can be broken up to record many different perspectives and can be used to capture animation and create a holographic scene from a sequence of two dimensional images.

Methods: In this paper, an image is fetched from gallery and displaying it in three or four in different rotation angles. Acrylic sheet used for experimental purpose is 2.0mm thick and application produces images in different rotation angles. Algorithms are tested and are designed on Android Studio 2.1.2 on x64-based processor.

Results: No overlapping of images is found in this algorithm except if Relative and Frame Layout are used. Hologram cannot be produced large virtual image if the screen display is smaller.

Conclusion: It is an independent of platform size application. Algorithms are successfully worked on different screen display size and density. The codes are tested and design for Android Mobile Operating System.

Keywords: Hologram, Acrylic sheet, java, Android Studio.

INTRODUCTION

Nowadays, In Mobile Application, Reflection Hologram gives Holographic effect chromatic photo with 3-D display. Three-dimensional holographic recording of images is one of the most spectacular applications of the phenomena interference and diffraction of light. The idea that one can record on a photographic film the interference pattern obtained from combining coherent light of a reference beam with the light reflected from an object and that this pattern then carries the complete (3-dimensional) visual information for the object occurred for the first time to Dennis Gabor in 1947. The hologram itself is a recording on a photographic plate of the interference pattern obtained from combining the laser light reflected from the object with the light of a beam coming directly from the same laser (this is called reference beam, see Fig.1).

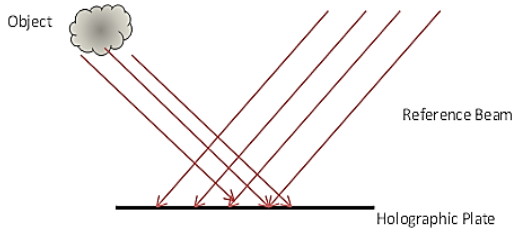


Figure 1: Principal of Transmission Holography

Nowadays, In Mobile Application we use 3-D stereoscopic display as Object, Reference Beam as Air and Holographic Plate as Acrylic Sheet for Reflecting Real Images in air as shown in Fig 2.

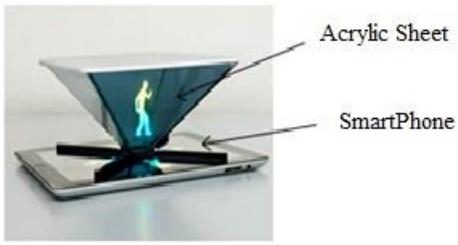


Figure 2: Projecting Image In air through smartphone display

METHODOLOGY

Reflection Hologram is new hologram technique in field of computer science to view in chromatic light that associates with a collection of some images. The lookup of reflection hologram is based on platform size in terms of diagonal and number of virtual images form by real image.

For Case I: (Displaying Hologram Four Times)

```
final ArrayList<ImageItem> imageItems = new ArrayList<>();
try {
   imageURI = Uri.parse(getIntent().getStringExtra("img"));
ParcelFileDescriptor parcelFileDescriptor = getContentResolver().openFileDescriptor(imageURI, "r");
   FileDescriptor fileDescriptor = null;
   if (parcelFileDescriptor != null) {
      fileDescriptor = parcelFileDescriptor.getFileDescriptor();
     bitmap = BitmapFactory.decodeFileDescriptor(fileDescriptor);
      parcelFileDescriptor.close();
   }
}
catch (Exception e)
{
   e.printStackTrace();
}
if (diagonal > d1 && diagonal <= d2) {
   for (int i = 0; i <= 7; i++) {
         if (i == 1 || i == 3 || i == 5 || i == 7)
{
            if (i == 1) {
               matrix = new Matrix();
               matrix.postRotate(0);
            }
            if (i == 3) {
               matrix = new Matrix();
               matrix.postRotate(90);
            }
            ……..// Image to be added
            scaledBitmap = Bitmap.createScaledBitmap(bitmap, 100, 100, true);
            bmp = Bitmap.createBitmap(scaledBitmap, 0, 0, scaledBitmap.getWidth(), scaledBitmap.getHeight(), matrix, true);
         }
if (i == 0 || i == 2 || i == 4 || i == 6) {
            Bitmap.Config conf = Bitmap.Config.ARGB_8888; // see other conf types
bmp = Bitmap.createBitmap(w, h, conf); // this creates a MUTABLE bitmap
}
```

```
    imageItems.add(new
ImageItem(bmp));
  }
```

For Case II: (Displaying Hologram Three Times)

```
protected void onActivityResult(int
requestCode, int resultCode, Intent data)
{
    super.onActivityResult(requestCode,
resultCode, data);
    try{
        if (diagonal > d1 && diagonal
<=d2)
                {
            protected void
onActivityResult(int requestCode, int
resultCode, Intent data)
        {
            super.onActivityResult(request
        Code, resultCode, data);
            try
            {

        imageView.setImageURI(imageUri
        );
                BitmapDrawable
        drawable = (BitmapDrawable)
        imageView.getDrawable();
                Bitmap bitmap =
        drawable.getBitmap();
                bitmap =
        Bitmap.createScaledBitmap(bitma
        p, sc1, sc2, true);

        imageView.setImageBitmap(bitma
        p);
                (---------) // More images
        to be scale
            }
            catch(Exception e)
            {
                e.printStackTrace();
            }
        }
```

The images in above code are viewed by same or different rotation angle by xml layout file. It is defined as

- Platform size independent since images are view according to diagonal (in inches) value and scaled by using values of variable sc1 and sc2.
- Only one image is shown in different rotation for generating 3-D image view.
- But, Limited size of virtual image is projected depending upon platform size.
- Can depend upon quality of acrylic sheet use for projecting images.

ALGORITHM

The steps for creating reflection hologram:

1) Case I:

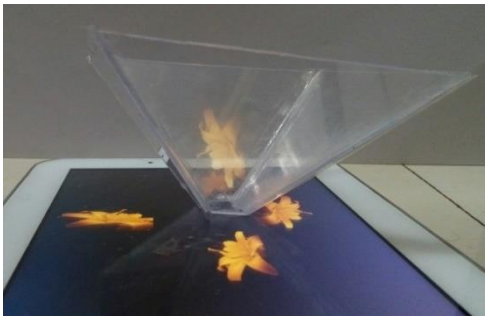
1. Open Gallery
2. Declare and initialize PICK_IMAGE as private static final int and 100 (for maximum pixel size).
3. Declare an object of class Uri.
4. Declare and Initialize an object of Intenet and parse image URI using putExtra.
5. Create a GridView using ArrayList
6. Using ParcelFileDescriptor and FileDescriptor create bitmap image.
7. Using matrix rotate images with different rotation angle.
8. Create default mutable bitmap for images not to be displayed.
9. Scale image according to diagonal size of image.
10. Add images to ArrayList.
11. Repeat step 10 for 7 times with different rotation angle.
12. Add this ArrayList to gridview layout.

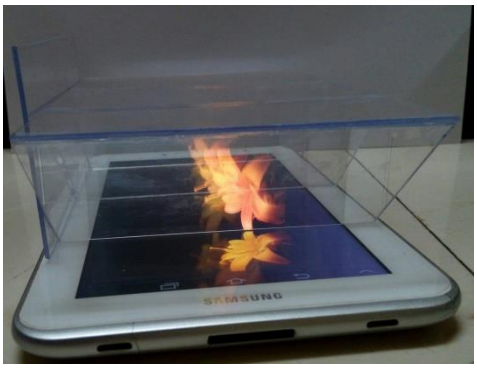
2) Case II:

1. Declare a four object of class ImageView

(package: android.widget.ImageView)
2. Declare and initialize PICK_IMAGE as private static final int and 100 (for maximum pixel size).
3. Declare an object of class Uri.
4. Initialize all object of ImageView class with respect to their ID in xml layout.
5. Declare and Initialize an object of Intent.
6. Using startActivityForResult() method display image through URI as setImageURI() method
7. Repeat step 6 for 2times with same rotation angle.

RESULTS





CONCULSION

Reflection Hologram in mobile application produces virtual images which are mirror images. Reflection Hologram can be produces multiple virtual images to give 3-D effect. Magnification in reflection hologram was not able to achieve. Large images could be displayed on acrylic sheet only if platform is larger in size.

Advantages of Reflection Hologram in mobile applications are

- Independent of monochromatic light source.
- Independent of platform size (in terms of diagonal in inches).
- No need of displaying image in dark, it is visible in light also.
- No need to spin mirror technique.
- Less electricity consumption.

Disadvantage: Images will be displayed according display device size.

LIMITATIONS OF SYSTEM

1. Magnification of images is not possible.
2. For viewing 3-D images angle between user and acrylic sheet must be between 45^0 and 135^0.
3. This is application is limited only to displaying images from user, not for moving pictures.
4. Viewing four images can be possible only with grid view layout.

FUTURE PROSPECTS

One of the most wide-ranging applications of 3-D Hologram is Mobile application based on Reflection Hologram. This problem is crucial in enlarging 3-D virtual images. But in future it may come with solution of enlarging images. Also projecting images in air may become multidimensional. Also we can view images using linear and relative layout for different platform size.

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HAND GESTURE RECOGNITION OF ENGLISH ALPHABETS

Ms. Sonia Deora and Ms. Mansi Pawar

Dept. of Computer Science, K.C. College, Churchgate, Mumbai-20
*Author for correspondence **Ms. Geeta N. Brijwani**

ABSTRACT

Objective: Controlling computers via hand gestures can make many applications work more intuitively. Hand gestures are an appealing way to interact with computer as they are natural part of how we communicate. The objective of this system is to take hand gesture images as an input from the user via a web camera and interpret the English alphabet corresponding to that gesture as per the ASL.

Methods: In this study, the hand gesture images are captured using *black gloves* via a *Webcam* and then are compared with the *benchmark images* in the database using an algorithm for comparing images using the attribute *pixel position.*

Results: It was observed that appropriate alphabet is obtained only if user captures images using *black gloves* along with white background. The difference in the *pixel positions* of captured images and the *benchmark images* varied typically hence, no range could be fixed.

Conclusion: The observed variation in the pixel positions of the captured images and the benchmark images played an important role in yielding unexpected results. However, it was found that expected results are obtained when the user captures images using the same angle in which the images are stored in the database.

Keywords: black gloves, Webcam, benchmark images, pixel position

INTRODUCTION

For the past few years, the common input computer devices did not change a lot. the computer input devices are restricted to mouse, keyboard, etc., then why can't we use our normal hand gestures to communicate with computer, just as we use them to communicate with humans? Controlling computers via hand gestures can make many applications work more intuitively. Hand gestures are an appealing way to interact with computer as they are natural part of how we communicate. Recently there has been a surge in interest in recognizing human gesture. They have applications in many computer games and various fields. Gesture recognition is a topic in computer science and language technology with the goal of interpreting human gestures via algorithms.

METHODOLOGY

Gesture Recognizing mechanism called as Similarity Finder is used here, which differentiates between the captured image and the images which are already being stored into the database for testing purpose, by finding out the least percentage difference (pixel wise) between the images and returns the corresponding English alphabet associated with the stored image in the database at prior.

- The user logins into the Gesture Recognition System.
- Webcam Panel gets displayed on the screen and user captures an image of a gesture (using black gloves) clicking on the capture button.
- By clicking on the Test button the user gets the corresponding English alphabet.

ANALYSIS

1. Benchmark (Standard Images for references)
   Inserts benchmark images into the database.

2. Login
   The login form is for security purposes, that no unauthorized user would login to the system.

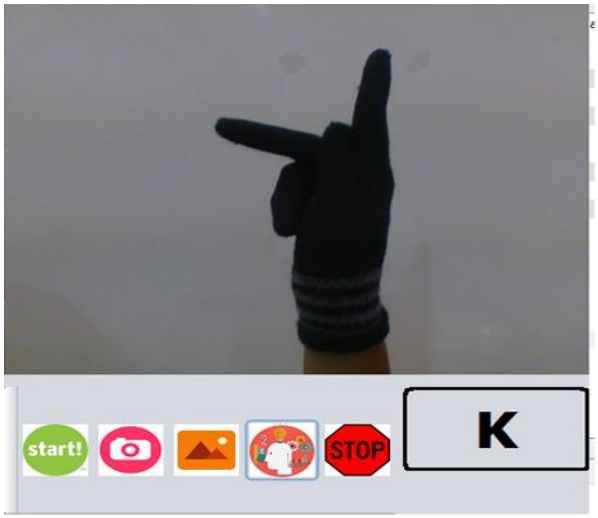
3. WebCamera
   Starts the webcam for the user to capture image.

4. Capture
   Captures the image and inserts it into the database.

5. Gallery
   Displays all the captured images on a new frame.

6. Test
   Tests the Captured Image with the benchmark images to generate the corresponding English Alphabet of the gesture shown in the captured image and display it as an output.

RESULT



CONCLUSION

This Windows Application has been made by considering all the security aspects. It ensures the no unauthenticated user can access the system.

We were successful in adding a new feature of viewing the gallery to the system. This image gallery views the total images captured by the user. This is just to keep a track of number of images captured by the user.

The observed variation in the pixel positions of the captured images and the benchmark images played an important role in yielding unexpected results. However, it was found that expected results are obtained when the user captures images using the same angle in which the images are stored in the database.

LIMITATIONS

- The application is limited to only English Alphabets i.e. the system can recognize only English Alphabets.
- The application recognizes gestures only when the user captures an image using black gloves.
- Approximations in result may occur if the background colour of the captured image is not kept to be white.
- The application is not flexible with respect to the Sign Language used i.e. it focuses specifically only on American Sign Language.

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OVERALL MODULE REPORT

"The Science of today is the technology of tomorrow"

-Edward Teller

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Module Number 1

Proposal Writing

Resource Person: Dr. Sagarika Damle
Date: 15th September 2015
Time: 9:30 AM- 10:30 AM
Venue: Life sciences Laboratory, KC College

Description:

Research proposal is one of the primary steps which explain the systematic time of your project with various parameters involved. A proposal starts with writing about the investigator, the key element of the research. Then we move on to discussing the subject and the title of the research with a keen reference to the institute and where the research will be undertaken, the guides and co-guides. The title should be short yet precise and informative. The introduction about the research should follow, which will contain the origin and nature of the problem the research will address and the interdisciplinary relevance, the application of the research in the fields other than the specified. The national and international status of the study, its significance and objectives to be achieved should be mentioned thereafter in a detailed manner. Then comes the major part, the methodology that is procedures, steps, durations, working, special techniques or expertise, sample procurement, relevant permissions, field work, target planning, availability of machinery, glassware, apparatus, chemicals and help and support needed from other departments in the institute. Finally a proposed budget and requirements should close the proposal. The main purpose of the proposal is to show that you are engaging in genuine enquiry, finding out about something worthwhile in a particular context.

Module Number 2

Time Management

Resource Person: Dr. Manju Nichani
Date: 13th October , 2015
Time: 11:30AM- 1:00PM
Venue: Seminar Hall, KC College

Description:

This module was one of the precious one, as everyone gets equal time, but only few can manage it. The resource person was our Principal Madam, who, very effectively taught us about managing our time. The important thing that one must learn is: **"Believe in today and learn to value time."**
For effective time management, one should follow the basic things as mentioned below:

- Give priority to work.
- Set clear goals.
- Learn to say **"NO"** to less prior things.
- Wake up early if you fall a shortage of time.

The main reason for the wastage of time is: People run behind things without goals. Thus, the primary thing which avoids wastage of time is, setting goals, and the goals should be **SMART (Specific Measurable Attainable Relevant and Time Bound)**. The **Pareto's Principle** also proved to be useful as it tells us about prioritizing our work. Another theory for setting priorities was **Stephen Covey's Time Management Matrix.**

Module Number 3

Interactive session

Resource Person: Dr. Sagarika Damle, Dr. Anupma Harshal
Date: 14th October, 2015
Time: 10:30 AM- 1:30 PM
Venue: Seminar Hall, KC College

Description:

The interactive session was conducted by our conveners Dr. Sagarika Damle and Dr. Anupma Harshal. According to me, this session gave us an insight into our own selves rather than the outside world.

It so happens that we often try to control people and situations according to our comfort level. This ultimately leads to frustration. This session encouraged us to analyse ourselves and our own thinking and adjust it according to the situations in life. We were divided into groups where we were all strangers to each other and we had to work together. This made us realize that we all have excellent potential to work as a team, but we often choose not to do so. We enacted different roles as a team. Some team made a caterpillar while another made a flowering tree. We scripted and acted out small role plays which had important morals. Also, we were encouraged to think different from the ordinary. We were asked questions like, is number 3 male or female and why? Or what comes to your mind when you think of the colour blue? There were different games

where students were blindfolded and given same instructions for a particular piece of paper. In spite of the same conditions and instructions, all the students made different designs. This explains the world is as we make it. No individual is right or wrong. It is all relative.

Module Number 4

Film Appreciation

Resource Person: Dr. Girija Gupte
Date: 14th October, 2015
Time: 02:30 PM- 04:30 PM
Venue: KC College

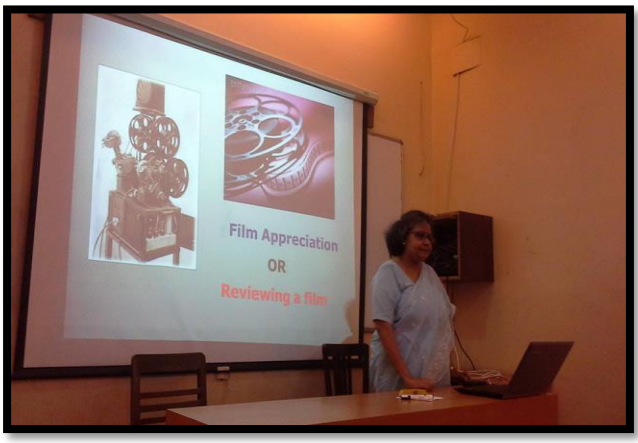
Description:

Film has a completely different attraction of its own and is a mass medium as it reaches many people at a time. In order to appreciate anything there needs to be a sense of understanding within us. This is what "Film Appreciation" is all about. It helps you understand the key dynamics of what film is made up of technology, art, industry. After watching a film, the next step would be to reflect on it and appreciate it.

The original term for "Cinema" is "Cinematograph" & "Movie" means moving images. It is closest to reality as it is audio visual & it moves. This session taught us everything right from when and how film production started.

Auguste and Louis Lumiere built a light weight hand held motion picture camera called cinematographe. They showed 10 films. Each film was 17 metres& lasted maximum of 50 seconds with a speed of 12 frames per second. Now 30 frames per second is the fastest. Slow motion camera requires high speed. We also learnt about the components of cinema that is direction, screenplay, narration, cinematography, art direction, acting, sound, editing. We also learnt about some very good old films such as "Dahan", "Patherpanchali", "Fiza", "Meghedhakatara" & actors as Rituparno Ghosh. Also the foreground and background, Mise-en-scence(put in the

scene) was studied carefully along with camera, tak& the different types of shots such as long shot, mid shot, short shot, close up, extreme close up. "Animals are beautiful" is one such movie which shows all of these shots and taks. Editing of a movie plays a vital role. Atlast we saw a movie called as "Harishchandrachi Factory". It was an amazing film.



Module Number 5

Communication Skills

Resource Person: Ms. Freyaz Shroff
Date: 15th October, 2015
Time: 10:00 PM- 12:0 PM
Venue: KC College

Description

The Communication Skills session was one of the most interactive and enjoyable session among those conducted.

Ms. Freyaz Shroff made us aware of certain facts about regular communication. For instance, 55% of communication depends on body language. Body language acts as a means of expressing the words used for communication and it largely determines the way the other person is going to perceive those words. 38% of communication depends on the tone used by the speaker. Only 7% communication depends upon he actual words used.

Listening plays an extremely important role in communication. Almost every time, the root of ineffective communication can be related to inattention, or not listening properly to the speaker. Through this session we realized that often we create problems in our own life simply because of ineffective communication or misunderstandings.

Module Number 6

Gender Sensitization

Resource Person: Ms. Shruti Chakravarty, Ms. Raj Merchant

Date: 15th October, 2015

Time: 2.00 PM- 4:30 PM

Venue: KC College

Description:

The name "Gender sensitization"itself suggests how sensitive this topic is. Gender sensitization refers to the modification of behavior by raising awareness of gender equality concerns. Itmeans that there is no discrimination on grounds of a person's sex in the allocation of resources or benefits, or in the access to services.Gender bias is very obvious when the world is viewed by gender lens. Gender bias is a social construct. The activities helped us in realizing some important facts about equality among both the gender. It also made me realize that it is the values inculcated within us that make a difference. From childhood it is being taught that girls wear pink and boys wear blue or girls play with doll and boys play cricket. But why it is so is a question that is never asked. It can be other way round as well. Both males and females should be treated equally. There was a discussion for which we were divided in to various groups& the topic was if there is no bias in the society than how would be the society? This was done on the basis of clothing/ appearance, family, public space, relationship, marriage, education, work place, etc.

For Example:

1) At home, a guy can stay out of house late night but a girl cannot.

2) In relation to work, Women

have longer working days than men, at work and then at home.
3) In valuation of work, it is most of the times undervalued.
4) In public spheres, Men hold high status positions and women tend to fill the roles of support persons.
5) In sharing of world resources, Women earn 1/10 of world's income for doing 2/3 of world's work.
Discussion on many other topics with respect to dressing sense, education, sports was done. This discussion made us realize that just by being literate the social thinking of the society didn't change. Change is supposed to be brought by creating awareness.

Module Number 7

Promoting Wellness

Resource Person: Dr. Avinash D'Souza
Date: 16th October, 2015
Time: 10.00 AM- 12.00PM
Venue: KC College

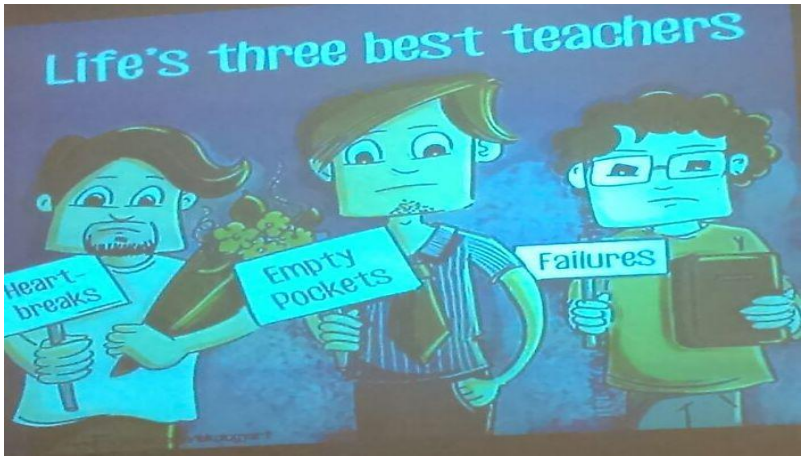
Description:

There was one statement made by Dr. Avinash which totally struck my mind and I agreed to it, and the statement was: **"We try to keep others happy except ourselves."** Most relationships fail because we spend too much of time pointing each other's mistakes rather than understanding their partner. We should never sacrifice our happiness for the sake of achievement. The real key to life is to happily achieve. We have 2 options:

- Live your life.
- Regret having not lived it.

Dr. Avinash also made us aware of a beautiful fact:
"Become friends with people, who aren't your age.

Hang out with people, whose first language is not the same as yours.
Get to know, who doesn't come from your social class.
This is how you see the world, this is how you grow."



Module Number 8

Awareness on 'Child Sexual Abuse'

Resource Person: NGO Arpan
Date: 17th October, 2015
Time: 10.30 AM- 01.00PM
Venue: KC College

Description

The session was conducted with an aim to create awareness among the students regarding Child Sexual Abuse. The volunteers working for NGO Arpan gave us an overview about this issue.

Statistics show that 53% children are sexually abused before they become 18 years old and out of these, 76% children are abused by other children, who may be elder to them. This statistics holds true for both girls as well as boys.

Sexual abuse is not necessarily physical. Displaying obscene material or using sexually graphic language are also forms of Sexual abuse. Also, sexual abuse usually occurs in three steps, which is Grooming (winning a child's trust), the actual Abuse, and finally Accommodation (the child slowly gets used to that particular touch).

The trauma of sexual abuse can be devastating. It can eat into the child's self-confidence, and make the victim feel guilty for something which is not their fault, especially if the abuser is a relative or a family member.

Parental intervention becomes absolutely essential in such cases, as the child may open up to the parents more easily than others. Parents need to be vigilant and observe the behaviour of their children, and talk to the children if they find something amiss.

Module Number 9

Laughter Yoga Session

Resource Person: Mr. Atmaram Bhau, Mr. Kishor Bhatt and Ms. Seema Bhate

Date: 23rd October, 2015
Time: 9.00 AM- 10.30 AM
Venue: MMR, K.C College

Description

The Laughter Yoga session was one of the most rejuvenating sessions amongst those conducted. Usually, one strives for excellence, perfection and creditability in life. However, being emotionally stable and happy is perhaps most important of all.

Atmaram Bhau and Team encouraged us to laugh- and laugh for no reason at all! Atmaram Bhau explained that if one keeps looking for reasons to be happy, that person shall keep searching forever. Also, it has been scientifically proved that the human body cannot differentiate between fake and genuine laughter. Both facilitate efficient blood circulation and act as stress busters.

Also, it is very important to be natural with one's own self, in spite of the innumerable facades we adopt in public. Atmaram Bhau demonstrated certain laughter exercises which exhilarated us physically as well as mentally.

Module Number 10

Literature Review

Resource Person: Mrs. Sharon D'souza

Date: 23rd October, 2015

Time: 10:30 AM- 12.00PM

Venue: MMR, K.C College

Description:

This module taught us about the referencing work and review of the literature. Referencing is mentioning or adding of some informative data, which is already existing. Referencing plays an important role in your research work, as 80% of your work is referencing, 20% of it is used in our project.

Literature Review is a solid written proof which proves that we have referenced. It gives a new interpretation of old work or combine new work with the existing one or might trace intellectual progression of field including major debates. The importance of referencing is:

- It acknowledges part of our work.

- Failure to acknowledge that some of our opinions and information have come from others, may be regarded as plagiarism.
- Show the reader, the range and nature of your source materials.

Sometimes, the literature review can prove to be a handy guide to your topic. No project can be completed without literature review. If you are expecting a quick research project than referencing and literature review can only make it possible. Doing proper reference of your work is important to trace the origin of the ideas, to indicate appreciation, to enable the readers to trace the sources you have used, etc.

Module Number 11

Fundamentals of Scientific Research

Resource Person: Dr. Vivek Patkar

Date: 23rd October, 2015

Time: 12.00 PM- 1.00 PM

Venue: MMR, K.C College

Description:

We all have our own definitions of science but what actually it is being understood in this session. Science basically is divided into mathematical science, natural science & social science. Divisions of science are theory, experiment & virtual. Eschatology is the study of end of universe.

Research aims should be to understand the properties of a given phenomenon, to understand relationship between variables, to predict outcome, to replicate research for validation, to develop new instruments & algorithms, to produce theory.

Research motives should be advancement of knowledge, commercial, policy formulation, investigative reporting, self satisfaction.

Research focus should be on generating novel but useful ideas, challenging the

existing knowledge, bringing a new creation to fruition, using creative capacity to discover or invent something new.

There are different types of research search as extension or generalisation of currently available results, explanatory- finding reasons by building theories& hypothesis & testing them, descriptive leading to prescription, comparative study, life history research.

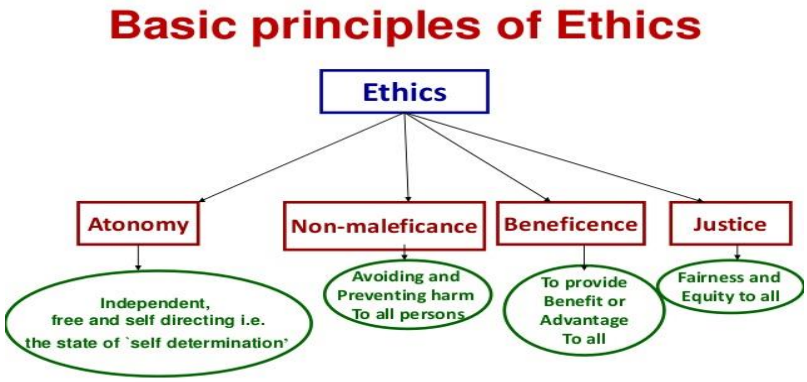
Research work starts with curiosity & cause to effect & vice versa relationship study. Various factors are taken into consideration for topic selection such as relevant to the institution & time, state of the current knowledge, emerging research data, personal confidence. Problem identification & the purpose of solving it. A standard research method is like Karl Popper's Method of Falsification. Clinical research works with diagnosis, prognosis, treatment & prevention. Research supporting tools are also important such as library, laboratory, computer, web & online sources, language, statistical techniques.

In individual research, be your own critic where as in group research, treat every partner equally.

The basic features of research are utility, patience, Impartial, effoerts, creativity, ethics, writing & reading. We also got good research tips.

Work on the SMART(ER) principle:

S- Specific
M-Measurable
A-Attainable
R-Relevant
T-Time bound
E-Evaluated
R-Recorded



Module Number 12

SGNP Nature Trail

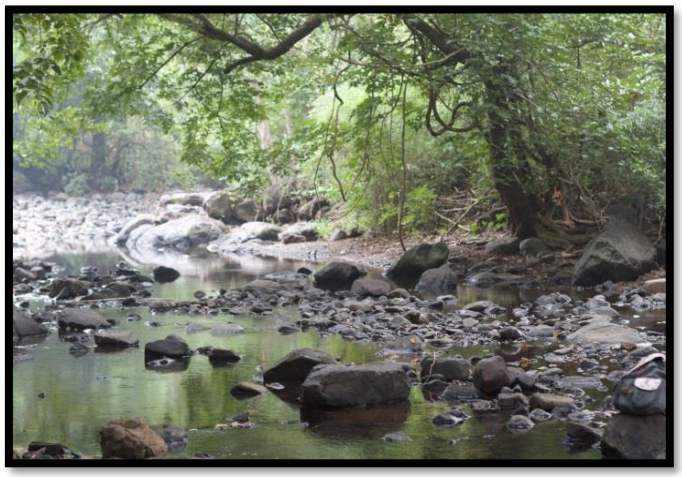
Resource Person: Dr. C.S Latoo, Dr. Leon Periera
Date: 25th October, 2015
Time: 8.00 AM- 4.00 PM
Venue: Sanjay Gandhi National Park, Mumbai

Description

The nature trail was led by Dr. Latoo and Dr. Leon Periera. The trail was extremely refreshing and we were very lucky to have such enthusiastic and energetic experts with us.
The Sanjay Gandhi National Park has an area of 103sq.km. It consists entirely of moist, deciduous forests. We were lucky enough to observe a variety of bird species such as the Drongo bird (*Dicrurus paradiseus*), the Golden Oriole, the White Breasted Kingfisher (*Haleyon smyrenensis*) and the Little Blue Kingfisher.

Also, we observed animal species including the Funnel-web spider (which weaves a cotton-like web), the Psyche Butterfly (it has a confused flight) and a large number of plant species including *Hoarrhena antidysenterica* (best drug for Diarrhoea). There were a number of plant species which are used widely in the Ayurveda.

Spotted deer, Orchids, Teak plants and medicinal herbs were also observed and thorough information on all was provided by the Resource persons.



Module Number 13

Research Scholar's Meet

Name of Chief Gust: Dr. Jacinta D'souza
Date: 09th February, 2016
Time: 08:30 AM-04:00 PM
Venue: KC College

Description

The Research Scholar's Meet scheduled for SHP Batch XI was an important lesson for us. We learnt event management, as the entire session was arranged by the students in all aspects. The students were hosting the session, as well as taking care of the hospitality of the participants.

Also, we had the opportunity of witnessing exactly how a research is presented and the sort of questions which could be asked upon it. All the judges who were invited for the Meet were renowned researchers and we had the opportunity of watching them judge the proceedings.

I would want to thank Dr. Sagarika Damle and Dr. Anupma Harshal for allowing us to be a part of the RSM.

Module Number 14

Men Against Violence and Abuse (MAVA)

Resource Person: Mr. Harish Sadani
Date: 1st April, 2016
Time: 10.00 AM- 2.00 PM
Venue: K.C College, Seminar Hall

Description

It so happens that we usually tend to think of Gender issues as Women's issues. However, today the crimes which are being committed on women are a gruesome result of the objectification of women. It is the thought process, and not the women, which needs to change.

Most of the bias against women is socially constructed. For example, the fact that the woman has to leave her own house after marriage is a socially constructed ritual. Nature does not differentiate between men and women. Also, patriarchal society pressurizes the men along with women. Men are expected to like sports and rough activities and it is considered that men do not cry.

It is this thought process which leads to crime against women. Violence and abuse is thus not just a women's issue. Men suffer equally because of this patriarchal society.

Module Number 15

Advanced Communication Skills

Resource Person: Ms. Freyaz Shroff
Date: 1st April, 2016
Time: 2:30 PM- 4.00 PM
Venue: K.C College

Description

This was the second session held concerning Communication Skills. In this session we were given different scenarios which we had to enact in 30 seconds. This made us determine exactly how strongly we can connect with the audience and deliver our message.

We were divided into groups and were given pieces of playing cards which we had to join to make full cards. This required negotiation, understanding and effective communication with the entire batch.

Also, Ms. Freyaz Shroff explained the concept of the neuron. If one forms a fear or phobia concerning a particular thing, then one has to decide and make a different choice to rid oneself of that phobia. Fear is very relative and should be dealt with as soon as it develops.

Module Number 16

Body Language Etiquettes

Resource Person: Ms. Manisha Mishra
Date: 2nd April, 2016
Time: 10.00 AM- 12.00PM
Venue: K.C College, Seminar Hall

Description

Usually, everyone attaches a lot of importance to one's image in today's world. Everyone was to be perceived a good or decent and accepted by society. However, it is not our image, but our self-image which takes us places in life. It is the way we perceive ourselves which determines our capabilities and sets our boundaries.

One's thought process becomes one's attitude, which governs our behaviour. Thus it is extremely important to be optimistic and open to changes at all times in life.

Ms. Manisha Mishra distributed sheets of paper upon which were listed various good qualities. We were asked to encircle the qualities which we possess, and work upon those which we did not. Personally, this proved to be a beneficial exercise for me. Whenever I feel I am not good enough, I take a look at the qualities I have listed for myself. It is more about proving to our own selves our capability, and not the rest of the world.

Module Number 17

Interactive Session

Resource Person: Dr. Sagarika Damle, Dr. Anupma Harshal

Date: 4th April, 2016

Time: 10.00 AM- 01.30PM

Venue: K.C College, Seminar Hall

Description:

Interactive sessions have always been the most interesting and fun-filled sessions. We had spiritual talk so as to one must pay attention to inward happiness rather than depending on others. It taught us that self-development should be physical, social, spiritual.

According to Maslow's theory, there are four images of self:

1) Who we think we are
2) What others think we are
3) What we want others to think we are
4) Who we really are

There are three steps of awareness: Information, Knowledge, wisdom.

Awareness of myself can be identified by:

1) What I am now? – Concern for self
2) What I want to be? – Setting of growth goals & pursue them
3) What I should be? – Confident of my ability
4) What I can't be? – Knowing my limitations.

Interactive sessions helped us in boosting our self-confidence. We had an activity of fashion show where we had to pass on a useful message without dialouges using newspapers & waste materials. These sessions were usually a part of stress reduction from our day to day busy schedule. We also learnt to keep the positivity going on in every situation. These sessions also included playing games and completing interesting tasks given to us. Each activity had a hidden value behind it. After the completion of every task we always learnt something new which was definitely very helpful to us. Each activity was unique. We enjoyed it alot.

Learn to grow, Grow to trust, Trust to change, Change to learn. This cycle goes on.

When I see different thing, I think differently.
When I think differently, I feel differently.
When I feel differently, I behave differently.

Module Number 18

Resume Writing Techniques

Resource Person: Ms. Kavita Peter
Date: 5th April 2016
Time: 10.00 AM- 12.30PM
Venue: K.C College, Seminar Hall

Description:

This was one of the most useful sessions. We learnt the basic difference between a resume & CV. We also learnt about the different types of resume such as Skill oriented resume, Chronological resume, Functional resume. We also learnt about the non-conventional resume types that is electronic resume, Email resume, Web resume. Components of a CV & resume differ a lot. CV is far more in detail compared to a resume. Resume comprises of name & contact information followed by a summary that includes your objective for joining that particular job. It also consists of your educational qualification in descending order along with skill sets. Also work experience in detail along with references in detail. One can add optional information about computer skills, certificates, languages, honors

and awards, etc. Each aspect should be mentioned in detail.

For eg: Educational qualification will include level of degree in descending order along

with the date of graduation, Institution were the degree was granted and the GPA. In a resume there should be no unnecessary capitalize, italicize and underline. One can also check the effectiveness of your resume by 20 seconds test in four quadrant method.
We were told about some basic soft skills as well as etiquettes which are the personal attributes that enable someone to interact effectively and harmoniously with other people.Soft skills revolve around personal relationships, character, and attitude. Soft skills are considered as a complement to hard skills, which refer to a person's knowledge and occupational skills.

Module Number 19

Malaria Awareness

Resource Person: Dr. Mira Ramaiya , Dr. Saroj Bafna
Date: 5th April, 2016
Time: 1.00 PM- 3.00PM
Venue: K.C College, Seminar Hall

Description:

Nowadays, mosquitoes are becoming a problem. The increasing number of mosquitoes has led to the threat of diseases like malaria, dengue and many more. Malaria is a mosquito-borne infectious disease of humans which is most commonly transmitted by infected female Anopheles mosquito. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood.

There are 5 species of Plasmodium which spread the malaria disease:

- Plasmodium falciparum

- Plasmodium vivax
- Plasmodium ovale
- Plasmodium malariae

From the above mentioned plasmodium, the Plasmodium falciparum is the most dangerous because it has the ability to affect all age of RBCs (Red Blood Cells).The symptoms are cold, headache, nausea, vomit, sweating, etc and they usually begin ten to fifteen days after being bitten. If not properly treated, people may have recurrences of the disease months later. As we know that **"Prevention is better than cure"** we must take certain measures. To control mosquitoes from breeding, we must make sure that sewage water does not collect in puddles in our locality. There are also certain indoor residual sprays available which restricts mosquitoes from breeding. Also, mosquito repelling creams like ODOMOS are available.

After all the discussion, there was a small MCQ test which we enjoyed a lot.

Module Number 19

Ethical Hacking

Resource Person: Mr. Sachin Dedhia
Date: 6th April 2016
Time: 10.00 AM- 12.00PM
Venue: K.C College, Seminar Hall

Description:

This session was one of the most interesting sessions. We learnt a lot of new things. Ethical hacking and ethical hacker are terms used to describe hacking performed by a

company or individual to help identify potential threats on a computer or network. An ethical hacker attempts to bypass system security and search for any weak points that could be exploited by malicious hackers. This information is then used by the organization to improve the system security, in an effort to minimize or eliminate any potential attacks.

Caller ID spoofing:

Caller ID spoofing is the practice of causing the telephone network to indicate to the receiver of a call that the originator of the call is a station other than the true originating station. For example, a Caller ID display might display a phone number different from that of the telephone from which the call was placed. The term is commonly used to describe situations in which the motivation is considered malicious by the speaker or writer.

Recuva:

Recuva is a data recovery program for Windows, developed by Piriform. It is able to recover files that have been "permanently" deleted and marked by the operating system as free space. The program can also be used to recover files deleted from USB flash drives, memory cards, or MP3 players. Recuva was described by vnunet.com as an "effective tool for undeleting or salvaging files we sent for recycling and deleted, in the past". The program works on both FAT and NTFS file systems. It is able to recover lost directory structure and automatically renames files when trying to recover two files of the same name. As of version 1.5.1 it can also recover files from Ext2 and Ext3 file systems. As with other file recovery programs Recuva works by looking for unreferenced data, but if the operating system has written new data over a deleted file then recovery will often not be possible.

Module Number 21

SEBI Visit

Date: 7th April 2016
Time: 02.00 AM- 04.30PM
Venue: SEBI, Bandra Kurla Complex, Mumbai.

Description

A visit to the Securities and Exchange Board of India was scheduled so that the students would get an idea about investment and financial markets. Financial Markets are of two types- Money market and Capital market. The money market is unorganized and is recommended for short term finance, whereas the Capital market is a security market.

We were informed about Investor Protection schemes so that we can make wise decisions about our investments in the future. We were shown a presentation regarding investment basics and savings, including the details of short term and long term investment.
Also, the session included information about Grievance Redressal for the investors and helplines for the same.

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Module Number 22

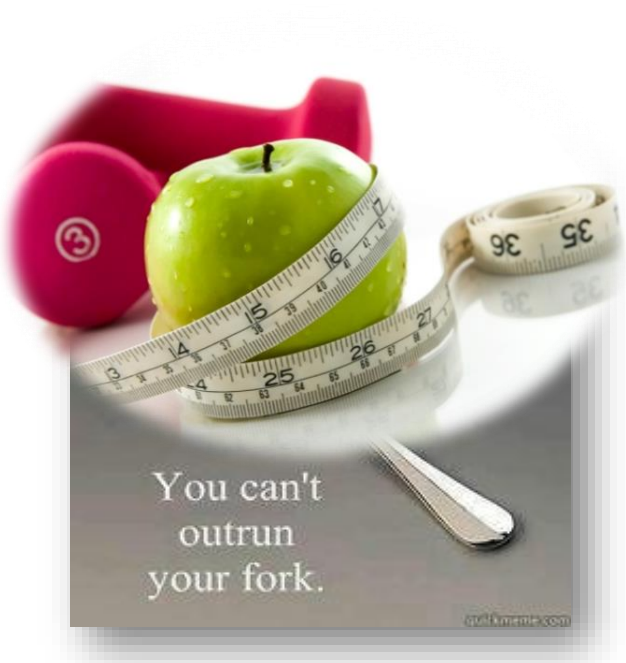
Nutrition, Diet and Exercise

Resource Person: Ms. Amruta Gogte
Date: 9th April 2016
Time: 10.00 AM- 12.00PM
Venue: K.C College, Seminar Hall

Description

This session dealt with the maintenance of a healthy body- both physically and mentally. Ms. Amruta Gogte explained that maintaining a balanced diet and a healthy lifestyle will benefit later in life. One ill-treats one's body when one is young, but the effects have to be borne later in life.

Physical exercise also helps to maintain a sense of well-being and positive outlook on life. One's self-image is boosted and one always remains prepared for challenges.



The most common mistakes related to health include emotional eating, perceived need for fast food and not following regular meal times. One needs to take special efforts to sort out mealtimes and follow them regularly. A slim person may feel that he/she does not need to exercise. However, exercise is essential to remain healthy and not just slim.

Along with physical exercise, emotional stability and happiness is also essential. Being contented with what one has and staying happy is another aspect of achieving a healthy body and mind.

Module Number 23

Photoshop

Resource Person: Mr.Paresh Pandya
Date: 9th April 2016
Time: 2:30 PM- 5.00 PM
Venue: K.C College, Computer Science Laboratory

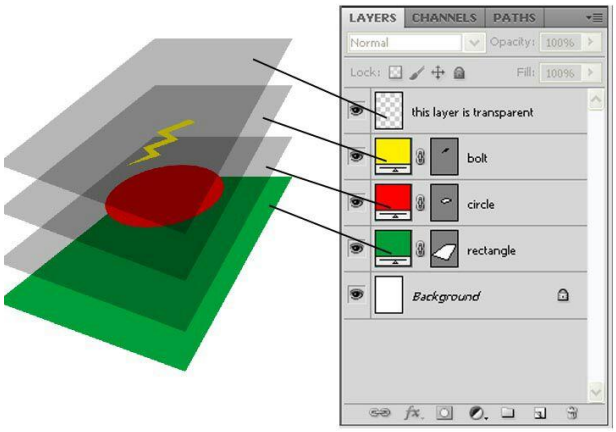
Description:

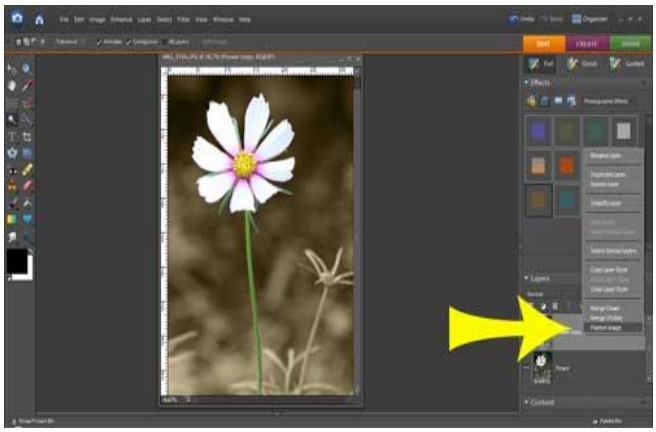
Adobe Photoshop is one of the most advanced photo manipulation software available till date. Adobe is also widely known for it's various photo, video and audio creative softwares today used by professionals across the film industries and more. The success of photoshop lies in it's simplicity of work environment. Major area of the work environment is occupied by the image in the centre that is to be edited, of course. It also serves as a canvas for any further additional images or effects that you may apply later on. A basic toolbar on the extreme left, a generalised task bar for rendering, saving or creating a new file on top, a miniature raw preview of the final image sits on top right followed down by further option of a particular tool or brush that is in use and finally, probably the most important one of all is the layers menu; pushed in the right bottom corner of the screen. The trick to quickly get started with the software is to understand the work flow of the "Layers".
As the name goes a layer can be considered as individual transparent canvas placed one above another. For instance if you copy an image over an already existing photo inside photoshop then it automatically forms a new layer. This means when a layer is selected, all the effects and editing that you may apply will affect the selected layer only. Ofcourse you can create a new transparent layer and work on them separately without damaging the rest of the work. This is something known as a "non-destructive-work-flow". It gives the artist a freedom to experiment with different styles without worrying about his work so far. If he's not happy with a particular effect, he may just delete that particular effect's layer and rest of the image stands untouched. However, more the layers, more the space it occupies and the final layers should be

merged down to one layer from time to time, to save space and processing power. The rest of the effects and manipulations requires skill, practice and patience to achieve the desired image.

In this session we were given an image and the necessary components that we were suppose to put together and make a new image using photoshop as sir directed us. It was fun.

With photoshop, creativity is limited by your imagination alone.





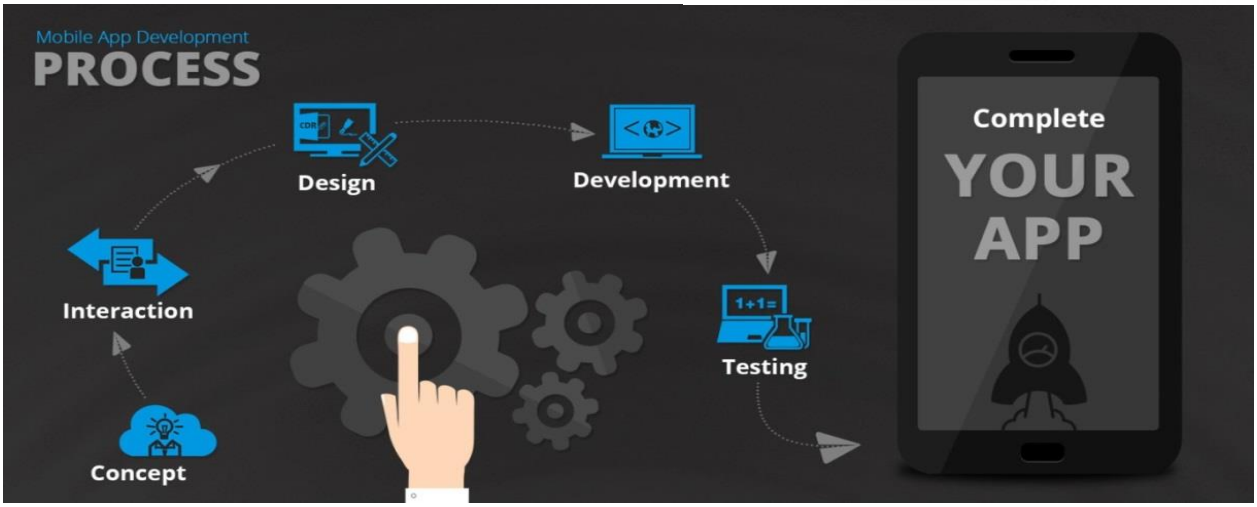
Module Number 24

Mobile App Development

Resource Person: Mr. Maunash Jani
Date: 11th April 2016
Time: 11:00 AM- 1.00 PM
Venue: K.C College, Computer Science Laboratory

Description

This was a thoroughly technical session. Mr. Maunash Jani introduced us to various technical languages required while creating an app- JavaScript, JSON, HTML5, and CSS3. We learnt about the various mobile operating systems including Nokia, Apple and Microsoft. Sir introduced us to the different types of apps which may be Native, Hybrid, JavaScript or Mobile web apps.



Mr. Maunash explained the necessity of app development. An app is developed to

make it convenient for the users to carry out certain actions which were inconvenient to perform.

It is necessary to identify the problem first and create wireframes for the particular app. The app is then tested and re-tested and can be launched after much deliberation.
We developed a simple calculator in our phone following Mr. Maunash Jani's instructions.

Module Number 25

Daawat-e-K.C

Date: 12th April 2016
Time: 10:00 AM- 1.00 PM
Venue: K.C College, Life Sciences Laboratory

Description

All the students were divided into 5 groups, each group consisted of 7 members. A week earlier, all the groups were given with 5 ingredients, which varied from group to group, out of which the group members had to decide minimum of 3 dishes that they can prepare from it. Each group had it's unique names.

My group was **Nutralacious (Nutrition+Delicious)** and our ingredients were: Apples, Avocado, Oats, Mushroom and Chocolates. The dishes that we had

prepared were: Stuffed Mushroom Gravy, Oats Roti, Apple fries, Chocolate Avocado Brownie and Apple Waldorf Salad. We made sure that all these dishes would be nutritious as well as delicious.

The judges for the competition were **Aashu Madam** and **Pratik Sir,** from the Life Science department and **Shailaja Madam** from the Statistics department. It was a difficult task for judges to select the winner as every group had worked efficiently for the competition. However, the judges had to choose one of the five groups.

With God's Grace our team was declared as winner later which is an unforgettable memory. We were not professional cooks, however we managed to cook tasty and **Nutralacious** food.

Module Number 26

WordPress

Resource Person: Mr. Maunash Jani
Date: 12th April 2016
Time: 02.30 PM- 04.30 PM
Venue: K.C College, Computer Science Laboratory

Description:

This session dealt with creating our own website on the internet. It is a blog which can be updated daily. This website is entirely personal and information can be provided on the internet in the form of pictures and videos concerning the particular topic.

Mr. Maunash Jani explained the entire procedure briefly and made us add some posts and format our websites. Also, Mr. Maunash helped us create shortcuts to access our websites and edit those further using wallpapers and images.

These websites can be very convenient to spread information about certain topics which are difficult to communicate but still require a personal touch during communication.

Module Number 27

Star Gazing

Organized By: Bramhand Organisation
Date: 13th April,2016 – 14th April,2016
Time: 08.00 PM- 4.00 AM
Venue: Sai Mandir site, Saphale.

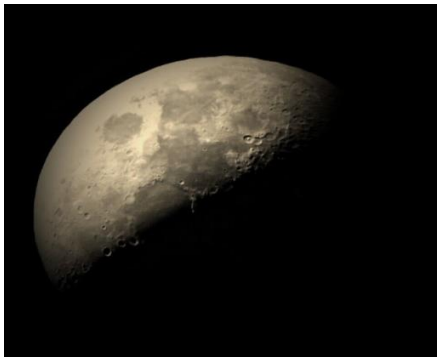
Description

This overnight field trip was one of the marvellous gift the SHP has given us. It was for the first time when all of us were going for an overnight field trip where whole night was to be spent just gazing the stars. All of us were super excited of this excursion.

As we reached Tandulwadi Village in Saphale, there was a small session on introduction to sky objects and satellites where they guided us about how to trace the sky maps. We enjoyed the magnificent look of the moon under the telescope as well as we spotted planets: **Venus, Mars, Jupitar (with its 4 moons)** and **Saturn with its amazing ring.** All of them seemed to be beautiful. We spotted the **Polaris** which is the centre of the Milky Way as well as the **Sirius (Dog Star)**.

The constellations that we spotted were: **Orion –the hunter, Hercules, The Great Bear (Ursa Major), Taurus, Gemini**, **Libra, Scorpio, Leo, Cancer** and **Virgo.**

Although it we had been there in summer, however the weather was pretty cool there at night. But all of us were engrossed in star gazing where no one was bothered about coldness. Throughout the whole star gazing session, the thing which struck my eye the most, was the **meteor shower**. This field trip was one of the most memorable one for everyone because the fun lies within staying awake the whole night and spotting the stars.



Module Number 28

Campus to Corporate

Resource Person: Mr. Dnyanesh
Date: 16th April 2016
Time: 02.30 PM- 04.30 PM
Venue: K.C College, Computer Science Laboratory

Description:

In this session we learnt the most essential thing for each one of us that is to learn the difference between the lifestyle of campus and corporate world. In corporate world, one has to work out of their comfort zone& be comfortable.Life has to be dynamic as life is all about changes & one who doesn't change, Life rots. We need to change our thinking, our behavior.What we know is **Values**and when these values are put in action it is called **Ethics.** You should be channelized towards positive attitude. Confidence matters a lot in interviews. One should focus on abundance & not on lack. These are some guidelines for corporate world.

Paradigm is a preformed thinking or ideology or image that we follow without thinking. There are four gifts by good: Awareness, Conscious, Power of visualization & Independence. During any sort of presentation or interview body language is one important thing. One must show their confidence by their body language, eye contact, firm feet, avoid fillers, avoid hand gestures, No body movement or sway. If you are

afraid to make eyes contact then don't look at the eyes, look at the forehead. You live only once so grab opportunities with both the hands. Second important thing is communication skills. Communication skills is a way of exchanging ideas, thoughts, expressions. Vocabulary includes 7% words, 38% tone, 55% body language. Communication must be done to express not impress & it should go by intent & not content. Communication skills includes soft skills that will always leave an impact & it comes with time. It is defined as skills, abilities & traits that pertain to personality, attitude & behavior. They are hardest to acquire, impossible to practice unless it becomes habits. One must put across your points effectively without offending. Soft skills are important as they help in decision making, development, handling interpersonal relations, etc. it also requires communication skills, interpersonal skills, problem solving skills, time management skills. Along with this emotional competence is the requirement to manage ambiquity & stress .

We had many activities one of which was assertiveness self assessment questionnaire which stated a person's personality& improvement for the same.

If it is to be, It is to me.

Day by day everyday I will become better & better.

Module Number 29

E.V.S Module

Resource Person: Dr. Tejashree Shanbaug
Date: 18th April 2016
Time: 10.00 AM- 2.00 PM
Venue: K.C College ,Seminar Hall & Life Sciences Laboratory

Description

The environment comprises of all living and non-living beings. There are various ecosystems that exist into the environment which consists of biotic and abiotic factors. All living beings can only survive until the natural resources are available, but what if these resources wouldn't exist?? Thus, it's duty of every human to conserve the natural resources which indirectly would save the environment. However, one can save environment only if he/she is aware about environment.

Thus, this module was held to inform us about the environment resources, adverse effects of human activities on environment, sustainable development and various types of pollution. Madam also shared the information about various types of ecosystems some of them are for coastal, grassland, freshwater and many more. Some of the principles that we should keep in mind to protect nature are: Think long term, Understand the system within which we live, Recognize the limits, Embrace creativity and lastly practice fairness.

Madam shared her views about the Rain Water Harvesting project that they had conducted in our college as well as the Water Audit project. At the end of the

session, the students were divided into group and the groups assigned with an activity, where our creativity was tested.

Module Number 30

Chai and Why ??

Date: 20th April 2016
Time: 10.00 AM- 2.00 PM
Venue: K.C College ,Seminar Hall

Description

All the modules were done until then, where various resource persons come and speak and we were audience. Unlike rest of the modules, this was the one, where we were the speakers and our mentor teachers were the audience. Yes, it was our turn where we had to present with our research work done till date. It was then, where we came to know about everyone's research projects. We had a short break in between where mentor teachers along with students enjoyed a cup of tea/coffee.

All of us also got some tips and some guidance from the mentor teachers present there. Overall, this session was quite interesting. All the students had an amazing experience. Also, we got an idea of how to present our work in the RSM.

Module Number 30

DAE Clean Technologies

Date: 29th April, 2016
Time: 09.30 AM- 05.00 PM
Venue: Bhabha Atomic Research Centre, Mumbai

Description

The entire lecture series consisted of short half hour lectures about the current technologies used for maintaining a clean and green environment.

The first lecture touched upon the excessive use of plastic bags and release of methane into the environment. The second lecture was given by Dr. Lalit Vasne which dealt with effective methods of treating sludge.

Dr. Mukherjee explained that plasma technology can be used to maintain healthy environment. Dr. Anand discussed the importance of sterilization of food products and electron beam technology to reduce the production of waste.

Dr. H.S. Sodaye spoke about Arsenic contamination in ground water which can be reduced by membrane separation process. Uranium contamination can be reduced using hybrid membrane technology. Dr. D.B Naik demonstrated that thermocol, which is a major pollutant, can be destroyed using acetone.

ENDNOTE

On the first day of Science Honours Program modules ma'am told us "LAB TO LIFE" & "WORLD WITHOUT WASTE" & now at the end of SHP I truly understood what she meant. SHP gave me a lot more then what I expected along with academics. For me now Research is creating new knowledge along with improvising your communication skills, time management, developing personality, behaviour among people, making new friends. Most of all I learned to be patient & started taking everything in a positive way. It give me the "Never Give Up" attitude & to always say that "I CAN & I WILL". It has been one of the most memorable journeys of my life and I'm surely going to miss it. As James Allen rightly says "No duty is more important than that or returning thanks." Thank you for helping me grow & learn.

Imagination is the highest form of Research.

– Albert Einstein

So Keep imagining.. .

Message for the superannuated mentors

Ms. Prabha Padmanabha, Dept. of Microbiology

A mentor is someone who sees more talent and ability within you than you see in yourself and helps bring it out of you. – Bob Proctor

This quote is so perfect for Prabha Ma'am who has been a part of the Science Honors Program right from its inception, as a member of its core committee. She has been guiding and mentoring the students with an incredible passion and dedication.

The SHP program aims at pruning and grooming young minds to bloom into flowering personalities. Ma'am has fulfilled this to the fullest by always being encouraging and motivating the students. This has led to the holistic development of several students.

It is also worthwhile to mention that she is an excellent team player and handled each and every situation with positive attitude. This attitude bridged the gaps between various departments in the form of interdisciplinary activities. Not only have the students benefitted, but also her colleagues have immensely benefitted with her guidance.

Dr. Sushil Puniyani, Dept. of Chemistry

Dr. Sushil Puniyani, an IITan and Associate Professor in Chemistry department has been the driving force among the students and teachers. Dr. Puniyani has been the part of science honors program since last three years. She has been guiding the students with full vigor and dedication.

The SHP is to encourage students to overcome their fears and develop confidence into them. Madam has helped the students to overcome their hurdles into their projects, and come out the winners. She is the best to develop positive attitude amongst the teachers and students.

Team KC with Students

Team KC with SHP Mentors

SHP Mentors Teachers

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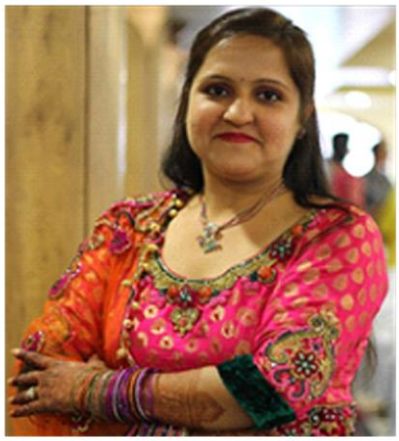

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