JIGYAASA

A Multidisciplinary
Research Initiative of KC College, Mumbai

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Chief Editors
Dr. Sagarika Damle
Dr. Shalini R Sinha

H(S)NC Board's
KISHINCHAND CHELLARAM COLLEGE

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Editorial Note

Following the principle stated by Raywin Connel, an Australian sociologist, 'Research is something that everyone can do and everyone ought to do', KC College, believes in training the young minds in research endeavors, in a systematic and disciplined manner. It therefore offers two major research platforms at KC College, the Science Honors Program-SHP and the Certificate Programme for Commerce and Arts-CPCA to its undergraduate students across Arts, Commerce and Science disciplines. 'Jigyaasa' is thus a compendium of selected research data compiled by the students enrolled in SHP and CPCA programs at KC. This 3rd volume of 'Jigyaasa' includes interesting research articles from different streams such as Biotechnology, Life Sciences, Microbiology, Chemistry, Computer Science, Social Sciences, Commerce and Humanities. These articles bear testimony to the efforts taken by the research guides and students who carried out a research project in a meticulous, scientific, and ethical manner. The articles include the methodology that can serve as a guide to the future batches of students who might be inspired to carry forward certain interesting possibilities of their predecessors and bring to light yet another uncovered part of the project.

The range of topics included in this 'Jigyaasa' volume III, would surely catch the attention of the young readers keen to work in the research arena. The Science section, emphasizes on the use of phyto-molecules to phyto-markers in making a Herbal toothpaste, Herbal lotion, Gene isolation from Aloe vera, Oral microbiome, tackling social issues such as Health of Police officers in view of Air pollution, authenticity of commercial detergents, Progesterone from Banana flowers and also studying mobile application for image projections. While under the section of Social Sciences, articles pertaining to levels of stress faced by Day-scholars and Impact of Covid 19 on Indian Economy etc. have all been included. The existence of such compendiums indicates the confidence generated in students who are publishing their research work to the outside world and are ready to defend the outcome in front of external scholars and scientists as the reviewers.

One of the noteworthy points in the new National Education Policy NEP 2020 is the provision for a research ecosystem under the stewardship of the National Research Fund (NRF), which will work towards seeding, funding, coordinating, and monitoring research and innovation initiatives. Therefore, the stage is perfectly set for well-equipped, able research- oriented young minds to pursue a bright career in research. K. C. College is thus harnessing the potential of such researchers, and encourages them towards further research by activities such as bringing out this book.

***Dr. Sagarika Damle** (Convener SHP)*
***Dr. Shalini R Sinha** (Vice Principal and Coordinator CPCA)*

Foreword

Malcolm Forbes had said that, "The best vision is insight".

'Jigyaasa' or curiosity leads to 'Shodh' or research which then gives 'Sookshma drishti' or clear insight. I am very happy to see Jigyaasa – A Multidisciplinary Research Initiative of K.C College, in its Third Publication Volume. Each year, the Jigyaasa- Science Honors Program (SHP) and Certificate Programme for Commerce & Arts (CPCA) have each been creating special groups of students who work hard under their Teacher Guides, all the while becoming researchers who develop confidence in not only their research abilities but also in academic writing abilities.

As another batch of SHP and CPCA complete their research viva, I can see the development in their overall personality. Each one of these students defends his or her research topic in the cross-examination by external judges and learns and grows while interacting with them, their peers and their teachers. The Researcher Student gains rich insight in his/her research topic and thus some quality research papers are generated.

The USP of the Jigyaasa compendium is its multidisciplinary nature. The readers can get an idea about various science related topics ranging from Biological to Physical sciences as well as the topics from social sciences fields such as Psychology and Economics. This cross read between the Science and Humanities is essential for breaking the silos and understanding the ethos of the research journey irrespective of the subject. Jigyaasa is one such effort in the direction of building bridges across the disciplines and offering students the possibilities of exploring transdisciplinary opportunities.

Research demands identification of problems and then the application of a thorough research methodology, sifting through quantitative data but also giving credence to a nuanced qualitative approach. The journey of this research and the achievement of these research papers by students and their teachers, is immensely satisfying and fruitful. The several intensive and interactive modules on research methodology, literature review, ethics of research and such sessions, help students understand not just the abstract concept of research but also apply it to the concrete research topic that they undertake. The intellectual and critical learning capabilities are enhanced for such students. Each of these research articles is a fresh insight into what might appear to be routine and mundane topics but actually help to transfer learning from the lab to life.

Dr. Hemlata K. Bagla
Principal,
Kishinchand Chellaram College, Mumbai

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SECTION I - LIFE SCIENCES

Chapter 1 - Development, Formulation and Evaluation of Herbal Teeth Whitening Toothpaste Containing *Cinnamomum tamala*

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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 tooth 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 colour 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 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 Tej pat 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 the toothpaste:

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 the toothpaste:

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 tooth guide 3D-Master. Volunteers were told to use the sample toothpaste once a day for 20 days. Their tooth shade after 20 days was checked and noted down again.

Selection of tooth shade: VITA Tooth guide 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.

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.

Table no. 1: Formulation per 100 gms of Toothpaste

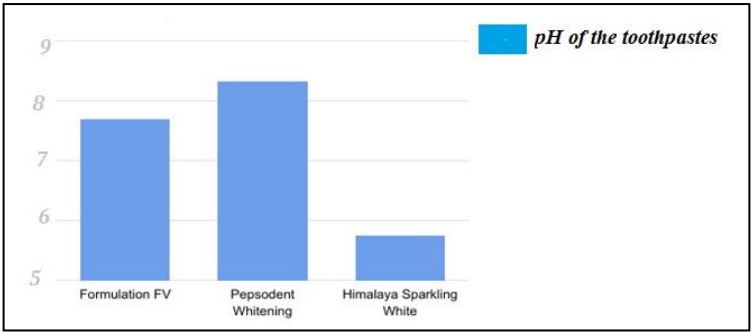
Ingredients	F1	F2	F3	F4	F5
70% Sorbitol	45.0 ml	45.0 ml	45.0 ml	45.0 ml	45.0 ml
Glycerin	5.0 ml	5.0 ml	5.0 ml	5.0 ml	5.0 ml
Distilled water	15.0 ml	14.25 ml	14.0 ml	14.25 ml	13.85 ml
Calcium Carbonate	20.0 gm	20.0 gm	20.0 gm	20.0 gm	20.0 gm
Tejpat Powder (A.P.I.)	10.0 gm	10.0 gm	10.0 gm	10.0 gm	10.0 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.

OBSERVATIONS AND RESULTS

The optimized formulation was Toothpaste Formulation 5. The 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. 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. 2: pH of the toothpastes

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

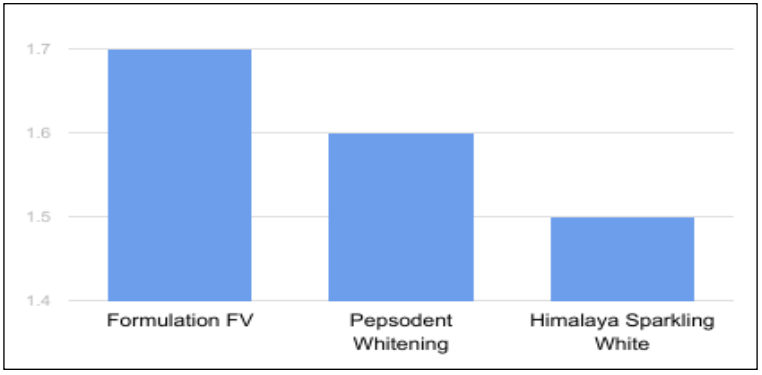


pH of the toothpaste

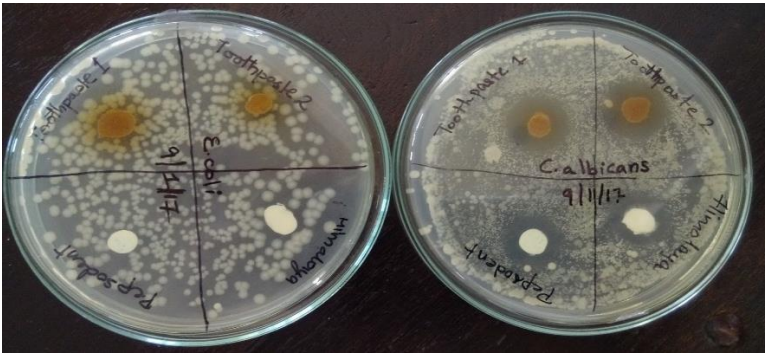
None of the toothpastes showed antimicrobial activity against *Escherichia coli*. The Anti-microbial activity results against *Candida albicans* were as follows:

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

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



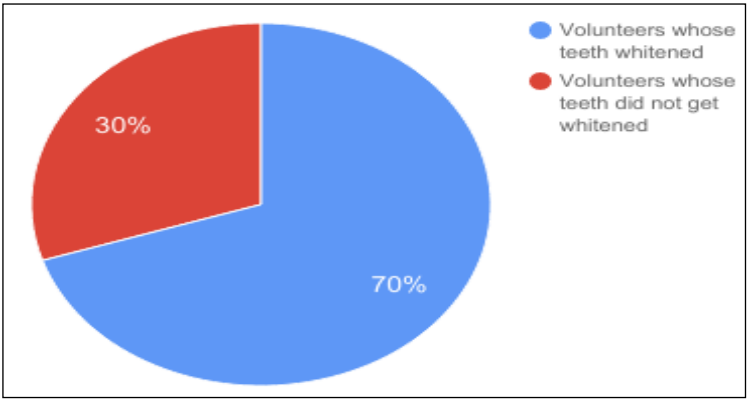
Antimicrobial activity against *C. albicans*



Antimicrobial activity of toothpaste against *E. coli* (left) and *C. albicans* (right)

Change in tooth shade

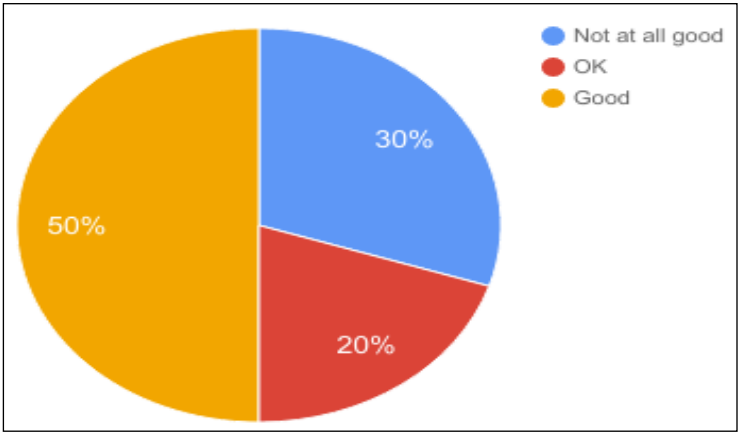
Out of the total volunteers 70% of the volunteers showed increase in tooth whiteness by one shade, in 20 days.



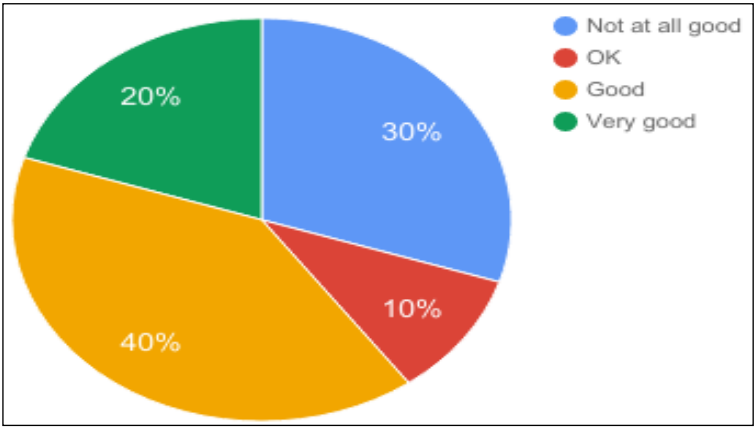
Teeth Whitening

Survey Observations and Results:

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



Taste



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 sit 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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Chapter 2 - 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. Asparaginase 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 asparaginase. 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 calorimetrically

at 500 nm by nesslerization. Enzyme activity was determined with reference to ammonia liberated using ammonium sulphate standard graph.

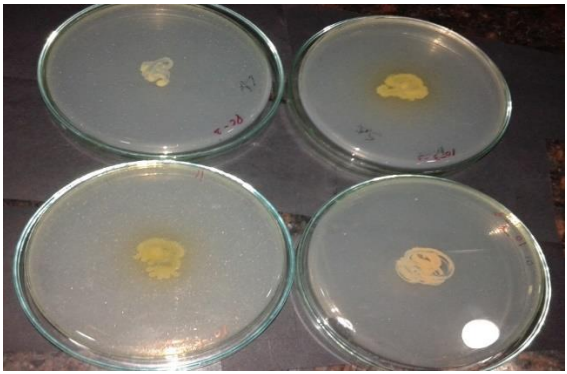
Identification of Hypersaline isolates

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 Determinative Bacteriology and also performed various biochemical tests.

Results

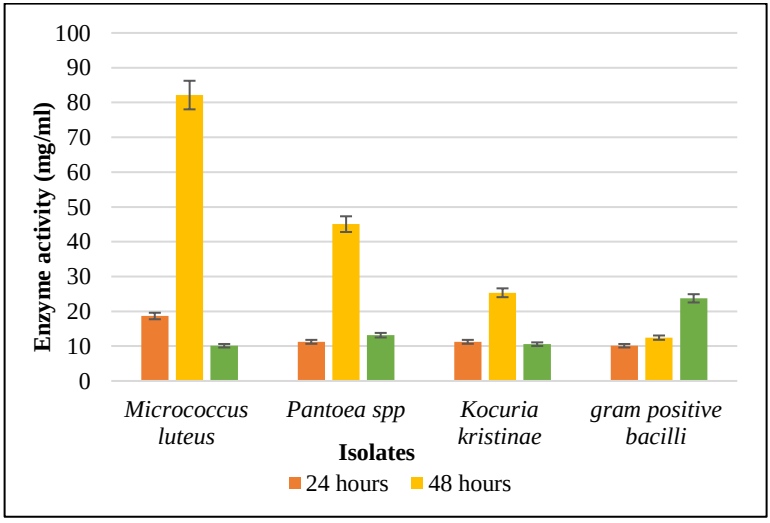
A total of 32 isolates were obtained from a marine soil sample of which 4 isolates (MI 1-MI 4) showed growth on Glucose asparagine agar were found to be positive in primary screening and also checked further using secondary screening.

The figure below shows 4 +ve 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 paracentral 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 discoveries 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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Chapter 3 - 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

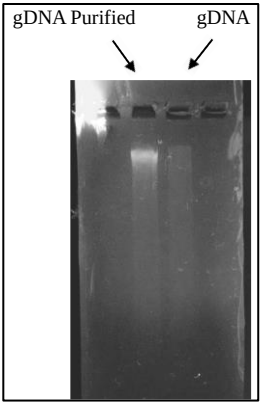
1. **Extraction of genomic DNA from Aloe vera-** The genomic DNA extracted from *Aloe vera* using CTAB method. The original protocol was standardized for *Aloe vera*, since it has a lot of gel content [7].

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.

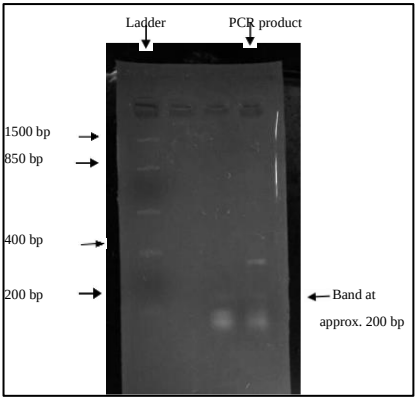
4. **Polymerase Chain Reaction -** The primers were synthesized from a Homolog of this gene in *A.thalaina*. The PCR was standardized at annealing temperature 54°C [9].

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 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. 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 (200bp).

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 cycles. [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 Red Taq, 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 obtained 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 reproducible 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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Chapter 4 - 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

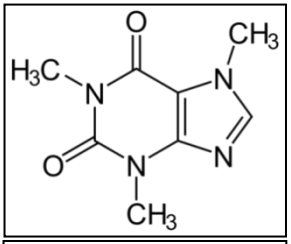
be spread that people who consume, more that 2-3 cups of instant coffee per day, should switch to filter coffee to avoid health hazards.

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.



Structure of Caffeine

1.2 Structure of 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

Chemical Formula: $C_8H_{10}N_4O_2$

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].

Plant of *Coffea arabica*(L.)

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 is repeated for 5 times.
4. Calcium sulphate is added to the separated chloroform layer to remove water. Shake well until fluffy, cloudy effect is observed.
5. The beaker holding the filtrate is weighed. Excess calcium sulphate is filtered out. The beaker with filtrate is placed into hot water bath to evaporate chloroform (Boiling Point: 61.2 degree Celsius).

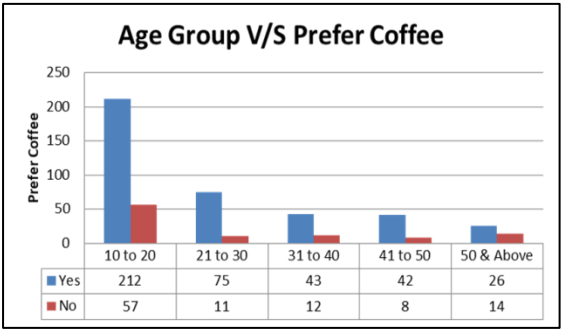
C) Re-crystallisation:

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.

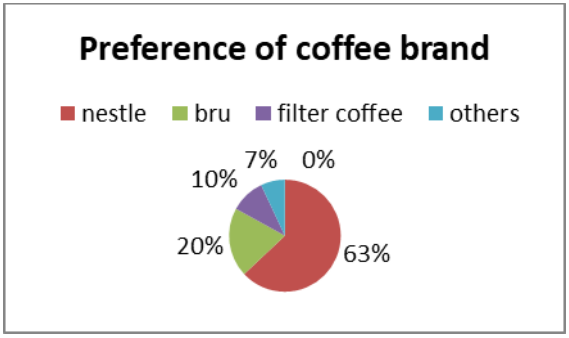
3. It is filtered while hot, using cotton plug.
4. The solution is further boiled to reduce the volume to 5ml.
5. The solution is cooled un till the formation of crystals is observed.
6. The crystals are powdered, weighed to calculate the amount of powder extracted.

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.



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 < 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].

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%

Discussion

A survey conducted with 500 individuals 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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Chapter 5 - 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.

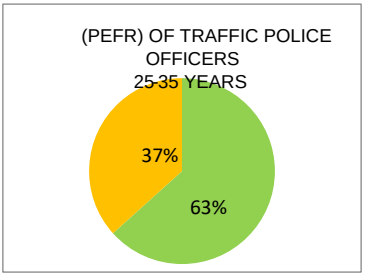
The questionnaire contained the following information- the traffic junction areas, the duration of their service (administrative office & traffic junction), whether they work at day/night & the average hours they work for.

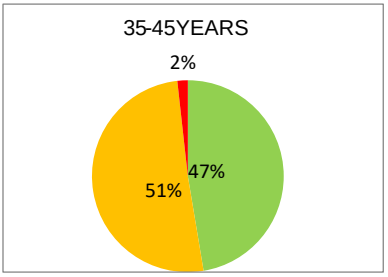
The other part of the questionnaire dealt with the problems they face during their duties such as :

- 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.

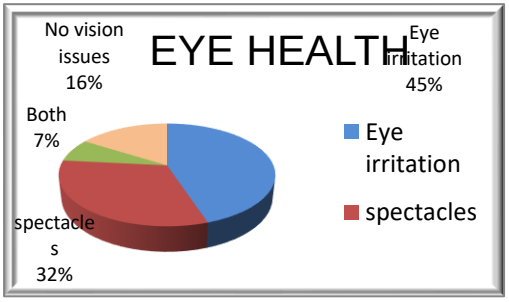
- One of the important questions included was whether they were provided with any protection masks and if they used it on their duty hours.

RESULTS

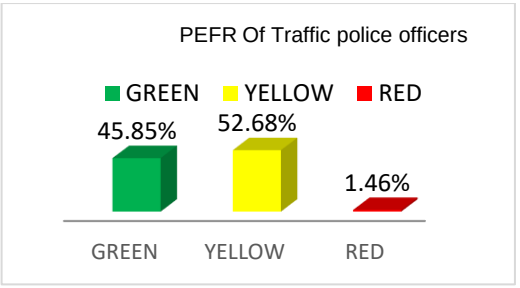


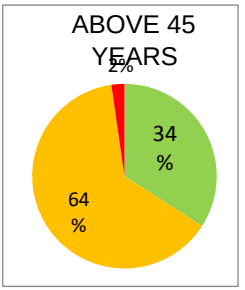


Our finding showed that, out of 205 traffic police personnel 16.58% reported family history of respiratory disorders amongst which 50% were suffering from hypersensitivity to dust or pollen. Overall, 32.68% had hypersensitivity problem amongst which (55.22%) were in yellow zone i.e. susceptible to asthma 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%.

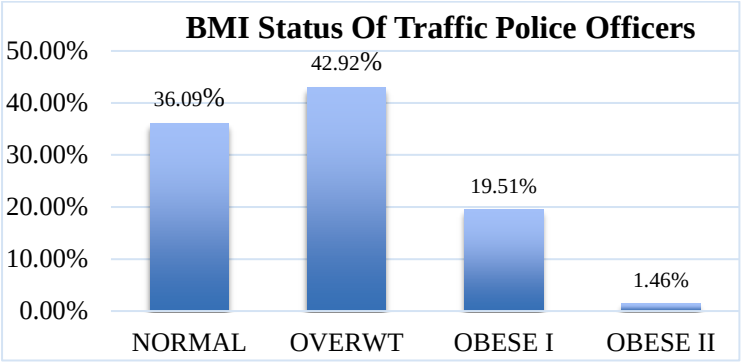


The data revealed that percentage of Yellow zone (52.68%) is greater than that of Green zone (45.85%) thus; there Lung capacity of traffic police personnel is at a high risk of various respiratory disorders (COPD). Some traffic police personnel come under Red zone (1.46%) this shows they are at immediate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





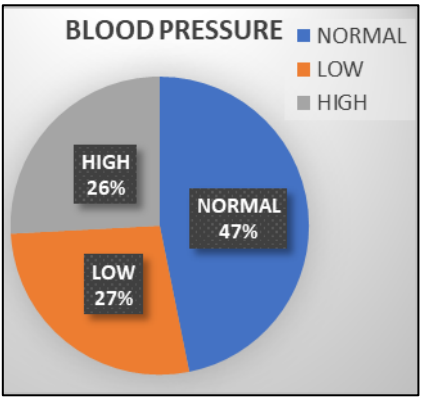
It was observed that 42.92% of people were overweight and 19.51% were obese class I (According to their BMI). Whereas, 1.46% people were obese class II and the rest 36.09% were normal.



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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SECTION II – CHEMISTRY

Chapter 6 - 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 number 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

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

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

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 ranges 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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Chapter 7 - 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 include alkylbenzenesulfonates, 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 37º 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 37º 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

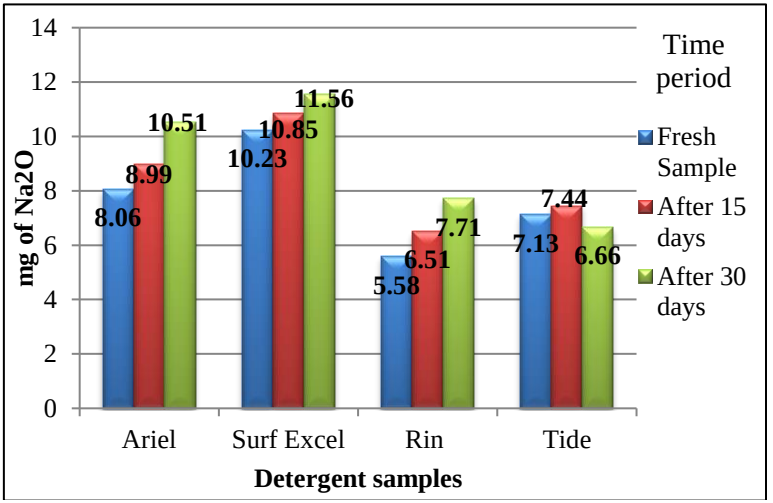
1. Moisture Content:

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.

Table: Moisture content of various detergents

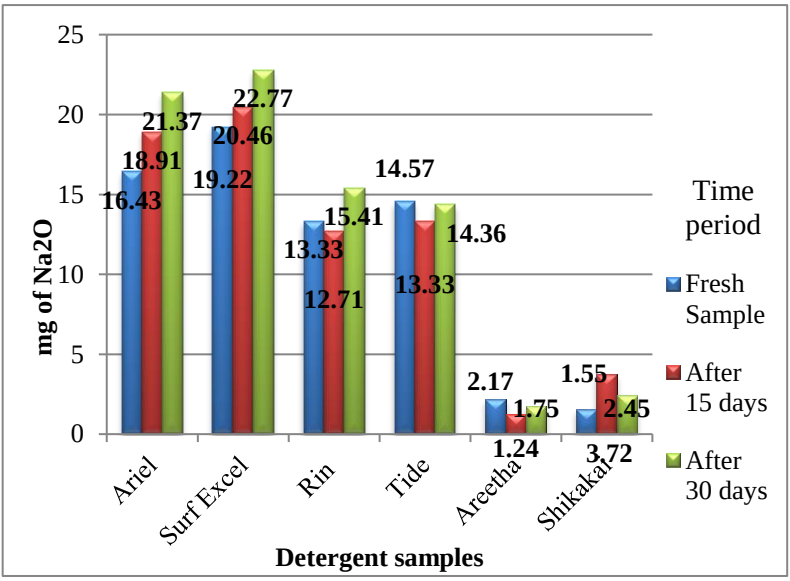
Sr. No.	Sample	Moisture content (in %)
1.	Ariel	2.639
2.	Surf Excel	6.312
3.	Rin	2.320
4.	Tide	2.881
5.	Areetha	6.390
6.	Shikakai	6.395

2. Alkalinity:



Total Alkalinity of Detergent Samples

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



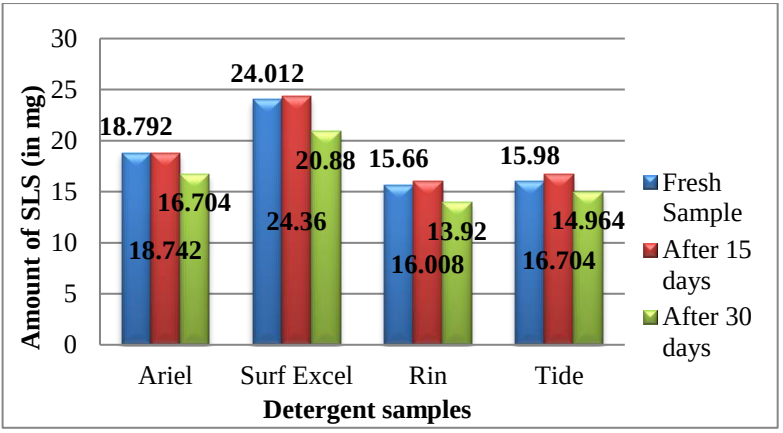
Total Alkalinity of Detergent Samples

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.

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



Active detergent matter of the samples of various detergents

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.

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

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.

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 37ºC. 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 Dhobighatand 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%

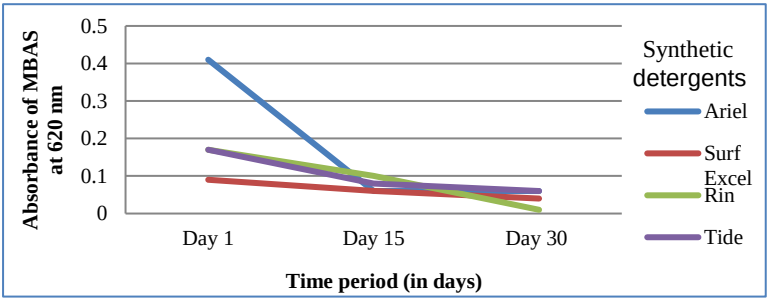
sample detergent solutions viz. Ariel, Surf Excel, Rin and Tide. This indicates that the active detergent matter present in them can be degraded slowly by the bacterial cultures isolated from the detergent rich soil in Dhobighat.

The Figure 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]

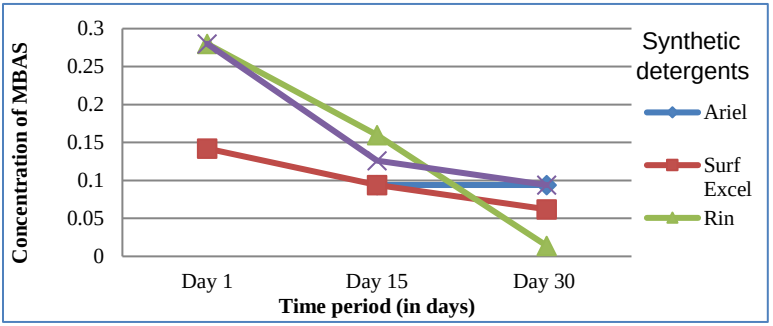
The Absorbance of MBAS (mg MBAS/L) on Day 1, Day 15 and Day 30 for various detergent samples

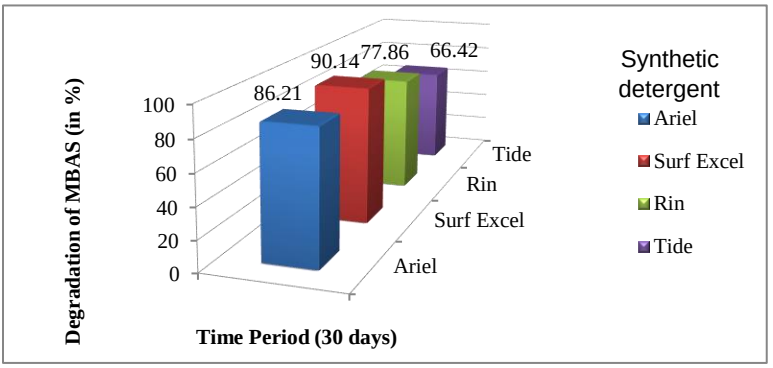
Sample (1% Detergent solution)	O. D. at 620 nm for Day 1	O. D. at 620 nm for Day 15	O. D. at 620 nm for Day 30
Ariel	0.41	0.06	0.06
Surf Excel	0.09	0.06	0.01
Rin	0.17	0.10	0.04
Tide	0.17	0.08	0.06



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





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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Chapter 8 - 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.

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 Whatman filter 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 for Lead, 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)

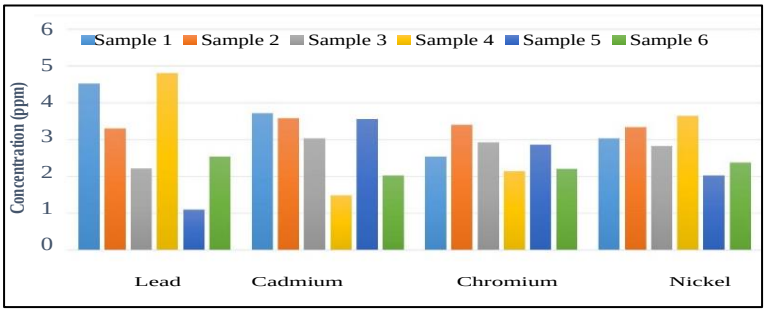

Figure 1: Concentration of Metals in Different brands of lipsticks

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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Chapter 9 - 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 its 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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Chapter 10 - 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-colored 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 oestrogen to "oppose oestrogen" as part of hormone replacement therapy. If oestrogen 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 HNO_3. 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^{o}C. The dried residue was then blended into powder and converted to ash by subjecting it to incineration in a muffle furnace maintained at 500^{o}C for 3:30 hours.

Samples digestions

Ash of 1.0g of powdered flower sample was digested in concentrated H_2SO_4 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 analysed 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:

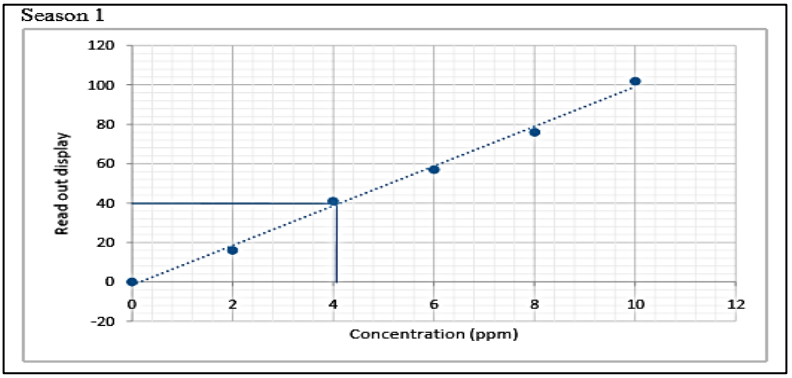
Lab: Analytical Chemistry Lab, Kishinchand Chellaram College. Vidyasagar K.M. Kundnani Chowk, Churchgate, Mumbai – 20. Sample Solution Preparation: Banana flower was Sun Dried for 48 hours. The dried flower was then blended in a mixer and a powder was obtained. 1 gram of powder was digested in distilled 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

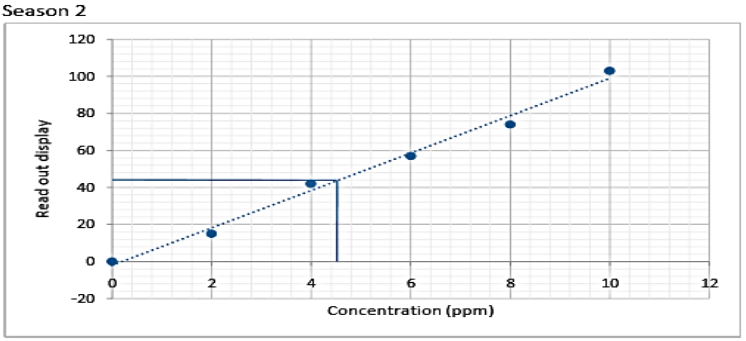
A) Estimation of Sodium

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:



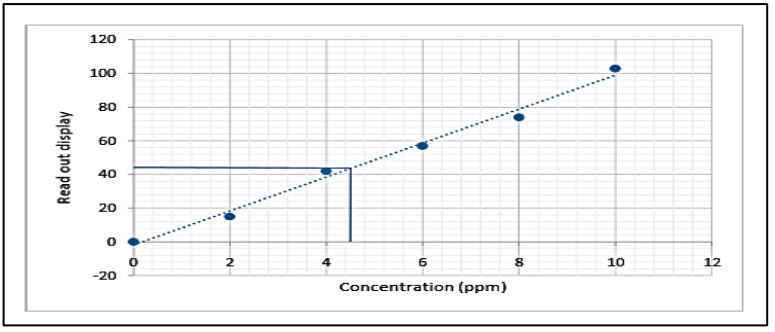
From graph, 1.0 g powdered sample in 100 ml contains 4.100 ppm Sodium. Therefore, Sodium content in flower sample is, 4.100 mg/100g.



B) Estimation of Potassium

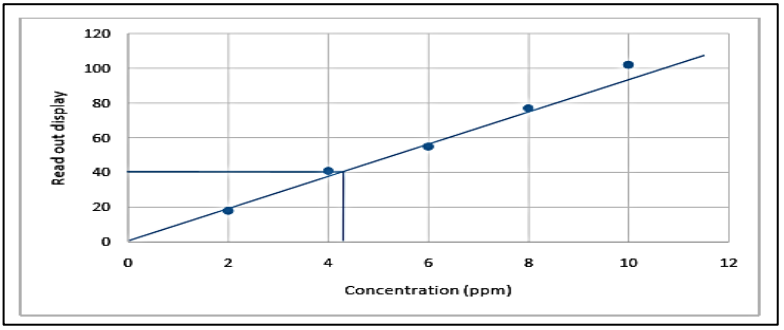
Concentration of Solution (Season1) (ppm)	Read-out Display		Concentration of Solution (Season2) (ppm)	Read-out Display
2	15		2	18
4	42		4	41
6	57		6	55
8	74		8	77
10	103		10	102
U.K. A1	44		U.K. A1	37
U.K. A2	42		U.K. A2	44

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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Chapter 11 - 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; standard milk 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 interplays 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. 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	MOTHERDAIRY	GOWARDHAN	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 1:10 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 to 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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SECTION III - BIOTECHNOLOGY

Chapter 12 - 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 nitroso proline. 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^{o} 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^{o}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 number 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		0.5	**Vortex each tube immediately**
1	0.1	10	0.9	3		0.5	
2	0.2	20	0.8	3		0.5	**Incubate at RT in the dark for 30 minutes**.
3	0.4	40	0.6	3		0.5	
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			

Mix the contents of the tube. Incubate for 10 minutes at RT

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.

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

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

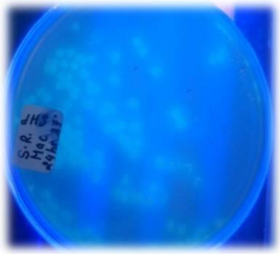

Fig 1. Colonies of *Pseudomonas aeruginosa* under UV transilluminator

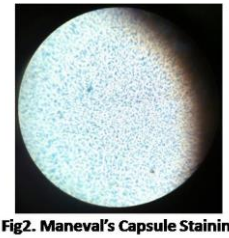

Fig2. Maneval's Capsule Staining

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]

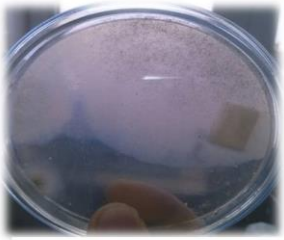

Fig 3.Organism name: *Rhizopus oryzae* strain WM 06.833

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.

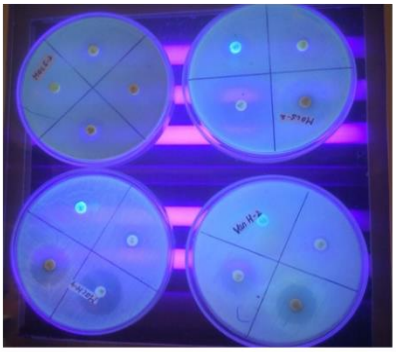

Fig 4 Antibiotic sensitivity profile of the isolates

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.

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 ($r= 0.48$, $P= 0.027$; $P< 0.05$)

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

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]

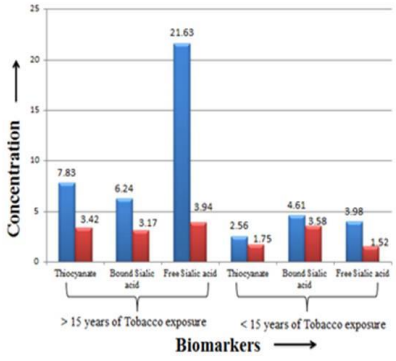

Fig 6: Biomarkers Estimate

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Chapter 13 - 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 on Nutrient Agar, MacConkey's Agar and Veillonella Agar**.**

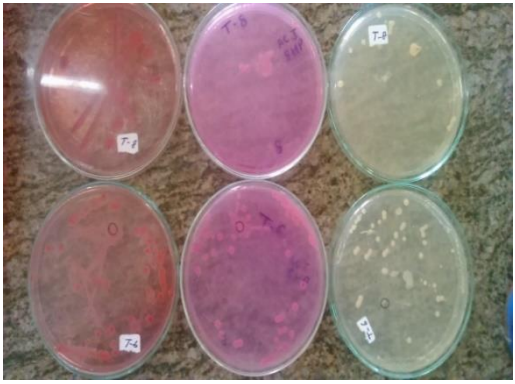


FIG-2 Gram staining of***Klebsiella sp***

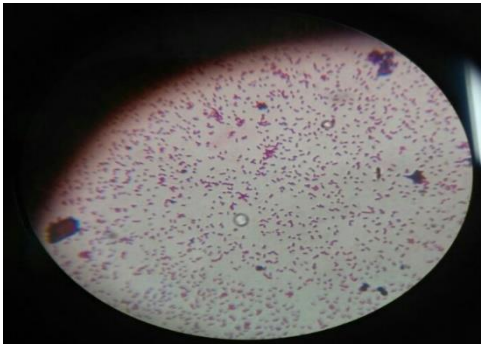
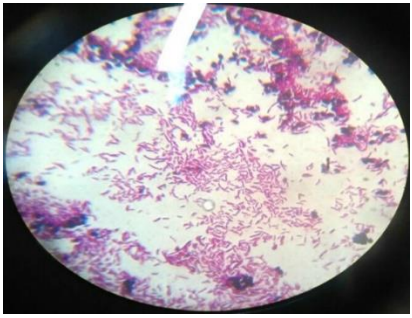


FIG-3 Gram staining ***beta proteobacterium***



Biochemical test-

Table-1*Klebsiella sp.*

Indole	Methyl red	Voges proskauer	Citrate test
-	-	+	-

ugar 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

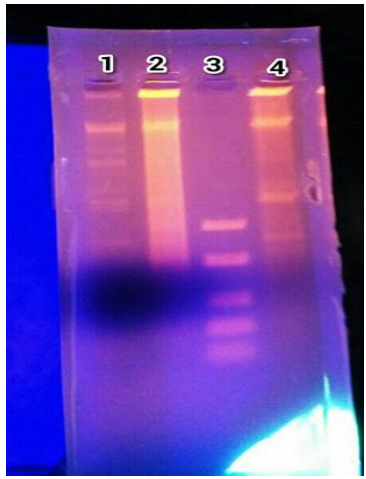
Smokeless Tobacco Product with following code	pH Reading
T1	8.6
T2	9.06
T3	5.77
T4	9.8
T5	5.5
T6	5.5
T7	5.5
T8	6
PM1	9.2
PM2	9
PM3	9.3
PM4	8.5
M1	6.5
G1	6.04
G2	8.0

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,photoautotrop-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	Retention time	Area	Height
Ethane,1,1,2-trichloro	12.021	54675	22060
1,4-Dioxane	10.374	321516	130481
Isoprothiolane	26.786	58354	15678
Diazoxon	34.877	90967	18020

FIG-5 (GC-MS) Chromatogram 1

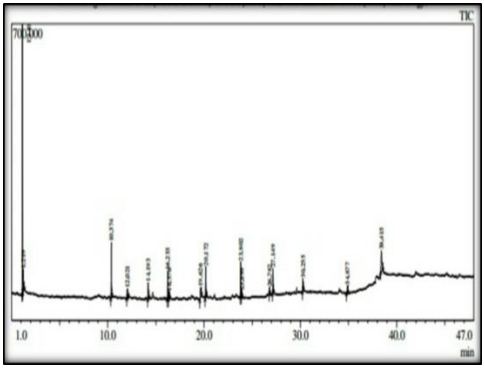
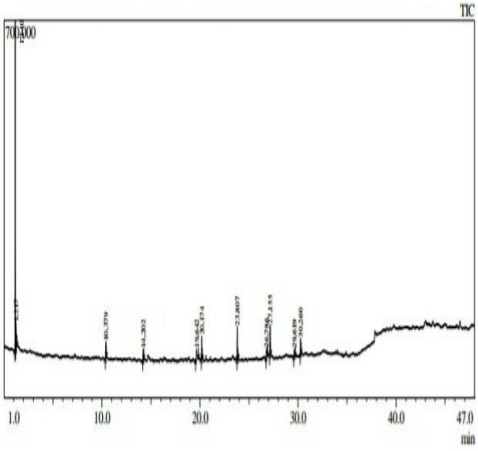


FIG- 6 (GC-MS) Chromatogram 2



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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Chapter 14 - 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 4ºC 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

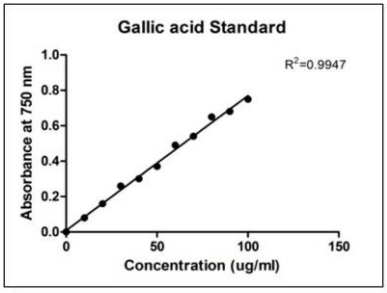
1. Qualitative Phytochemical Analysis

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.*

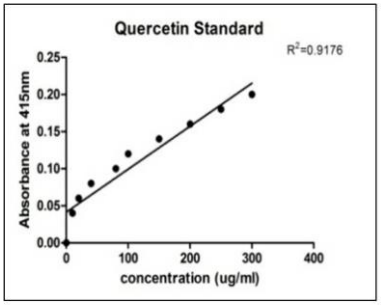
2. Quatitative Phytochemical analysis

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

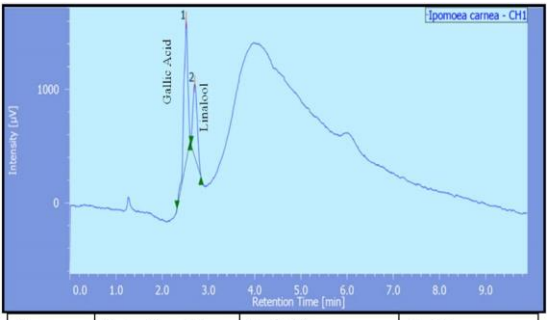


Graph 1: Standard calibration plot of Gallic acid

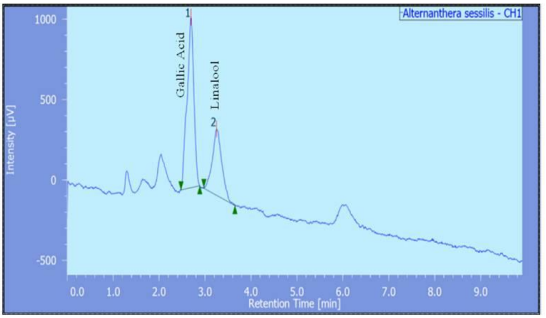


Graph 2: Standard calibration plot of Quercetin

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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Chapter 15 - 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:

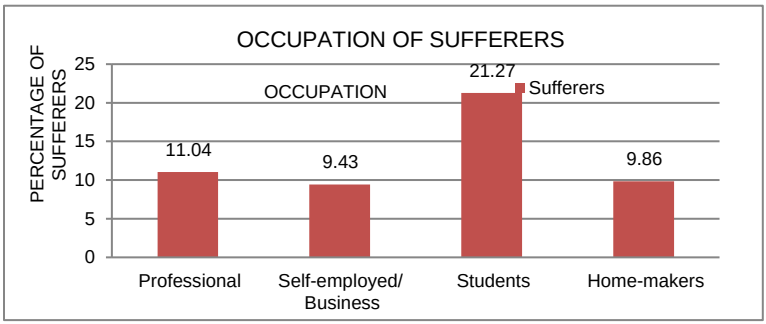
This test provides insight into the correlation between two attributes, their association, disassociation or complete independence. Yule's coefficient of association has the advantage of simplicity. If the attributes are independent of each other, the coefficient of association will be zero. If the attributes 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.

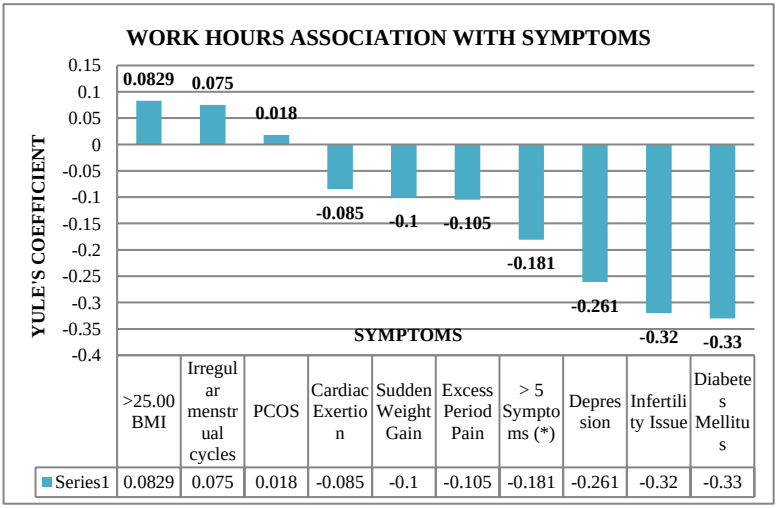
RESULT

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: χ^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	**χ^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 worked 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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SECTION IV - MICROBIOLOGY

Chapter 16 - 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+ 3.3 mm and 12.3 +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

Tinospora cordifolia, commonly known as Guduchi, is the species of Menispermaceae family. 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 37^0C 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.

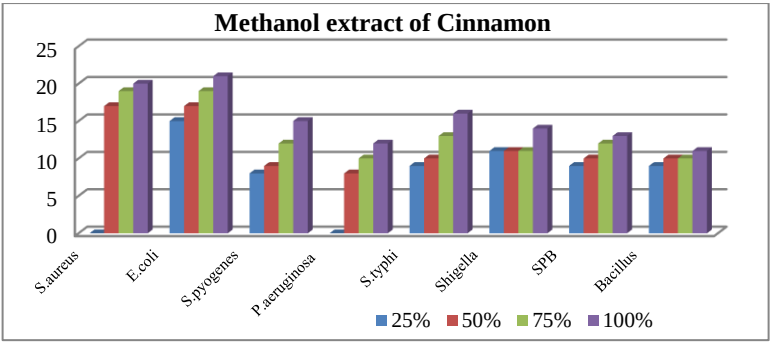
Synergistic activity of Cinnamon and Guduchi: For synergism, agar well diffusion performed.All the 4 concentrations (i.e. 25%, 50%, 75% and 100%) of the extract of Guduchi and cinnamon were mixed and the extracts were added to the agar wells and the activity was checked against individual test organisms 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

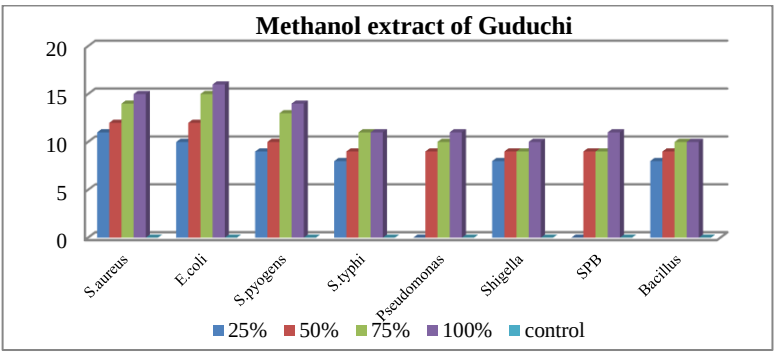
From the test organisms, methanol extract of Cinnamon at 100% has showed best activity against E. coli (21mm) as compared to Bacillus (11 mm) in agar well diffusion method. All organisms except S. aureus showed inhibition at 25% concentration. The activity of 100% Cinnamon extract in methanol was maximum 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 andShigella (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.



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.

Concentration (mg/ml)	*E.coli*	*S.aureus*	*S.typhi*	*P.aeruginosa*	*SPB*	*Shigella*
6.25	+	+	+	+	+	+
12.5	+	+	+	+	+	+
25.5	+	+	+	+	+	-
50.5	-	-	-	-	-	-

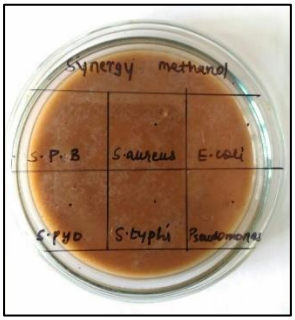
Table.1- MIC of Cinnamon by Agar well diffusion method.

Concentration (mg/ml)	*E.coli*	*S.aureus*	*S.typhi*	*P.aeruginosa*	*SP B*	*Shigella*
6.25	+	+	+	+	+	+
12.5	+	+	+	+	+	+
25.5	+	+	+	+	+	-
50.5	-	-	-	-	-	-

Table.2- MIC of Guduchi by Agar well diffusion method.

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 shows Synergy by agar dilution method

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SECTION V – COMPUTER SCIENCE

Chapter 17 - 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).

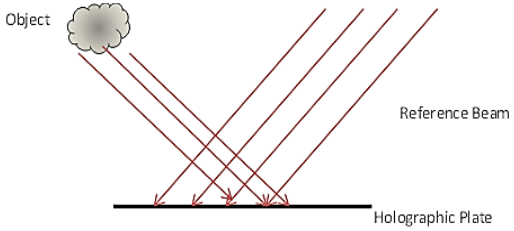


Figure 1: Principal of Transmission Holography

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.

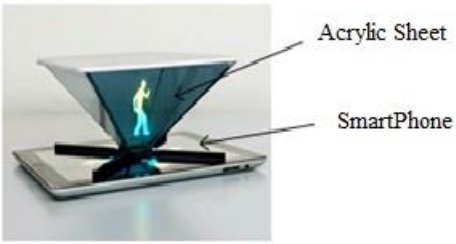


Figure 2: Projecting Image In air through smartphone display

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 Array List<Image Item> image Items = new Array List<>();
try {imageURI = Uri.parse(getIntent().getStringExtra("img"));
Parcel File Descriptor

parcel File Descriptor get Content Resolver () . open File Descriptor
(image URI, "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(), scaled Bitmap.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(requestCode, resultCode, data);
        try
        {
            imageView.setImageURI(imageUri);
            BitmapDrawable drawable = (BitmapDrawable)
    imageView.getDrawable();
            Bitmap bitmap = drawable.getBitmap();
            bitmap = Bitmap.createScaledBitmap(bitmap, sc1,
    sc2, true);


            imageView.setImageBitmap(bitmap);
              (---------) // 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:

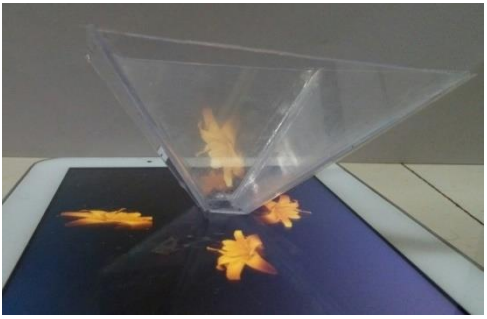
1. Declare a four object of class ImageView

(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 start Activity For Result () method display image through URI as set Image URI () 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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Chapter 18 - Hand Gesture Recognition of English Alphabets

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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. Web Camera

Starts the webcam for the user to capture image.

4. Capture

Captures the image and inserts it into the database.

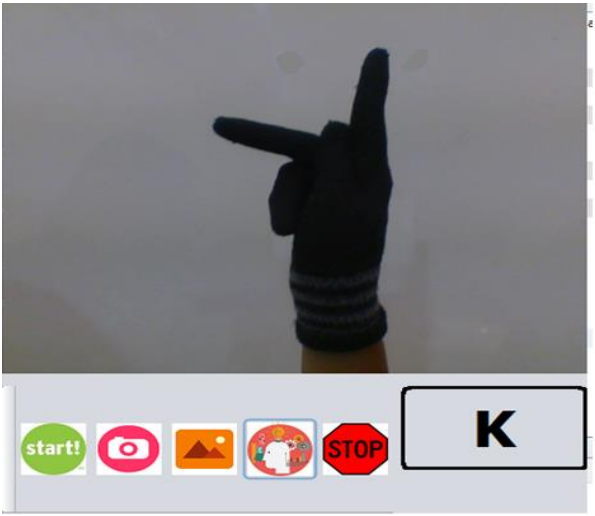
5. Gallery

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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SECTION VI - PSYCHOLOGY

Chapter 19 - A Comparative Study of Level of Stress Faced by Day Scholar & Outstation Students and Their Parents

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Abstract

The stressors faced by people lead to differences in the level of stress experienced by them based on their perception. Thus, the aim of the study is to investigate the significant difference in the level of stress experienced by outstation students and their parents, and day scholar students and their parents. A random group design was used, which comprised of 28 students and 56 parents from different cities in India. Self-devised questionnaires were used to measure the level of stress faced by students and their parents. To analyze the significance of difference between the level of stress faced by parents & students as a whole, outstation students & day scholars, day scholars' parents & outstations' parents (mother & father separately), outstation males & females, outstation females' parents & outstation males' parents, and males & females as a whole; a parametric t-test was used and several t-values were calculated. Results indicated that a t-value of 2.2874 for parents vs. students significant at .05 level was found. The other t-values, 0.0247 for outstation parents vs. day scholar parents, 0.5796 for outstation mothers vs. day scholar mothers, 0.5574 for outstation fathers vs. day scholar fathers, 1.3652 for outstation students vs. day scholar students, 0.3643 for parents of outstation females vs. parents of outstation males, 0.4817 for females vs. males and 1.1715 for outstation males vs. outstation females were not found to be significant at .05 and .01 level. Certain factors which emerged as the main stressors are irregular sleeping habits and separation anxiety.

KEYWORDS*:* Stress, Outstation students, Day scholar students, Parents.

"It's not stress that kills us; it is our reaction to it."

~ Hans Selye

Stress is defined as “the physiological or psychological response to internal or external stressors.” (APA Dictionary) Every group of society faces stress and has different perspective on stress. Thus, stressors generally vary from individual to individual making stress difficult to define as it does not mean the same thing to everybody. Nonetheless, most people acknowledge that the experience of stress has three components. The first component is the stressor, which is the events in one's surroundings, mind or body that produces stress. The second component is perception, most of the events occurring around oneself are neutral, it is dependent on the person if he perceives the event as stressful or not. The third component is the nature and degree of reaction to the event; it is how one responds to a circumstance that really depicts the experience of stress. Thus, one experiences stress when circumstances disturb one's normal functioning and necessitates one to put in extra effort to re-establish one's equilibrium. Since different segments of society experience unique circumstances, the quality and type of stress they encounter is also distinct from each other. For instance, stress faced by students is relatively different from that experienced by their parents.

STUDENT STRESS

Students may face stress related to their academic performance, personal relationships, finances, career, higher education, daily hassles etc. which may impact their physical health, emotional wellness and academic achievements which again may reduce their quality of life.

Supporting research studies have shown how stress negatively affects students psychologically and physiologically. It affects a student's academic performance (Mannuel, 1982), impairs learning (Montague, 1953) and arouses confusion (Lucas, 1952). As founded by Atkinson and Litwin (1960), Cox (1962), Singh (1966) and Speilberger (1962) stress also affects students’ intellectual performance and their ability to solve problems (Brown, 1953). In addition, it was found that fear of failure in academics is the major source of stress among undergraduate students (Marwan Zaid Bataineh, 2013). These common stressors may be the same for all the students.

Students' stress may vary on the basis of their lifestyles. Students staying away from their home are known as outstation students. They have a very different lifestyle when compared to day scholars. Staying away from family and living independently may bring additional stress to their lives. Outstation students face many difficulties and hurdles which day scholar

students may not have experienced such as financial crises and running out of monthly budget, adjustment issues with mates, personal helplessness, alteration in eating and sleeping schedules, and many other issues which may impact and increase outstation students' stress.

PARENTAL STRESS

It is overwhelming for parents to fulfil the requirements of parenting which causes stress about their children's well-being. Parents face stress regarding every facet of their children's lives such as children's growth, safety, relationships, studies, lifestyle, daily routine and so on.

Research findings suggest that a stressful environment and limited financial resources act as a hindrance to managing parental stress. One of the sources of stress in the child-parent relationship is culture (Sidebotham and ALSPAC, 2001). Consequently, societal and cultural impositions make parenting stressful. It is found that there is an increase in stress due to lower-income, education levels and less social support (Charlie Stelle, Christine A. Fruhauf et al., 2002). Finding suggests that parents felt more competent and that fewer behavioural issues were reported in children when parents reported greater levels of social support (Marshall et al., 2001).

Thus, although both parents and students are stressed about common factors, there is a mismatch when it comes to the level of stress experienced by them on these factors which is what the current paper wishes to investigate. Hence, if the research findings suggest that the mismatch in the perceived level of stress do exist, it can help parents and students realise it. This may in turn help them to employ better coping strategies and inculcate a healthier relationship with each other.

LITERATURE REVIEW

Several research studies have been conducted focusing on stress experienced by students and the repercussions of the same.

The specific factors of stress in individuals encompass difficulties in financial management, changes in the living atmosphere, difficulties in managing personal and academic life, etc. (Byron, Brun & Ivers, 2008; Chernomas & Shapiro, 2013; Goff, 2011; Jimenez, Navia-Osorio & Diaz, 2010; Moscaritolo, 2009). The study conducted by Chandrashekhar T & Sreeramareddy, et al. (2007) highlighted the most frequently occurring sources of stress among students which includes quality of food in mess, high parental expectations, dissatisfaction with

the class lectures, the vastness of academic curriculum/syllabus, worrying about the future, lack of entertainment in the institution, frequency of examinations, becoming a doctor (expectations on all fronts), and lack of time for recreation. Other studies on stressors found that in academic settings, some of the common stressors reported include excessive assignments, poor time management and social skills, peer competition, etc. (Fairbrother & Warn, 2003).

Therefore, understanding the sources of stress will help in developing customised interventions targeted to reduce the stress levels of students. This approach will contribute towards the overall well-being of the students. (K. Jayasankara Reddy et al., 2018).

Furthermore, it was found that gender too can influence stress as in one study it was found that although adolescent girls and boys of class X and IX may experience the same level of stress, boys experience a greater level of parental pressure in academic work. Also, parents with lower education level were found to pressurise their children more than parents who were well educated. (Zeetha M Sangma et.al., 2018).

In another study on gender conducted by Latha R. and Kantha S. (2006) on the 'Effect of gender and hostel life on stress and performance in medical students' to assess the stress levels in 100 first-year medical students before and after an important examination. The study showed that the females and day scholars were less stressed and had performed significantly better than males and hostlers respectively. The reasons that the study suggests for the lower stress level in females can be the better coping strategies, neuro endocrine mechanisms and Tend and Befriend theory adapted by the females. According to this study, outstation students have shown poorer performance and more stress because they are living away from their homes and might be facing adjustment issues in new surroundings which has taken a toll on their well-being.

Research on outstation students suggests that they learn how to live independently and how to compromise with the other students and roommates (Khozaei et al., 2010). Undoubtedly, they experience many difficulties and problems such as financial crises, adjustment issues, personal helplessness, distress, changes in eating and sleep habits, and other issues. On the other hand, research shows that empathy, altruistic behaviour, emotional stability will be higher in outstation/hostel students.

The environment in hostels gives an opportunity for socialization among students (Mimrot, 2012). Hostel life also makes students more ambitious, more self-reliant and confident than other students and they learn courage and spirit from other students, and this may help students to face the practical life more confidently (Ahmad, 2006). Another study on outstation/hostel students by Iftikhar, A & Ajmal A (2015) shows that living in a hostel makes students more punctual, independent, confident, social, realistic, disciplined, and sharp. The study also deduced that male hostel students are more vulnerable to be affected negatively during hostel stay as most of them indulge in drug addiction.

A study on the topic "Stress and Resilience: A Comparative Study Between female Hostellers and female Day Scholars" (Singh R.S, 2014) showed that both of them face an equal amount of stress irrespective of their accommodations but the measure of resilience showed a significant difference in day scholars and hostellers. It was established that resilience is higher in hostellers which proved their tendency to cope better when exposed to severe stress, trauma and adversity.

In a similar study which took place in 3 different medical colleges in Pakistan to assess psychological depression, anxiety and stress. The findings depicted that there is a higher level of stress amongst students living at home compared to students living in hostels. (Hafsa Laiqat, Usama Khalid Choudry, et al.,2017).

Additionally, another study conducted by Ravichandran (2015) on the topic of "Measuring stress in hostelites and day scholars" portrayed similar results. To assess the psychological state of dental students who were in their first year of college in Chennai. The results showed that although the stress portrayed by the anxiety score was present in both groups, the stress was found to be greater in day scholar students than hostelite students statistically. It was explained that although hostelites face separation stress and stress-related to moving to a completely new environment etc, the hostel life helps students to become more social, outspoken and allows them for new friends as well as help them develop into more independent individuals. Whereas day scholar students are exhausted due to long hours of travelling, lack of time to complete their tasks and are unable to have any social interaction with their peers which adds on to their stress.

In contrast, a study conducted in the University of Porto, Portugal aimed to measure the intensity and sources of stress, understand the stress and

lifestyle of medical students and explore the discrepancies in academic stress and psychosocial variables in regards with the gender of students, course year and displacement of home. The results indicated 58.2% of clinically significant stress amongst students who are living away from home. Adding to that, the study revealed that female students perceived higher levels of academic stress and stress responses and 47% of the students had health risk behaviours; some of which were eating habits, alcohol and drug consumption etc. (Elizabete Loureiro et.al, 2008).

Stress is also experienced among parents in relation to their children.

Parkes A., Sweeting H., & Wight D. (2015) conducted a study on "Parenting stress and parent support among mothers with high and low education" in Scotland in which they took 5,865 mothers of 10-months-old children as participants. This study attempted to investigate the correlation between the parenting stress of mothers and the SEP of the mothers. The SEP of the mothers was measured using the Scottish Credit and Qualifications framework and the parenting stress was measured using an abbreviated version of the Parental Stress Scale by Berry & Jones (1995). The results revealed that there is a higher level of parenting stress among both most and least educated mothers compared to intermediate educated mothers. Moreover, it was found that grandparents' support for the mother is crucial as less frequent contact grandparent contact resulted in higher levels of parenting stress for both high and low educated mothers, especially if they are migrants. Not only the marital status had an impact on the level of parenting stress as single-parent mothers experienced higher levels of parenting stress but also, migrant mothers faced higher levels of stress compared to non-migrant mothers.

Thus, both students and parents experience stress though the level may differ.

OBJECTIVES

Aim

The aim of the research is to compare the stressors and the level of stress among the day scholars and outstation students and their parents respectively & to find the areas of mismatch between the level of stress experienced for same stressors by parents & students of each category.

Rationale

In today's time where migration is quite mainstream and around 3.7 million students migrate every decade for their education, this present study wants to understand the impact of moving to a new place on the students and their parents' stress. In order to have a better understanding of outstation students and parents' stress, the study attempts to compare their stress with that of day scholar students and their parents'. Moreover, only after the comparison is done, it can help both the day scholar and the outstation students and parents to know if a mismatch exists in their level of stress, then they can develop better coping mechanism to manage the stress levels. Furthermore, the study wishes to learn more about the severity of the stress experienced by outstation students and day scholar students.

Additionally, this study is an attempt to help parents and students have a better understanding of their level of stress. Not to mention, living away from home actually develops students holistically. They learn not only to live independently but also to manage their overall life more properly. Previous studies have shown that outstation students are more resilient than day scholar students hence this study attempts to help parents of outstation students understand that although there is the physical distance between parents and the students, their child is coping well and is able to adapt to a new environment by inculcating better life skills. Lastly, the researchers did not come across any study conducted on the perception of stress between parents and students.

Hypotheses

Null Hypothesis 1: There will be no significant difference between the level of stress of parents and students.

Null Hypothesis 2: There will be no significant difference between the level of stress of parents of outstation students and parents of day scholar students regarding their children.

Null Hypothesis 3: There will be no significant difference between the level of stress of mothers of outstation students and mothers of day scholars.

Null Hypothesis 4: There will be no significant difference between the level of stress of fathers of outstation students and fathers of day scholars.

Null Hypothesis 5: There will be no significant difference between the level of stress of outstation students and day scholar students.

Null Hypothesis 6: There will be no significant difference between the level of stress of parents of outstation females and parents of the outstation males.

Null Hypothesis 7: There will be no significant difference between the level of stress of female and male students as a whole.

Null Hypothesis 8: There will be no significant difference between the level of stress of outstation males and outstation females.

METHOD

Independent Variables

The place of living (Home or hostel)

Operational definition

Outstation students who have migrated from their hometowns for further education to different metropolitan cities in India Mumbai, Delhi, Indore, Chennai, Bangalore and Jaipur for the past 2-3 years.

Day scholar students living in different cities of India for more than 10 years; Jodhpur, Kalyan Ahmedabad, Mumbai, Bangalore, and Delhi.

Dependent Variables

Level of stress in day scholars, outstation students & parents of each

In terms of following Stressors -

General Well-Being which includes finance, accommodation quality, safety of living area, nitty-gritty of daily living, food and nutrition and ease of commute,

Institution which includes quality of education, college students, faculty and their grade performance.

Social life stressors include quality of friends, alcohol and drug use and abuse, late-night parties and love affairs.

Physical and Mental health stressors include medical emergencies, natural calamities, riots, sleeping habits, separation anxiety, emotional stress.

Operational definition

Level of the stressors measured by scores on a five-point rating scale where 1 = no stress, 2 = least stress, 3 = moderate stress, 4 = high stress and 5 = highest stress.

CONTROLS

- All participants taken in the research are from India and are residing in different cities of India.
- Students' age group ranges from 19 to 21 years. Day Scholars are staying in their residential cities for more than 10 years, whereas outstation students are staying in the migrated city for 2-3 years.
- Parents' age group ranges from 40 to 60 years.
- Parents are living together.
- Equal number of males and females are included in the sample.

SAMPLE

The data was collected from different colleges and cities in India. The sample size is 24 students & 56 parents. It includes 14-day scholar students that are equally divided on the basis of gender (M=7, F=7) and both of their parents (Mothers=14, Fathers=14) as well as 14 outstation students and their parents who are also divided as mentioned above. The age of students was from 19 to 21 years and for parents from 40 to 60 years.

PROCEDURE

Online google forms were sent through email to people residing in different cities of India. Informed consent from the participants was taken and confidentiality of information provided by them was also assured.

DATA ANALYSIS

Descriptive statistics were used to describe the sample and the data collected.

Several t-tests were calculated to find out whether there were any significant differences between the variables under study to test the hypothesis.

Tables & graphs were utilised to explain the data better.

RESULTS AND DISCUSSION

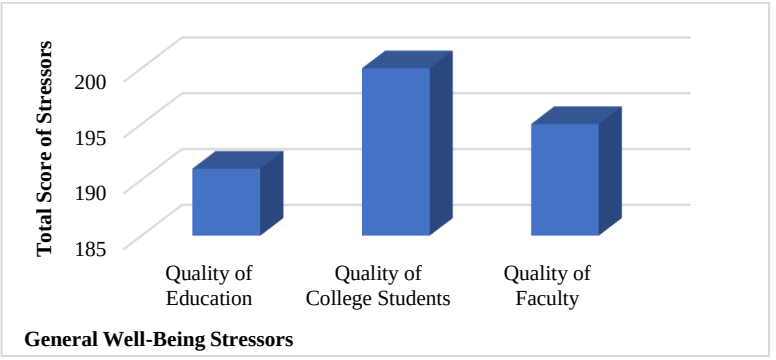
The study attempted to measure the level of stress amongst the parents and their children who were either day scholar or outstation students. The sample for the study was collected from different geographical parts of India to make it representative of the whole population. The outstation

students were from Mumbai, Raipur, Ahmedabad, Jodhpur, Vadodara, Bikaner and Hampi. The cities to where they migrated were Mumbai, Delhi, Indore, Chennai, Bangalore and Jaipur. The day scholar students were from Ahmedabad, Bangalore, Jodhpur, Delhi, Mumbai and Kalyan. The study took 28 students. The students were equally divided into 14 outstation and 14-day scholar students and they were further divided on the basis of their gender M=7, F=7 in each category. There were 28 parents each from outstation students and day scholar students. The age range for students was 19-21 years and for parents, it was 40-60 years. The mean age of male students was 19.21 as for female students, the average age was 19.07. Additionally, the mean age of mothers was 45.93 and for fathers, it was 49.18.

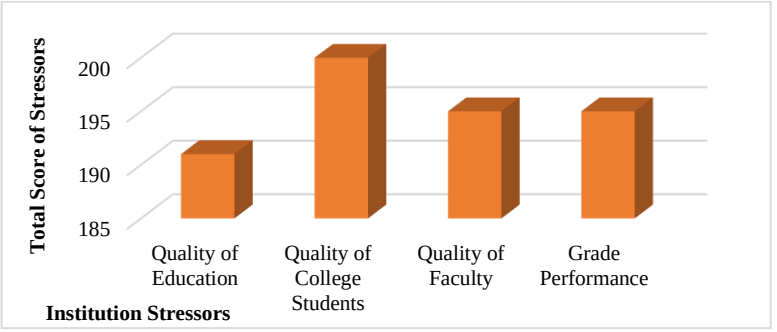
The researchers investigated several stressors and divided them into general well-being, institution, social life and physical and mental health factors.

In general well-being, the factors common to both outstation students and day scholar students and their parents were aspects of finance, quality of food and ease of commute, amongst them, the highest stressful factor was quality of food, second highest was aspects of finance and lowest was safety and ease of commute within the city (Graph 1). Under institution, the factors were quality of education, quality of college students, quality of faculty and grade performance. Amongst them, the highest was the quality of college students, the second highest was quality of faculty and the lowest was quality of education (Graph 2). In social life, the factors were quality of friends, late-night parties, alcohol or drug use/ abuse and love affairs. Out of these, the most stressful factor was late-night parties, the second-most stressful factor was quality of friends and least stressful factor was loving affairs (Graph 3). Whereas, in physical and mental health the factors studied were medical emergencies, natural calamities/riots, irregular sleeping habits, separation anxiety due to migration and emotional stress. Out of these, the most stressful factor was irregular sleeping habits, the second most stressful was emotional stress and the least stressful was natural calamities/riots (Graph 4).

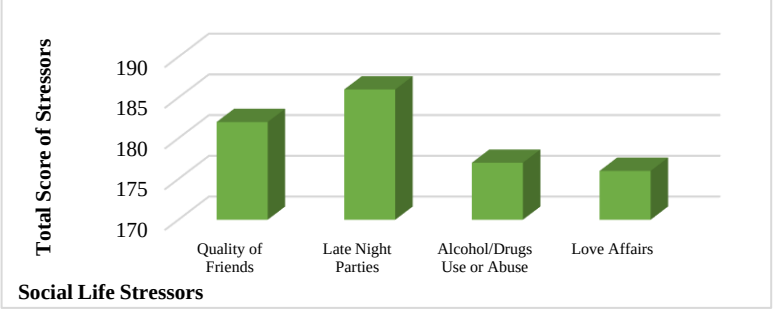
Graph 1: *General Well-Being Factors of Stress for Parents and Students*



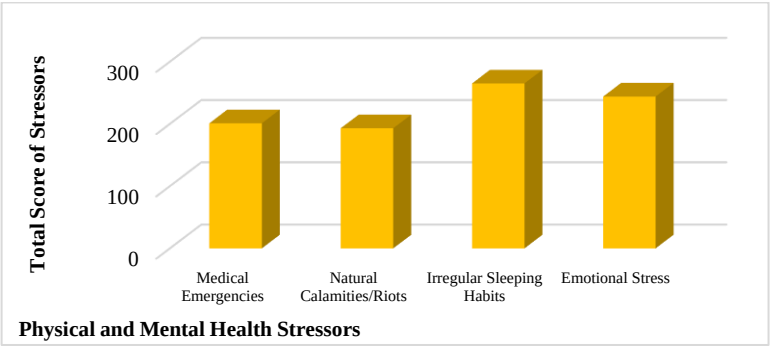
Graph 2*: Institution Factors of Stress for Parents and Students*



Graph 3*: Social Life Factors of Stress for Parents and Students*

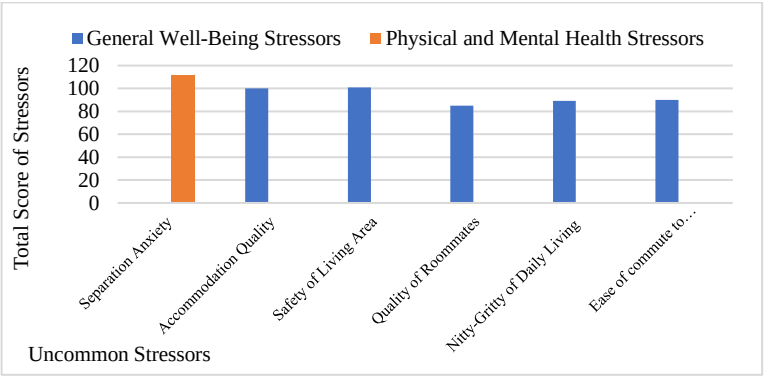


Graph 4: *Physical and Mental Health Factors of Stress for Parents and Students*



However, there are some factors under the general well-being and physical and mental health which are exclusive to only outstation students' and their parents. These are accommodation quality, safety of living area, nitty-gritty of daily living, ease of commute to hometown and other cities under general well-being and separation anxiety under physical and mental health. The most stressful factor under general well-being amongst outstation students and their parents was safety of living area followed by accommodation quality, commute to hometown and other cities, nitty-gritty of daily life and the quality of roommates. Amongst all the exceptional factors for outstation students and their parents, the highest stress was observed in separation anxiety (Graph 5).

Graph 5: *Uncommon Factors of Stress for Outstation Students and their Parents*



In order to compare the stress faced by day scholars & outstation students and their parents, the researchers formulated several null hypotheses.

The first hypothesis stated that there will be no significant difference between the level of stress of parents and students. The total score of stress faced by parents was 2424 & by students was 1416. The t-value (Table 7) indicated that there is a significant difference in the stress faced by parents & students as a whole and the null hypothesis was rejected and the alternative hypothesis was accepted.

Table 7: *T value for Hypothesis 1*

Variable	Parents as a whole	Students as a whole	t value	p-value
Level of stress	M= 43.2857 SD= 14.0424	M= 50.5714 SD= 13.1696	2.2874	>0.05

The second hypothesis stated that there will be no significant difference between the stress faced by the parents of outstation students and the parents of day scholars with respect to their children. The total score of outstation parents was 1021 and day scholar parents was 1019. However, the t-value (Table 8) revealed that there is no significant difference between the stress experienced by both of them. Therefore, the null hypothesis was accepted and the alternative hypothesis was rejected.

Table 8*: T value for Hypothesis 2*

Variable	Parents of outstation students	Parents of day scholar students	t value	p-value
Level of stress	M= 36.4643 SD= 9.7087	M= 36.3929 SD= 11.8489	0.0247	<0.05

The third hypothesis was that there will be no significant difference between the stress faced by the mothers of outstation students and the mothers of day scholars with respect to their children. The total score of mothers of outstation students was 530 and mothers of day scholar students was 496. At an apparent level, the mothers of outstation students seemed to face slightly higher levels of stress when compared to the mothers of day scholars. The t-value (Table 9) revealed that there is no significant difference between the stress experienced by both of them. Hence, the null hypothesis was accepted and the alternative hypothesis was rejected.

Table 9: *T value for Hypothesis 3*

Variable	Mothers of outstation students	Mothers of day scholar students	t value	p-value
Level of Stress	M= 37.8671 SD= 10.8547	M= 35.4286 SD= 11.3118	0.5796	<0.05

The fourth hypothesis was that there will be no significant difference between the stress faced by the fathers of outstation students and the fathers of day scholar students with respect to their children. The total score of fathers of outstation students was 491 and fathers of day scholar students was 523. The t-value (Table 10) indicated that there is no significant difference between the stress experienced by both of them. Therefore, the null hypothesis was accepted and the alternative hypothesis was rejected.

Table 10: *T value for Hypothesis 4*

Variable	Fathers of outstation students	Fathers of day scholar students	t value	p-value
Level of stress	M= 35.0714 SD= 8.5886	M= 37.3571 SD= 12.7135	0.5574	<0.05

The fifth hypothesis was that there will be no significant difference between the stress experienced by outstation students and day scholars. The total score of outstation students was 519 and day scholars was 453. The t-value (Table 11) indicated that there is no significant difference between the stress experienced by both of them. Hence, the null hypothesis was accepted and the alternative hypothesis was rejected. This finding is inconsistent with the finding from the study conducted by Usama Khalid Choudhary et.al in Pakistan who revealed that severe stress was considerably higher among students living at home than students living in hostels.

Table 11: *T value for Hypothesis 5*

Variable	Outstation students	Day scholar students	t value	p-value
Level of stress	M= 37.0714 SD= 10.0343	M= 32.3571 SD= 8.1392	1.3652	<0.05

The sixth hypothesis was that there will be no significant difference between the stress experienced by the parents of outstation females and the parents of outstation males. The total score of the parents of outstation females was 715 and the parents of outstation males was 690.

The t-value (Table 12) indicated that there is no significant difference between the stress experienced by both of them. Thus, the null hypothesis was accepted and the alternative hypothesis was rejected.

Table 12*: T value for Hypothesis 6*

Variable	Parents of outstation females	Parents of outstation males	t value	p-value
Level of stress	M= 51.0714 SD= 15.0867	M= 49.2857 SD= 10.425	0.3643	<0.05

The seventh hypothesis stated that there will be no significant difference between the stress experienced by female and male students as a whole. The total score of the females was 474 and males was 498. The t-value (Table 13) indicated that there is no significant difference between the stress experienced by both of them. Therefore, the null hypothesis was accepted and the alternative hypothesis was rejected. The present study' results indicate that the level of stress is similar between boys and girls including outstation and day scholars both, which is contradictory to the findings of the study conducted by Elizabete Loureiro et.al in Portugal which indicated that there is a higher level of perceived stress among females.

Table 13: *T value for Hypothesis 7*

Variable	Females	Males	t value	p-value
Level of stress	M= 33.8571 SD= 10.1288	M= 35.5714 SD= 8.6444	0.4817the	<0.05

The eighth hypothesis stated that there will be no significant difference between the stress experienced by outstation females and outstation males. The total score of the outstation females was 383 and outstation males was 325. The t-value (Table 14) revealed that there is no significant difference between the stress experienced by both of them. Consequently, the null hypothesis was accepted and the alternative hypothesis was rejected.

Table 14: *T value for Hypothesis 8*

Variable	Outstation males	Outstation females	t value	p-value
Level of stress	M= 46.4286 SD= 3.1511	M= 54.7143 SD= 13.3131	1.1715	<0.05

CONCLUSION

The main aim of this current study was to scrutinize the sources of stress and their impact on day scholar & outstation students and their parents. It was presumed that there will be no significant difference in the stress experienced amongst parents and students, parents of day scholar and outstation students, mothers of outstation and day scholar students, fathers of outstation and day scholar students, parents of outstation males and females.

Moreover, it was also presumed there will be no significant difference between students based on their gender and whether they are outstation or day scholars. The research findings suggest that there is a significant difference only in the level of stress experienced by parents and students at .05 level and that there is no significant difference in any other hypotheses. In addition, the mean for the level of stress for total parents is 43 and mean for the level of stress for total students is 50.

Thus, students experience stress more than parents and the mismatch does exist here. However, it is a healthy sign that parents are confident and less stressed about their children's well-being and lifestyle but since the findings suggest that students stress more, students should employ better coping strategies to manage their stress.

LIMITATIONS

- The sample size is too small so it cannot be generalised to the rest of the population.
- Online google form was used due to the pandemic, though a face-to-face survey or interview would have yielded in-depth results.
- Two self-devised questionnaires were used which may lack validity and reliability.
- The questionnaires were a self-report measure which can lead to social desirability bias.
- Only two genders (male and female) were chosen in the study.

FURTHER RESEARCH

- The students belonged to different fields of study so further research could be studied focusing on one field or comparison across different fields.

- The marital status of parents i.e., whether divorced, separated or single could also impact the stress level of parents and their children, further research could focus on that.
- The outstation students in the study were living in different settings like hostels, PGs, flats. Thus, further research can do a comparative study on the basis of accommodation of outstation students and its impact on them.

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Chapter 20 - Anticipatory Grief and its Link to Locus of Control During the Covid-19 Pandemic

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Abstract

The COVID-19 pandemic has led to a wide range of losses across the world, physically, financially and socially. With the increasing prospects of future losses, anticipatory grief emerges as a specific grief response during this time. This study aims to understand the relationship between anticipatory grief and locus of control. The sample consisted of 140 participants namely 70 participants each in two age groups of 16-26 years and 45-55 years residing in India. Rotter's Locus of Control scale and a self- devised Anticipatory Grief scale were used to collect the data. Pearson's product moment correlation test was used to determine whether there was any significant correlation between anticipatory grief and locus of control. The correlation between anticipatory grief and internal (0.22) and external (-0.068) locus of control was insignificant. T tests were used to compare the level of anticipatory grief and the degree of locus of control (internal and external) between the two groups. The T value for level of anticipatory grief (3.70) showed significant difference between the two groups whereas the degree of internal (0.48) and external (-0.21) locus of control were insignificant. Results revealed that no conclusive relationship could be formulated between the variables under study.

Key Words: Anticipatory Grief, Internal Locus of Control, External Locus of Control, COVID-19

Introduction

The Covid- 19 (SARS-CoV-2) pandemic has taken the world by storm. With its first human case reported by officials in Wuhan city, China in December 2019, it has now emerged as a global health crisis. With the number of positive cases becoming increasingly ubiquitous, this novel virus has brought unprecedented social, economic, physical and mental concerns to the forefront. Millions of lives world over have been negatively impacted by Covid- 19 related suffering at both a community

and individual level. The course of the virus also advanced to India with the first confirmed case arising in the state of Kerala on 30th January, 2020, and soon spread to various parts of the country. 50 days after the first reported case in the country, the Prime Minister of India, Narendra Modi, announced a series of phased nationwide voluntary lockdowns which resulted in disruption of daily routine, economic and social activities. While these measures of self- quarantine may be important to mitigate the spread of the infection, they have resulted in consequences affecting the mental well- being of the population as a whole.

Researchers have said that COVID-19 pandemic would "have dire consequences for mental health". Jonathan Porteus, a licensed clinical psychologist commented that "Our society is in a collective state of trauma". People are facing many psychological problems including stress, anxiety and frustration as they are worried about issues like their financial situation, struggling between work from home and managing the house, co-parenting, strained relationships, health of family members, future education and scholarships. There has also been a consequent rise in the number of calls to crisis and suicide hotlines.. People are also unable to use their usual coping mechanisms like socialising, making the situation more difficult than ever. Apart from this, the nationwide worries of shortages of resources for testing, uncertainty of a vaccine, growing financial uncertainty, lack of complete information from authorities and also new challenges of rumours and misinformation in this age of social media have created a wide range of losses to bear. In such a situation that is predominantly characterised by loss in various forms, both immediate and anticipated, the study of grief comes to the forefront.

Thus, in the dynamic world of the present day, grief has emerged as a complex emotional concept. Grief is a common human response to loss. David Kessler, the world's foremost expert on grief, mentioned that an emotional response people are facing in the midst of this pandemic is Anticipatory Grief. Anticipatory grief has been the subject of much speculation since Lindemann coined the term to describe premature mourning and is described as "a range of intensified emotional responses that may include separation anxiety, existential aloneness, denial, sadness, disappointment, anger, resentment, guilt, exhaustion, and desperation". Anticipatory grief is amplified in the pandemic as people have lost a sense of normalcy and connection and are uncertain of their

future. People are grieving what is expected to come such as the financial losses they will have to incur due to the markets crashing and the economy coming to a standstill, being laid off and jobless by the end of the pandemic, changes in the educational sector regarding admissions, graduations and tweaks in curriculum, and most importantly coming in contact with the virus and health concerns. There is still a persistent sense of worry that more loss is yet to come.

While one cannot control the demanding and stressful situation one is facing, research suggests that people do have the capacity to reduce the intensity of their anxiety and despair by altering their internal narratives . Locus of control is a psychological concept that refers to how strongly people believe they have control over the situations and experiences that affect their lives. Developed by Julian B. Rotter in 1954, the locus of control theory refers to an individual's beliefs about the extent of control that they have over things that happen to them. There are two parameters of locus of control, internal and external. People with an Internal Locus of Control attribute responsibility for what happens to themselves, they feel responsible for controlling most of the situations they face and believe that they can interfere in the results of these situations (Coleta, D 1987; Tavares, 2006). They have a strong belief in themselves, are self-motivated to achieve their goals in life and feel less anxiety and worry. People with an External Locus of Control, on the other hand, tend to attribute responsibility for what happens to others or to what is external (luck and fate), they do not feel able to control the situations they go through (Callado et al., 2006) People with this personality style are more anxious.

This locus of control theory is of great significance to understand individual reactions to the pandemic. Research suggests that those with a strong internal locus of control who feel that their choices will affect their health and safety in the pandemic will maintain social distancing, wear a face mask and wash their hands frequently. On the other hand, those with an external locus of control who believe that they have no control over their health and safety in the pandemic will be less likely to embrace recommendations to limit the spread of the infection.

Research (Gidi Rubinsten, 2004) also suggests that sudden losses are less threatening for individuals with a high internal locus of control as they believe that although those losses cannot be anticipated, they can be dealt with, should they arise. In contrast, unexpected losses result in higher levels of depression and somatic complaints in those who have an

external locus of control and believe that they have little control over their lives.

The current research topic was selected keeping in mind its pertinence and applicability. The novel Covid- 19 has resulted in unprecedented loss which has led to increasing anticipation of further losses, socially, financially and personally. The locus of control theory also plays a principal role in adapting and maintaining quality of life and mental well-being in times like these.

This research paper aims to study the correlation between anticipatory grief and locus of control during the Covid- 19 pandemic in India among two age groups of 16- 26 years and 45- 55

Literature Review

Several research studies linking locus of control and anticipatory grief to a large number of factors have been conducted pointing at a plethora of findings. Research was conducted with the aim of studying locus of control, interest in schooling and self-efficacy as predictors of academic achievement of Junior Secondary School Students. (Tellaa, Tella and Adeniyi, 2009) The population of the study consisted of 500 students (300 boys and 200 girls) selected from 25 secondary schools through stratified random techniques. Instruments used were Trice's academic LOC scale (1985), Interest in Schooling Scale by Mitchell (1993), Self – Efficacy Scale by Morgan and Jinks (1999). The result showed that the three variables positively correlated with and significantly predicted the criterion i.e. academic achievement. A significant impact of locus of control i.e. better achievement of a student in school work was related to internal locus of control.

Another research (Pavalache-Ilie, Barsan and Truţa , 2012) aimed to study how internal and external locus of control was related to pro environmental attitudes. 112 student participants from third and fourth year of college with an age average of 21.7 years filled the Environmental Attitudes Inventory (Milfont & Duckitt, 2010) and Levenson's Multidimensional Locus of Control Scale (Levenson, 1973, 1981). Participants with high scores on the Internality scale supported conservation policies and had a tendency towards preservation of nature while externality was correlated with utilization of nature. Also, students who were involved in ecological activities had internal locus of control which means that they were more likely to act towards nature preservation.

A correlation study (Türk-Kurtça and Kocatürk , 2020) between the role of childhood traumas, emotional self-efficacy and the internal-external locus of control and psychological resilience was conducted. 291 participants (208 females and 83 males) filled the Childhood Trauma Scale, Emotional Self-Efficacy Scale by Kirk, Schutte and Hine (2008), Psychological Resilience Scale - III R by Maddi and Khoshaba (1994) and Rotter Internal-External Locus of Control Scale (1966). Results of this quantitative study showed that childhood trauma was negatively and significantly correlated, emotional self-efficacy was positively correlated and locus of control was negatively correlated to psychological resilience. People with internal locus of control are more resilient and empowered, are unlikely to feel weak and have control over their lives.

A research (Berglund, Erik & Lytsy, Per & Westerling, Ragnar. (2014)) studied the relation of locus of control and disease burden with self-rated health among patients with risk of cardiovascular disease. 414 subjects filled in Wallson's multidimensional health Locus of Control scale and self-assessed five-point scale for self- rated health (very good, good, neither good nor poor, poor, very poor). Positive relationship was found between self-rated health and internal health locus of control. Health locus of control in chance lowers self-rated health and increases disease burden and so is the case with health locus of control in powerful others.

Another study (Burger, J.M. (1984)) aimed to see the relationship between locus of control and depression. 71 undergraduate students filled in the Levenson's locus of control questionnaire and Beck's Depression Inventory(BDI) in the first month and after 6 months filled BDI again and answered 4 additional questions to see the stability in the relationship of personality measures and depression. The results showed a significant positive relationship between locus of control of chance and depression and similar results were found between locus of control of powerful others and depression both during the original testing and after six months. Weak relationships were established between internal locus of control and bouts of depression both the times.

In another study on the relationship between locus of control and 3 kinds of anxiety (Archer, Robert. (1980)) Eighteen out of twenty studies showed a significant relationship between general trait anxiety and locus of control. A strong relationship between internal locus of control and lower trait anxiety has been established. Situation specific anxiety is tested in 2 categories either test anxiety or death anxiety. 7 out of 10

studies have shown that higher test anxiety is seen among people with external locus of control. The relation between death anxiety and locus of control are still obscure and more research is needed. Generalised locus of control scores are related to the scores of the state anxiety in situations where no clear cues about the control about the stressful situation is given and no relation is found between the two variables in situations where there are clear cues regarding the control of the situation.

In an article published by Yusen Zhai and Xue Du in "Brain, Behavior and Immunity" (Volume 87, July 2020) grief is a concept that is discovered to be inevitable and multidimensional for individuals with losses during the Covid- 19 pandemic. Individuals with patients or family with severe complications of the virus may experience anticipatory grief, knowing that their loved ones are suffering and they could probably miss out on their final moments. Frontline health care providers, whose mental and physical well-being is severely affected during this time, as the huge surge in deaths is distressing. However, the article states that it is particularly difficult for them both emotionally and psychologically since they have little time of mourning due to the intensifying situation.

"Pre Loss Grief is a grief response that occurs as someone struggles to come to terms with both the potential loss of a loved one and the changes that result from a loved one's health decline" This research (Singer, J., Spiegel, J. A., & Papa, A. (2020)) paper elaborated on pre loss grief and how it is likely to be experienced by a family member of the patients suffering from Covid - 19 and the expectations of their potential death. Preparedness usually reduces pre loss grief and prolonged grief. Open communication, misinformation and misunderstanding results in decreased preparedness, thereby increasing the intensity of pre-loss grief. By challenging the misinformation, giving evidence based information about Covid- 19 in layman language so it is understood by all, use of telemedicine platforms for communication increasing preparedness, finding creative solutions for social support and restorative activities (e.g. funerals) and finally meaning- centred therapy may help family member make sense and meaning in life and lessen Pre Loss Grief.

In a commentary addressing the role of grief in mental health outcomes relating to the pandemic, Bertuccio and Runion (2020) highlighted the manifestations of grief and loss as being the primary outcomes of Covid-19. "Uncertainty distress" was discussed as it emphasizes the "unknown-

ness" to be the main cause of distress and its subsequent impact on functioning. Three grief responses were discussed, providing a relevant framework – ambiguous loss, anticipatory grief and complicated grief. Anticipatory grief was described as the phenomenon of grieving what is yet to come in terms of potential health scares and fatal outcomes. The expansive and around- the- clock news coverage may also lead to anxious questioning of whether they or a loved one will inevitably acquire or even die from the virus.

Holm, Alvariza, Fürst, Öhlen, and Årestedt conducted a research study to evaluate the psychometric properties, focusing on anticipatory grief, in a sample of family caregivers in palliative care as they are often faced with experiences of grief in anticipation of the loss of a close person. In this cross-sectional study, data was collected from 270 family caregivers based in 10 palliative care settings between 2013 and 2014. The questionnaires administered included demographic questions and self-rating scales, including a Swedish version of the Anticipatory Grief Scale (AGS), the Hospital Anxiety and Depression Scale (HADS), and the Texas Revised Inventory of Grief (TRIG). Even though the AGS has been used in multiple studies in various contexts, it has not been validated. No subscales or cut- off scores have been reported for the AGS, and the present study made several attempts to communicate the validation process with the original authors, however these attempts were unsuccessful. In a parallel analysis carried out, 14 items were removed from the AGS, and the construct validity was supported making it the first study that has validated the Anticipatory Grief Scale. It was renamed AGS – 13 and consisted of two sub scales called the behavioral reactions and emotional reactions, thus providing fewer questions that do not overburden the family caregivers by asking unnecessary questions and easily capture the emotional and behavioural reactions of grief.

Neyshabouri, Tabibi, Maghsoudi, and Shahbakhsh (2018) conducted a research to compare anticipatory grief of mothers of children diagnosed with cancer within 1 month and those whose children were diagnosed 6-12 months earlier. In this cross-sectional study, the sample consisted of 70 mothers living in Mashhad; the children of 35 mothers were diagnosed with cancer within the previous 1 month, and the children of 35 mothers were diagnosed with cancer 6 to 12 months earlier. Anticipatory grief was assessed using the MM-CGI Childhood Cancer (Al-Gamal, Long, & Livesley, 2009). The results of the study show that mothers of children diagnosed with cancer within the previous 1 month

reported a higher level of anticipatory grief and it's components in comparison to those whose children were diagnosed 6-12 months earlier. It means that cancer diagnosis in children has an acute and intensive effect on the psychological state of mothers, and over time, this effect becomes milder. This signifies that as time passes, they adapt to the illness and the anticipatory grief fades off. This can open up further avenues for anticipatory grief during the time of Covid- 19 as the present research study was undertaken in Phase 3 and 4 of the nationwide lockdowns, and can prove to be a precursor to understanding whether anticipatory grief has faded off as time has progressed.

Methodology

Aim

The aim of this research is to study the relationship between anticipatory grief and locus of control amongst two age groups of 16- 26 years and 45- 55 years.

Hypothesis

- Hypothesis 1:There will be negative correlation between anticipatory grief and internal locus of control.
- Hypothesis 2: There will be positive correlation between anticipatory grief and external locus of control.
- Hypothesis 3: There will be no difference between the degree of locus of control (Internal and External) of the two age groups.
- Hypothesis 4: There will be no difference between the level of anticipatory grief of the two groups

Variables under study

Anticipatory grief - Anticipatory grief is an active psychological process of thoughts and emotions that connotes a range of intensified emotional responses towards an impending loss.

Locus of control - refers to an individual's beliefs about the extent of control that they have over things that happen to them.

- People with internal locus of control attribute the consequences in their lives to their own actions and abilities.
- People with external locus of control attribute the consequences in their lives to factors external to them like luck or fate **.**

Sample

140 participants were taken for this study. 70 participants from each age group of 16-26 years of age and 45 – 55 years of age were evaluated, hailing from different regions of India. Samples were chosen randomly and consent was taken prior to data collection.

Controls

- Participants with physical disabilities/illness & mental disorders were discarded from the sample.
- Age limits of both the groups in the sample were controlled.

Tools

Locus of control

Rotter's Internal-External Locus of Control Scale (1966) was used to measure Locus of Control. Rotter's Locus of Control Scale is the most widely used measure to score locus of control. There are 29 alternative items in the test and the participant had to answer all of them. Rotter reported "the test- retest reliability of the scale to range from 0.49 to 0.83 depending on the time period and particular population.."

Anticipatory grief

A 12 item self- devised scale on Anticipatory Grief was used, making use of the symptoms, in which the participants rated the degree to which they experienced the respective symptoms of Anticipatory Grief. This 5-point Likert Scale consisted of answers ranging from strongly disagree to strongly agree to understand the extent to which they were grieving.

Procedure

Online forms were randomly sent through email to people residing in different regions across India. Following informed consent, participants were also assured of confidentiality of the information provided by them. A total number of 214 participants filled out the questionnaire. 23 samples were discarded as they were invalid after which the first 70 valid participants were selected from each age group for further evaluation.

Data Analysis

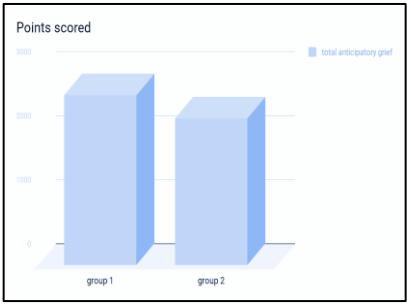
- Descriptive statistics was used to describe the data.
- Pearson correlation coefficient test was used to study the relationship between locus of control and anticipatory grief.

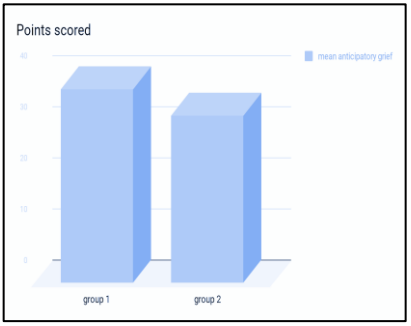
- T- tests were used to study if there is any significant difference between the level of anticipatory grief and the degree of locus of control of both the age groups.
- Tables and graphs were utilized to explain the data better .

Discussion

Age	**16-26 years**	**45-55 years**
Mean	**19.34**	**48.36**

The aim of the research was to study the relationship between anticipatory grief and locus of control and see the difference between levels of variables among two age groups. The study was conducted online during the 3rd and 4th phase of the lockdown.The sample consisted of 140 participants with 70 participants each in two age groups of 16-26 year olds and 45-55 year olds from different regions of India . The mean age of both the age groups was calculated and is written in the table below.





Total and mean score of anticipatory grief of both the age groups were calculated. Results showed that the total score of anticipatory grief of age group 1 (16 - 26 year) is 2659 and mean score is 37.98 whereas for age group 2 (45-55 years), 2287 is the total score and 32.67 is the mean score.

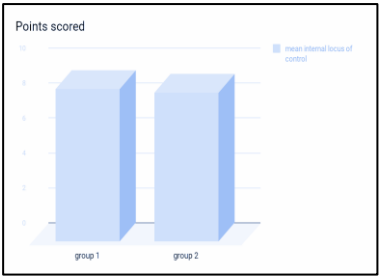
Total anticipatory grief (graph 1)

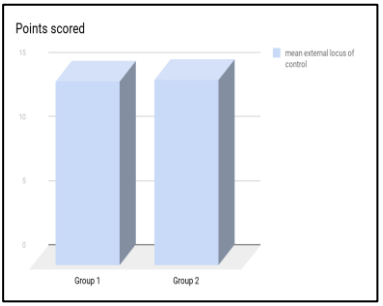
Mean anticipatory grief (graph 2)

Total and mean score of Internal locus of control of both the age groups were calculated .

Mean internal locus of control (graph 3)

Mean external locus of control (graph 4)





Results showed that the total score of Internal locus of control of age group 1 (16 - 26 years) is 262 and mean score is 8.73 whereas for age group 2 (45-55 years) ,298 is the total score and 8.51is the mean score.

Total and mean score of external locus of control of both the age groups were calculated . Results showed that the total score of the external locus of control of age group 1 (16 - 26 years) is 574 and mean score is 14.35 whereas for age group 2 (45-55 years) 506 is the total score and 14.46 is the mean score.

Pearson's product moment correlation coefficient was carried out to determine the relation between anticipatory grief and locus of control . The correlation value for anticipatory grief and internal locus of control was found to be 0.22. This value is insignificant at .05 and .01 level which implies that there is no correlation between both the variables thus the hypothesis of negative correlation between internal locus of control and anticipatory grief was not accepted.The correlation value for external locus of control was found to be -0.068 which is also insignificant at both the levels which implies that there is no correlation between the two variables. The hypothesis of positive correlation between the two variables is also not validated.

Variable	**Internal locus of control**	**External locus of control**
Anticipatory grief	0.22	-0.068

The result of the study performed provides insufficient evidence for any correlation between anticipatory grief and locus of control .

The mean anticipatory grief score for 16 to 26 year olds was 37.98 and the mean anticipatory grief score for 45 to 55 year olds was 32.67 therefore at an apparent level age group 1 (16 - 26 year olds) has higher anticipatory grief. In order to find out whether the difference was significant T test was used The t value was found to be 3.6958 for anticipatory grief which is significant at both .05 and .01 values therefore the null hypothesis was not accepted .

T value

Variable	16-26 years	45-55 years	t value	p value
Anticipatory Grief	M= 37.98 SD= 7.801	M= 32.67 SD= 9.1584	3.6958	> 0.05

The mean of internal locus of control for 16 to 26 year olds was 8.73 and for 45 to 55 year olds was 8.51 at an apparent level group 1 (16- 26 years) has higher mean and thus in order to find out if there was any significant difference in the level of locus of control experienced by both the age groups a t test was formulated .The t value for internal locus of control came out to be 0.48 which is insignificant at both .05 and .01 levels therefore the null hypothesis was accepted .

T value

Variable	16-26 years	45-55 years	t value	p value
Internal Locus of Control	M= 8.7333 SD=1.9464	M= 8.5143 SD= 1.7213	0.4815	>0.05

The mean of external locus of control for 16 to 26 year olds was 14.35 and for 45 to 55 year olds was 14.46 at an apparent level group 2 (45-55 years) has higher mean and thus in order to find out if there was any significant difference in the level of locus of control experienced by both the age groups a t test was formulated ,The t value for external locus of control it was -0.21 which is insignificant at both .05 and .01 level therefore the null hypothesis was accepted .

T value

Variable	16-26 years	45-55 years	t value	p value
External Locus of Control	M= 14.35 SD= 2.0823	M= 14.4571 SD= 2.318	-0.2109	>0.05

CONCLUSION

The aim of the research was to study the relationship between anticipatory grief and locus of control . It presumes that there will be a negative correlation between anticipatory grief and internal locus of control and a positive correlation between anticipatory grief and external locus of control . The findings of the study show no correlation between anticipatory grief and both internal and external Locus of Control.The present study also presumes that there will be no significant difference in the level of anticipatory grief and degree of locus of control of both the age groups .The result for this hypothesis shows a significant difference between the level of anticipatory grief experienced by the two age groups as group 1 (16 - 26 year olds)experience higher anticipatory grief whereas for the degree of locus of control , both internal and external , the difference between the age groups came out to be insignificant.

Limitations

1. The sample is limited to the upper middle class strata of the society only.Further studies could be conducted on different strata and a comparative study could be conducted.
2. The sample size is small and hence results cannot be generalised to a larger population.
3. The study was conducted in phase 3 and 4 of lockdown only .Further studies can be conducted comparing different phases of unlockdown.
4. Sample consists of only two genders male and female hence is not a very inclusive study.
5. Anticipatory Grief is a self devised questionnaire , the reliability and validity have not been measured.

Future studies

1. Data can be collected from more socio- economic groups .

2. Another personality trait that affects anticipatory grief can be explored.
3. As Anticipatory grief is subjective qualitative research can be conducted.

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Chapter 21 - Sexting and its Link to Sensation Seeking and Self-Esteem

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Abstract

Sexting, defined as sending, receiving, or forwarding sexually explicit messages and images via the Internet or a mobile phone, has become a recent cause for concern as it is known to lead to harmful consequences of privacy misuse. With increasing use of technology by youth, this behaviour may emerge as a specific concern in this age. The present study aims to understand the practice of sexting among youth along with its link to two variables i.e. sensation seeking and self-esteem, in order to help explain this behaviour and inform decisions about whether and how to address it. The sample consisted of a group of 76 participants in the age group 18-21 year olds who were initially asked about their frequency of engaging in sexting. Arnett Inventory of Sensation Seeking was used to determine the levels of sensation seeking of the participants and the Rosenberg Self Esteem Scale was used to determine the level of self-esteem of the participants. Correlational analysis (Spearman's Rho) was carried out to determine the nature of relationships between the variables. Results revealed no statistically significant association between sexting behaviour, sensation seeking and self-esteem indicating that no conclusive relationship could be formulated between the variables under study.

KEY WORDS: Sexting, Self-Esteem, Sensation Seeking

Introduction

One of the most debated questions of the present time asks whether technology and social media is a boon or a curse. Do they work to benefit people and our society or result in the detriment of it? While there are many issues that have emerged due to the increasing use of the internet and social media, sexting as a concern is a relatively new and serious one. Communication via technology has become a significant part of relationships. Although technology is being widely used by all age groups, teenagers and young adults have begun to use various forms of technology and social media, like emails, messages, videos, Facebook WhatsApp to initiate, manage and end relationships. Sexting can be

defined as sending, receiving, or forwarding sexually explicit messages and images via the Internet or a mobile phone. Sexting can be categorized in six ways: sending (1) sexually suggestive photos or videos, (2) photos or videos wearing lingerie, (3) nude photos or videos, (4) sexually suggestive text messages, (5) text messages propositioning sex, and (6) forwarding on or showing others sexts which were meant to be kept private. (Balog, Francke, Hemphill, 2016)

Research on sexting leads us to understand the point of views that people hold about various topics surrounding sexting like technology and sexual behaviour among young adults. One of the most commonly held stereotypes about sexting is heavily gendered. Many people believe that girls are the protagonists and boys are passive recipients in this particular scenario which is a problematic mind set. As adolescents and early adults are in the midst of major developmental changes: physical, cognitive, and social ones, and in a period of sexual exploration, understanding of sexting among adolescents is important. Media often reports sexting among adolescents as problematic. Mitchell, Finklehor, Jones, and Wolak (2012) found that, in a national sample of 1500 youth aged 10–17, 9.6% had appeared in or created nude or nearly nude images or receiving such images in the past year. In a study of over 600 high school students, between 9% and 27% had ever sent a sexually explicit cell phone picture and between 24% and 65% had received a sexually explicit cell phone picture (Strassberg, McKinnon, Sustaíta, & Rullo, 2013). It has been found that sexting behaviours were more frequent as participants increased by age. Dake, Price, Maziarz, and Ward (2012) indicated that 17% of their middle school and high school sample had engaged in sexting. According to ‘‘Sex and Tech’’ (2008), 20% of teenagers (aged 13–19 years) and 33% of young adults (aged 20–26 years) in their sample, sent or posted nude or semi-nude pictures or videos of themselves, and 38% of teens and 58% of young adults sent or posted sexually suggestive messages. In a sample of over 200 Hispanic female undergraduates, 20.5% had sent erotic or nude photos of themselves and 34.5% had received photos from someone else (Ferguson, 2011)

Similar to other sexual activities, sexting is heavily influenced by the individual characteristics such as sexual drives, sexual experience, personality, etc, and social context, for example family, peers and social norms. However in the present study we try to understand how two

different personality factors, “Self Esteem” and “Sensation Seeking”, affect the sexting behaviour of adolescents.

The term self-esteem is used to describe a person's overall sense of self-worth or personal value. In other words, how much you appreciate and like yourself. Self-esteem is often seen as a personality trait, which means that it tends to be stable and enduring. Self-esteem can involve a variety of beliefs about yourself, such as the appraisal of your own appearance, beliefs, emotions, and behaviours. (Cherry, 2019)

While high self-esteem may refer to an accurate, justified, balanced appreciation of one’s self- worth and one’s successes and competencies, it can also refer to an inflated, arrogant, grandiose sense of superiority over others. Similarly, low self-esteem can be either an accurate, well-founded understanding of one’s shortcomings as a person or a distorted, even pathological sense of insecurity and inferiority. (Baumeister, 2003)

Lower level of self-esteem can lead people to feel depressed and defeated at different points in time. Lowered self-esteem leads for people to make bad decisions and it can lead them to remain in toxic relationship or to fail to accomplish their highest potential. On the other hand, high level of self-esteem can be exhibited in narcissistic personality disorder which can damage personal relationships.

Sensation seeking, also called excitement-seeking, is the tendency to pursue sensory pleasure and excitement. It's the trait of people who go after novelty, complexity, and intense sensations, who love experience for its own sake, and who may take risks in the pursuit of such experience. Sensation seekers are known to be easily bored without high levels of stimulation. According to psychologist Sam Gosling, "They love the hustle and bustle and like to take risks and seek thrills." Sensation seekers look for immediate gratification which sensory experiences can provide. During this process they tend to overlook the dangers accompanying risky behaviours such as gambling, drugs, reckless driving etc.

The current topic was selected, keeping in mind the relevance of the topic in present times. Technology is an integral part of our day to day living and needless to mention, young adults are one of the first sections of the society to adapt to any new technology. This makes them highly vulnerable to the negative aspect of the emerging technology, one of the by-products being “sexting”. Sexting has emerged as a concern because it encroaches upon an individual’s privacy which can lead to many anti-

social practices like child pornography, revenge pornography, cyber bullying etc.

The following research is aimed at studying the relation between sexting behaviour, self-esteem and sensation seeking of young adults between the ages of 18-21 years. Sensation seeking as a personality trait is possessed by many people, however, technology and the internet gives people a platform to explore the endless possibilities in a comparatively safe environment as it gives them a mask in the form of the computer or mobile screen.

LITERATURE REVIEW

Messer, Bauermiester, Grodzinski and Zimmerman (2012) conducted a study to understand the sexting behaviour among young adults which aimed at examining the mental well-being and the sexting behaviours among young adults of the United States. The Participants' sexting behaviour was recorded with the help of an online respondent-driven sampling, wherein the respondents ranged from 18-24 years of age. The sexing behaviour was calculated on the basis of 4 sub-categories of sexting namely: non-sexters, senders, receivers and two-way sexters (Messer, Bauermeister, Grodzinski, Zimmerman, 2012) On the basis of these categories relationships were drawn out between socio-demographic categories, sexual behaviour and psychological well-being. Based on the results of the research, 57% of the participants were recorded as non-sexters, 28.2% as two-way sexters, 12.6% were receivers and 2% were senders. Male respondents were more likely to be on the receiving end of a sexually suggestive message/photo as compared to the female respondents. Results also show that sexually active respondents were more likely to be two-way sexters. The researchers were unable to find any relation between sexting behaviour and psychological well-being. (Messer, Bauermeister, Grodzinski, Zimmerman, 2012)

In another research, sexting has been defined as sending, receiving, or forwarding on sexually explicit messages and images via the Internet or a mobile phone (Scholes-Balog, Francke, Hemphill, 2016). In this study sexting has been divided into 6 sub-categories as follows: sexually suggestive, photos or videos, photos or videos wearing lingerie, nude photos or videos, sexually suggestive text messages, text messages propositioning sex and forwarding on or showing other sexts which were meant to be kept private .(Scholes-Balog, Francke, Hemphill, 2016).

Being in a relationship was one of the highest predictors of engaging in all the types of sexting behaviour which have been mentioned above. From the perspective of personality traits, sensation seeking and self-esteem have varying impacts on the sexting behaviour of young adults. The research suggests that higher self-esteem links to lower tendency of engaging in sexting and lower self-esteem links to higher chances of engaging in sexting behaviour. On the other hand, higher sensation seeking level predicts a higher chance of an individual to engage in sexting behaviour, while lower sensation seeking level suggests the opposite trend.

Benotsch, Snipes, Martin & Bull (2012) studied the correlation between sexting, substance abuse and sexual risk behaviour. An observation made by the researchers was that, young adults are usually early adopters of new technologies, including cell phones, messaging services, photographs etc. While most of the previous research on the topic of sexting has focused on the legal and social consequences of this behaviour, this study focuses on public health implications of sexting by examining the correlation between sexting, substance abuse and sexual risk behaviour in youth. 273 young adults completed online surveys assessing demographics, cell phone including texting and sexting as well as sexual risk behaviour. (Benotsch, Snipesa, Martin, Bull, 2012).

The results of the research showed that only about 44% of the participants reported engaging in sexting. However, those engaging in sexting also reported engaging in recent substance use and high-risk sexual behaviours which includes unprotected sex and sex with multiple partners. About 31.8% of participants who engaged in sexting admitted having sex with new a partner for the first time after engaging in sexting with that person. (Benotsch, Snipesa, Martin, Bull, 2012).

Results suggest that sexting is strongly associated with high risk sexual behaviour. Many individuals share explicit or provocative photos with long term sexual partners, however some participants incurred new sexual risks after engaging in sexting.

Ngo, K. Jaishankar and Agustina conducted a research which provides us with current research gaps and the legislative issues revolving around the sexting behaviour of Individuals. According to this research, Sexting is defined as sending, receiving, or forwarding sexually explicit messages or nude, partially nude, or sexually suggestive digital images of one's self or others via a cell phone, e-mail, Internet, or Social

Networking Service (Brown et al., 2009; Calvert, 2009; Corbett, 2009; Dilberto & Mattey, 2009; Halder & Jaishankar, 2014; Jaishankar, 2009, Walker & Moak, 2010).

According to prior research conducted by the above mentioned researchers, evidence suggests that adults tend to engage in sexting more often as compared to adolescents. Evidence also suggests that sexting tends to take place in committed relationships among adolescents. Some of the factors which can lead individuals to engage in sexting are, being sexually active, having multiple sex partners, having unprotected sex etc. previous research also states that those who engage in sexting report higher frequency of consuming alcohol, abusing drugs, smoking cigarettes, being bullied, experiencing depression, having suicidal tendency and displaying impulsive behaviour. Many people who sext, confess to facing some negative legal and social consequences of engaging in this activity. With regards to the prosecution of minors who engage in sexting, in a study involving a sample of college students, the authors report that one third of the participants would endorse the prosecution of teen sexting, another third would oppose such prosecution, and the final third expressed that the prosecution of teen sexting should depend on certain factors (Strohmaier et al., 2014).

As sexting is considerably a newly emerging phenomenon state legislatures and prosecutors of many countries are having a hard time deciding on how to punish the offender, especially when the offender falls in the category of an adolescent. Many jurisdictions rely on different archaic laws and piece them together to deal with this new technological phenomenon. Many legal scholars debate that when the current child pornography laws were being written, the possibility of sexting did not cross even the imaginations of the law makers. Also the phenomenon wherein the adolescents engage in self-produced sexual images could not be considered as child pornography as the pure definition of child pornography is, subjecting children to unimaginable harm in creation of sexual images. However, in the case of sexting, most of the times children are not subjected to physical harm, and mostly adolescents have created such images out of consent. Such glitches in the legal definitions of various phenomenon creates a confusion while forming laws of such recent technological crimes.

Ehrenberg, Juckes, White and Walsh conducted a research to understand if Personality and self-esteem are the true predictors of young people's technology use. According to the researchers, a heavy reliance on

technology for the validation of social connections may have certain psychological implications like developing technological addiction. The researchers have used 5 personality traits to predict participants' mobile behaviour, the 5 traits being: Extraversion (depth and intensity of inter-personal interactions), Neuroticism (Level of emotional stability and adjustment), Agreeableness (Difference in level of co-operation and social harmony), Openness to experience (pursuing and enjoying new experiences) and conscientiousness (control, regulation and direction of goals and impulses) (Ehrenberg, Juckes, White, Walsh 2008). Along with these 5 personality traits, individual difference variable of self-esteem was also used as a predictor. The researchers have linked the personality traits of Disagreeableness and Extraversion with higher mobile phone use while low conscientiousness and higher neuroticism have predicted more engagement in Short Message Service (SMS). Participants with lower self-esteem have reported higher and problematic mobile phone use. This is how various personality traits have been linked to and have been used to predict the engagement of young people with mobile phones and technology.

In their study, researchers, Joris, Ellen, Koen, Michel have tried to establish an association between adolescents' characteristics and their engagement in sexting. The emotional and legal baggage that comes along with the engagement of adolescents' in sending sexually explicit pictures through the internet or mobile phones have caused significant concern about adolescents' behaviour. Current study draws a comparison between the characteristics of adolescents who engage in sexting and those who do not. This study was conducted in Belgium. The data which was collected throughout the study and the analysis states that, sexting is significantly linked with sensation seeking, experimental thinking styles and depression. However, some contradictory results have been found in the previous research conducted on similar topics, which makes the future researchers keen to continue to drill out more findings establishing the co-relation of sexting and sensation seeking.

Klettke, Mellor, Silva-Myles, Clancy and Sharma conducted a study to find out the relationship between sexting and mental health was conducted on Indian and Australian young adults. In this study, the engagement in sexting by young adults of Indian and Australian nationalities has been studied in relation to depression, anxiety and stress considered as risk factors. A total of 298 Australian young adults and 300 Indian young adults were asked to fill out an online as well as a

hardcopy survey, recording the sending and receiving of sexts and the mental health of participants. (Klettke, Mellor, Myles, Clncy and Sharma-2018).

Results found that Australian Male participants were more likely to send sexts than their Indian Male counterparts. Australian females were more likely to both send and receive sexts, as compared to their Indian female counterparts. Lastly, Indian males were found to be more likely to send and receive sexts as compared to Indian females.

High level of stress was found to be associated with sending sexts for all the participants. For male respondents, in all, higher level of stress and lower level of depression was found to be associated with sending more sexts. While on the other hand, no association was found between the mental health and sexting levels among the females. However, higher age of the females was associated with sending sexts. In a traditional and a cultural context, sexting behaviour can be associated with cultural values and can vary on the basis of gender.

Based on the above research literature we aim to study the relation between sexting behaviour, self-esteem and sensation seeking of young adults between the ages of 18-21 years. We hypothesize that there would be a negative correlation between self esteem and engagement in sexting behavior, which means we expect that lower the self-esteem level of the young adult, higher would be the tendency of the individual to engage in sexting behaviour. And on the other hand, we predict a positive correlation between sensation seeking and engagement in sexting behaviour, i.e., higher the sensation seeking behaviour of the young adult, higher would be the tendency of the individual to engage in sexting behaviour.

METHODOLOGY

Participants

The sample consists of 76 participants in the age group of 18-21 years of age, hailing from different areas in Mumbai. All the participants gave their consent prior to data collection.

Measures

Self Esteem:

Self Esteem level of the participants was calculated using the Rosenberg Self Esteem Scale (RSE). The scale was developed by Morris Rosenberg.

The test makes use of a 10item scale which can measure the global self-worth of an individual, by calculating both positive and negative feelings about the self. All items are answered using a 4-point scale ranging from strongly agree to strongly disagree. A higher score on the scale indicates higher self-esteem whereas a lower score determines lower self-esteem. It measures global self-esteem thereby providing good indication of general rather than specific views of the self (Baumeister et al., 2003). The RSE is a scale with high internal reliability (alpha .92). Rosenberg (1979) reported that the scale is correlated modestly with mood measures.

Sensation Seeking:

Arnett Inventory of Sensation Seeking (AISS), was used to calculate the levels of sensation seeking of the participants. Sensation seeking can be understood as a need for novel and intense stimulation. The test makes use of 20 items using a four point scale as follows: 1= Describes me very well to 4= Does not describe me at all. Higher score determines higher sensation seeking level whereas lower score determines lower sensation seeking level. The AISS has adequate internal consistency ($\alpha = 0.70$) and has been validated through relationships with other sensation seeking scales and self-reported risky behaviour, including aggression and reckless driving (Arnett, 1994, 1996; Arnett et al., 1997).

Sexting:

Making use of a previous study conducted on a similar topic, a self-devised scale on sexting was used. In this 5 point scale, the participants rated the frequency to which they engage in sexting ranging from very frequently to never. this scale consists of 6 items which makes an attempt to understand the sexting behaviour of young adults.

Procedure

The questionnaire was administered to participants all over Mumbai through an online survey. Following informed consent, the participants were asked to fill out a preliminary survey followed by the 3 study measures. The participants were guaranteed confidentiality about the information that they were providing.

Data Analysis

After the descriptive statistics were computed for all variables, the distribution of scores were tested for normality to determine the appropriate correlational analysis method.

RESULTS

Descriptive Statistics

As depicted in Table 1, 52 Female participants and 24 Male participants comprised of the sample (n=76). Means and Standard Deviation for all the 3 variables are mentioned below.

Table 1: Mean and Standard Deviation scores for all the participants for all the 3 Variables.

Variables	**N**	**Mean**	**Standard Deviation**
Sexting	76	4.07	5.38
Self-Esteem	76	17.58	4.63
Sensation Seeking	76	53.46	4.86

For Sexting, the Mean Score for all the participants on the Sexting Index was (M=4.0658, SD=5.38). For the Self-Esteem Variable, the Mean score for all the participants was (M=17.5789, SD=4.63397) and for the sensation seeking variable, the Mean score for all the participants was (M=53.4605, SD=4.86194)

Table 2: Mean scores of female and male participants on all variables

Variable	**Female**		**Male**	
	Mean	**SD**	**Mean**	**SD**
Sexting	4.48	6.02	3.17	3.55
Self-Esteem	18.04	4.46	16.58	4.94
Sensation	53.54	4.88	53.29	4.92

As illustrated in Table 2, The mean scores of females in sexting behaviour was higher than males, i.e. (M = 4.48, SD=6.02) and (M = 3.17, SD = 3.55) respectively.

The mean score for self-esteem, was higher for Females (M=18.04, SD= 4.46) than for males. (M= 16.58, SD= 4.94) The differences were less noticeable in case of sensation-seeking with (M=53.54, SD= 4.88) and (M= 53.29, SD= 4.92) for females and males respectively.

However further inferential statistics were not computed to identify if the differences found were statistically significant.

Table 3: Results for Shapiro-Wilk Test for Normality

Variable	**Statistic**	**p- value**
Sexting Behaviour	0.76	0.00
Sensation Seeking	0.98	0.35
Self Esteem	0.98	0.35

The data, however, was tested for normality before inferential statistics were calculated to test the inter-variable relationship. The Shapiro-Wilk Test was used to check for normality, and as shown in Table no. 3 the data for sensation seeking and self esteem were found to be normally distributed, since the null hypothesis of the test, that the data is normally distributed, was retained for both the variables. Data for sexting behaviour was found to be not normally distributed, since the null hypothesis had to be rejected for the normality test based on the statistics obtained. Since sexting behaviour was the common variable for correlation analysis with both sensation seeking and self esteem, non-parametric statistics, in this case, Spearman's Rho was to be used to test the hypotheses of the study. Since non-parametric tests were being used, bivariate normality was not calculated.

Analysis of the Relationship between Sexting Behaviour and Self-Esteem

Table 4: Correlation between Sexting Behaviour and Self-Esteem

		Self-Esteem
Sexting Behaviour	Spearman’s rho	-0.07
	Sig. (2-tailed)	0.56
	N	76

Spearman's rho Correlation Coefficient was computed to examine the relationship between sexting behaviour and self-esteem. Interpretation of the correlational analysis reveals that the relationship between sexting behaviour and self-esteem was found to be not significant (r_s = -0.07., n.s).

Analysis of the Relationship between Sexting Behaviour and Self-Esteem

Table 5: Correlation between Sexting Behaviour and Sensation Seeking

		Sensation
Sexting Behaviour	Spearman's rho	0.0997
	Sig. (2-tailed)	0.392
	N	76

Spearman's rho Correlation Coefficient was computed to examine the relationship between sexting behaviour and sensation seeking. Interpretation of the correlational analysis reveals that the relationship between sexting behaviour and sensation seeking ($r_{(87)}$ = 0.0997, n.s) was found to be not significant

Therefore, no statistically significant correlation indices were found for sexting behaviour with either self-esteem or sensation seeking.

DISCUSSIONS

The results of the present study provide insufficient evidence for the existence of a significant relationship between sexting behaviour, self-esteem and sensation seeking.

It seems like the reason for the non-significant statistics is primarily the distribution of data for sexting behaviour which appears to be exhibiting floor effect. Even though Spearman's Rho tests for presence of a monotonic relationship, i.e. seeing if increase or decrease in levels of one variable seems to accompany a non-linear increase or decrease in another variable, variance in both variables is necessary, which data for sexting behaviour doesn't exhibit, rendering the scatterplot distribution to look rather parallel to one of the axes.

One reason for such a distribution could be attributed to social desirability bias amongst the respondents where low frequencies of sexting behaviour were reported. Because topics related to sexuality are generally considered more private, modest estimates of frequency of sexting behaviour might have been deemed as appropriate by the

respondents to report. Another reason for floor effect in the data could be that sexting behaviour would generally be seen as more risky in the Indian social context, where expression of sexual desires and pursuit of its fulfilment outside the institution of marriage is looked down upon, and thus exchanging sexually explicit content, besides being seen as inappropriate, could also be seen as a potential source of unpleasant events if they were to be discovered, especially by conservative authority figures in one's life.

Another reason could be lack of stratified sampling based on sex, gender, and sexuality. Since majority of the sample consists of female participants, a floor effect might have occurred for the same reasons as mentioned above, social desirability and aversion to risks inherent with sexting behaviour. Effects of both would be compounded especially among female, women participants due to the presence of structural oppression of women's sexual expression that is common to women all around the world.

LIMITATIONS AND RECOMMENDATIONS

- The results should be interpreted with caution when generalising to the larger population of Indian young adults as the sample size was limited.
- The three questionnaires used to collect the data were self-report measures and therefore there was no way to determine if the answers given were honest or socially correct.
- This research was conducted on the participants of Mumbai only. A similar research can be conducted in different parts of India to understand the relationships between sexting behaviour, sensation seeking and self-esteem.
- This research was only limited to young adults but can be conducted on other age groups in India to understand sexting behaviour as prevalent among adolescents and older adults.
- For further studies attention can be drawn to other personality traits that influence sexting, apart from sensation seeking and self-esteem.
- The current research focused on sending sexually explicit messages rather than receiving such messages. Further research could focus on both facets of sexting behaviour.

- Since the topic of sexting is a very private concern, a qualitative analysis research study may help understand the nuances of this behaviour and how it differs culturally within our country.
- Further research could also focus on the relationship status of the participants and assess the sexting behaviour of people within relationships and those who are not in a relationship.

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SECTION VII - ECONOMICS

Chapter 22 - Impact of Covid-19 on Indian Economy

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Abstract

This research paper is an attempt to study impact of COVID-19 on Indian economy. India reported its very first case on 30th January 2020 in the state of Kerala. At that time China was reporting the highest 9,692 cases in the world. Now India is on 3rd position and China is on the 36th position (as of 30 .08. 2020). This research paper will analyse impact of COVID 19 on Indian economy during the lockdown. It will study the impact in 4 phases of lockdown. It will also study the impact on three sectors of the economy (primary, secondary, and tertiary), GDP, unemployment. As it is a very vast topic this research paper has shown the impact of COVID-19 on the sectors and variables mentioned above. This research paper is also going to study the situation of Indian economy before the lockdown period. After a prolonged lockdown, government come up with unlock 1 guideline in June for different sectors of the economy for the revival and recovery of the economy and put it back on the track of growth and development.

Keywords: COVID-19, economy, sectors, lockdown, unlock,

Introduction

"We are in this together and will get through this, together."-UN Secretary-General António Guterres

Let's start our discussion with biological aspects i.e. What does COVID 19 means: Coronaviruses are a large family of viruses, including some that cause common cold to some, that cause major diseases such as the Severe Acute Respiratory Syndrome (SARS) and the Middle East Respiratory Syndrome (MERS).

The coronavirus (COVID-19) outbreak came to light, on December 31, 2019, when China informed the World Health Organization (WHO) of a cluster of cases of pneumonia of an unknown cause in Wuhan City in Hubei province. On January 9, 2020, the WHO issued a statement saying Chinese researchers have made "preliminary determination" of the virus

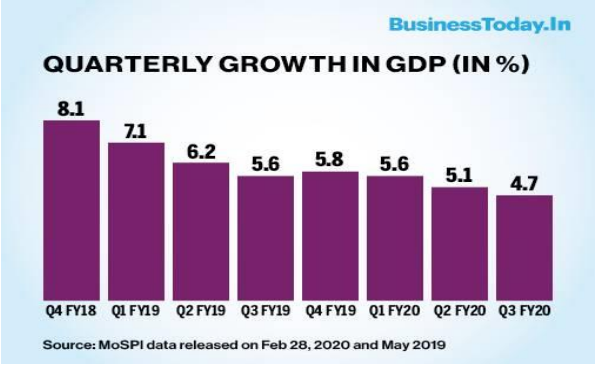
as a novel coronavirus. The virus has acquired the ability to spread among humans, with cases of human-to-human transmissions being reported first in Vietnam and Germany. It has been recognized as a pandemic by the World Health Organization (WHO) on 11 March 2020. India is widely affected by this pandemic.

OBJECTIVES OF THE RESEARCH STUDY

- ¶ To study the situation of Indian economy before COVID-19.
- ¶ To analyse the effect on several sectors, GDP, unemployment of the Indian economy during lockdown phase of COVID-19.
- ¶ To review government policies and relief programmes to handle COVID-19 situation.

RESEARCH METHODOLOGY

The research paper is based on secondary data sources newspapers, reports, and research articles.



Economic Scenario In India Before Lockdown In 2019-20

India's growth outlook to 1.8% from 3.5% for fiscal 2021.The quarterly GDP growth has consistently fallen since Q4 of FY18. It has fallen from 8% to 7.1% for Q1; From 7% to 6.2% in Q2 and 6.6% to 5.6% in Q3. The magnitude of the slowdown became clear in the fall of 2019, in the third quarter of the fiscal year – which runs from April 1 to March 31 – when the annual growth rate fell to 4.5%. Growth in the gross domestic product (GDP) falling to a 11-year low of 4.2 percent in 2019-2020. The economy grew by 3.1 percent in the January-March quarter of 2019-

2020, against 5.7 percent at the same time a year ago, the slowest growth in at least eight years.

INDIAN ECONOMY DURING COVID-19 LOCKDOWN: IMPACT ON DIFFERENT SECTORS, GDP, AND UNEMPLOYMENT

The United Nations Conference on Trade and Development (UNCTAD), has suggested that India's trade impact due to the COVID-19 outbreak could be around USD 348 million. Let us now study COVID-19 impact on the different sectors of Indian economy.

PRIMARY SECTOR

The primary sector is one of the sectors from most affected sectors. For the agriculture sector, (March and April) is the peak of rabi season in India, and crops like wheat, gram, lentil, mustard, etc. (including paddy in irrigated tracts) are at the harvestable stage or almost reaching maturity. This is also the time when the farm harvests reach the mandis (market yards) for assured procurement operations by designated government agencies. Moreover, any severe disruption to the supply of perishable fruits and vegetables, dairy products, fish, etc. having mobilized to meet the increasing demand from a bulging middle class as well as urban and rural consumers, may create irreparable damage to all actors in the supply chain. Making the food grains, fruits and vegetables, and other essential items available to consumers, both in rural and urban areas, is the most critical challenge for Government machinery during the lockdown period. The agriculture and allied sector's growth stood at 3.7 percent during 2019-20 fiscal year.

SECONDARY SECTOR

The lockdown is expected to have a serious impact on both the industrial and services sectors, which are facing a double whammy from a slump in export demand and hit to domestic demand. The Purchasing Managers Index (PMI) for manufacturing fell to 51.8 in March, from a peak of 54.5 in February. Services contracted, as PMI slipped below 50.0 to 49.3 from 57.5 in February. In the industrial sector, subsectors such as food products, cement, steel, other items used in construction, export items such as gems and jewellery, and textiles, face the threat of permanent loss of demand, or a scenario where even pent-up demand may not compensate for the loss. In other subsectors, such as consumer durables,

cars retailing, and discretionary goods, there could be demand postponement.

TERTIARY SECTOR

The services sector – which accounts for 41% of total exports – has been resilient. But a recession in the advanced economies would dampen the prospects for information technology (IT)-IT-enabled services and tourism, and bring down service-exports growth. Some domestically-led services could continue to bear the brunt for an extended period even after the lockdown is lifted, as consumers will be averse to public transport (including air transport), and places of entertainment business such as malls and cinema halls, hotels and restaurants, among others.

GROSS DOMESTIC PRODUCT

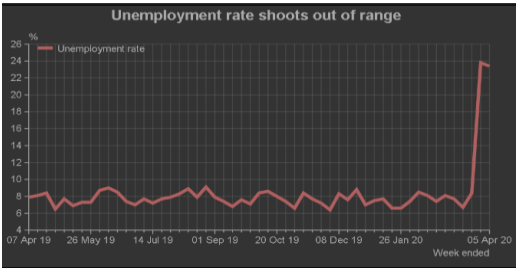
The loss of GDP in nominal terms is Rs 10 lakh crore or ~Rs 7,000 per capita. Acuity Ratings & Research Ltd earlier this month estimated that the lockdown will cost the Indian economy almost USD 4.64 billion (over Rs 35,000 crore) every day and the entire 21-day lockdown will result in a GDP loss of almost USD 98 billion (about Rs 7.5 lakh crore). Fitch Ratings puts its estimate for India growth at 2 percent while India Ratings & Research has revised its FY21 forecast to 3.6 percent from 5.5 percent earlier. Acuite Ratings believes there is a risk of a contraction of April-June (2020-21 fiscal) GDP to the extent of 5-6 percent, with Q2 (July-September) also likely to post modest growth in a best-case scenario. It expects the overall GDP growth for 2020-21 to be in the band of 2-3 percent which considers a significant economic revival in the second half of the financial year.

12 April 2020, a World Bank report focusing on South Asia said that India's economy is expected to grow 1.5% to 2.8% for FY21. The World Bank report said that the pandemic has "magnified pre-existing risks to India's economic outlook". In mid-April, the International Monetary Fund projection for India for the FY21 of 1.9% GDP growth was still the highest among G-20 nations. The Confederation of Indian Industry (CII) estimated that India's GDP for FY21 will be between 0.9% and 1.5%.

UNEMPLOYMENT RATES

According to the Centre for Monitoring Indian Economy (CMIE) , latest estimates show that the employment situation worsened from the beginning of March 2020, before the lockdown was put in place, and then rapidly spiked in the last week of the month and the first week of

April 2020."In March 2020, the labour participation rate fell to an all-time low, the unemployment rate shot up sharply and the employment rate fell to its all-time low," wrote Mahesh Vyas, CMIE's chief executive, on the organization's website. “The unemployment rate in March was 8.7%. The 'unemployment rate' essentially represents the percentage of people who were looking for jobs who failed to find one.



Source: CMIE,2020

According to CMIE reports, the unemployment rate in India increased by 14.8% in April reaching to 23.55%.

GOVERNMENT’S FINANCIAL PACKAGE FOR REVIVAL OF THE ECONOMY

To mitigate the economic fallout, Prime Minister Narendra Modi’s government had announced a $266 billion support package containing both fiscal and monetary measures, said to be worth around 10% of India’s GDP. The complete package of 20 lakh crore was announced by the Prime Minister for revival of the economy and to deal with this economic crisis. Finance Minister Nirmala Sitharaman announced the details of the special economic package. “This will play an important role in the ‘Atmanirbhar Bharat Abhiyan" said by PM. The Prime Minister emphasized on achieving self-reliance for the country at a time when the pandemic has brought the economy to a standstill. “This era of self-reliance will be our new pledge; we have to move on with a new resolve.”

Let us have a look at the summarized details of the financial package:

1ST TRANCHE (ATMANIRBHAR BHARAT ABHIYAAN):

On 13 May the Finance Minister, Nirmala Sitharaman, and the Minister of State for Finance and Corporate Affairs, Anurag Thakur, elaborated

on the financial package. The announcements included Rs 3 lakh crores collateral-free loans for businesses, including MSMEs. For non-bank lenders a liquidity scheme and partial credit guarantee scheme. Tax deadlines were extended. Rs30,000 crore special liquidity scheme for NBFCs /HFCs/MFIs. Under this scheme shall be reduced by 25% of the existing rates. This reduction shall be applicable for the remaining part of the FY 2020-21 i.e., from 14th May 2020 to 31st March 2021.

ATMANIRBHAR TRANCHE 2:

The 2nd trench focused on migrant and small workers, small farmers, street vendors, and self-employed people. Free supply of food to migrants: Migrants who are neither the beneficiaries under the National Food Security Act and nor are the holder of state-level cards will be given 5 kg of grains per person per family and 1 kg of chana per family per month for 2 months.8 crore migrants will be benefitted with government spending of Rs 35,000crore.

One Nation One Ration Card: The technology system to be used to access PDS from any fair ration shop will be covered the national probability scheme by August 2020.

Affordable Rental housing complex to migrant workers and urban poor. The government will provide affordable rental housing to urban poor under Pradhan Mantri Awas Yojana.

3rd TRANCHE OF FINANCIAL PACKAGE:

Operation Greens was extended from tomatoes, onion, and potatoes (TOP) to all fruits and vegetables. Cereals, edible oils, oilseeds, potato, and onion were deregulated (except in exceptional circumstances) and no stock limit shall apply for storage as was proposed Amendment in Essential Commodities Act (1958). Matsya Sampada Yojana was announced for fisheries and animal husbandry infrastructure fund was announced. The Agri-infrastructure fund, agricultural marketing reforms for farmers, and fair price legal framework support for farmers were among other things covered.

4th TRANCHE-NEW HORIZONS OF GROWTH:

Focuses on:

a) Fast track investment clearance through Empowered group of secretaries.

b) Ranking of states on investment attractiveness to compete for new investments.

A fund for farm-gate infrastructure was announced, amendments to the Essential Commodities Act, as well as the opening up of the defence sector, power sector, and space sector for privatization. Technology development cum incubation centres will be set up for synergy between research facilities and tech -entrepreneurs. While not all the measures in the package provided immediate relief, the finance minister said that the immediate needs of the country had also been addressed.

5th TRANCHE: GOVERNMENT REFORMS AND ENABLERS

On 17 May the finance minister concluded the announcement of the economic package. Taking the total amount of stimulus package to Rs.20,97, 053cr.An additional amount of Rs.40000 cr. was allotted for migrants under MGNREGA. Additional Rs.40000cr. has been allotted to provide job security to the farmers during the monsoon. This part was especially focused on MGNREGA, healthcare and education, and ease of doing business.

CONCLUSION

The coronavirus pandemic has triggered a massive reverse migration from the urban to rural areas in large parts of the country. Times of crisis is a great teacher as along with the inherent challenges it can throw open many new opportunities. The government, through its various interventions specifically through the Prime Minister Garib Kalyan Yojana and MNREGA and financial packages has provided timely relief in these difficult times. The Government of India through it 'Atmanirbhar' package has rolled out many path breaking reforms. It is now important for all institutions to step up and engage all stakeholders to take forward the vision of a rejuvenated, ambitious and self-reliant Bharat.

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Chapter 23 - Discussion on the Global Financial Crisis and The Covid-19 Pandemic with reference to Nifty Bank

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Abstract

We live in a world where an activity in a foreign land has an implication on our economy. In such an economic world, recession is a big affair. It affects the people and organizations not only financially but also physically and emotionally. Key research questions which are addressed in the study are; what exactly is meant by recession? How does the equity market face the heat of recession, with reference to the banking sector? Why do the share prices collapse? How is the pandemic related to economic activities? In such times, the shares of banks are adversely affected. As the aggregate demand falls, banks have to act as a provider as well as a protector for people's misery. In the two recessions i.e. The Global Financial Crisis and the COVID-19 Pandemic, bank shares have suffered. There are patterns followed by five bank share prices in both the periods and the causes of it can be found out in the study. It can be said that the COVID-19 Pandemic is more severe than the Global Financial Crisis of 2008-09 as the growth projections made by the International Monetary Fund are nearly negative for most of the economies of the world.

Keywords: *Recession, Nifty bank, COVID-19 Pandemic, equity market, crisis*

Introduction

Background:

Where there is demand, there is supply. Where there is money, there is income and expenditure. Where there is trade, there are profits and losses. These all activities come under the umbrella of economy. Market of goods and services is the fuel for efficient working of the economy. When the markets fluctuate, the economy also goes through ups and downs. Economy experiences business cycles i.e. prosperity and recession or depression.

The Business Cycle Dating Committee of the National Bureau of Economic Research (NBER) defines recession as "A significant decline

in economic activity spread across the economy, lasting more than a few months, normally visible in production, employment, real income, and other indicators. A recession begins when the economy reaches the peak of activity and ends when the economy reaches its trough". In other words, a recession is an over-all slowdown in economic activities and considerable fall in aggregate demand.

In recessionary periods, the stock market i.e., a forum for trading of shares of listed companies is highly affected. It experiences bearishness from earlier achieved bullishness. The share prices fall to their lowest level, break the low circuit and spread a wave of pessimism in the equity market. As the scenario improves, the equity market or stock market also improves and people start to gain from the trading.

Past Recessions:

Recession is not a recent phenomenon, but it has a long history:

1) The Credit Crisis of 1772-

The Credit Crisis of 1772 emerged in London. It was due to over-optimism and over expansion of credit and it was also the chief cause of the Boston Tea Party protest and resulted in the American Revolution.

2) The Great Depression of 1929-39-

Next in the chronology comes the Great Depression of 1929-39. This can be termed as the worst financial crisis which lasted for 10 long years. It started with the Wall Street crash in 1929 and was accelerated by poor policy measures of the American government.

3) OPEC Oil Price Shock of 1973-

The Arab countries under the Organization of the Petroleum Exporting Countries (OPEC) halted their oil exports as revenge from the US for sending their arms supply to Israel in the 4th Arab-Israeli war. This created an acute shortage of oil and mounted the oil prices in the US. This crisis is also known as "Stagflation" (Stagnation + Inflation)

4) The Asian Crisis of 1997-

It was a repercussion of over-extension of credit and over-debt accumulation of the economies of Thailand, Indonesia, Malaysia, Singapore, South- Korea and Hong Kong. It created a wave of panic in Asian economies and wiped the foreign investments to a large extent.

5) The Global Financial Crisis of 2008

It was the most devastating recession after the Great Depression. After the collapse of the Housing Bubble in the US, it saw the fall of Lehman Brothers and bankruptcy of many businesses and investment banks. It wiped out millions of jobs and the government had to come out with large and unprecedented bailouts. Recession is a part of the business cycle and the present crisis which we are facing is also a recession caused by the COVID-19 Pandemic.

OBJECTIVES

- The following objectives are to be fulfilled through this research study:
- To know about recession as a concept and the past recessions which impacted the global economy.
- To gain knowledge about the Global Financial Crisis of 2008-09.
- To explain the impact of COVID-19 Pandemic on Global economy.
- To analyse the impact of recession on equity markets.
- To examine the causes and repercussions of both the recessions on the equity market. To speculate the new normal scenario and the progressive changes already taken by the world during the pandemic and in the aftermath of the pandemic.

Research Methodology

This research study is an amalgamation of descriptive as well as analytical methods of research. Secondary resources like research works of other researchers, articles from credible websites and blogs published by renowned financial institutions like International Monetary Fund and Reserve Bank of India are taken into consideration.

The data comprising share prices of the selected stocks is taken from the money control site and this data is being plotted into graphs using Microsoft Excel. While analyzing these graphs and their movements, various profitability ratios, leverage ratio and efficiency ratio have been utilized. Profitability ratios which comprise this research study are Operating Profits (OP), Net Profit Margin (NPM), Return to Net Worth; efficiency ratio comprises Total Asset Turnover. Debt-Equity ratio is a leverage ratio which is computed here by the following formulae:

Debt-Equity ratio= Total Debt/ Total Equity

The financial data was obtained from the balance sheets of the banks for the year 2008 and 2009 only.

REVIEW OF LITERATURE

Mohanty D, 'The Global Financial Crisis: Genesis, Impact and Lessons'.

The research study satisfies the thirst to know about the 'Global Financial Crisis' as it covers the background, genesis, causes, response, and lessons for India. The roots of the Global Financial Crisis are possibly as follows:

1. Global Imbalances

There were imbalances at the global level and a large amount of discrepancies were seen in terms of monetary policy in advanced economies and emerging market economies. Easy monetary policy was practiced in advanced economies which provided easy credit or cheap credit at a considerably low rate of interest. The level of savings was also low in advanced economies as compared to emerging market economies.

2. Excessive leverage

Low interest rates with easy credit provided a path for excessive leverage by investors and other financial institutions. Consequently, the use of credit accelerated while deteriorating the credit standards. The concept of NINJA (No Income, No Job, No Asset) loans emerged.

3. Underpricing of Risk

The increase in liquidity due to easy credit developed a behaviour of underpricing the risks which created speculative bubbles in the real estate. This, probably, was the warning signal of the sub-prime mortgage crisis which ultimately led to the recession.

Consequently, the emerging market economies were adversely impacted due to increased deleverage by foreign investors and shortage of US Dollars ($). The reverse capital flows led to significant losses in the equity market which also resulted in currency depreciation in the later years. In response to the crisis, the US government and many developing countries started Counter Cyclical fiscal policy. In addition, the Reserve

Bank of India used conventional and non conventional policy measures like the repo rate was cut down to 4.75%, Cash Reserve Ratio (CRR) was reduced to 5% and correspondingly the central government announced the fiscal stimulus package which helped the economy to revive.

OVERVIEW

GLOBAL FINANCIAL CRISIS 2008-09

The Global Financial Crisis of 2008-09 is considered the worst economic downturn since the Great Depression of 1930. The financial crisis or Credit crisis was a man-made recession whose prime cause sub-prime mortgage crisis or bursting of the 'housing bubble'. It led to bankruptcy and unemployment in bulk. But what was this 'housing bubble'?

It is considered as well as a reality that the prices of houses always go up and everybody needs a house but not necessarily everybody owns a house without loans or mortgages. This was the case in the 2006 period in the United States. There was a chain of mortgage brokers, lenders, investment banks and investors. All mortgages are bundled together and given to investment banks, which are known as Collateral Mortgage Obligations (CMO). These CMOs are distributed among investors under three categories namely safe, medium risky and risky. These investors get interest payments from the house owners which are known as Collateral Debt Obligations (CDO). The scenario was going well but eventually, the demand for houses was declining.

To make more money, investment banks and brokers started with sub-prime mortgages with incentives like no down payments, no requirement of income proof, etc. Thus, irresponsible people were given mortgaged loans. Eventually, they defaulted from paying interests and lost their houses to the investors. The housing prices also began to fall. Consequently, the regular payers also refused to pay interests as their houses were worth less than what they were paying as interest.

As a result, the investors went bankrupt and also the investment banks. It resulted in the burst of the housing bubble and the sub-prime mortgage crisis which ultimately led to the collapse of Wall Street and fall of Lehman Brothers. By August 2007, the Federal Reserve liquidated *$24 million* in the banking system, but it was inadequate. The crisis succumbed to the entire economy in the 3rd quarter of 2008 when GDP fell by 0.3%.

Coming to the stock market, it crashed on 29th September 2008. Dow Jones Industrial Average dropped by 777.68 points in Intra-day trading, which was the highest point drop in history. The drop was a consequence of the rejection of a bank bailout bill by the Congress. The shock of the stock market made the situation even worse. In October 2008, Congress came out with a $700 *billion* bank bailout bill. In February 2009, *a $787 billion* stimulus package was announced by the Congress. In this period, unemployment reached 10% with 6 million job losses.

In India, although interest rates were low, the fiscal spending raised which caused inflation. The stock market experienced a drop of 10.96% in Sensex and 13.11% in Nifty and unemployment rose by 2.48%. The credit problem of the Indian Corporate firms complicated when the External Commercial Borrowing (ECB) got dried up and they chose the domestic market to fulfill their credit needs. As we live in a globalized world, the adverse impact of the Global Financial Crisis was even experienced by developing countries like India.

PANDEMIC CRISIS 2020

The Corona pandemic or the Great Lockdown can be compared to the Great Depression of 1930. The pandemic is severe in its intensity as the vaccination or drug has not yet been found to save the lives of human-beings. Not a single country is spared by the Corona Virus Pandemic. The Giant economies are at a vulnerable stage and underdeveloped economies are facing dying misery. According to the latest World Economic Growth Projections made by International Monetary Fund, the real GDP and annual percent change are as follows:

Country	**`2019**	**2020**	**2021**
United States	2.3	-5.9	4.7
Germany	0.6	-7.0	5.2
China	6.1	1.2	9.2
India	4.2	1.9	7.4
World Output	2.9	-3.0	5.8

(Source: *International Monetary Fund, World Economic Outlook, April 2020, figures in %)*

The virus has given massive shocks to the major sectors and financial markets of the world. The indices of equity markets faced major downturns like Nikkei was affected by -14.7%, Dow Jones by -18.5% and FTSE 100 by -24.5%. Further, 6.8 million people have filed for

unemployment benefits just in the US in April 2020 and 3.8 million in May 2020. For the first time in the history of the United States, the Oil price crashed and went to negative value of - 37.63. It's aptly said by Ms. Gita Gopinath, Chief Economist of IMF that, "the crisis could knock $9 trillion off the global GDP over the next two years".

In India, Nifty 50 slipped by -15.04% with a downturn of around 4900 points whereas Sensex got affected by -15.31% with a crash of around 16000 points. It is a major fall in the equity markets of India. The sectors like MSME, small businesses, tourism, food industry etc. got affected by the lockdown. This lockdown has unemployed many people. For instance, food delivery company *Swiggy* announced layoff of 14% of its total workforce; *Uber* Cab Company has decided to sack 6500 employees globally (500-700 from India).

The Great Lockdown has caused damages to the economic structure of firms and industries with businesses going bankrupt and stagnation of economic activities. There is huge expenditure incurred by the governments of all the countries on medical infrastructure, restarting economic activities, stimulating businesses, and giving life to the economy post lockdown.

The nations have to come up with massive fiscal stimulus packages to boost the economy and bring the activities to the new normal. Finland has the largest fiscal stimulus of 30.0% of its GDP, followed by Japan (21.1%), India (20.0%), Singapore (13.0%) and USA (11.0%). Such packages work as oxygen to the stagnated economic activities and boost the economic growth.

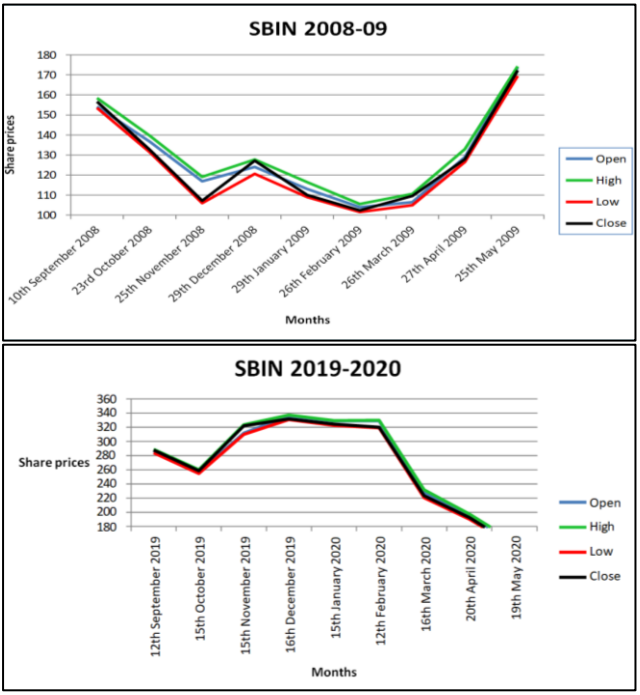
NIFTY BANK IN BEARISH TRAP SBIN 2008-09

Open	High	Low	Close	
10th September 2008	154.1	158.37	153.5	156.67
23rd October 2008	136.6	139.7	131.4	132.63
25th November 2008	116.78	119	106	107.19
29th December 2008	124	127.65	120.56	127.18
29th January 2009	113	116.4	108.87	109.93
26th February 2009	103.9	105.5	101.5	102.31
26th March 2009	106.38	110.5	104.83	109.55
27th April 2009	128.9	133	126.5	127.82
25th May 2009	173.68	174.27	169.5	172.24

2019-2020

Open	High	Low	Close	
12th September 2019	286.75	288.8	284.15	287.85
15th October 2019	256.45	259.9	254.8	258.45
15th November 2019	312	323.7	310.05	321.9
16th December 2019	335.95	337.25	330.8	331.85
15th January 2020	329	329.2	322.35	324.25
12th February 2020	329	329.9	319.25	320.2
16th March 2020	227.9	231.4	220.6	223.35
20th April 2020	194	197.35	190.05	192.5
19th May 2020	157.5	157.85	152.4	152.8

SBI is a subsidiary of RBI even though it suffered the Bearish trend in both the periods.



The share prices in 2008 -09 period show a dip in February 2009 while in 2020 it drops from the month of March 2020.

KOTAK MAHINDRA

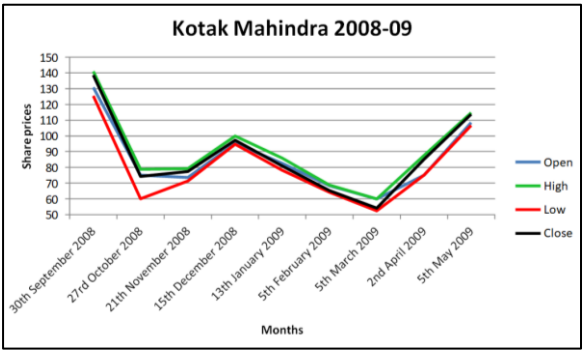
2008-09

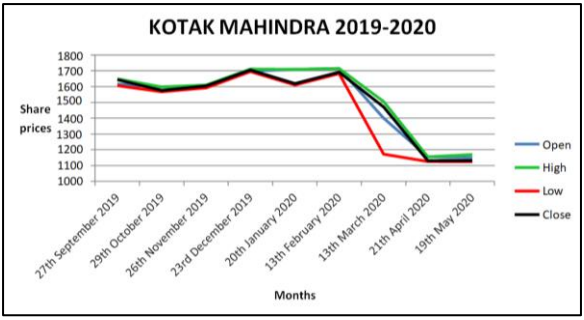
Open	High	Low	Close	
30th September 2008	130.75	140.95	125.39	138.54
October 2008	75	78.75	60	74.3
21th November 2008	73.5	79.24	71.39	77.4
15th December 2008	95.25	100	94.75	97.1
13th January 2009	82.6	85.88	78.13	80.82
5th February 2009	68.35	68.75	64.25	65.24
5th March 2009	59.95	59.98	52.38	54.1
2nd April 2009	75.07	87.5	75.07	85.12
5th May 2009	108.5	114.75	106.5	113.59

2019-2020

Open	High	Low	Close	
27th September 2019	1615.85	1649.4	1605.95	1643.5
29th October 2019	1597.4	1597.4	1568	1575.5
26th November 2019	1601	1610.5	1591.3	1604.4
23rd December 2019	1699	1711.2	1695.1	1704.9
20th January 2020	1709	1709	1611	1617.9
13th February 2020	1714.7	1714.7	1682.3	1689.8
13th March 2020	1400	1505	1172.05	1470.05
21th April 2020	1155	1155	1125	1130.15
19th May 2020	1150	1169.9	1125.3	1130.45

It is the 2nd largest Indian private sector bank by market capitalization as of April 2019





Kotak Bank hit the low circuit in March 2009 and in April 2020 during the pandemic.

HDFC

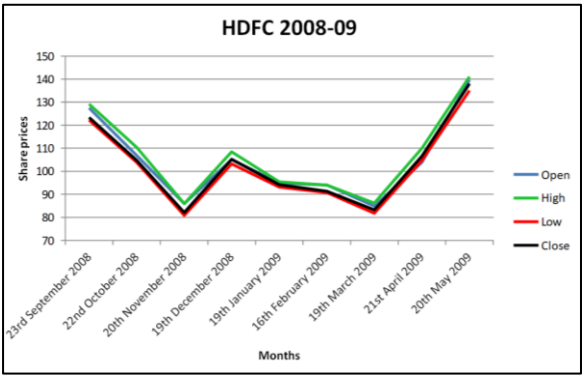
2008-09

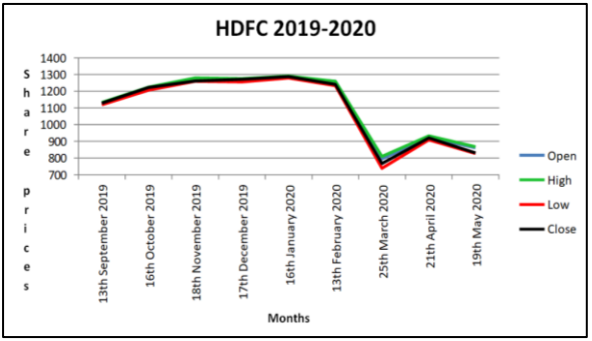
Open	High	Low	Close	
23rd September 2008	127.51	129.18	122.23	123.38
22nd October 2008	107	110.5	104	104.85
20th November 2008	85.97	85.97	80.9	82.12
19th December 2008	105.1	108.44	103.26	105.21
19th January 2009	94.9	95.45	93.15	94.11
16th February 2009	94	94	90.8	91.4
19th March 2009	84.87	86.3	81.9	83.24
21st April 2009	104	110	104.83	106.71
20th May 2009	139.7	141.1	135.1	138.06

2019-2020

Open	High	Low	Close	
13th September 2019	1125.5	1133.73	1119	1128.78
16th October 2019	1207	1225	1206	1223.05
18th November 2019	1277	1279.45	1258.7	1262.05
17th December 2019	1260	1274.8	1255.35	1271.1
16th January 2020	1282.05	1291	1279.35	1287.65
13th February 2020	1259.9	1259	1233.6	1241.4
25th March 2020	795.25	810	738.75	767.7
21th April 2020	925	934	908	921.65
19th May 2020	860.85	867.3	826.1	830.65

HDFC Bank is the largest Indian private sector bank by assets.





HDFC Bank broke its lower resistance in November 2008 and again in March 2009. In 2020, the situation will worsen in March 2020.

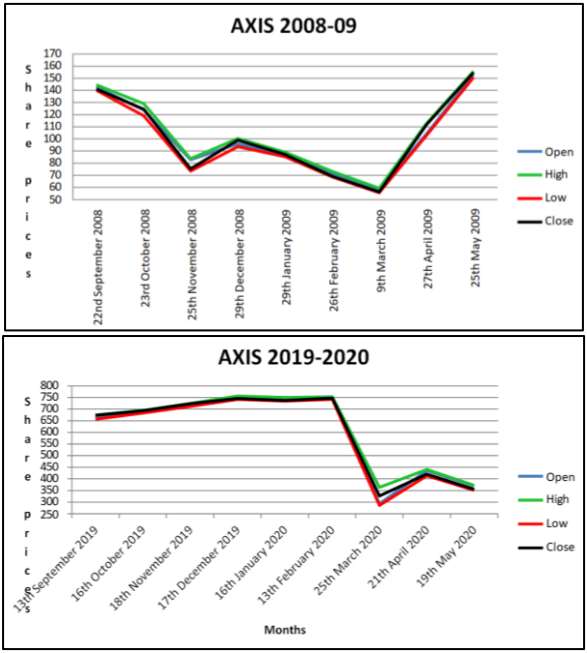
AXIS - 2008-09

Open	High	Low	Close	
22nd September 2008	142.99	144.56	140	140.98
23rd October 2008	124	129	119	124.48
25th November 2008	82.96	83.74	73.4	75.31
29th December 2008	96	100.36	93.6	98.99
29th January 2009	86	89	85.22	87.13
26th February 2009	72	73.2	68.6	69.26
9th March 2009	58.52	59.34	55.65	56.28
27th April 2009	104	112.67	102.67	111.5
25th May 2009	153	155.72	150.72	154.8

2019-2020

Open	High	Low	Close	
13th September 2019	663.55	675.4	657.05	674.25
16th October 2019	693.4	695.4	683.55	693.4
18th November 2019	717.8	725.5	712.2	722.95
17th December 2019	747.6	756.5	741.35	745.65
16th January 2020	746.5	750.4	735.1	737.3
13th February 2020	751.1	753.75	742.3	747.8
25th March 2020	293.5	363.75	286	326.8
21th April 2020	436	439.95	412.95	420.65
19th May 2020	368.8	372.5	350.1	354.5

Axis Bank is the 3rd largest private sector bank in India.



We see the graph touching its lowest point in the month of March in 2009 as well as 2020.

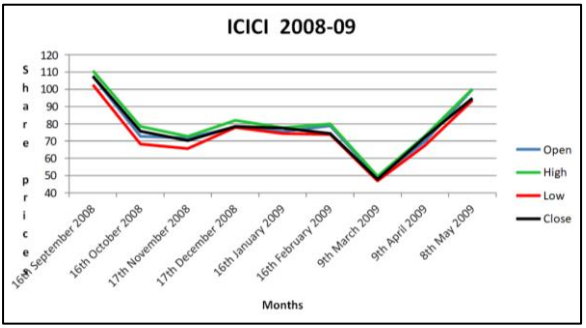
ICICI - 2008-09

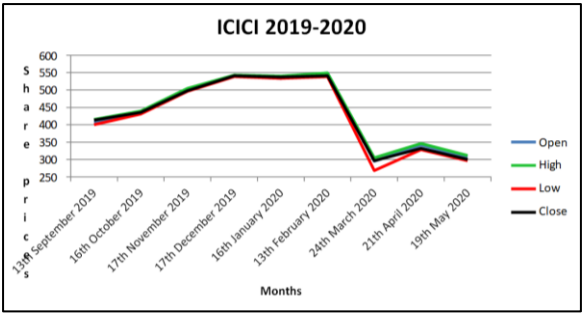
Open	High	Low	Close	
16th September 2008	107.47	110.73	102.6	107.57
16th October 2008	72.62	78.36	68.18	75.66
17th November 2008	71.82	72.73	65.57	70.26
17th December 2008	78	81.87	77.81	78.51
16th January 2009	75.45	77.76	74.3	77.5
16th February 2009	78.82	79.98	73.81	74.36
9th March 2009	48.88	49.53	46.91	47.81
9th April 2009	69.82	73.09	67.49	72.33
8th May 2009	100	100.16	93.66	94.68

2019-2020

Open	High	Low	Close	
13th September 2019	406.8	414.9	400.65	413.4
16th October 2019	434.5	439.5	431.15	435.5
17th November 2019	498.75	504.45	496.65	498.35
17th December 2019	540.95	543	538.75	541.15
16th January 2020	533.9	540	533.2	537.15
13th February 2020	549	549	538	541
24th March 2020	295.3	305	268.3	296.5
21th April 2020	341	346.2	328	331.85
19th May 2020	307.05	311.5	297.1	300.3

ICICI Bank is a multinational banking and financial services company with its network in 17 countries.





Again, the month of March is the pressurized month for ICICI as well.

ANALYSIS

In the analysis of the two recessionary periods, we find a relationship between the two. In the Global Financial Crisis, the financial sector was first targeted due to the sub-prime mortgage.

The crisis and the housing bubble and the real economy bore the brunt due to bankruptcy and resulted in massive unemployment. However, in the Corona Virus Pandemic, the real economy was first hit due to the surge in COVID-19 cases and imposition of country-wide lockdown which has its impact on the financial sector of the economy.

From the graphs it is clearly visible that the share prices had a rough patch to go in 2008-09 as well as the 2019-2020 scenario. It can be inferred from the data that prices had declined due to the Global Financial Crisis and pandemic crisis. However, there can be other reasons as well for the downfall of the share prices of five bank stocks taken for data collection. The primary area of comparison is the share prices between the two periods of recession.

An observation can be aptly made by looking at the graphs that a similar pattern is seen in each stock for the particular time period. In the 2008-09 scenario, a w-shaped curve is observed very clearly in all the five nifty-bank stocks. On the other hand, a trend is seen in the same stocks in the 2019-2020 period. There is a trend of constant movement with little fluctuations till half the period of study followed by a sharp drop in the prices and instincts of recovery towards the end of the period of study.

W-Shaped Curve of 2008-09

The w-shaped curve of the stocks seems to be a bit unusual as the prices go down initially till October and November 2008, then rise towards December 2008 and again fall till the period of March 2009 and finally show recovery towards May 2009. The initial fall can be attributed to the impact of the Global Financial Crisis, but the problem arises when the curve reaches December 2008. In December 2008, the government of India had declared the fiscal stimulus package to boost up the economy. The prices should rise after the announcement of the fiscal stimulus package however a reverse trend was observed as the share prices declined further till March 2009. The reasons for this decline could possibly be in the financials or the balance sheets of these banks for 2008 and 2009- (data from the balance sheets of the banks)

SBI BANK			**HDFC BANK**		
(Figures in Rs. Cr.)	**2009**	**2008**	(Figures in Rs. Cr.)	**2009**	**2008**
Operating Profits	230.04	173.61	Operating Profits	92.36	107.32
Net Profit Margin	12.18	11.84	Net Profit Margin	11.35	12.87
Total Asset Turnover ratio	0.09	0.09	Total Asset Turnover ratio	0.13	0.11
D/E Ratio	1253.44	932.95	D/E Ratio	342.04	296.94
Return to Net worth (%)	15.74	13.72	Return to Net worth (%)	15.32	13.83

AXIS BANK		
(Figures in Rs. Cr.)	**2009**	**2008**
Operating Profits	83.56	56.88
Net Profit Margin	13.39	12.23

Total Asset Turnover ratio 0.11 0.1

D/E Ratio	355.3	260.68
Return to Net worth (%)	17.77	12.21

KOTAK BANK			**ICICI BANK**		
(Figures in Rs. Cr.)	**2009**	**2008**	(Figures in Rs. Cr.)	**2009**	**2008**
Operating Profits	13.08	16.32	Operating Profits	48.58	51.29
Net Profit Margin	8.46	10.42	Net Profit Margin	9.82	10.53
Total Asset Turnover ratio	0.11	0.12	Total Asset Turnover ratio	0.1	0.11
D/E Ratio	62.33	62.5	D/E Ratio	314.6	324.93
Return to Net worth (%)	7.06	8.17	Return to Net worth (%)	7.58	8.94

From the tables above, let's focus on the Debt-Equity ratio i.e. D/E ratio of SBI, HDFC and AXIS banks. It is seen that the D/E ratio for SBI has increased from 932.95 Cr. in 2008 to 1253.44 Cr. in 2009. Again, in HDFC, the ratio increased from 296.94 Cr. to 342.04 Cr. in 2009. Similarly, Axis bank also showed an increase from 260.68 Cr. to 355.3 in 2009. This clearly shows an increase in debt due to aggressive debt financing. Higher D/E ratio tends to indicate higher risk anticipated by shareholders for a stock. This anticipated risk can impact the prices adversely and a decline in prices can be seen. On the other hand, Kotak bank and ICICI bank show a different picture. In both the banks the other profitability ratios like operating profit, net profit margin, return to net worth and total asset turnover have decreased as compared to FY 2008. This indicates decline in performance which could have affected their share prices.

Let's come to the COVID-19 Pandemic crisis. The initial trend was usual equity market conditions. However, when India reported its first Corona patient on 30th January 2020 from Kerala, the virus started its attack on the equity market. As the cases increased in the country, the drop in the equity market intensified. Due to the lockdown, all businesses got shut; transportation was halted which advanced a wave of pessimism in the share market. On March 23, 2020 Indian equity markets hit the low circuit and trading was halted for some time and again resumed. Nifty-bank experienced a sharp drop of 16496.85 points from the earlier achieved height. A ray of hope was seen after May 2020 and the revival of the equity market is taking place at a slow pace.

The main cause for the sharp drop in the nifty index is due to the lockdown which resulted in decline of consumer demand and major sectors of tourism, transportation, aviation, hotel and restaurants, MSMEs and local industries were hit hard as they were closed for nearly 3 months. As the local, state and national boundaries were sealed, inter-state or cross-border trade was hampered. On the other hand when consumers had very few options of spending , investment in gold achieved its new highs with gold crossing the 50000 mark. Nonetheless, the equity markets are slowly and gradually recovering and they are progressing with little corrections.

TOWARDS A NEW PARADIGM

"Behind every cloud of darkness, there is a silver lining of opportunities"

All crises, be it pandemic or financial distress, have a huge impact on our lives and the economy. Moreover, as we live in a globalized world the impact is multifaceted and multi dimensional in its intensity. However, there is always something positive in all negative situations that drive us towards prosperity. There are certain lessons which we need to take from such recessions and pandemics.

The Global Financial Crisis of 2008-09 was a warning bell for repairing the malignance in the financial systems of advanced as well as emerging market economics. There is no doubt that it reduced the growth rate but what is the use of such increased growth rate which is fueled by wrecks in the system. It gave a chance of introspection and improvisation on the credit standards and financial security measures. It taught the advanced economies that developed cannot be accelerated through low credit standards whereas emerging market economies learnt how to thrive the external shocks on the financial system.

In the present time of COVID-19 pandemic, knowingly or unknowingly, we are having a shift towards a new paradigm. This paradigm is about efficiency, innovation, and prosperity of the local economy. We may be very pessimistic about the future circumstances as there is uncertainty regarding the drug or vaccination to fight the virus. Nevertheless, being from economics we need to think beyond this time.

Due to the standstill stance of many economies, globalization is slowing down, and the governments are also focusing on local industries which is leading towards protectionism in trade. Some countries may even impose higher import tariffs to promote domestic industries. Although

the implications of protectionism are debatable, countries like India have an advantage due to its huge and young population. As a result, Aatma Nirbhar Bharat scheme is a golden opportunity if exploited properly and economically. Indian industries and service sector have a deserving chance to prosper and in leading the way forwards we have companies like Tata Consultancy Services (TCS) who is planning to hire 40000 freshers in India. We need more such companies to come forward in achieving the aim of self-reliance.

Coming to the consumer demand, consumers are afraid to spend as there is a trust aversion in the minds of consumers. Moreover, the lay-off of employees by many companies may have an impact on consumer demand in future. Anyways, consumer demand is gradually coming back with the restart of many businesses in the unlocking phase. We also observe a surge in consumer spending on online media with the help of digital modes of payments.

In this new paradigm, countries are moving towards Green Energy because COVID-19 has made us realize the value of life. With cities like London and Milan increasing the cycling infrastructure it is quite clear that the objective this time is to heal the planet rather than hurt the planet. This transition is an effective step towards achieving the goal of Green GDP. Green GDP simply means growth will sustainability and preservation of the environment.

Coming to the common man, I see my father who is a government employee working from home. I see my neighbor who is a professor teaching her students online and I too attend my lectures regularly on my phone. Surprisingly, most people have accepted this tradition while many people still face problems in accepting this change due to scarce resources. Remote working has added the element of efficiency in the workforce. It has also led to less traffic congestion on roads and eventually reducing pollution in major cities and towns. This, indeed, is a road to the Fourth Industrial Revolution.

While mentioning about the fourth Industrial revolution, we need to keep in mind that we want to achieve efficiency in this phase which may not accelerate the GDP growth. GDP growth should not be the goal of this revolution because there is no value of such growth if the resources are wastefully utilized. This also raises the question on how to interpret the GDP in future.

At the end, it is quite clear that we are moving towards a new paradigm

and consumers have a huge role to play in this period to help the economy to grow. Corresponding to it, the industries and governments should willingly try to meet the demands of the consumers while preserving the natural resources.

CONCLUSION

It is conceivable to say that the COVID-19 Pandemic led recession is different from the Global Financial Crisis of 2008-09. In the financial crisis of 2008, money and jobs were at stake while in the present scenario, with money and jobs, health and lives of people are at stake. The 2008 recession had its impact on few sectors of the economy whereas the COVID 19 pandemic has hit nearly every sector hard and made the economies of the world stagnant. It can be feasibly concluded from our analysis that the Corona pandemic is far more severe than the Global Financial Crisis and as devastating as the Great Depression of the 1930s.

Such crises have a direct relationship with pessimism in the market of equity that is seen in almost every recession. Whether it is a Wall Street crash or a Main Street Crash, it has led a bearish trend for a considerable period of time. Equity markets are speculative, and people are optimistically speculating the market to regain its bullish trend but still they are hesitant to invest in the market even though this period is a golden time to invest. This has resulted in less injection of liquidity in the market which is halting the growth of the equity market.

To sum up, the world has not ended, businesses and trade have not shut permanently, the desires and needs of people have not died. Recession is a part of business cycles and furthermore any pandemic cannot last forever. There is no doubt that many have lost their lives, and many have faced dire miseries due to the pandemic and lockdown, but this time shall also pass. This period has given a chance to improve in the field of medical infrastructure, resource management, finance, and business. We are very hopeful regarding the invention of vaccines or drugs which will save many lives and the old normalcy shall come back.

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