JIGYAASA

A Multidisciplinary
Research Initiative of KC College, Mumbai
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H(S)NC Board's
KISHINCHAND CHELLARAM COLLEGE

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Editorial

The successful reception of Jigyaasa Vol 1 has inspired the creation Jigyaasa Vol 2 this year. The students enrolled in Science Honors Program (SHP) and Certificate Programme for Commerce & Arts (CPCA) were highly enthused towards producing quality research in the hope that the best research papers would be published in this volume. The above two research-based programmes of K.C College have encouraged not just students but even teachers to engage enthusiastically in the creative, critical and scientific pursuit that makes up research. SHP and CPCA programmes at K.C. College, train their young researchers to communicate effectively- first with the community while gathering research data, then with their peers while collating and analysing that data and finally with the research experts while presenting and defending their finished research results.

This 2nd volume of Jigyaasa is a compendium of such selected research papers by students enrolled in SHP and CPCA and their teachers. The chapters on different fields such as Biotechnology, Life Sciences, Microbiology, Chemistry, Physics, Computer Science, Statistics, Social Sciences, Commerce and Humanities, reveal insightful research efforts.

We are sure that the readers would be interested in the chapters that include topics ranging from the extraction of pharmaceutically important enzyme Bromelain, Microbial analysis of water samples across Mumbai region, Smart Mirrors in the Science section to the Comparative analysis of themes of Marginalization and Patriarchal oppression and Comparison of Bollywood movies, in the Humanities and Social Sciences section. Research in K.C College has inculcated strong critical thinking and analytical abilities in the concerned students and also refreshed the teacher mentors and revived their love for research.

Experiential learning is the passport to changing the future of not just an individual but that of the community. Thus, we strongly hope that these research compendiums of Jigyaasa would act as a guiding light to all the future students of KC and other educational institutions who wish to travel the path from Lab to Life.

Foreword

Research not only motivates young students but also makes them socially conscious and inculcates critical thinking ability in them. The success of our research compendium - Jigyaasa Vol I has made me proud of our two innovative research-oriented initiatives of Science Honors Program (SHP) and Certificate Programme for Commerce & Arts (CPCA).

Both these programmes are not only research centric but also enhance communication skills and confidence level of the participants, besides improving their academic writing ability and computer skills and making them gender sensitive. The research component of the two programmes helps imbibe both quantitative and qualitative research methodology, training the young researchers to critically analyse. Students formulate their thesis topics, learn to review the literature, observe, survey, analyse and then draw conclusions. This step-by-step process is undertaken under the guiding hands of their Teacher Guides. It gives me great pleasure to go through another collection of diverse research papers and bring out the 2nd research volume of Jigyaasa, which is a compendium of the tireless efforts of the researchers. Like Vol 1, this volume too has interesting and insightful research chapters from different fields such as Biotechnology, Life Sciences, Microbiology, Chemistry, Physics, Computer Science, Statistics, Social Sciences, and Humanities.

In this growing age of Interdisciplinarity, Jigyaasa volumes with their diverse research chapters become a true example of how seemingly different worlds overlap and intersect. Experiential learning is the passport to identifying problems and formulating new perspectives. The path of knowledge travelled by the present ' Jigyaasus' will illuminate those who take this same road in the future. Thus, these research compendiums will act as a guiding light to all those who will climb aboard the research vehicle in the future and will be well-versed with the tools of research methodology to be able to connect the lab to life.

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

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SECTION I - LIFE SCIENCES

Chapter 1 - Bioprospecting bromelain as a candidate for management of infections: A pilot study

Sukaina Abbas, Maitri Trivedi, Dr.Tejashree Shanbhag*, Dr. Amruta Kothare **

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Abstract

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

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

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

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

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

Introduction

Pineapple *(Ananas comosus)* has been used as a traditional medicine by several cultures throughout time and Bromelain had been established in 1876. Bromelain gets its properties mainly due to the presence of its sulfhydryl proteolytic enzymes. Bromelain is classified as stem bromelain or either fruit bromelain depending on the origin of the protease. (Rowan et al, 1990). Bromelain is present throughout the pineapple plant however the concentration and composition may vary depending on the part of the fruit and its variety. (Gautam S, 2010).

Protease enzyme bromelain is important in health care and industry. The purification methods increased percentage recovery and fold purification. It supplements elderly patients' protein intake. Bromelain is a category of sulfhydryl proteolytic enzymes and cysteine proteases isolated from pineapple stems and fruits. Bromelain treats acne, wrinkles, edoema, bruising, and skin dryness. Bromelain breaks down the top skin cell layer's protein, allowing new cells to grow. The antimicrobial action of Bromelain against several bacteria motivated its study against acne causing pathogens.

Acne is a skin condition affecting the skin’s oil glands is a very prominent condition. It can create a lot of psychological disturbances and stress on the individual that it effects. P. acne is an opportunistic pathogen that plays an important role in the growth and cause of acne. S. aureus is a part of the normal skin microbiota and is associated with skin conditions such as folliculitis and also reported to enhance the effect of other microbes in acne lesions. (B. Kumar, et all, 2006). Based on this background study, we decided to explore the possible effects of Bromelain as a treatment plan for acne.

Materials:

Biological material: Pineapple *(Ananas comosus)*, Aloe vera, Turmeric *(Curcuma aromatica), S. aureus, E. coli, P. aeruginosa, P. acne* (MTCC: 1951) and *C. diphtheria*.

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

Media: Nutrient agar and Nutrient Broth: Peptone, Beef extract, Yeast Extract, NaCl, Agar

Methodology:

1. Sample preparation:

1.1 Crude Extract Preparation: The pineapple parts were categorized as fruit, stem, peel, core and crown. Small pieces were made and they were homogenized and ground with 0.1M of sodium acetate buffer (pH – 7.0). The juice obtained was filtered and centrifuged at 6000 rpm for 20 mins at 4ºC. This extract was used as the crude Bromelain.

Fig 1: Waste parts of the pineapple

1.2 Ammonium Sulphate Precipitation: The crude extract was subjected to Ammonium sulphate at 40% saturation. Dialysis: The fractionate from ammonium precipitation was loaded into dialysis bag and tagged. It was loaded into a beaker filled with dialysis buffer. The process of dialysis was carried out in cold conditions by keeping it over ice and using a magnetic stirrer.

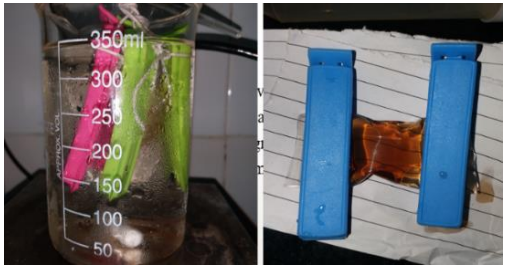

Fig 2: Dialysis chamber and Dialysis bag loaded with sample (L-R)

2. Measurement of Protein content: Crude bromelain and fraction protein content was diluted appropriately and was determined by using the Folin-Lowry method. BSA was used as a standard for protein estimation. The optical density was read at 660nm.

3. Antibacterial activity assay:

3.1 Bacterial maintenance: *S. aureus, C. diphtheriae, E. coli, Pseudomonas aeruginosa, P. acne* (MTCC: 1951) were maintained on Nutrient Agar slants and incubated at 37ºC.

3.2 Ditch plate technique: Antimicrobial susceptibility of the organisms towards the crude extract was observed by using the ditch plate technique. The plates were left overnight to incubate at 37ºC. The Zone of Inhibition was measured.

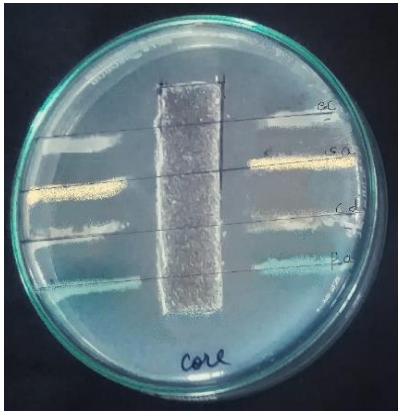

Fig3: Ditch plate against core crude

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

Percent inhibition=$\frac{-}{\quad}$ 100 where A is OD of blank and B is OD of sample. (Khalaf, 2008; Lee-Fong,2014)

5. Formulation: Three different face wash formulations were made the only difference being with the source of bromelain in each, stem bromelain, crown bromelain and peel bromelain.

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

5.2 Antibacterial testing of formulation: The antimicrobial activity of the crude extract was determined using the disc diffusion method. Nutrient agar plates were cotton swabbed with the cultures. Sterile Whatman discs impregnated with the test samples were placed. The samples were tested in four concentrations of (v/v) percentage at 25%, 50%, 75% and 100%. They were left overnight to incubate at 37ºC. The Zone of Inhibition was measured.

6. Comparison of commercial products: Four herbal facewashes were selected at random and their antimicrobial action against bacterium were tested at four (v/v) % concentration levels (i.e. 25%, 50%, 75%, 100%)

7. Statistical comparison of commercial products against Bromelain formulations: ANOVA testing was done at 5% level of significance using Excel Data toolpak.

Observation and Result:

1. Estimation of protein content: Fruit contained the highest concentration of Bromelain as crude extract and even after purification. The protein yield in stem increased with purification. However, in peel, bromelain yield decreased with purification leading to the possibility of other protein contaminants present in the peel. The results are depicted in Fig 5.

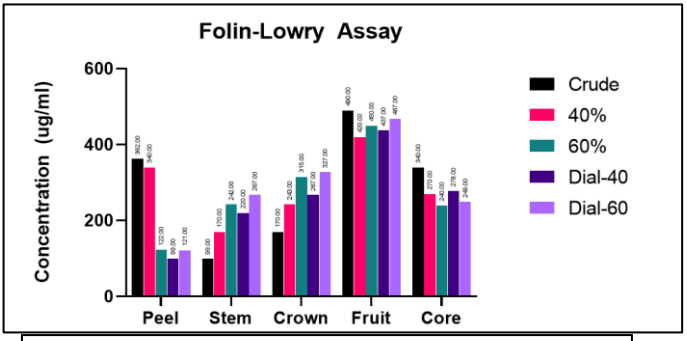

Fig5: Graphical representation of protein content in Crude bromelain and its purified fractions

2. Antimicrobial studies of Crude Bromelain by ditch plate method: The results are graphically depicted in Fig 6. *P. acne* growth was considerably inhibited by Crude Bromelain, highest by Fruit extract.

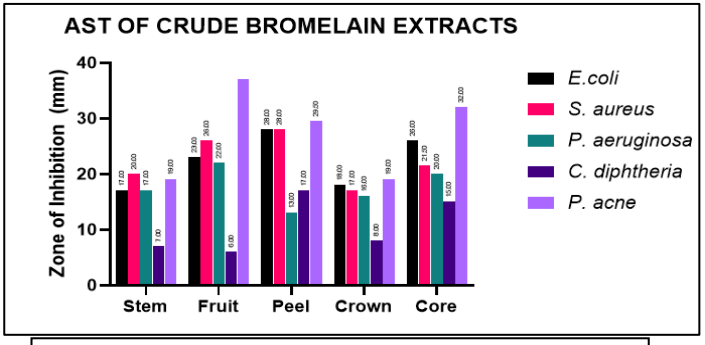

Fig6: Graphical representation of zone of inhibitions of crude bromelain against microbes

3. Antioxidant activity of crude Bromelain extracts: natural antioxidants are present in Bromelain that contribute to preventing oxidative stress. By statistical analysis, there is clear difference between activity of Ascorbic acid and crude bromelain extracts. While lesser than ascorbic acid, there is still considerable amount of activity. As we can see (Fig.7), above 20ug/ml the activity is comparable to higher range of concentrations also.
4. Physical characteristics of formulation:
5. Antimicrobial studies of the formulation: the formulation was tested by disc diffusion against *P. acne, E. coli, C. diphtheriae, S. aureus*.
6. The zone of inhibition is graphically represented and tabulated. By statistical analysis (ANOVA) it is proven that the three formulations are similar to each other.

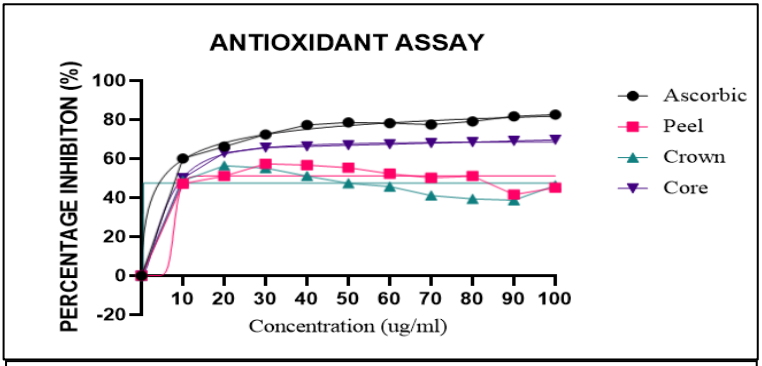

Fig7: Graphical representation of antioxidant assay performed by DPPH assay

Factor	PEEL FORMULATION	STEM FORMULATION	CROWN FORMULATION
pH	8.5	8.9	8.3
Color	Golden yellow	Brownish yellow	Pale green
Consistency	Semi-solid	Semi-solid	Semi-solid
Washability	Good	Good	Good

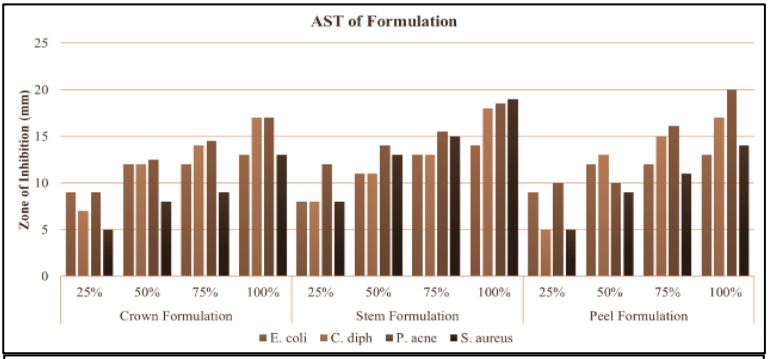

Fig.9: Graphical representation of zone of inhibitions of bromelain formulation against microbes

7. Comparison of commercial products: all the facewashes were found to be quite effective against P. acne. However Brand 2 and brand 3 showed no inhibition against E. coli.

8. Statistical Analysis of data: For *S. aureus and P. acn*e the formulations were found to have comparable and similar effects to the commercially available formulations. For E. coli the bromelain formulations were statistically better. For, *C. diphtheria* the commercial formulations proved to be more effective

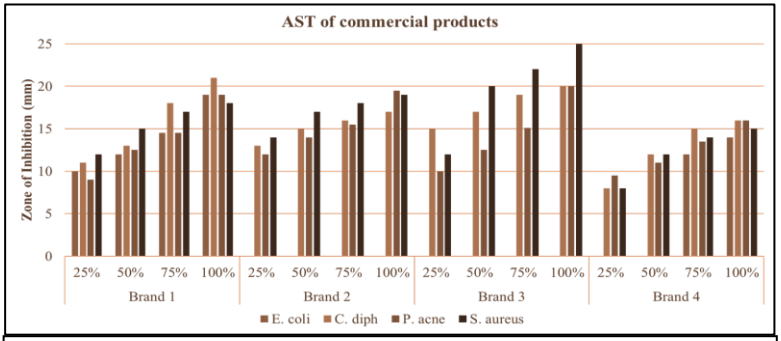

Fig10: Graphical representation of zone of inhibitions of commercial products against microbes

DISCUSSION

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

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

Conclusions:

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

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Chapter 2 - Phytochemical Analysis and Antioxidant Analysis of fruit rind of *Couroupita guianensis* Aubl.

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Abstract

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

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

Introduction

Cannonball tree, also known as ‘Kailashpati’ is a tree that belongs to family Lecythidaceae and is native to Central and South America. The tree is religiously and culturally significant in The Republic of India. The flowers of the tree appear to resemble a ‘Shivling’ which is protected by a hooded Naga. Due to this characteristic feature and the sweet fragrance that it produces, these trees are often seen planted near Shiva Temples. The tree is commonly spotted in Mumbai and is seen flowering

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

Materials and Methods

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

Preparation of Extract - 20g of the powder was weighed and then it was tied into a thimble using new sheet of muslin cloth. The thimbles were inserted into the Soxhlet extractor (Hot continuous extraction). The extraction was carried out at 80°C using ethanol (Cooper-Driver & Harborne, 2007). The extraction was carried out for 10 cycles and then the sample was sample was concentrated using a rotatory evaporator. The extracts obtained were subjected to phytochemical tests, and antioxidant assay by DPPH method.

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

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

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

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

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

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

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

Thin Layer Chromatography

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

spotted with a capillary tube. A solvents like Petroleum Ether, Acetone, Benzene, and Methanol were used. The plates were kept in pre-saturated chromatography chamber and allowed to run. The best separation was observed in 8:3(Pet Ether:Acetone). The spots were visualized in Iodine gas chamber and yellow to brown spots were observed.

Column Chromatography

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

Antioxidant Assay by DPPH Method

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

Results and Discussion

The preliminary phytochemical analysis of the sample showed the presence of Tannins, Terpenoids, and Flavonoids in the Inner shell, and the presence of Triterpenes, Terpenoids, Flavonoids, Tannins, Phlobatannins in the Outer shell. This indicates that there is a difference in the phytochemical composition of the Inner and Outer shell. Natural antioxidants present in herbs, spices, etc are responsible for the reduction

or prevention of negative consequences caused by oxidative stress. They contain free radical scavenging

Table 1: - Detection of Phytochemicals

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

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

Conclusion

The phytochemical analysis was carried out by TLC, and column chromatography. The composition of the inner and the outer shell were compared using these techniques. The antioxidant property of both

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

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SECTION II – CHEMISTRY

Chapter 3 - Effect of A.C. Water on Kota Tile

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Abstract

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

Introduction

KOTA TILE:

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

A.C. WATER:

- Literature studies reveals that the AC water is same as distilled water chemically.

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

Background

- Observation of the effect of AC water on Kota tiles during passed three years.
- Kota tile becomes rough indicating erosion.
- Rough surface also accelerates algal growth

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

Objectives:

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

Tools: pH and Conductance

Methodology: Procedure: - (Replicate analysis)

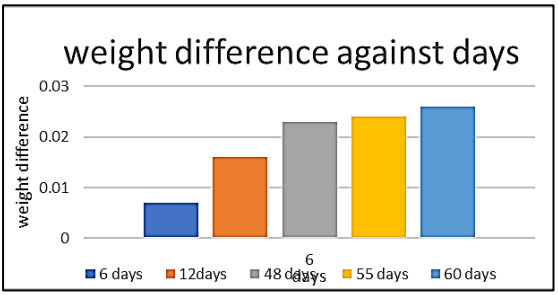
1. Collection of water from AC.

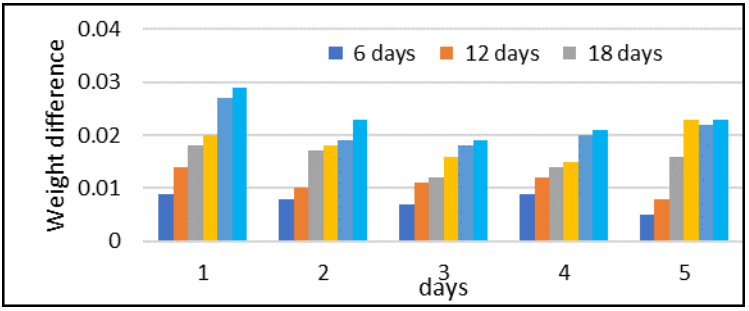
2. Take 6 beakers containing piece of Kota tile of dimension 1cm x 1cm

RESULTS

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

Graph:

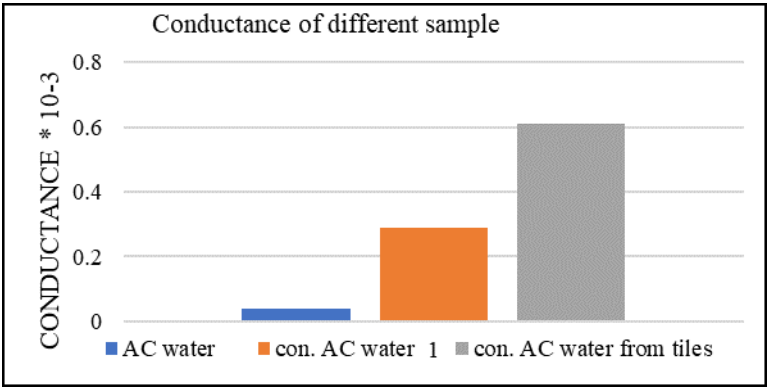




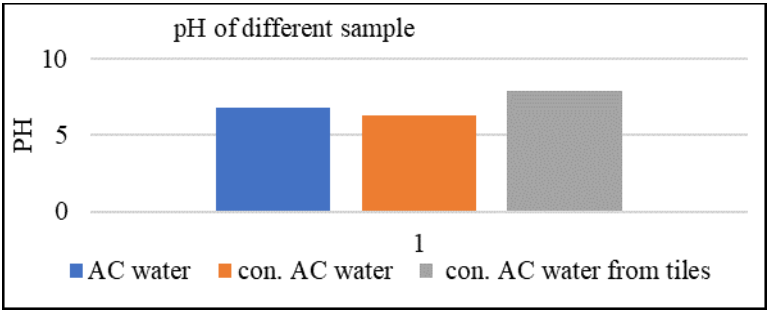
Methodology: CASE 2: Procedure: Concentration of A.C water sample.

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

Results:



- To determine calcium content of con. AC water sample after flowing through Kota tile by flame photometry.



Ion Exchange Chromatography

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

Hardness comparison

- Hardness of con. A/C water is 22 ppm.
- Hardness of con. A/C water drain from Kota tile is 85 ppm.
    - Result: Hardness of con. A/C water is less than Hardness of con. A/C water drain from Kota tile
- Analysis of A.C. sample which is concentrated after drain from Kota tile and diluted Kota tile sample using Inductively Coupled Plasma Atomic Emission Spectroscopy.

Vol. Flask no.	Vol. Of 1000 ppm ca2+ sol.	Total volume	Con. Of ca2+ mg/l	Emission intensity
1	20	100	20	17.7
2	40	100	40	40.1, 41.0
3	60	100	60	65.4
4	80	100	80	80.5
5	100	100	100	89.4
6	Unknown 1%	100	1%	0.9, 0.0
7	Unknown 5%	100	5%	35.2, 22.5, 17, 22.5
8	Unknown 10%	100	10%	91.6, 49.7, 52.2, 58

Results

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

Conclusion:

- The release of Calcium ion from tile in the water.
- Increase in conductance of AC water.
- The AC water become alkaline after flowing from Kota tiles as pH is increase.
- Erosion of Kota tiles due to AC water.
- Hardness of con. Ac water which is drain from Kota tile is more than con. A/C water.

Future Goals: Rate of erosion determination.

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Chapter 4 - Study of Reactive Dyes and their Biodegradation

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Abstract:

Coming from a background which deals with the dyeing and processing of the textile, the disposal of the wastewater of dyeing is a big concern. This Wastewater from the industries which are toxic in nature is released directly in the environment. They are released in the surface waters through drainage system, seeps into the ground water and soil too near the water bodies. Hence, cheaper method like Biodegradation of the dyes is to be carried out.

Keywords- Dyes, Textile, Reactive, Biodegradation, Disposal

Introduction

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

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

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

Amongst many other dyes. Certain Azo dyes are carcinogenic in nature and hence are banned all over the world. There are 112 Azo and Benzidine containing dyes which are banned in India. The Dyes that are most commonly used in textile industries are Reactive Dyes.The Reactive Dyes can be classified into further types:

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

Advantages:

- Reactive dyes have various advantages over other dyes and hence are most commonly used.
- Permanency in color: Reactive Dyes can be easily said to be most permanent of all dyes. This is because of the unique quality of the dye to form a covalent bond with the substrate of the cellulose.
- Easy washing: The fibre that are dyed can be safely washed with white garments without danger of coloring it.
- Chemical bonding: Chemical bond significantly improve product's color, stability and wash ability
- Wide range of color: These dyes provide a large range of colors.
- Lower Cost: They are comparatively cheaper than the other dyes.

Covalency: Reactive Dyes are the only class of dyes which makes covalent bond with the fibre and becomes part of it

General formula is S—F—T—X

Where, S: Solubilizing Group,

F: Chromophore,

T: Bridging group;

X: Reactive system.

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

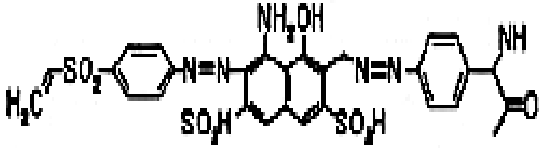
Problem to the Environment:

The wastewater from the industries mostly from the small scale which are not treated before releasing in the environment. Although it has many

advantages and is most commonly used, in most case almost 50% of thedye is lost through hydrolysis during the dyeing process and therefore, appears as wastewater. The textile industries are considered one of the most important industries all over the world and are considered as 5th largest source of foreign currency, but are also considered as main source of water pollution. They are also not easily scale industries have softener plants that treats this wastewater. Small scale industries do not have it as it is expensive and hence, they cannot afford it. It undergoes Nucleophilic substitution or addition.

As the other method being expensive, there should be an alternative affordable to these small-scale industries. Therefore, the idea of Biodegradation can be used for the degradation and decolourisation of the dyes.

This can also be reusing the water if the degradation occurs.



Navy blue HE2R (Reactive Blue 172)

Aim and Objective: Process:

The process of fungal dye degradation consists of two stages. The first stage involves the reductive cleavage of the dyes' azo bond (-N=N-), resulting in the formation of aromatic amines, that is generally colorless but potentially hazardous. The second stage involves degradation of the aromatic amines under aerobic conditions. There are factors affecting the decolorising of dyes. Various Microorganism that decolorizes reactive dyes. The dye here used is reactive dye Navy Blue HE2R.

This contains diazo group. It has structure as follows:

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

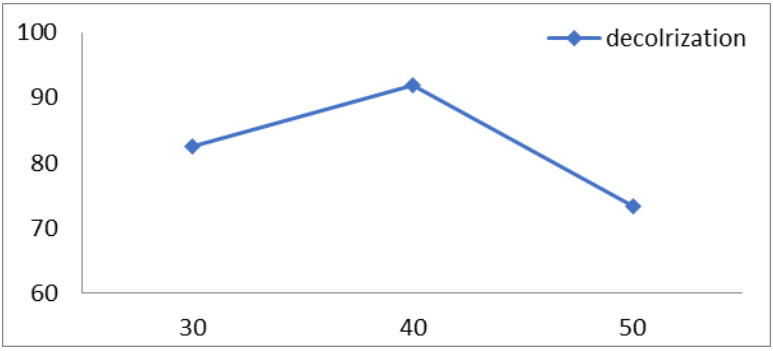
Organisms	Dyes	Reference
Fungi:		
Trametes villosa	Drimaren Brilliant Blue	Machado *et al*., 2006
Pycnoporus sanguineus		
Pleurotus ostreatus	Remazol Brilliant Blue R	Palmieri *et al* ., 2005
Funalia trogii	Remazol Brilliant Blue R	Deveci ., 2004
Aspergillus ochraaceus	Reactive Blue - 25	Parshetti *et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

Decolorization of Navy Blue HE2R:

Biodegradation was carried out by the White Rot fungi. The strain was maintained on the Potato dextrose agar plate at 30ºC and stored at 4ºC. Decolorization experiment was conducted in 250ml conical flask. This fungus was tested for its ability to decolorize selected Navy Blue HE2R dye over a period of 24 hours.

DECOLOURISATION%=INITIAL-FINALx100

Effect of Various Concentrations:

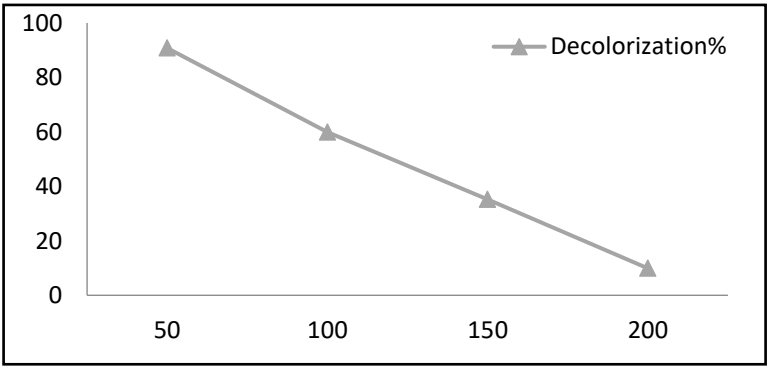


Method:

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

Observation:

The rate and extent of decolourization was affected by the ranging concentration. For the Navy Blue HE2R at concentration, 50 ppm, the decolorization was 90.75%, for 100ppm it was found out to be 59.90%, followed by 150 ppm (35.25%) and 200ppm (9.90%)



Effect of different Temperatures

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

Observation: The decolorisation was found to differ at different temperatures. It was maximum at 40^{o}C i.e., 91.90.%, then 82.50% at 30^{0}C and 73.33% at 50^{o}C

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

Observation:

It was observed that the dye before decolorization was toxic to the microbial cells hence it showed inhibition on the microbial growth. Whereas, the decolorized product showed no inhibition in the growth of the microbial cells and hence were not toxic to the microorganisms.

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

Conclusion: In conclusion, discharge of effluent into water bodies is a serious environmental problem. Reactive dyes are azo based dyes and they are recalcitrant to degrade by conventional treatment method.

The biological treatment is an effective alternate method to decolorize and mineralize the dyes in effluent without leaving harmful by-products.

Future Aspects: Other toxicity tests like phytotoxicity can be done. Further, different parameters can also be taken into consideration with more range of dyes and microbes.

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Chapter 5 - Qualitative and Quantitative Analysis of Organic Juices and Industrial Beverages

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Abstract

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

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

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

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

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

Introduction:

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

In addition to basic food items, people also store frozen or preserved garden-grown fruits, vegetables and freeze- dried or canned produce. Food can be classified as fast food, junk food, whole food, organic food out of this whole food is unprocessed, unrefined so it has very short shelf life. Nowadays Mostly all food products have food preservatives. The purpose is generally to preserve the natural characteristics of food and to increase the shelf life of food, and inhibit natural ageing and discoloration that can occur during food preparation such as the enzymatic browning reaction in apples after they are cut.

Natural methods of preservation usually aim to exclude air, moisture, and microorganisms, or to provide environments in which organisms that might cause spoilage cannot survive. Natural way of Food Preservation can be done by Boiling, freezing, pasteurizing, dehydrating, smoking, pickling. Adding Sugar, sometimes sugar is combined with alcohol for preservation of luxury products such as fruit in brandy or other spirits, salt, alcohol, vinegar are also often used as food preservatives. They very efficiently drop the growth of bacteria in food. Coffee powder and soup

are dehydrated, dried for preservation.

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

Sodium benzoate, Benzoic acid, Sodium sorbate, Potassium sorbate, Sodium nitrite are used as antimicrobial agents they inhibit the growth of bacteria, molds, insects and other microorganisms. Some substances used as Antioxidants (that act as free radical scavengers) are Vitamin E, Vitamin C, Pine Bark Extract, Grape Seed Extract, Sodium Erythorbate Sodium Diacetate, Sodium Succinate, Sodium Dehydro Acetate, Succinic Acid and Ascorbic Acid, Parabens, Erythorbic Acid, Proplyphenols.

Also, some Chelating agents work as preservatives for example Disodium ethylenediaminetetraacetic acid (EDTA), Polyphosphates, Citric acid and Ascorbic acid Monosodium Glutamate (MSG) Disodium Guanylate and Disodium Inosinate are used as food flavouring agents.

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

Nitrates and Nitrites: These additives are used as curing agents in meat products.it gets converted into nitrous acid when consumed and is suspected of causing stomach cancer. Benzoates are used in foods as antimicrobial preservatives, and have been suspected to cause allergies, asthma and skin rashes.

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

contact dermatitis. Also, a nuclear radiation when used for preservation does not make foods radioactive, but may cause changes in food color or texture. After consuming certain foods if it causes allergy that can be noticed but some people develop the symptoms of allergy day or two later, so it is difficult to know what is causing the problem. People consume variety of foods so it is difficult to find out the exact substance which causes allergy. For this reason people have to go on an elimination diet. They stop eating all foods that might be problematic and introduce one at a time to see if side reaction occurs. Side reactions of these preservatives can be immediate or build up in the body over time. Only in recent years have researchers seriously considered the physical impact of these additives over the long term.

Objectives/Scope of the Research`

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

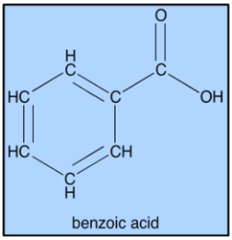
Practical Research Undertaken

1. Preservatives used in different Commercial Beverages:

Levels of Benzoic Acid in soft drinks and fruit juices:

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

Introduction:



benzoic acid

The use of chemical preservatives has become important in food industries in INDIA. Benzoic acid is one of the commonly used chemical preservatives in the food and beverage industry.

India has become a middle-income country and as a result the production of fast, processed and convenient products especially in food and

beverage industry by indigenous manufacturers has increased over the past decade due to lifestyle changes and improved standard of living. This has led to a lot of proliferation of soft and fruit drink industries.

A survey conducted showed that some manufacturers prepared the products with low quality assurance and few accredited laboratories. The use of benzoic acid as an antimicrobial agent has been observed to have adverse effects such as metabolic acidosis, convulsion, hyperactive and hyperpnoea in experimental animals and humans given very high doses of benzoic acid. The development of allergic reactions to benzoates in humans, such as urticaria, non- immunological contact urticaria and asthma, has also been reported in some studies.

Benzene which is carcinogenic can be formed at very low level (ppb level) in soft and fruit drinks containing both benzoates and ascorbic acid. Exposure to heat and light further stimulate the reaction. The use of food additives is limited by specific regulations. India, as many countries, follows the recommendations by the joint FAO/WHO Expert Committee on Food Additives on the safe use of food additives and limit of 150 mg/L benzoic acid has been established.

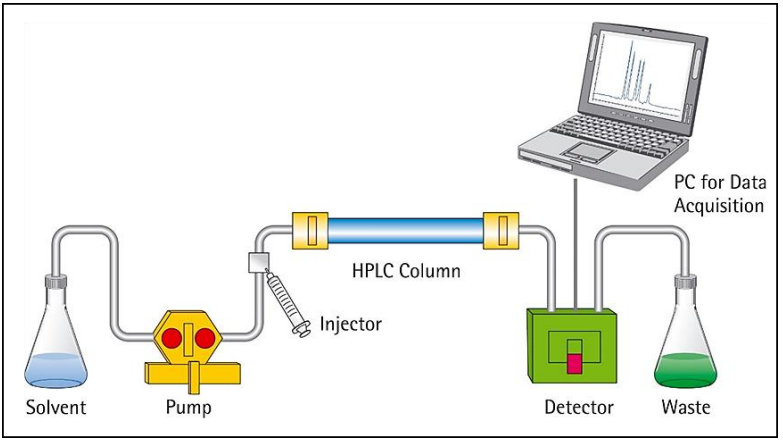
Although there are several analytical methods for the determination of levels of benzoic acid, such as spectrophotometric and gas chromatographic (GC) method, in this study we used high performance liquid chromatography (HPLC), which has a high specificity and minimum sample preparation and does not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

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

Materials and Methods:

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

High Performance Liquid Chromatography



Chromatographic Conditions - The chromatographic analysis was carried out by high -performance liquid chromatography. The analytical column was 4µ polar – RP 80A 150×2mm 4-micron column with 325 UV detector. The mobile phase contained a mixture of acetonitrile-ammonium acetate buffer adjusted to pH 4.4. The chromatographic separation was achieved with isocratic elution condition at a flow rate of 0.4 mL/min and 20 µL of sample were injected. The UV-spectrophotometer was used to determine the wavelength of maximum absorption of the benzoic acid prior to the analysis.

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

Sample Preparation - Five different brands of soft drinks (Coca-Cola, Pepsi, Sprite, Thumbs-Up and Mirinda) and 5 fruit juices (Orange,

Apple, Cranberry and Mixed Fruit Juices of Real Brand and Tropicana Orange Juice) were purchased from the open markets in three batches. No extraction was done, but the samples were filtered and centrifuged at 3000 rpm for 30 minutes and the supernatant taken. Samples were diluted prior to the analysis.

Results and Discussion

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

For the soft drink analysis, the concentration of benzoic acid detected in the samples ranged from not detected (nd) to 564.00 mg/L. Out of the 5 soft drink samples analysed only one sample had no levels of benzoic acid, 1 sample showed levels above the set standard for benzoic acid or benzoates in soft drinks (150 mg/L) with the rest within the range of zero to 150 mg/L. Thumbs-up had a level of Benzoic Acid of 152 mg/L.

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

abundance of natural fruits in the country. The potential daily intake of benzoic acid calculated are within the range of 0 – 5 mg/L body weight per day.5 samples of fruit juices were analysed for the levels of benzoic acid. The concentration of the samples ranged from not detected to 148 mg/L with a mean of 31.00 mg/L. One product had no level of benzoic acid. Most of the fruit juices that had labels which displayed as ***ó n o p r e s e r v a t i v e ô*** actually had some levels of benzoic acid in them.

Conclusion

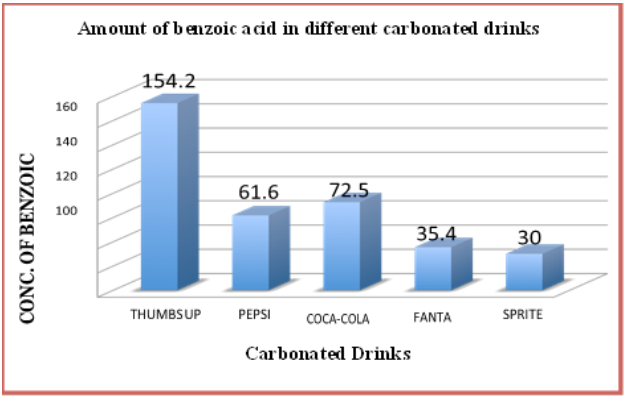
The analytical determination of benzoic acid as preservative is not only important for quality assurance purposes but also for consumer interest and protection. This work used a simple, fast HPLC method for the determination of benzoic acid in soft and fruit drinks samples in India. The results showed that the benzoic acid concentration varied between different kinds of soft drink samples, with some levels above the maximum values established by national and international legislation and authorities. Most of the products that declared ***ñ n o p r e s e r v a t i v e*** some of the fruit juice were not in accordance with their label claims. There was a great significant difference between the levels of benzoic acid in the soft drinks and fruit juice as carried out by the one-way analysis of variance at a significant level of 5% with the soft drinks having high levels than the fruit juice. This may be attributed to the fact that most of the fruit juice manufacturers pasteurized their products and therefore tend to add little of the benzoic acid. In conclusion, for producers whose products exceed the legislation limit, it is recommended that whenever possible there should be judicious use of additives and benzoic acid levels should be regulated and only used in the control of yeast and bacteria in food products. A regular monitoring and education should be organized for soft drinks and fruit juice manufacturers in India. This work has given us a first-hand information to the levels of benzoic acid in soft and fruit drinks on the Indian market and also the daily intake of benzoic acid by children and adults.

Estimated Daily Intake (Edi) Of Benzoic Acid

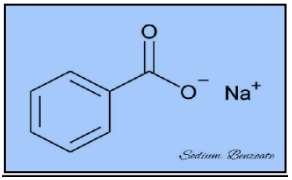
Category	Concentration [mg/L]	EDI for Adults [mg/L]	EDI for Children[mg/L]
Soft Drinks	70.48	0.19	1.14
Fruit Juice	31.00	0.00072	0.0043
		Total = 0.19072	Total = 1.1443

EFFECTS OF BENZOIC ACID

- The use of Benzoic acid as an antimicrobial agent has been observed to have adverse effects such as metabolic acidosis, hyperactivity in humans with high doses.
- The development to allergic reactions to benzoates such as asthma has also been reported in some studies.
- When benzoates are mixed with Vitamin C (Ascorbic Acid), they form benzene, a known carcinogen and DNA damage.
- Like most chemicals that have toxic properties, the effects of benzoic acid depend on its concentration. At the low concentrations found in plants and foods, the chemical isn't dangerous for most people. Once it's eaten or produced, benzoic acid is absorbed through the lining of the digestive tract and eventually converted into Hippuric acid, which is excreted in the urine.
- Benzoic acid can irritate the eyes, skin, lungs, and digestive tract. Most people aren't exposed to high enough concentrations to experience this irritation, but people who work with the pure chemical or with concentrated mixtures need to be careful. Benzoic acid can enter the body through the skin as well as the digestive and respiratory tracts.



Determination of preservative sodium benzoate in selected samples of beverages.



Introduction:

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

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

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

Determination of Sodium Benzoate in Mango Fruit Drink;

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

In a study conducted, Different brands of Mango soft drinks, available in the market were taken. These were FROOTI, MAAZA AND SLICE.

Benzoic and Sorbic acids were analyzed by high performance liquid chromatography (HPLC). Only one sample presented a preservative level above that permitted by the legislation that was SLICE. This study was conducted so that awareness could be created among consumers regarding safety of processed fruit juices and as far as the use of sodium benzoate is concerned.

Methods:

Quantitative research was undertaken to determine the amount of preservative sodium benzoate in selected samples of fruit juices. Various brands of fruit juices available in the market were taken for analysis. Fruit juice flavor included was mango whereas brands included were FROOTI, SLICE and MAAZA. Six samples were subjected to determination of sodium benzoate content as preservative in fruit juices. The samples were analyzed for sodium benzoate content twice. All of the samples of the drinks were procured from the market in the month of March for analysis of sodium benzoate content. Titrimetric method was used to determine amount of sodium benzoate as a preservative in samples of drinks as described in A.O.A.C (Association of Official Analytical Chemists) Manual. Sodium benzoate was calculated as: 1ml of 0.05 N NaOH = 0.0072 gm of anhydrous sodium benzoate. That is titrated value multiplied by 0.0072. Sodium Benzoate content is obtained in grams. To obtain sodium benzoate content in milligrams multiply it with 1000. The data collected was compiled and tabulated using computerized data base management programs. MS Excel was used for the interpretation and analysis of the data. Sodium benzoate values of the sample were compared with the standards i.e. 0.1% as set by International Standards of Health.

Results:

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

the table that mango juice of SLICE brand contained 0.102% sodium benzoate which slightly exceeded the standard value of 0.1% maximum during winter season due to non-availability of fresh fruit in the market. It was further observed that pack of the mango juice was labeled as "Preservative" without specifying its proper name. In mango juice of FROOTI and MAAZA brands both values were found to be within limits i.e. 0.092% in winter season and 0.083% in summer season. The label of mango juice FROOTI brand provided no information about any preservative. Determination of sodium benzoate in samples showed that sodium benzoate was extracted in very less amounts in the brands of MAAZA. On label of FROOTI brand of mango juice the preservative " sodium benzoate E211" was mentioned but it certainly did not contain sodium benzoate, whereas on label of mango juice of SLICE brand word "permitted preservative" was mentioned but sodium benzoate of mango juice showed the mango juice exceeded the standard value in winter season that is 0.10

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

Determination of Sodium Benzoate Preservative in Orange Juice;

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

The same analysis procedure was carried out as done for Mango Drinks and certain results were drawn. Sodium benzoate as a preservative was extracted to safe level in four samples of orange juice of above brands. Sodium benzoate was not extracted in the orange juice of RAW brand. The analysis of orange juice samples of REAL and TROPICANA

showed that sodium benzoate extraction varied from one season to another due to seasonal availability of fruit. It is evident from the table that sodium benzoate was extracted at higher amount in summer season and relatively at a lower amount in winter season due to non-availability of fruit in the market. The values found from the analysis conducted during winter and summer season lied within the limit as set by international standards of Health to 0.1% maximum. It was further observed that pack of the orange juice was labeled as "Preservative" without specifying its proper name in TROPICANA brand. In orange juice of REAL brand "All Pure No Preservative" was written but sample analyzed during winter and summer season indicate that sodium benzoate was extracted below 0.1% maximum. On label of PULPY ORANGE brand of orange juice the preservative "sodium benzoate E211" was mentioned. On label of orange juice of RAW brand "100% Pure No Preservative" was mentioned and it did not contain preservative

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

Discussion;

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

different method of detection (HPLC) was used in them while in current study the method of detection used was titrimetric.

Conclusion

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

Effects of Sodium Benzoate on Human Health:

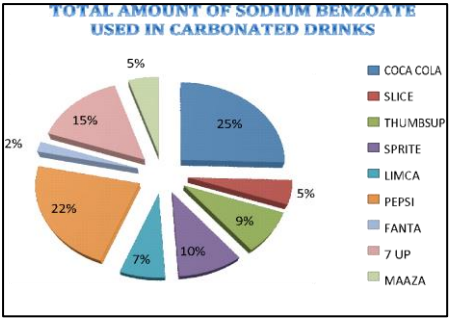
Studies suggest that acceptable amount of sodium benzoate is around 10-15g in a month. Consumption of sodium benzoate more than 12g has adverse effects on human health which include the following:

- Severe headache, fainting and blurred vision.
- Chest pains and slow heart rate.
- Asthma attacks and high blood pressure
- Causes damage to important parts of DNA.

Facts:

- Average intake of sodium benzoate due to juices is 13g.
- A person drinking only one glass of juice everyday consume 1.3g of sodium benzoate in just 3 days.
- Certain fruit juices contain around 1.5g/l of sodium benzoate rather than 1.3g/l.

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



Detection of Sodium Benzoate in different Carbonated drinks:

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

Estimation of Total Sugars in apple juice:

Sample Preparation:

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

Analysis:

Place 10 ml of distilled water, and 1 ml of the correspondingly diluted sample in the test vessel and stir to mix. Press the START button of the Reflectometer and at the same time immerse both reaction zones of the test strip in the measurement sample for approx. 2 s. Allow excess liquid to run off via the long edge of the strip onto an absorbent paper towel. Immediately insert the test strip into the strip adapter. After 10 minutes the strip is measured in the Reflectometer. The result [mg/l] is automatically stored.

Serial Measurements:

After the first measurement, any number of further measurements can be made by pressing the START button. This, however, requires the use of

a stopwatch, since the countdown function of the Reflectometer is available only for the first measurement. The detection zones of the test strip must be appropriately protected from light during the reaction time.

Calculation Scheme:

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

Results:

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

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

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

Contents of Total Sugars in other fruit Juices:

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

Conclusion

From the above tests, experiments and studies carried out, one thing is clear that every commercially sold fruit juices, and carbonated soft drinks do contain heavy quantities of numerous chemical preservatives

in them. Though these preservatives do increase the shelf- life of the beverages and are commercially feasible, health wise, excessive consumption of these beverages in harmful for human health and metabolism.

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

Future Aspects:

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

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SECTION III - BIOTECHNOLOGY

Chapter 6 - Production of Lactic Acid via Fermentation of Leaves of *Amaranthus* Species

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

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

Introduction

In this modern age, we highly rely on use of polymers in our day-to-day life, be it meagre packaging materials or exceptional auto ancillaries. Lactic acid is a widely used monomer for making organic polymers, also known as poly-lactic acid (PLA)1. It is an optically active organic acid with three carbon atoms. This hygroscopic compound is colourless in its

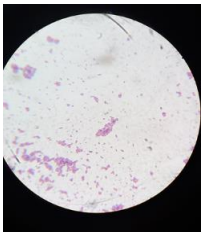
liquid state and is miscible in water and ethanol. The market for this organic acid is exponentially increasing with the growth of industrial sector. It is mainly used in food industries but also is making its way into pharmaceuticals, cosmeceuticals, healthcare and biodegradables2. The worldwide production on year 2005 was approximately 120000 tonnes per year3. Asia-Pacific region is projected to be the fastest growing market for the same. Naturally, this calls for better and cheaper techniques to meet the ever-growing requirements.

Lactic acid can be produced both naturally and synthetically. Chemical synthesis being a cruder method, mainly produces racemic lactic acid is eclipsed by the biological synthesis which offers the advantage of enantiopure products. This study proposes use of leaves of Amaranthus spp. as a substrate for lactic acid bacteria (LAB) to metabolise and produce lactic acid in the process. The amaranth primarily consists of wild, rapidly growing and spreading weeds. These weeds have been known to be a big problem for farmers and they eventually burn the entire growth to get rid of it. The study provides a commercial alternative to that.

Material and Methods - Isolation of LAB:

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

Streptococci



Lactobacilli

Hydrolysis of sugars in leaves:

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

The leaves were powdered, dried & mixed in 1% H_2SO_4. The mixture was then autoclaved for 40-60 minutes & then the centrifuged to remove leaf powder. The solution now contains hydrolysed simpler sugars.

Fermentation:

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

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

Extraction of lactic acid:

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

Due to presence of impurities from fermentation broth, the solution formed after acidification process was distilled through a mini distillation apparatus. A clear colourless solution was removed at 94^{o}C. A brackish black solution was left behind which showed to have

hygroscopic properties. Lactic acid is known to be hygroscopic in nature so it is suspected that the solution contains lactic acid.

Detection of lactic acid:

The lactic acid can be detected easily by using paper chromatography technique. The hygroscopic solution & lactic acid standard was spotted onto paper. The solvent system consisted of ethanol: liquor ammonia: water (80:4:16). The paper was dipped into solvent system and allow to run. After about 45 minutes, the chromatogram was developed with bromocresol purple.

Result

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

Discussion

The hydrolysis method used here was non-enzymatic hence inefficient at breaking down the cellulose into glucose[5]. It also consisted of larger quantities of pentose sugars which won't yield lactic acid when metabolized. However, in order to maintain simplicity of the procedure for practical application in farms, this procedure was used despite of its shortcomings. Another study focused on using rice straws as a substrate for fermentation due to its mass production in China[6]. It involved use of LAB community with capability of enhanced lactic acid production. It emphasized on quality of product over quantity since substrate was ample & cheap. This study tackles a similar problem pertaining weeds, however due to simplicity of the procedure, quality of product obtained is not as good, however quantity is not an issue. Provided more research is done in developing a more efficient and suitable consortia is done, the issue with the quality of product can be dealt with.

Conclusion

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



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Chapter 7 - Comparison of Culture Independent and Culture Dependent Techniques to Enumerate the Number of Microorganisms from Water Samples in Mumbai

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Abstract

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

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

Introduction

Microorganisms are extremely tiny but yet can be highly pathogenic, they are present all over the biosphere and are impossible to eradicate completely. Some organisms maybe also be present as the normal flora on and inside our body systems. Water is an integral part of a developing country like India, it is therefore essential to test the microbial load of the water, assess the nature of the microbes within it and correlate it to the type of diseases they cause. The vast majority of microbes are leeched into our water systems due to industrial and public activities; like

unchecked effluent dumping, non-compliance to effluent treatment and irresponsible citizen activities. Unchecked hospital and municipal wastewaters may contain pathogenic viruses, multiple drug resistant mutants, yeasts, protozoa, parasite eggs and a host of other deadly microorganisms.

This study assesses the microbial load of water samples collected from various sites in Mumbai which is important for the people who come in contact with these waters on a daily basis directly or indirectly on a sanitation standpoint. The use of simple techniques for both Culture Dependent and Culture independent techniques are advocated, for they serve as economical and yet reliable options for assessing the water quality. Culture Independent techniques especially are further elaborated and prioritized for the simple reason being that they are superior and accurate for organisms that fail to be cultured on laboratory-based media, such as VBNC (Viable but Non Culturable) bacteria.

Material and Methods

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

The techniques employed for this study involve two categories namely – Culture Dependent methods and Culture Independent methods. Firstly, the Culture Dependent methods used here are the Viable Count Methods which includes both Spread plate and Pour plate techniques. Secondly, the Culture Independent method used here is the complete DNA Extraction via the TRIZOL Method followed by Spectrophotometric analysis and comparison with standard data from research papers.

The Culture Dependent technique for Viable Counts involves –

(i) Spread plate technique – This technique involves serial dilution of the water sample in concentrations ranging 10-1 to 10-8. Followed by spreading a 0.1ml aliquot aseptically onto Nutrient Agar plates with a sterile glass spreader, until completely dry. Incubation of the plates at 37°C for 24h. Post-incubation, calculation of the CFU (Colony Forming Units)/ml is done. [1]

(ii) Pour plate technique – This technique also involves serial dilutions in a similar 10-1 to 10-8 fashion. Followed by the addition of 0.3ml aliquot into sterile molten Nutrient Agar butts, at a favorable temperature with caution. Next, these butts are poured into sterile glass petri plates and are allowed to solidify. Incubation succeeds this step at 37°C for 24h. Post-incubation, calculation of the CFU (Colony Forming Units)/ml is done and Results data is documented. [2]. Simultaneously, for the same given water sample being studied, Culture Independent techniques are also carried out.

The Culture Independent technique involves –

Millipore Filtration - of the water sample with Cellulose Nitrate Paper for a volume of 1litre. Carried out in sterile conditions aided by a vacuum pump and Millipore Filtration Assembly.

Transfer of the filter paper to eppendorf tubes – after cutting the filter paper in a 1cm x 1cm fragment. DNA extraction with the TRIZOL Reagent (1ml) – which contains phenol and guanidine isothiocynate as two of the most crucial chemicals for the lysis and extraction of the DNA. Also, 200µl chloroform (Centrifuge for 15mins at 12,000 rpm at 4°C) and 300µl absolute ethanol (Centrifuge for 5min at 3,000 rpm at 4°C). Pelleting and resuspending DNA (in DEPC water) [3] Spectrophotometric analysis of the DNA pelleted with DEPC Water (Diethyl pyrocarbonate) (serves as blank) at 260nm.

Enumerating the total number of cells present for the given sample using appropriate formulae and calculations. (As discussed further in the Results section). The above two techniques are carried out on the same

day for a given sample to avoid any discrepancies in and during result data evaluation.

Results

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

Formulae employed for Calculation - Culture Dependent techniques:

CFU/ml = ——————————————

Culture Independent techniques:

Conc of DNA = A260 x Dilution factor x 50

Where, 50 = A260 (1.0 for pure DNA)

No. of cells = $\frac{}{2.5 \quad 10^{-9}}$ [4]

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

Source of Origin	**Culture Dependent (CFU/ml)**	**Culture Independent (cells/ml)**
Chowpatty	3.60 x 108	8.50 x 109
Vasai	2.13 x 107	7.00 x 107
Powai	9.25 x 108	1.50 x 109

The graph provided below shows the relation between the Culture Dependent and Culture Independent techniques for the various water samples of natural origin. A higher count can be observed in Culture Independent readings owing to the fact that both viable and dead cells; culturable and non-culturable cells are accounted for here. The overall water quality can also be considered “poor” according to the U.S.

Environmental Protection Agency's (EPA) Standards. For Commercial Sources – the average number of microbial cells per ml are as follows:

Source of Origin (Tap water)	Culture Dependent (CFU/ml)	Culture Independent (cells/ml)
Households	9.70 x 104	1.05 x 106
Colleges	1.50 x 105	9.70 x 105

The graph provided below shows the relation between the Culture Dependent and Culture Independent techniques for the various water samples of commercial origin. These water samples are not as contaminated as the natural sources for they have been treated by the general municipal treatment facilities before being supplied to the houses and colleges. However, it surely will require sufficient in-house treatment using appropriate water filtration systems.

Discussion

Culture Dependent and Culture Independent techniques comparison has been demonstrated for water samples of natural and commercial origin collected from Mumbai. The Significance of this study – Unconventional culture-independent techniques have been brought into the limelight along with their comparison to culture dependent techniques. Culture dependent techniques have also been proven to be more efficient and quicker in terms of delivering quality results. They may or may not be economical in certain circumstances.

Some microbes cannot be cultured in laboratories, they can be enumerated by Culture Independent techniques. Eg: VBNC (Viable but Non Culturable bacteria) which are alive, but they do not grow or divide hence cannot be cultured. E.g. – *Vibrio* spp, *Listeria* spp, etc. [6] On a sanitation standpoint, a large number on individuals (children and workers) come in contact with these waters directly or even indirectly. It is important to assess the microbial load of the water samples for this reason. Large number of industries being close to the water bodies dump their semi- or un-treated wastes into the water bodies. These can both

harbor pathogenic microbes as well as destroy the natural marine flora and fauna.

Future Prospects include but are not limited to the following:

Widening the sample study-set by surveying other water bodies in and around Mumbai. Employing the use of other Culture Dependent methods. Employing the use of other Culture Independent techniques such as Direct Microscopic Count, Flow Cytometry, qPCR (real time quantification PCR), RT-PCR (Reverse Transcriptase PCR), FISH (Fluorescent *in Situ* Hybridization), and triplicate PCR reactions of partial 16S rDNA.[7][8]. Phenotyping the microorganisms enumerated and correlating it to the probable diseases they cause. Determining the level of antibiotic resistance of the microorganisms enumerated. Determining the sources of intrusion of the microorganisms into the water bodies.

Acknowledgement

I would like to conclude by extending my warm thanks to my mentors – Dr. Sejal Rathod and Ms. Amina Dholkawala for their undivided attention and constant support. Also, my parents for them being there for me during the ups and downs along the course of this research experiment.

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SECTION IV - MICROBIOLOGY

Chapter 8 - Isolation and Characterization of Lytic Coliphage and its Application as Biocontrol Agents in Food Industry

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Abstract

Bacteriophages are viruses that infect bacterial cells. Bacteriophages infecting *E. coli* are called coliphages. In this study we have isolated coliphage from sewage sample, using E. coli as the host. The presence of coliphage was checked by qualitative plaque method. After enrichment, the phages were isolated and enumerated by quantitative plaque method. The phage titre was found to be 7.1×1015 pfu/ml. The isolated coliphage was characterized by studying its host diversity, the effect of pH, temperature and cations by qualitative and quantitative plaque method. It showed narrow host range and could only infect E. coli and *E. coli* 113-3D. The coliphage remained stable at a wide pH (3–12) and temperature range (4–45°C). Studies were conducted to determine the effect of Ca+2, Mg+2, K+ cations (1mM) on coliphage propagation. In the presence of K+ ions, the phage showed the highest rate of infectivity. The objective of this study was to isolate and characterize a coliphage to effectively control food contamination due to *E.coli*. The coliphage was found to be effective in controlling *E.coli* contamination on meat products. This could be the first step towards exploiting the potential of coliphages as biocontrol agents against *E.coli*.

Keywords: Coliphage, Biocontrol agent, cations.

Introduction

Bacteriophages are viruses that infect bacteria (bacteriophage means bacteria eater) (12)(13). Bacteriophage, do not attack bacteria

indiscriminately; they usually attack only one specific host. Most phages are 24-200 nm in length. Every phage has a head structure, which can vary in size and shape. Some are icosahedral (20 sides), others are filamentous. The head (or capsid) is composed of many copies of one or more type of protein, and it contains the phage's genetic material (i.e. nucleic acid). The genetic material can be ssRNA, dsRNA, ssDNA, or dsDNA between 5 and 500-kilo base pairs (kbp) long in either a circular or linear arrangement (14)(15)(16).

Many, but not all phage have tails attached to the phage head, the tail is a hollow tube through which the nucleic acid passes during infection, and its size can vary considerably. In the more complex phages, like T4, the tail is surrounded by a contractile sheath which contracts during infection of the bacterium, at the end of the tail, some phages have a base plate and one or more tail fibres attached to it; these structures are involved in the attachment of the phage to the bacterial cell (17) to enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins or even flagella.

This specificity means that a bacteriophage can only enter bacteria that bear the certain types of receptors that they can bind to, and it is these portals of entry that determine the phage's 'host range'. Bacteriophage that infects *E. coli* sometimes are referred to coliphage generally, the bacteriophage is referred to simply as phage. As is true for all viruses, the phage can replicate only within host cells. In other words, coliphage can replicate only within *E.coli*.

Phage must attach to a receptor on the surface of a bacterial cell in order to initiate an infection. This interaction between the phage and receptor is very specific – a given phage type only will bind to a specific receptor molecule. Thus, all phage are not alike. The aim of the present work was characterization of E.coli phage isolated from sewage and studies its characteristics like host density, temperature, pH, and the effect of cations by qualitative and quantitative method. We also studied the use of coliphage as a biocontrol agent in reducing the load of E.coli on raw meat.

Material and Methods

Isolation of phage from sewage sample. Three samples were taken from different places in Mumbai. All three samples were subjected to enrichment. One gram of sample was mixed with 10ml phage buffer (10mM Tris, pH7.5, 10mM $MgCl_2$, 68mM NaCl, and 1ml $CaCl_2$) and centrifuged at 3000rpm for 10 min. The supernatant was syringe filtered (nylon membrane, 0.2µ). 10 ml of above filtrate was added to *E.coli* (host) grown in 100 ml nutrient broth & further incubated at 48hours. Aliquots of the enriched sample were taken, centrifuged at 3000 rpm for 10mins and the supernatant was syringe filtered. The filtrate obtained was tested for the presence of coliphage against *E.coli* by qualitative plaque assay. One of the samples showed a zone of clearance indicating the presence of coliphage.

Isolation of phages:

Isolated phages were obtained from the above sample using quantitative plaque method. One of the lytic plaques was picked up and was enriched by the previous procedure. Aliquots from incubated media were again centrifuged and syringe filtered. The filtrate was again tested by qualitative phage assay against *E.coli* to confirm the presence of coliphage. The phage filtrate was stored at 4°C, in the presence of chloroform and was then used for further characterization study.

Enumeration of phages

The above enriched coliphage sample was enumerated by quantitative plaque assay using dilutions 10^{-1} to 10^{-13} of which 10^{-11}, 10^{-12},10^{-13} were plated to determine phage titre.

Study of Host diversity

Isolated coliphage was enriched with the different bacterial host (*E.coli, E.coli 113-3D, Klebsiellae, S.typhi, S.paratyphi B*) for 48 hours and then checked for infectivity by qualitative plaque assay with the same host organism.

Effect of pH

A range of pH from pH2 to pH12 was adjusted in nutrient broth. 0.1ml of coliphage was added into the broth & incubated at 37℃. Aliquots were collected after every interval of 2 hours up to 8 hours and 24 hours. The aliquots were checked for the presence of coliphage by qualitative plaque assay.

Effect of Temperature

Sterile tubes with 10 ml of nutrient broth were inoculated with coliphage and incubated at various temperatures (4℃, RT, 37℃, 45℃, 80℃). Tested for the viability of coliphage by qualitative plaque assay at intervals of 2 hours up to 8 hours.

Qualitative and Quantitative effect of cations

Different types of cations i.e. 1mM of Ca^{+2}, Mg^{+2}, K^{+} each were added to sterile nutrient agar butts. Qualitative and quantitative plaque assay was performed to check the presence of phage with cations. For quantitative plaque method, the dilutions used were 10^{-5}, 10^{-6} and 10^{-7} and was compared with control (absence of cation).

Application of coliphage as bio control agent

3 meat pieces approx. (2cm x 2cm) were cut and used as a sample. In CONTROL 1 sample was inoculated with 10^{6} suspension of *E.coli*, which was used as a host control. CONTROL 2 was inoculated with 0.1ml isolated phage (10^{-4}), which was used as a phage control. The TEST was inoculated with 10^{6} suspension of *E.coli* + 0.1ml of isolated phage (10^{-4}). Samples were incubated at 37℃ for 6 hours. Further processing of samples was carried out by addition of 10ml sterile saline to the tubes followed by vigorous vortexing. (Phage control: chloroform was added to kill bacterial cells). All the 3 tubes were serially diluted and enumeration of host control and test was done by spread plate technique on sterile Mac Conkey's Agar plate. The phage control was enumerated by Quantitative Plaque assay technique.

Observations

Table 2: Determination of host range.

HOST	RESULTS
E. coli	+
E. coli 113-3D	+
S. typhi	-
S. paratyphi B	-
Klebsiella	-

Key:

'+' presence of plaque

'-'absence of plaque

Table 3: Effect of pH.

pH	TIME					
	0 hrs.	2hrs	4hrs	6hrs	8hrs	24 hrs.
2	+	-	-	-	-	-
3	+	+	-	-	-	-
4	+	+	+	+	+	+
5	+	+	+	+	+	+
6	+	+	+	+	+	+
7.4	+	+	+	+	+	+
9	+	+	+	+	+	+
10	+	+	+	+	+	+
11	+	+	+	+	+	+
12	+	+	+	+	+	+

Table 4: Effect of Temperature

TEMPERATURE	TIME				
	0hrs	2hrs	4hrs	6hrs	8hrs
4℃	+	+	+	+	+
RT	+	+	+	+	+
37℃	+	+	+	+	+
45℃	+	+	+	+	+
80℃	+	-	-	-	-

Table 5: Qualitative effect of Cations

CATIONS	RESULT
Ca+2	+
Mg+2	+
K+	+

Table 6: Quantitative effect of cations

CATIONS	PHAGE COUNT pfu/ml	DIFFERENCE IN PHAGE COUNT
Ca^{+2}	$6.35x10^{15}$	10.56% ↓
Mg^{+2}	$9.84x10^{15}$	27.84% ↑
K^{+}	$1.7x10^{16}$	58.23% ↑
Without ions	7.1×10^{15}	-

Table 7: Phage as bio control agent

SR.NO	TUBE	COUNT (0 HOURS)	COUNT (6 HOURS)
1.	Host control	10^6cfu/ml	1.6×10^{11} cfu/ml
2.	Phage control	10^3pfu/ml	3.1×10^6 pfu/ml
3.	Test	10^6cfu/ml (*E.coli*) + 10^3pfu/ml (Phage)	5.5×10^7cfu/ml (*E.coli*)

Results and Conclusion

Three sewage samples were enriched for the presence of coliphage out of which 1 showed the presence of coliphage. This sample was further subjected to quantitative plaque method and one clear plaque (phage) was picked up for further study. The phage was once again enriched and its lytic ability was detected by qualitative plaque method.

Enumeration of the isolated phage was performed by quantitative plaque assay technique using dilutions 10^{-1} to 10^{-13} of which 10^{-11}, 10^{-12} and 10^{-13} were plated to determine phage titre which was found to be 7.1×10^{15} pfu/ml. Characterization of the isolated coliphage was done by checking host range, the effect of pH, effect of temperature, qualitative and quantitative effect of cations on the isolated phage.

Qualitative plaque assay was performed with different bacterial hosts (E. coli, E.coli 113-3D, Klebsiellae, S.typhi, S.paratyphi B) to determine the host range of isolated coliphage. The isolated phage could infect only

E.coli and E.coli113-3D whereas it was unable to infect Klebsiellae, S.typhi and S.paratyphi B (refer table1). This indicates that the phage is highly host-specific and has a narrow range of infectivity.

The isolated phage was tested for its viability using qualitative plaque assay at pH ranging from pH2 to pH12. It was observed that the phage was viable from pH 4 to pH 12 up to 24hours and lost its viability at pH 2 and at pH3 after 2 hours. (table 2). Similarly, the isolated phage was tested for its viability using qualitative plaque assay at different temperatures. (4℃, RT, 37℃, 45℃, 80℃) The phage was viable at 4°C, RT, 37°C, 45°C up to 8 hours and lost its viability at 80°C (table 3)

Effect of different cations (Ca^{+2}, Mg^{+2}, K^{+}) on infectivity of isolated coliphage was tested with using Qualitative plaque assay technique. Plaques were obtained in presence of calcium, magnesium and potassium ions (table 4). Quantitative phage assay was performed in presence of (Ca^{+2}, Mg^{+2}, K^{+}) using dilutions 10^{-5}, 10^{-6} and 10^{-7}. The phage titre obtained in presence of Calcium, magnesium, and potassium was 6.35×10^{15} pfu/ml, 9.84×10^{15} pfu/ml and 1.7×10^{16} pfu/ml respectively. An increase in phage titer by 58.23% was observed in presence of potassium ions and calcium showed a decrease in phage titer by 10.56 %. (Refer table 5)

The count obtained in host control and phage control was 1.6×10^{11} cfu/ml and 3.1×10^{6} pfu/ml and test showed a count of 5.5×10^{7} cfu/ml. On comparing the host control and test a decrease in host count from 10^{11} to 10^{7}cfu/ml was recorded.

Discussion

The objective of this study was to isolate and enumerate coliphage against *E.coli*, its characterization, and application as a biocontrol agent in the food industry. Stagnant sewage outlet sample was found to be the best source for coliphage. Coliphage was isolated from one of the totals of 3 sewage samples tested.The phage titer was found to be 7.1×10^{15} pfu/ml.The coliphage has narrow range infecting only *E.coli* and *E.coli 113-3D*.

The phage was stable at a wide range of pH from pH 4 to pH 12. Phages are often quite sensitive to protein denaturation in an acidic environment, which may result in a loss of viability of the phage (Hazen, 2002). Maximum phage viability was seen between pH4 to pH12.Isolated coliphage was found to be stable between temperatures 4℃ to 45℃. Phage viability was lost at 80℃. Phage viability can be checked between 45℃ to 80℃ to check the thermal inactivation temperature. Loss of viability due to high temperature is due to disruption of head proteins and subsequent release of nucleic acid.

Isolated coliphage showed lytic activity in presence of Ca^{+2}, Mg^{+2}, K^{+} ions. However, maximum infectivity of coliphage was observed in presence of K^{+} ions i.e. 58.23% increase, whereas Ca^{+2} ions showed a decrease in phage titer by 10.56%. An increase in plaque forming units was observed in presence of K+ ions, maybe due to the enhancement of phage adsorption on host cells due to the presence of K^{+} ions.

Isolated coliphage was found to be an effective biocontrol agent with respect to raw meat as the *E.coli* count was decreased after phage treatment on infected meat. We also need to test whether the isolated coliphage is capable of lysing against food pathogens such as *E. coli O157:H7* using same techniques.Observing the structure of coliphage by transmission electron microscopy can be done. This will help us in identify the family of the phage.Also further genomic analysis needs to be done for the identification of isolated coliphage to know if the phage is a novel or known. Isolation of coliphages against other species of coliform group can be done in order to formulate coliphage spray to decrease the load of pathogenic coliforms in local butcher shops.

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Chapter 9 - Screening of Antimicrobial and Antioxidant properties of Plant extracts for the formulation of Cosmetic products

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Abstract

Plants are rich source of phytochemicals that have been used as the ingredients in traditional systems of medicines, nutraceuticals, herbal cosmetic and bioactive principles in pharmaceuticals. The chemicals used in skin care products are often very harsh on the skin and too abrasive for people with sensitive skin. Skincare product containing plant extract can nourish and help treat skin problems. The research aims to select a few commonly available plants as a source of Antimicrobial and Antioxidant agent for formulation of a cosmetic product. The plant sources selected are *Solanum tuberosum*, *Coriandrum sativum*, *Murraya koenigii*. Processing involved drying of the plant sources, followed by preparation of methanol and aqueous extract. These extracts were subjected to various Antimicrobial analysis by disc diffusion against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium diphtheriae* Minimum Inhibitory Concentration (MIC) to study the individual activity, while the Agar dilution was used to study the synergistic activity and Antioxidant analysis such as DPPH and FRAP. The *Solanum tuberosum* failed to show any antimicrobial activity MIC of *Coriandrum sativum* was observed to be 2% and for *Murraya koenigii* it was 0.5%. Good Anti-oxidant properties were observed for both the extracts and were used in preparation of an herbal skin cosmetic.

Keywords- Antioxidant, Antimicrobial, Plant extract, Skin

Introduction:

Cosmetic products are used to cleanse and enhance the external appearance of the skin. The key ingredients include: water, emulsifier,

preservative, humectant etc. ingredients of cosmetic products can be synthetic or natural in nature. [1]

Industrialization made availability and affordability to cosmetic products with synthetic components easy. Our ancestors trusted in the naturally derived components to boost and maintain good skin health. After learning the environmental and health issues associated with synthetic product, the belief in natural products is regenerated. The peels *Solanum tuberosum* contain vitamin B, Vitamin C, calcium and iron. [2] *Coriandrum sativum* leaves are loaded with

Vitamin C and antimicrobial compounds. *Murraya koenigii* contains calcium, vitamin A, vitamin B, vitamin C and is known to have antifungal and antibacterial properties. [3] Oxygen is essential to life, but as the body uses oxygen generate product known as Reactive Oxygen Species and common free radical. Radical called Reactive oxygen species (ROS) have important cell functions like cell signaling, ion transportation, apoptosis etc. However, excess of ROS attacks the nucleic acid, amino acid side chains, proteins etc. and cause DNA, RNA, protein and lipids resulting in increased risk of cancer, cardiovascular disease and other disease. [4] [5] Antioxidants counteract or neutralize the effect of free radical. Antimicrobial is an agent that kills the organism or stops the growth [6] and are required to decrease the burden of microbial disease.

Material and Methods

Selection and collection of plant materials: Three plant sources were chosen for evaluation of antimicrobial and antioxidant source such as *Solanum tuberosum* (potato peels), *Coriandrum sativum* (leaves), *Murraya koenigii* (curry leaves).

Preparation of Sample: The samples was washed, air dried followed by heating in the hot air oven at 60°C, then ground to obtain the powder.

Preparation of the Extract: Hot water, cold water, methanol and oil extracts were prepared. Methanol extract was obtained by continues hot percolation technique using Soxhlet apparatus. Hot water extract was

prepared by boiling the sample in distilled water. Cold water extract was prepared using sterile distilled water and kept in shaker for 8 hours. Oil extract was made similarly as hot water extract but instead distilled water is replaced by sterile paraffin oil.

Antimicrobial ability: Methanol extract obtain by extraction is dissolved in 50% DMSO and incorporated into molten Nutrient agar. Antimicrobial activity was studied in two ways individually and in combination. Individual activities were studied using methods like Disc diffusion, Agar well [(7)] and Agar dilution [(8)]. Combined activity was performed by Agar dilution and filter paper strip method. MIC was determined by Agar dilution using different concentration. 3 Gram positive skin pathogenic organisms namely *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Corynebacterium diphtheriae* was used in the analysis.

Antioxidant activity: The ability to scavenge free oxygen radical was determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) antioxidant assay and FRAP (Ferric Reducing Antioxidant Power) assay.

Formulation of a Cosmetic Products: Three cosmetic products were formulated namely face wash, Scrub and cream.

Cream[(3)]: An Oil-in-water (O/W) emulsion-based cream formulated. The formulation components for the preparation such as Stearic acid, Lanolin, Mineral oil were taken in porcelain dish and melted in boiling water bath. To that Potassium Sorbate (Preservative) and the oil mixture is slowly mixed with water. The oil extract of curry leaves and coriander leaves was added in equal quantity. When it is in the cream form essential oil is added.

Face Wash [(9)]: A cosmetic preparation was made using components such as Xanthum gum, Propylene glycerol, Sodium lauryl ether sulfate, Sodium Chloride. All these components are mixed making it a Soap Base and then water extracts of curry leaves and coriander leaves are mixed. When the facewash is ready then Preservative and Essential oil is added.

Scrub [9]: Scrub a semi-abrasive cosmetic lotion includes components such as Beeswax, Borax, Almond oil. The ingredients are mixed in proper proportion and water extract and powdered sample of curry leaves and coriander leaves was added. After the formulation is ready Preservative and Essential oil is added to the preparation.

Physical Analysis of Cosmetic Products:

These formulations were subjected to various analysis such as physical analysis which include color, odor, appearance, pH, washability, etc.

Results

Antimicrobial Activity - Individual analysis:

By disc diffusion method: The samples when analyzed for the antimicrobial activity, *Solanum tuberosum* peels were observed to inhibit *S. aureus* and *Corynebacterium diphtheriae*, but no inhibition was observed against *S. pyogenes*, whereas the other extracts of *Coriandrum sativum* and *Murraya koenigii* inhibited all the three test organisms.

Table. 1: Tabular representation of the antimicrobial ability of the plant extract in terms of zone size.

ORGANISM	**HOT (mm)**	**COLD (mm)**	**METHANOL (mm)**	**CONTROL (mm)**
Coriandrum sativum				
c.diphtheriae	10.25	11.5	10.5	0
S. aureus	9	11.75	9.75	0
S. pyogenes	8	11.25	10.75	0
Murraya koenigii				
C.diphtheriae	13	13.3	14	0
S. aureus	0	0	13.5	0
S. pyogenes	0	0	14	0
Solanum tuberosum				
C.diphtheriae	11.25	12.16	13.6	0
S. aureus	7.5	0	12.5	0
S. pyogenes	0	0	0	0

Fig. 1: Activity in terms of zone size, shown by *Coriandrum sativum* against *S. pyogenes* (left), *S. aurues* (center) *and C. diphtheriae* (right).

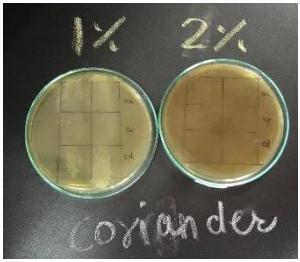
By agar dilution method: The MIC of *C. sativum* was found to be 2% and that of *M. koenigii* was found to be 0.5%.

Table 2: tabular representation of the antimicrobial ability by Agar dilution (mic determination)

ORG.	**CONCENTRATION**				
	0.1%	**0.5%**	**1%**	**2%**	**CONTROL**
Murraya koenigii					
S. aureus	+	-	-	-	-
S. pyogenes	+	-	-	-	-
C. diphtheriae	+	-	-	-	-
Coriandrum sativum					
S. aureus	+	+	+	-	-
S. pyogenes	+	+	+	-	-
C. diphtheriae	+	+	+	-	-

Key: (+) growth observed; (-) no growth observed

Fig. 2: Inhibition shown by *Coriandrum sativum* extract of 1% concentration (left) and 2% (right).



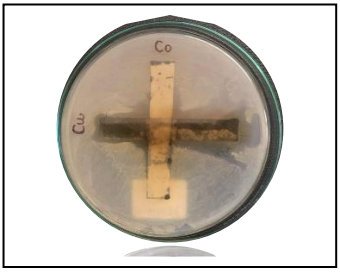
Combined analysis:

By filter paper strip method: The *M. koenigii* and *M. koenigii* showed synergistic activity.

Table 3: tabular representation of combined activity of *C. sativum* and *M. koenigii.*

Plant extract	**Size of zone of Inhibition**
C. sativum	12mm
M. koenigii	12mm
C. sativum + M. koenigii	26mm

Fig. 3: Combined Antimicrobial Activity shown by *Coriandrum sativum* and *Murraya koenigii* against *S. pyogenes* (right) and *S. aureus* (left).



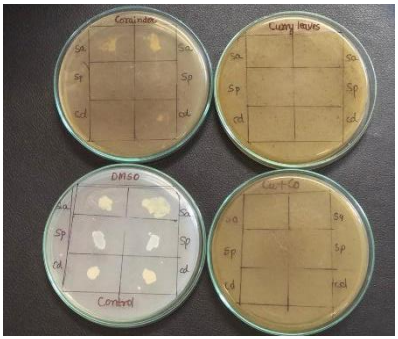
By Agar Dilution method: 0.25% of *C. sativum* and 0.25% of *M. koenigii* together where observed to inhibit *S. aureus, S. pyogenes, C. diphtheriae*.

Table 4: tabular representation of combined activity of *C. sativum* and *M. koenigii* by Agar Dilution method.

Organisms	**0.5% *C. sativum***	**0.5% *M. koenigii***	**0.25% *C. sativum* + *0.25% M. koenigii***
S. aureus	+	-	-
S. pyogenes	-	-	-
C. diphtheriae	+	-	-

Key: (+) growth observed; (-) no growth observed

Fig.4: Combined inhibitory Activity shown by *Coriandrum sativum* and *Murraya koenigii*, by Agar Dilution Method.

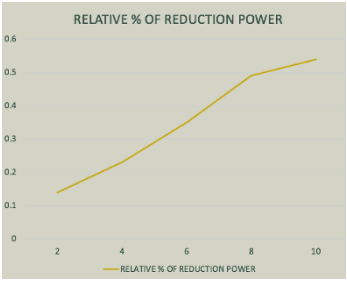


Antioxidant Activity - FRAP Assay:

Table 4: FRAP values of dyes in ascending order.

EXTRACT	O.D. (700nm)	ACTIVITY OBS.
M. koenigii	0.29	1000
C. sativum	0.40	1380

Fig 5: Graphical representation of FRAP values of Ascorbic Acid



Cosmetic Production

Evaluation of the formulated cosmetic products: the result of formulation of scrub is presented in Table 5. The overall evaluation report reveals an

ideal tropical formulation containing extracts of *Coriandrum sativum* and *Murraya koenigii.*

Table 5: evaluation of the formulated cosmetics

Parameter	**Observation for scrub**	**Observation for facewash**	**Observation for cream**
Appearance	Coarse semisolid	Homogeneous, smooth	Semisolid
Color	Dark green	Pale green	Cream color
Odor	Pleasant	Pleasant	Pleasant
pH	8	7.6	8
After feel	No residue left behind	No residue left behind	No residue left behind, no staining of skin
Washability	Easily washed off when placed under running water	Easily washed off when placed under running water	Easily washed off when placed under running water

Discussion

The plants extracted from natural sources are rich in phytochemicals and phenolic compound. Plants like *Coriander sativum* leaves, *Solanum tuberosum* (potato peel), *Murraya koeniggi* (curry leaves) are known to have good antimicrobial and antioxidants properties. They also have antioxidant, anti-fungal, antiseptic and disinfectant properties that help in clearing up your skin from many problems. They are rich in Vitamin C, Vitamin B, Vitamin A, Vitamin E, folate and beta carotene. It helps in maintaining smooth skin, prevent in from acne and also in getting rid of dead skin cells and makes the skin healthy. It also helps in cures cut, burns, and rashes

The antimicrobial activity of plant extracts were tested against *Staphylococcus aureus, Streptococci pyogenes, Corynebacterium diphtheria* using agar dilution, Agar well and Disc diffusion methods. The plant extracts showed inhibition to common pathogenic organisms like *Staphylococcus aureus, Streptococci pyogenes, Corynebacterium diphtheria* but *Solanum tuberosum* did not show appreciable

antimicrobial activity. The antioxidants activity was studied by FRAP assay using spectrophotometrically as that of *Coriander sativum* was 1380. The Other plant extracts also showed good antioxidants activity. Cosmetic products such as face wash, face cream, face scrub was found to be refreshing, soothing and glowing effect to the skin. Organic beauty products have

advantages over conventional cosmetics such as Naturally scented, environmentally friendly, Avoid irritants, no side effects. Good antioxidants activity was shown indicates that prevent it from oxidative damage of skin. The ability to inhibit the test organisms and prevent it from any skin disease and improves the skin glowing. Soothing and prevent from acne. Thus, use of natural plant extracts cosmetics over conventional cosmetics is of future benefit.

According to study by Hanan Al Harbi, Dr. Uma Irfan and Dr. Sarah Ali of European journal of pharmaceutical and medical research show that the antimicrobial activity is showing more inhibition of Staphylococcus aureus is show and our is showing less inhibition to methanol extract of curry leaves. According to study by Krutika Patel and Mita Vakilwala shows that the antimicrobial activity is showing more and our study is showing less zone if inhibition to methanol extract of *Coriander sativum*.

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Chapter 10 - Analysis of Biodegradation of Azo Dyes

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Abstract:

Azo dyes, very commonly found in various industrial effluents, have a deleterious effect on the environment. In the current study, antibiotic susceptibility test (AST) against 12 antibiotics was carried out for 11 potent strains of azo dye (crystal violet) degrading bacteria previously isolated from textile effluent using the Kirby Bauer's disc diffusion method. Two best dye degrading isolates were screened for plasmid by alkaline lysis followed by agarose gel electrophoresis, wherein one of the strains was found to possess a plasmid. Their AST results indicated that they were both resistant to beta-lactam antibiotics. Plasmid curing by sodium dodecyl sulphate (confirmed by replica plate technique) was followed by dye degradation assay and AST of the cured strain. Antibiotic susceptibility increased but dye degradation remained unaffected, indicating that the ability to decolorize the azo dye probably did not originate from the plasmid DNA. Furthermore, cell-free crude enzyme assays indicated the presence of an extracellular dye degrading enzyme complex with similar degradation rate as that of whole cells; a good prospect for commercial manufacturing of a cell-free dye degrading system. Moreover, immobilization of the enzyme could be an influential future prospect in large-scale bioremediation and treatment of industrial effluents.

Keywords: Azo dye, degradation, enzyme, plasmid

Introduction:

Synthetic dyes are extensively used in textile, paper, food, colour photography, paper printing, plastic, cosmetics, pharmaceutical; leather and toy industries [1, 22]. Azo dyes, considered to be the most prevalent group of synthetic dyes, are electron-deficient xenobiotic compounds due to the presence of azo (N=N) and sulfonic (SO3) electron-

withdrawing groups. Hence, azo dyes are prone to endure oxidative bacterial catabolism under aerobic environmental conditions [16].

Thousands of azo dyes are available in the commercial market, out of which, at least 500 have carcinogenic aromatic amines as part of their chemical structure [15]. After entering the human body through ingestion or skin contact, these compounds are metabolized to aromatic amines by azoreductases in the gastrointestinal tract and in the mammalian liver [4, 15]. Approximately 90% of 4,000 dyes analysed in a survey had LD50 values greater than 2000 mg/kg with highest toxicities found among basic and diazo groups of dyes [18] Azo dyes are considered to be rather persistent pollutants for the reason that they are not readily degraded under aerobic conditions [11, 21]. Under anaerobic conditions, azo dyes can be reduced by intestinal bacteria and various environmental microorganisms to colourless amines, which however, may be toxic, mutagenic, and carcinogenic to humans and animals [3,5].

The textile industry is one of the most chemically intensive industries and the major source of contamination of potable water. The direct discharge of large quantities of unused dyestuff having complex chemical properties into water bodies affects their ecological environment [12]. The treatment of textile wastewater is essential before discharging the wastewater into a receiving water body [13]. During the past two decades, several physicochemical decolorization techniques have been reported; however, few have been accepted by the textile industries [14]. Biological methods are generally considered environmentally friendly as they can lead to complete mineralization of organic pollutants at low cost. Moreover, there is a need to develop novel biological decolorization processes leading to more effective cleaning up of azo dyes using cell-free systems that are as efficient as whole cells.

Thus, this work aims to (I) screen for pathogenicity of azo dye degrading isolates, (II) study the potential of these isolates in azo dye degradation, (III) detection of plasmids associated with degradation. (IV) determination of dye degradation efficiency of cell-free extracts.

Material and Methods:

Azo dye decolorization assay: To study the decolorization efficiency of 11 strains previously isolated and characterized from a textile effluent [25], 0.1 mL of the culture (24 hours old culture of density 0.1 O.D. at 540nm) was inoculated into 3 different concentrations of Crystal Violet [16] (azo dye), viz. 50ppm, 75ppm and 100ppm, prepared in Luria Bertani (LB) broth. Uninoculated medium without the dye was used as control. 1 mL aliquots, withdrawn after 48 hours of incubation at 37°C, were centrifuged at 10,000 rpm for 15 minutes. The absorbance of the centrifuged, 1:10 diluted supernatant and the control was read at 540nm using a colorimeter. The decolorization efficiency (%) of different isolates was calculated as follows:

$$\% \text{ Decolorization} = (A_{blank} - A_{test} / A_{blank}) X\ 100$$

where, A_{blank} : Absorbance of blank; and A_{test}: Absorbance of test

Antibiotic Susceptibility Test (AST): All the 11 strains previously isolated from the textile effluent [25] were screened for their susceptibility towards 12 antibiotics belonging to different groups by Kirby Bauer's disc diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [8]. The 12 commercially available antimicrobial discs used, obtained from HI-Media, were from the beta lactam (Amikacin 30µg/ml, Cefepime 30µg/ml, Cefotaxime 30µg/ml, Aztreonam 30µg/ml, Imipenem 10µg/ml), carboxyfluoroquinoline (Ciproflaxin), polymyxin (Colistin 10µg/ml), nitrofuran (Nitrofuratoin 300µg/ml), sulfonamide (Trimethoprim 30µg/ml), polyketide (Tetracycline 30µg/ml), aminoglycoside (Streptomycin 10 µg/ml) and glycopeptide (Vancomycin 30µg/ml) groups. Post 24 hours of incubation at 37°C, the zone sizes were measured in mm and were interpreted on the basis of the standard CLSI chart for AST.

Plasmid Extraction: On the basis of their decolorization efficiency at 100ppm, 2 most potent dye degrading strains were selected and screened for plasmid using Alkaline Lysis Method [7]. This was done to detect whether the dye degradation gene was present on plasmid or was chromosomally mediated. 24-hour old cultures grown on LB Agar slants

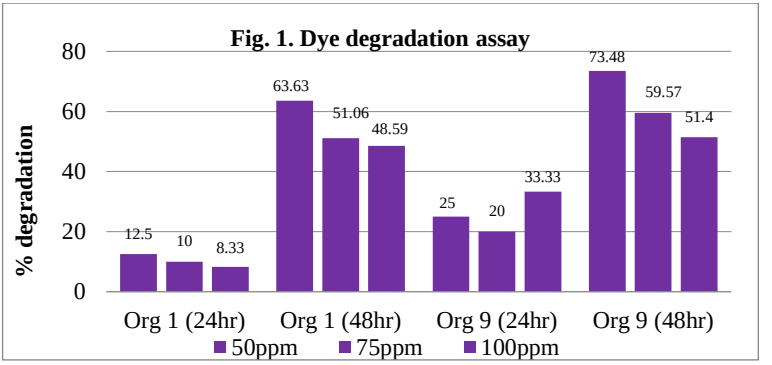
containing 300µg/mL of ampicillin were used. The plasmids were separated using 1% Agarose Gel Electrophoresis; visualization was done under a UV Trans-illuminator [2].

Plasmid Curing: Plasmid curing of the isolate was carried out using Sodium Dodecyl Sulphate (SDS) [6, 19] at its highest sub-lethal concentration i.e., 0.5%. Curing was confirmed using Replica Plate Technique on LB agar. Colonies that failed to grow on the replica plate (LB agar having 300µg/mL of Ampicillin) but exhibited growth on the master plate (LB agar without Ampicillin) were postulated to be cured of plasmid.

Preparation of Cell-Free Extract and Comparative Dye Degradation Assay: To study the dye degradation efficiency of the cell free extract, 1mL of 24-hour old culture (density 0.1 O.D. at 540nm) was inoculated in 100mL of LB Broth (having 50ppm of Crystal Violet) for 48 hours under shaker conditions. The broth was then divided into 2 parts of 50 ml each; one part was treated with 30 drops of Toluene (which would lyse the cells and release intracellular enzymes) [20] while the remainder was kept untreated. Both parts were centrifuged at 10,000 rpm for 15 minutes and the supernatants were used as cell-free extracts. These were used instead of whole cells for a comparative dye degradation assay.

Results:

Azo dye decolorization assay: The rate of degradation against different concentrations (50 ppm, 75 ppm, 100 ppm) of crystal violet by the 11 isolated organisms from textile effluents was estimated at 48 hrs. Isolate no. 1 and isolate no. 9 showed maximum degradation at a concentration of 100 ppm of crystal violet. Rate of degradation was calculated as % decolorization. (fig. 1.)



Antibiotic Susceptibility Test (AST): The susceptibility of the 11 isolates towards 12 different antibiotics was checked by the Kirby Bauer's method. The zone sizes were measured and interpreted by comparing with the CLSI Standard chart for AST. Isolates no. 1 and 9 exhibited resistances towards a wide range of antibiotics, especially those belonging to the β-lactam group.

Plasmid isolation, visualization & curing: Isolates no. 1 and 9 were screened for plasmid. Visualization was done using a UV trans-illuminator. Isolate 9 showed a super-coiled plasmid on the agarose gel medium. Isolate 1 did not show any plasmid. (refer fig. 2.)

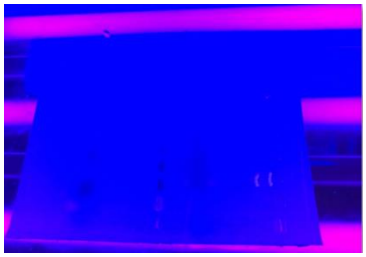
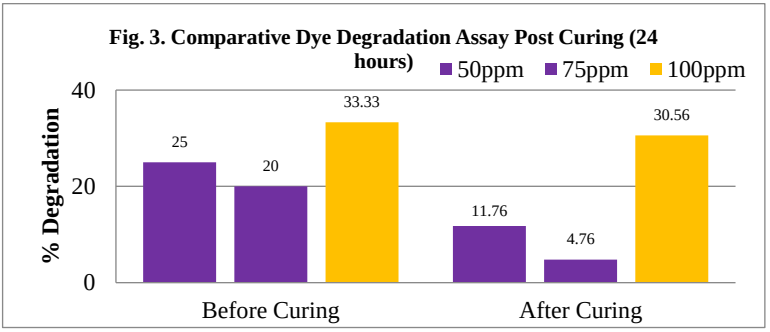


Fig. 2. Organism 9; Supercoiled Plasma

Degradation assays post-curing for isolate no. 9: Dye degradation rate checked for the cured colonies of isolate 9 showed no significant difference in the rate of decolorization. T test was carried out and the difference in dye degradation before and after curing was found to be

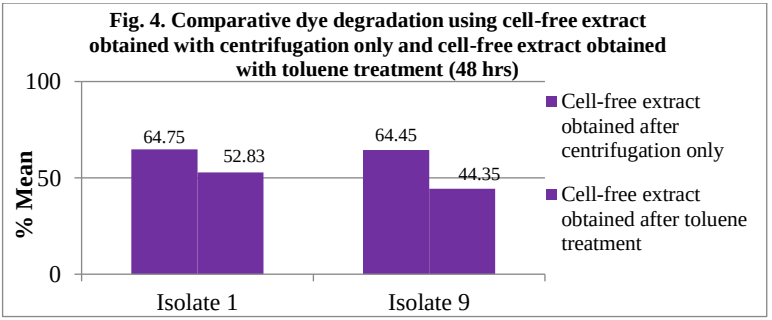
statistically insignificant; (paired t test, P >0.05). This indicates that the factor responsible for degradation was not plasmid mediated. (fig. 3.)



Antibiotic Susceptibility Testing for the plasmid cured isolate no.9: Susceptibility of the plasmid cured isolate 9 against 12 antibiotics was checked by Kirby-Bauer method and zone sizes were measured and interpreted by comparison with CLSI standard chart. The isolate no.9 exhibited susceptibility against all the antibiotics to which it was resistant. This indicated that the antibiotic resistance was plasmid mediated.

Comparative Dye Degradation Assay of cell free extract: The dye degradation assay carried out for the cell free extract of isolates 1 and 9 was carried out and the effect of toluene on the cells was studied. There was no significant difference in the rate of degradation in the cell-free extract post centrifugation; however, degradation rates for the cells treated with toluene decreased indicating a probable interference of toluene with enzyme activity (fig. 4.)

Statistical t test was carried out and the mean difference was found to be statistically significant (unpaired t test, $P < 0.05$). This suggests that the enzyme responsible for degradation could be extracellular.



Discussion:

In the current study, the cell-free crude extract (obtained from strain 9) exhibited a degradation rate of 73.06% for 100ppm crystal violet dye concentration in 48 hours. From a previous study by Lade et al., it was found that bacterial dye degraders could decolourize 12-22% of 100ppm concentration of azo dye in 48 hours, which is comparatively lower than the present study. Similarly, another study [23] with a Gram-Positive bacterium gave a maximum dye degradation rate of 40% in 96 hours, which too is lower than the degradation efficiency observed in the present study. In addition to this, it was also found that azo dye degradation (for strains 1 and 9) is mediated by extracellular enzymes, which is similar to a study [24]. Since industrial effluents usually contain a complex mixture of dyes, dye degradation efficiency of the cell-free crude extract for a combination of recalcitrant dyes needs to be analysed for their effective in situ bioremediation. Moreover, optimization and immobilization of the cell-free extract could be an influential future prospect in large-scale bioremediation and treatment of industrial effluents. Phytotoxicity studies of the azo dye degradation products can help determine the dye mineralization efficiency of the cell-free extract and whether the bioremediation process is generating any toxic end products. Lastly, strain improvement of the other nine isolates followed by strain stability studies could help generate safer, more stable and better choices for large scale manufacture of dye degrading enzyme(s).

Conclusion:

From 11 isolates, two best dye degrading isolates were found to be antibiotic resistant (β-lactam, polyketide and glycopeptide groups). On plasmid isolation, strain 9 was found to contain plasmid, whereas it was absent in strain 1. Upon curing of plasmid of strain 9, it was analyzed that the dye degradation was not plasmid mediated but antibiotic resistance was plasmid mediated. By the present study, it was found that the enzyme(s) responsible for degradation were extracellular. The degradation efficiency was retained with crude cell-free extracts when compared with whole cells; a good prospect for commercial manufacturing of a cell free dye degrading system.

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SECTION V – COMPUTER SCIENCE

Chapter 11 - Iot Based Smart Home Using Raspberry Pi

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Abstract

In this paper, our objective is to implement the Internet of Things Technology in making a smart home. Internet of Things (IoT) allows us to control devices through the internet. The Main propose is to use Raspberry Pi in order to control home appliances, thus automating modern homes via mobile or computer. Internet of Things (IoT) allows us to control devices through the internet. We will be using Raspberry Pi for controlling the devices and various sensors for data collection. The working can be monitored using a GUI (Graphical User Interface) would be used to monitor and control the elements. The Automation of the house is observed. The user was able to control various house elements, a smart home for smart people and the smart city is being achieved. This system saves time and manpower as it can be accessed and controlled by the authorized user from anywhere and makes our life a lot easier.

Keyword: Smart home, IoT, automation, Raspberry Pi, Sensors, Pi camera, GUI, wireless control

Introduction

IoT or Internet of things is an up-coming technology that allows us to control hardware devices through the internet. So here we propose to use IoT in order to control home appliances, thus automating modern homes via mobile or computer. It involves controlling the lighting, ventilation, fan, AC and security systems in-house as well some of the other home appliances. This is a step towards smart homes or smart house. Smart homes are a very board term and home automation is a step towards it. These ways save time as it can be accessed and controlled by

the authorized user from anywhere and make our life a little easier. To detect changes in the background using different sensors (light sensor, temperature sensor, motion sensor, gas sensor, moisture sensors etc.)

Methodology

In this, the automation is done by using Raspberry Pi (a packet sized single-board computer) In this there will be an application that will control all the system of the house we can switch on and off the devices(lights, fans, etc.) and other devices through it. We can power on and off a device from another location through remote control. The device (lights, fans, etc.) is connected to raspberry pi which can be controlled wirelessly. Whether you give that command by voice, remote control, tablet or smartphone, the home reacts.

The device is controlled via a Node-Mcu Module. NodeMCU is an open source IoT platform. It includes firmware which runs on the ESP8266 Wi-Fi SoC from Espressif Systems, and hardware which is based on the ESP-12 module.

The user sets up and Node Mcu that is and the server. The auth token helps the server to distinguish between different devices in a large network pool. When the command is given the Node Mcu receives the command and then works on the next part of the circuit with help of a Relay channel. Relays are switching that open and close circuits electromechanically or electronically. Relays control one electrical circuit by opening and closing contacts in another circuit.

Analysis and Codes: Auth Token

A security token (sometimes called an authentication token) is a small hardware device that the owner carries to authorize access to a network service. The device may be in the form of a smart card or may be embedded in a commonly used object such as a key fob.

- Python GUI (Graphical User Interface): Helps in controlling the system (Raspberry Pi)

- Google Assistant: Google Assistant is an artificial intelligence-powered virtual assistant developed by Google that is primarily available on mobile and smart home devices. The automation is purely based on voice command.
- Intruder Detection System: An intrusion detection system (IDS) is a device that monitors a smart house system for intruders.

Conclusion

There are many home automation companies that promise to automate your house, but they do charge a lot of money. Our aim is to automate the house with much cheaper products so that everyone will be able to afford this home automation system. Raspberry Pi is used in this version of the product. We also used a Node Mcu that replaces raspberry pi in network-based communication.

I have successfully built a cheap Smart home model which controls the full house and we have also used Google assistant as a medium to send updates to the relay channel to turn on and off the home lights. For future enhancement and improvising the use of product, we are using IFTTT. We will also using different other sensors.

Limitations

- The application doesn't support other voice assistants.
- The Scope of control is only limited to few devices.
- Few sensors have been only tested.

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Chapter 12 - Smart Mirror and Automation

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Abstract

In this paper, Smart Mirror displays date and time, weather forecast and reports, headlines with respect to the device location with power saving feature. Acrylic mirror sheet will be used for object reflection. The program will automatically detect the device location and displays various information based on its location accordingly. It can be accessed remotely by the user from an Android application. The remote application allows user to register and activate their product. Also, it can be used to turn off Smart Mirror and change location, country news headlines and weather. Along with that AR (Augmented Reality) technology has been integrated, application named as "Virtual Newspaper" that superimposes a media file on a user's view of the real world. To keep track on user's activity, an admin panel is there which displays the user's activity performed over Mirror or Application. It will display the registered customer details and their activity records. Administrator is only allowed to modify the customer details record. This system will reduce the human efforts, saves energy and time.

Keywords: Raspberry Pi, security module application, acrylic mirror sheet, android app, web application.

Introduction

A mirror is an object found in most people's homes. In mirrors we see our reflections. But what happens when you combine the idea of a mirror with technology? How smart a mirror could be? A Smart Mirror usually consists of a one-way mirror with a screen attached to it that displays a static user interface. The display can show the viewer different kinds of information in the form of widgets, such as weather, time, date, and news updates. This product would be useful for busy individuals that want to

multitask and stay informed while on the go. Instead of constantly pulling out a device, one could get informed while finishing daily grooming task. Lots of Smart Mirror includes various hardware components combined with different technologies. This version of Smart Mirror uses less and essential hardware components and displays maximum information which can be controlled by a remote application.

Methodology

Smart Mirror uses only two hardware components i.e. electronic display and raspberry pi. Raspberry Pi is a micro-controller It displays information from API hosted on AWS (Amazon Web Services) which assembles and provides data from other services. The information on Smart Mirror can be modify using a remote application namely 'Smart Controller'. The modification performed from Smart Controller reflects the database. An AR (Augmented Reality) program is also integrated in it which enhances the experience of reading newspaper. Smart Mirror uses polling technique to check the updated values in database. A Smart Admin Dashboard is also introduced in order to keep a track on user statistics.

The user sign-ups into the Smart Controller using unique product key displayed by the Smart Mirror. User interface gets displayed on the Smart Mirror which will show date, time, location, weather, last updated time of weather, weather description and news updates.

The user logins into Smart Controller which allows to modify data such as location, news type, country code, news category. It also allows to turn off the mirror. The second panel in Smart Controller displays a list of news updates with description and link to the source. It also includes button to start AR program named as 'Virtual Newspaper'.

The admin logins into the Smart Admin Dashboard to view user details and statistics of usage of device and application.

Analysis and Codes

Product Key - Generates a random and unique product key of 16 characters assigned to each product.

Python GUI (Graphical User Interface) - Gets the data from API and displays in form of widgets.

Login - The login form restricts unauthorized user from accessing controls.

Signup - The signup form registers the product key of the Smart Mirror.

Home - Home panel in Smart Controller includes Location, News Type (top-headlines or specific category), Country Code (country specific news updates) and Mirror Off button (turn off mirror).

News - News panel in Smart Controller includes list of top-headlines with description and links to the source website and AR (Virtual Newspaper) application.

Virtual Newspaper - Superimposes a streaming video relative to the images printed on newspaper.

Admin Dashboard - Requires Login and displays user details which can be modify and user activities.

Conclusion

This product has been made at cheaper price using only essential components required to build a Smart Mirror. An Acrylic Smoke Mirror Sheet is used for reflection rather than two-way thin Acrylic Mirror Sheet which has reduced the cost price. Raspberry Pi is used in this version of the product but it can be replaced with Arduino which will save 35% of the total cost price.

I have successfully built a cheap Smart Mirror which displays basic information without use of any external sensors or hardware component. Also, it ensures that no unauthenticated user can access the system. It is remotely accessible and uses less packet data. Also, Virtual Newspaper is developed using AR which is integrated to the remote application, enhances the way of reading newspaper by playing a video over the targeted image printed on newspaper.

For future enhancement and improvising the use of product, an Admin Dashboard is created to display all the user records and application statistics in tabular format.

Limitations

- The application doesn't support encryption methods while transmitting data to the API.
- The product cannot be turned on from remote application.
- Admin Dashboard doesn't display user details in confidential way.

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SECTION VI – STATISTICS

Chapter 13 - Comparison and Analysis of Bollywood Movies of Year 2008 and 2018.

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Abstract

This research project focuses on comparison and analysis of Bollywood (Hindi) movies of 2008 and 2018 inspired by #10yearchallenge.Which year had better and more diverse collection of movies? Are the ratings of 2008 better than 2018? How much has the Bollywood industry changed? What kind of Bollywood movies do people prefer to watch? Etc. Such type of questions will be answered from this research.

The aim of this research project is to know how much Bollywood has changed in past 10 years. Primary data was collected using Google forms to know the view of public on Bollywood movies. Secondary data was taken from IMDb sites and google.

A comparison of 2008 with 2018 Bollywood movies was done using statistical techniques like Welsch t-test, two-sample test for equality of proportions with continuity correction, regression and sentiment analysis. This project also includes interview analysis of people who are working in Bollywood Industry. The data shows that there has been changes in Bollywood movies produced in 2008 and 2018. Movies of 2018 are better as compared to movies of 2018 according to the analysis.

Keywords: Comparing, Change, 10 years, Bollywood, IMDb.

Introduction

#10yearchallenge was trending on social media recently. This project is inspired by this challenge, 'How Hard Did Aging Hit You' challenge was started on Facebook. It is believed that the first prominent user to partake in the challenge was Damon Lane, chief meteorologist for KOCO News 5 in Oklahoma. The post appeared on his Facebook page on January 11th, 2019. This challenge has several names: *2009 vs. 2019, How Did Age Hit You or 10-year challenge.*

Bollywood is a major entertainment industry. Most of the people in India watch Bollywood movies for entertainment. Bollywood is a word given by Indian Media, it refers to the Hindi language movie industry in India. The word is often used to describe Indian cinema as a whole, but more precisely it means Hindi language movies only. These movies are made with different concept and ideas, some movies are hit and some are flop at box office.

As the years have passed, people and their mind set has changed. Since India is a secular country with people from diverse backgrounds Bollywood has to produce movies which majority of people will like to watch. This task is very hard as no one exactly knows what kind of movies may entice people.

Methodology

- Google Forms
- Google analysis
- Visualization
- MS Excel:
- Sort and Filter
- Sorting
- Classification
- Cluster analysis
- Graphical Analysis
- T-test from Analysis Add-in

R-programming:

- Two sample tests for equality of proportions with continuity correction.
- Regression analysis
- Sentiment analysis - Using the following online resource we calculated the sentiment score:
- https://www.danielsoper.com/sentimentanalysis/default.aspx

Data Collection

- **Primary Data: Interview**
    - S. Ashwin (**Director**)
    - Omkar Shirgaoukar (**Script writer**)
    - Anand Naidu (**Producer**)
- **Questionnaire**
- Google Form https://docs.google.com/document/d/1UG2YlgQ4kQTh1kD7uUxylm7jWG3jysksswwm3Q9IfLo/edit?usp=sharing

Secondary Data - IMDb (Internet Movie Database)

IMDb offers a rating scale that allows users to rate films on a scale of one to ten. IMDb indicates that submitted ratings are filtered and weighted in various ways in order to produce a weighted mean that is displayed for each film, series, and so on.

Google Rating - It calculates an overall rating based on user ratings and a variety of other signals to ensure that the overall score best reflects the quality of the establishment.

Data Analysis

1) Welsch T - Test: Two-Sample Assuming Unequal Variances

Using Analysis Add-in of MS-Excel we obtained the following result:

(Gupta V, 2002) (Lu, 2010)

For AVG RUNTIME of 2008 and 2018

μ1 = mean runtime of 2008.

μ2 = mean runtime of 2018.

H_0: μ1=μ2 (there is no significant difference)

H_1: μ1 ≠ μ2 (there is significant difference)

H_2: μ1>μ2

p value (two tail) = **0.000183258**

p value (one tail) = **9.16289E-05**

Reject Ho if p value< 0.05

Conclusion: Average run time of 2018 movies is less as compared to average runtime of 2008 movies.

For IMDb ratings of 2008 and 2018

μ1 = mean of IMDb ratings of 2008

μ2 = mean of IMDb ratings of 2018

H_0: μ1=μ2 (there is no significant difference)

H_1: μ1 ≠ μ2 (there is significant difference)

H_2: μ1<μ2

p value (two tail) = **0.00309192**

p value (one tail) = **0.00154596**

Reject Ho if p value< 0.05

Conclusion: IMDb ratings of 2018 movies is more as compared to IMDb ratings of 2008 movies.

For GOOGLE ratings of 2008 and 2018

μ1 = mean of GOOGLE ratings of 2008

μ2 = mean of GOOGLE ratings of 2018

H_0: μ1=μ2 (there is no significant difference)

H_1: μ1 ≠ μ2 (there is significant difference)

H_2: μ1<μ2

p value (two tail) = **0.011076615**

p value (one tail) = **0.005538308**

Reject Ho if p value< 0.05

Conclusion: GOOGLE ratings of 2018 movies is more as compared to GOOGLE ratings of 2008 movies.

For GOOGLE ratings and IMDb ratings of 2008 Movies.

μ1 = mean of google ratings

μ2 = mean of IMDb ratings.

H_0: μ1=μ2 (there is no significant difference)

H_1: μ1 ≠ μ2 (there is significant difference)

H_2: μ1>μ2

p value (two tail) = **7.2773E-21**

p value (one tail) = **3.63866E-21**

Reject Ho if p value< 0.05

Conclusion: GOOGLE Ratings are more than IMDb ratings for 2008 movies.

For GOOGLE ratings and IMDb ratings of 2018 Movies.

μ1 = mean of google ratings

μ2 = mean of IMDb ratings.

H_0: μ1=μ2 (there is no significant difference)

H_1: $\mu 1 \neq \mu 2$ (there is significant difference)

H_2: $\mu 1 > \mu 2$

p value (two tail) = **1.27243E-26**

p value (one tail) = **6.36215E-27**

Reject Ho if p value< 0.05

Conclusion: GOOGLE Ratings are more than IMDb ratings for 2008 Movies.

2) Two-sample test for Equality of Proportions with Continuity Correction (Gupta v., 2002)

To compare the genres of movies released in 2008 and 2018.

TWO TAIL TEST

P1= Proportion of a particular genre in 2008 movies.

P2=Proportion of a particular genre in 2018 movies.

H0: P1 = P2 (The proportion of movies for a particular genre is equal in 2008 as well as in 2018)

H1: P1 ≠ P2 (The proportion of movies for a particular genre is not equal in proportion in 2008 as well as in 2018).

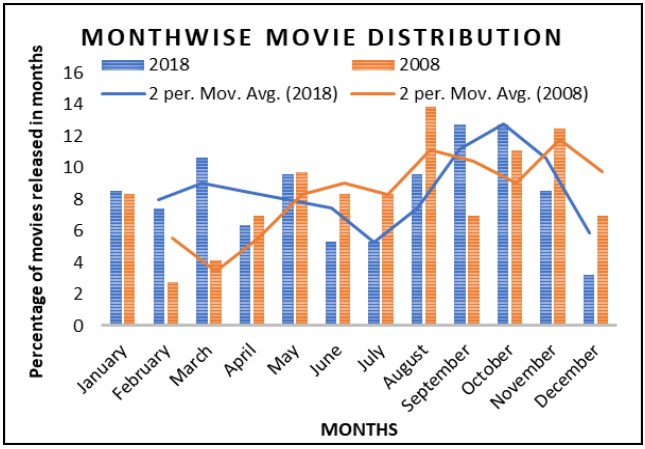
Conclusion: There is no significant difference in the proportion of above given genre in 2008 and 2018 respectively, except for the genre of Romance and others

Conclusion: More romantic movies were released in 2008 as compared to 2018 and others i.e. Psychological, Social, Political, Historical etc. movies were more in 2018.

Proportionality Test (For Two Tailed)				**Decision criteria Reject H_0 when p value <0.05**
Genre	**prop 1**	**prop 2**	**p-value**	**Conclusion**
Comedy	0.221374	0.213333	0.9856	**Do not reject H_0**
Horror	0.229008	0.12	0.4523	**Do not reject H_0**
Biopic	0.007634	0.026667	1	**Do not reject H_0**
Thriller	0.053435	0.08	0.4385	**Do not reject H_0**
Drama	0.045802	0.006667	1	**Do not reject H_0**
Adventure	0.038168	0.02	0.08614	**Do not reject H_0**
Crime	0.022901	0.113333	0.5796	**Do not reject H_0**
Romance	0.229008	0.12	0.02362	**Reject H_0**
Others	0.022901	0.113333	0.006756	**Reject H_0**

One tailed test		**Hypothesis**	**Decision Criteria Reject H_0 if p value < 0.05**
Genre	**p-value**	H_0:P1=P2	Conclusion
Romance	0.02362	H_1: P1>P2	Reject H_0
Others	0.9966	H_1: P1<P2	Reject H_0

3) Visual Analysis of month wise movie distribution.



Conclusion: 2018 - Maximum number of movie released in month of September and October. Minimum number of movies released in the month of December. 2008. Maximum number of movie released in month of August. Minimum number of movies released in the month of February

SENTIMENT ANALYSIS (Sentiment Analyzer n.d.)

Q) What you see and hear affects your manners and how you react. Is that true in case of movies?

Interpretation: This text has a sentiment score of -67.5. This means that the overall sentiment or tone of This text is quite negative / serious.

Conclusion: Mannerism is not affected by what we hear and see in movies.

Q) What will be the scenario of Bollywood after 10 years?

Interpretation: This text has a sentiment score of -26.2. This means that the overall sentiment or tone of this text is somewhat negative / serious.

Conclusion: Scenario of Bollywood will not be good in coming years. Most preferred movies of Bollywood Favourite Artists of Bollywood movies.

	Movie 2008	**Movie 2018**
IMDb	A Wednesday	Andhadhun
Survey	A Wednesday	Andhadhun
Interview	A Wednesday	Newton

	Actor	**Actress**	**Director**
IMDb	Amitabh Bacchan	Deepika Padukone	Rajkumar Hirani
Survey	Ranveer Singh	Deepika Padukone	Rohit Shetty
Interview	Shahrukh Khan	_	Imtiaz Ali

Analysis - Secondary Data (Google and IMDB Ratings)

IMDb 2018 rating are better than IMDb 2008. Google 2018 rating are better than 2008. Avg run time of 2018 movies is less as compared to average runtime of 2008 movies. Google has comparatively rated more than IMDb ratings in 2008 as well as in 2018. In 2008 and 2018 the proportion of genres (movies) are similar for comedy, action, drama, etc. except for the genre of romance are less and movies with new concept are more in 2018.

Analysis Based on Questionnaire (Made On Google Forms)

Comedy is most preferred genre in Bollywood movies with 69% which implies that the box office collection of these movies will be high and the chances of comedy movies going flop are less.29.1% respondents like watching movies in evening that means theatres will be more filled during evening time and least preferred is night (16.4%). Rarely respondents go to watch movies in theatre (42.7%). Movies do affect mentality of respondents\but not much according to 29% respondents who rated 7/10.

60% respondents prefer to watch movies with friends. Movies do have a strong impact on people according to 58% respondents.54% of respondents voted saying that the censor board should not have rights to decide what the respondents want to see in movies.75.5% of respondents do not want to enter Bollywood. 40% of respondents think that movies collect good money because of storyline. 72.7% of respondents feels that their community is represented in Bollywood movies.

61.8% of respondents prefer to watch Bollywood movies in theatre. 23.6% of respondents have rated 7/10 for changes observed in mentality of people because of movies. 64.5 % of respondents think there is improvement in selection of movies since 2008. 42.7% of respondents prefer to watch Bollywood movies on Netflix. 86.4% respondents said that more movies should be made on social causes. 20% respondents rated Bollywood 6/10.

Interview Analysis - S.Ashwin (Asst. Dir. of Pink)

Favourite movies are Newton, Lunchbox and Masaan. Favourite actor is Shahrukh Khan. Perception building is 20% from Bollywood movies. Preferred genre romcom.

Omkar Shirgaoukar Script Writer (for Dene Mai Kya Harz Hai)

Favourite movie: Dil Chahta Hai. In his opinion masala movies work more on screen. Movies are more preferable than web series. His preferred genre is reality depicting human emotions.

Anand Naidu (Production)

According to him storyline is the most important factor for a movie's success. 2018 movies are going in wrong direction. Most preferred genre is action.

Conclusion

People's perception and preference towards Bollywood has improved. People have rated more on Google than IMDB for movie ratings. Major change what our generation wants is that more social movies should be made. Storyline is the most important factor for a movie's success. Most preferred genre in the coming years will be biopic and comedy are the most watched genre of movie with drama being constant in 2008 and 2018. Mostly people check reviews before going for a movie. Box office collection of movies mostly depend on the trailer and review. People like Bollywood movies which has good storyline. Mannerism is not affected by what we hear and see in movies. Scenario of Bollywood will not be good in coming years.

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SECTION VII - PSYCHOLOGY

Chapter 14 - A Study on Differing Effects of Phases of Menstrual Cycle on Mindfulness And Mood

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Abstract

The rise and fall of the level of the hormones throughout the 3 phases of menstrual cycle [i.e., pre-menstrual phase, menstrual phase, and ovulation phase] cause mood swings and emotional upheavals for women of childbearing age. This is what present research purpose to measure i.e., how does mood and level of mindfulness differ in the 3 phases of menstrual cycle.

The sample consisted of 30 participants in the age group of 19 to 21years, having a regular menstrual cycle. The Mindfulness scale – Day-to-Day experiences -MAAS Scale (Brown KW & Ryan R.M, 2003) to measure mindfulness and The Mood Scale – Patient Health Questionnaire (PHQ) – (Pfizer Inc, 1990) to measure mood in terms of Depression and Functionality was administered to the same participant during the 3 phases of menstrual cycle.

The mean score for mood scale on the factor of depression were 7.733, 8.3 and 6.733 and for second factor i.e., functionality was 1.766, 1.7 and 1.766 in the 1st, 2nd and 3rd phase respectively; the mean score for mindfulness were 4.093, 4.157 and 4.326 for the 1st, 2nd and 3rd phase respectively. Thus, the highest mean for mindfulness was in third phase, the mean for depression was highest in the second phase and the mean for functionality was the same for first and third phase.

A one-way ANOVA repeated measures design was calculated for mood swing score for depression and for functionality and also score of mindfulness comparing the 3 stages of menstrual cycle. The F values were 26.12, 34.69 and 36.15 respectively. All F values were found to be

significant at .01 level of significance. Therefore, to conclude the phases of menstrual cycle do impact mindfulness and mood.

Key words: Menstruation, Mindfulness, Mood.

INTRODUCTION

"I begin feeling like everything in my life is wrong and that I am leading the wrong life" says Sheets, a marketing director.

Sheets is just one of the many women who experiences premenstrual syndrome which is a collection of unpleasant symptoms such as anxiety and bloating that typically occur one or two weeks before menstruation and might influence behaviour throughout the menstrual cycle. An estimated 85% of women experience at least one symptom of PMS- Pre-Menstrual Syndrome per month, according to The American College of Obstetricians and Gynaecologists.

Though only a few hormones are known to fluctuate throughout the month, there are corresponding fluctuations in other hormones that affect emotions, processes of thought and physical well-being throughout the menstrual cycle. The various stages in menstrual cycle and their hormonal fluctuations can be described as follows-

The Follicular Phase: This phase starts on the 1st day of menstruating and lasts for 10 to 14 days. During this time the hormone estradiol begins to rise. Follicle stimulating hormone is secreted, stimulating the production of follicles in the ovaries that contain eggs. This is more likely to be "happy" time of the month. This has a biological basis as the good feelings may be because of a more sensitive brain. The Estradiol hormone rising in the body helps to reduce the effects of the stress hormone adrenaline and cortisol that could also help in preserving the happy mood.

The Ovulatory Phase: It is the midpoint of the menstrual cycle. In this phase the following events occur i.e., the rise in estrogen from the follicle triggers a surge in the luteinizing hormone produced by the brain causing

the release of egg in fallopian tube. This hormone increases and enhances the libido.

The Luteal Phase: During this phase the progesterone hormone increases and enhances the amount of cortical hormone in the body which is high in people experiencing stress. Thus, women experiences moodiness and irritability during this stage.

Moods: A mood is an emotional state. In contrast to emotions, feelings and affects, moods are less specific, less intense and less likely to be provoked by a particular stimulus or event. Moods are typically described as having either a positive or negative valence. Positive mood can affect one's mind in a good way as it aids individuals in situations which involve heavy thinking and brainstorming. Negative moods can affect an individual's judgement and perception of objects and events. Negative moods have been connected with depression, anxiety, aggression, poor self-esteem, physiological stress and decrease in sexual arousal. Since the various stages of menstrual cycle experience hormonal fluctuations, they impact the mood of women and in turn their behaviour in drastic ways.

Mindfulness: It is the psychological process of bringing one's attention to experiences occurring in the present moment. People who ruminate and worry contribute to mental illnesses such as depression and anxiety, thus to reduce the same, mindfulness-based interventions are effective. If people are emotionally upset or in a negative mood mindfulness reduces. Mindfulness has also been shown to influence attitudes about menstruation and premenstrual symptom severity.

Thus, mindfulness is- Letting go of taking things for granted. Mindfulness means to return to the present moment. Mindfulness is the self-regulation of attention with an attitude of curiosity, openness and acceptance.

Mindfulness has become integrated in cognitive and behavioural approaches such as Dialectical behaviour therapy, Acceptance and commitment therapy and mindfulness based cognitive therapy. Positive

psychology movements have also focused on mindfulness to focus on growth and optimal functioning.

LITERATURE REVIEW

Several studies have investigated the impact of hormonal fluctuations during the various phases of menstrual cycle on depression, anxiety and mood of women.

A review of 47 studies that followed women's mood across the menstrual cycle (Romans S, 1990) found that in 15% of the studies women experienced classic Pre-Menstrual Syndrome (PMS) mood that worsened as the menstrual period approached and lifted when menstruation occurred however 38% of the studies found no association between mood and any particular phase of the cycle, 9% found that the worst moods actually occurred outside of the pre-menstrual phase.

In another research results indicated that women who were ovulating performed better on the verbal memory task. On the other hand, women tested in their pre-menstrual phase were better at solving spatial navigating tasks. That proves women tend to use different strategies to solve tasks such as navigating a maze or remembering a list of words depending on where they are in the menstrual cycle.

Essentially the study shows that the hormonal changes women experience throughout their cycle have a broader impact than previously believed and have significant effects on how women approach and solve problems. 90% of all women of reproductive age report negative, physical, psychological or relational symptoms in the days leading up to menses. (Campagne and Campagne, 2007). These symptoms range from mild and unobtrusive to absolutely debilitating (Kahn and Halbrich, 2003; Ross and Stenier, 2003).

Pre-Menstrual Syndrome (PMS)/PMDD is significantly related to numerous health risks and health behaviours (Lustyk, Gerrish, Douglas, Bowen and Marlatt, under review). The cyclical pattern of these symptoms results in unique quality of impairment across multiple domains (Lustyk, and Gerrish, 2008). Multiple regression analysis traces

the effects of body time as indexed by the female menstrual cycle upon mood. Positive mood peaked in the ovulatory phase and on weekends, while negative mood peaked in the luteal phase of the menstrual cycle (Rossi, 1977). Research examined differences in sleep patterns in relation to the menstrual cycle. No difference in percentage of REM sleep were found (Lee et al, 1990).

METHODOLOGY

Rationale: The rise and fall of hormonal levels throughout the three phases of menstrual cycle cause mood changes and emotional upheavals which impact women in all spheres of their lives.

Therefore, the present study was undertaken to study the differential impact of phases of menstrual cycle on two important aspects of their lives i.e., mindfulness and mood.

Problem: To investigate whether there is a differential impact of the three phases of menstrual cycle on mindfulness and mood.

Null Hypothesis: There will be no differential impact of three phases of menstrual cycle on mindfulness and mood.

Independent Variable: The phases of menstrual cycle (Phase 1: Follicular Phase, Phase 2: Ovulatory Phase and Phase 3: Luteal Phase).

Dependent Variable: The level of Mindfulness and Mood.

Control Variable:

Women having regular menstrual cycle.

Survey was taken in the months of May and June for all participants.

Sample: A total of 30 participants.

Tool: Electronic Google Questionnaire was used.

Mindfulness Scale- Day-to-Day Experience - MAAS Scale (Brown KW & Ryan R.M, 2003).

Mood Scale- Patient Health Questionnaire (PHQ) – Pfizer Inc, 1990.

Data Analysis: A one-way ANOVA repeated measures design was calculated.

RESULTS AND DISCUSSION

The study was conducted on 30 participants in the age group of 19 to 21 years. The Mindfulness scale – Day-to-Day experiences -MAAS Scale (Brown KW & Ryan R.M, 2003) was administered on the participants in the each of the three phases of the menstrual cycle and the mean scores were 4.09, 4.15, and 4.32 respectively.

The Mood scale - Patient Health Questionnaire (PHQ) – (Pfizer Inc, 1990) generated two scores- Depression and Functionality. The mean scores for depression were 7.73, 8.3, and 6.73 for the three phases respectively and the mean scores for functionality were 1.76, 1.7 and 1.76 respectively for the three phases of menstrual cycle.

Thus, the mean score for mindfulness was the highest for the third phase i.e., 4.32. The mean score for depression was highest for second phase and mean score for functionality was the same for first and second phase. Thus, at an apparent level participants were high on depression and low on functionality in the second phase as indicated by their scores and high on mindfulness in third phase.

Table 1: Mean of entire group for mindfulness and mood factors of depression and functionality.

	Phase 1	**Phase 2**	**Phase 3**
Mindfulness	4.093	4.159	4.326
Depression	7.733	8.300	6.733
Functionality	1.766	1.700	1.766

In order to find out whether there was a significant difference in the mean score of Mindfulness and two factors of Mood i.e., depression and functionality an ANOVA was calculated for each of them separately.

Table 2: Indicating the F value for phases of menstrual cycle and mindfulness. *p<.01

	SS	df	MS	F
Between groups	4694.215	2	2347.107	**39.15***
Within groups	1618.595	27	59.94	
Total	6312.81	29		

Table 3: Indicating the F value for phases of menstrual cycle and depression factor of mood. *p<.01

	SS	df	MS	F
Between groups	15373.7	2	7687.85	**26.12***
Within groups	7945.07	27	294.26	
Total	23320.77	29		

Table 4: Indicating the F value for phases of menstrual cycle and functionality factor of mood. *p<.01

	SS	df	MS	F
Between groups	812.56	2	406.28	**34.69***
Within groups	316.2	27	11.71	
Total	1128.76	29		

The F value for mindfulness and the two factors of mood i.e., depression and functionality were found to be 39.15, 26.12 and 34.69 respectively. All the three F values were found to be significant at .01 level of significance. This indicates that the various phases of menstrual cycle impacts depression and functionality in different ways.

These results are somewhat in line with research conducted by Sanders et al, 1983 where self-ratings of "well-being" reached their maximum in the late follicular phase declining throughout the luteal half of the cycle. Also, physical distress increased during the second half of the cycle to reach a maximum in the late luteal phase. A clear temporal relationship was therefore demonstrated between mood, physical state and hormonal phases of the cycle.

In research by Endicott, J (1993) it was indicated that at least for a subset of women with mood disorders the premenstrual phase of the cycle was a period of increased vulnerability for the appearance of a period of severe depression or worsening of an on-going period of depression.

In another longitudinal study by Moob et al (1969) on fifteen married women changes in menstrual cycle and its impact on mood, sexual arousal was measured. Results indicated cyclic changes in mood, sexual arousal in relation to the menstrual cycle.

Another research which could be cited in support of result of current research is the research conducted by Lane and Francis (2003) where relationships between premenstrual symptomatology, locus of control, anxiety and depression in women with normal menstrual cycle was investigated. Both overall and specific subtypes of premenstrual phases were found to correlate with external locus of control, anxiety and depression.

A study on 203 women nurses mood responsiveness on work days and off days during various phases of the menstrual cycle indicated differences depending upon hormonal levels throughout the three phases. A study conducted on thirteen healthy girls' functional performance it was found that phases of menstrual cycle impacted scores on functional performance test (Kami et al 2017). Thus, differential impact of phases of menstrual cycle on Mindfulness and two factors of Mood i.e., depression and functionality have been substantiated by earlier research findings.

CONCLUSION

Since the results were found to be significant at the level of .01 level of significance i.e., the results are more to experimental manipulation and less due to chance so, we reject the null hypothesis. The three phases of menstrual cycle do impact Mindfulness &Depression and Functionality factors of Mood.

Thus, this research could be helpful for laws regarding special leave during this period.

It is important to know about how the hormones affect women during pre or post or even during menstruation because our hormones follow a predictable pattern every cycle, that means the effect they have on us are same cycle after cycle and thus it allows us to know ahead of time that what our mood, health and behaviour will be. Thus, it also helps us plan our week and month ahead of time and help regulate our emotions. It is helpful even to people around us as it helps them to understand the cause behind women's unreasonable behaviour such as irritation, agitation, forgetfulness, crying, depression etc. Certain chronic and recurring health issues such as asthma, eczema and irritable bowel syndrome worsens during the 4^{th} week due to low level of estrogen. Thus, it helps our healthcare provider with information of symptoms that reappear regularly during the cycle and thus helps in further planning the treatments or prevention of such attacks.

LIMITATIONS

- Limited sample size, so generalization is not possible.
- Only a particular age group was included.
- Only one month measurement was taken.
- No proper measurement of hormonal fluctuations was conducted.
- Participants experienced practice and fatigue effects.

FURTHER RESEARCH

- Large sample size.
- Comparison across different age group.
- Measurement across several months.
- It can be done on various other aspects such as aggression, urges of sex, hunger, specific food craving, etc…

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Chapter 15 - A Study Investigating the Impact of Quality of Sibling Relationship on Peer Relationship

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Abstract

Sibling relationships provide an important context for the development of children's understandings of their social, emotional, moral and cognitive worlds. (Howe et al, 2005) Since peer relationships are an integral aspect of a child's social world, sibling relationships would also impact the quality of peer relationships. This is what the present research purports to measure, i.e., the impact of quality of sibling relationship on quality of peer relationship.

A sample of 36 participants in the age range of 18-25 years were selected, 12 each belonged to Brother-Brother, Sister-Sister, Brother- Sister pairs out of which 6 were elder siblings and 6 were younger siblings in each pair. The Children's perceptions of the qualities of sibling relationship scale (Furman, W. & Buhrmester, D. (1990) to measure the quality of sibling relationship was a dministered which measured 4 factors namely Warmth/Closeness, Power/Relative Status, Conflict and Rivalry. The quality of peer relationship was measured using the McGill Friendship questionnaire (Mendelson, M. J. & Aboud, F) which measured 4 factors namely Stimulating Companionship, Intimacy, Emotional Security and Self validation.

A correlation study among the 4 factors of sibling relationship and the 4 factors of peer relationship was conducted for the entire sample of 36 participants. The significant correlations were found between Power/Relative Status on one hand and Stimulating Companionship, Emotional Security and Self Validation at the 0.01 level of significance and Intimacy at the 0.05 level of significance on the other hand and A negative correlation between warmth and closeness and Intimacy at the 0.05 level of significance and Emotional Security at a level of 0.01 level

of significance was found. The mean for Warmth/Closeness for Brother-Brother, Brother- Sister and Sister-Sister pair was 8.84, 10.49 and 11.50 respectively. The mean for Relative Status/Power was 2.42, 0.67 and 0.33 for Brother-Brother, Brother-Sister and Sister-Sister pair respectively. The mean for Conflict was 9.31, 8.88 and 10.01 for Brother- Brother, Brother- Sister and Sister-Sister pair respectively. The mean for Rivalry was 1.83, 1.75 and 1.41 for Brother- Brother, Brother-Sister and Sister –Sister pair respectively. Overall means of the 4 factors for the entire sample were 10.28 on Warmth/Closeness, 0.916 on Relative Status/Power, 9.4006 on Conflict and 1.66 on Rivalry. Therefore, to conclude, in our sample the Warmth/Closeness and Conflict factors were the highest and sibling relationship did impact peer relationship to a certain extent.

KEY WORDS: Sibling Relationship, Peer Relationship

INTRODUCTION

'Like branches on a tree, we grow in different directions, yet our roots remain as one.'

The above quote clearly defines siblings who are two or more individuals who have one or both parents in common. While it rightly defines what it means to be siblings, the definition is far from capturing the nature and fugacity of the relationship itself.

As recent research has indicated, characteristics of sibling relationship are a source for support, solidarity, companionship and well-being (Bedford, 1995) and also conflict, competition and anti-violence (Connidis, 2007). Sibling relationship is also considered critical in the development of young children's social and emotional understanding (Dunn 1983). As siblings grow up, their relationships go through developmental transformations (Dunn, 1983) and turn out to be more egalitarian and more asymmetrical (Buhrmester and Fuman, 1990). But even though the distance between the siblings grows and their common activities decrease, their emotional attachment to each other remains moderately strong throughout adolescence (Cole and Kerns, 2001). As

they seek to develop their own identity, siblings detach themselves emotionally from the family and show greater interest in the wider world of friends/peers and romantic partners (Dunn, Slomkowski, Beardsall and Rende, 1994). Peers can be defined as individuals of the same/similar age, sharing similarities in areas of interest. Several theories hold that same age peers contribute to the development of children's interpersonal competence, conflict resolution skills, perspective taking and social reasoning skills (Piaget 1965, Sullivan, 1953, Youniss, 1980).

Thus, sibling and peer relationships are an important aspect in a child's life. Few studies have addressed the interplay between children's friendship and sibling relationships. Recent research has focused on the linkages between family and peer relationships (Pettit, Dodge, & Brown,1988, Pettit, Harriot, Bates and Dodge, 1991, Parke and Ladd,1992, Putallaz, 1987) but much of it has examined links with peer-group acceptance and competence in areas such a social functioning rather than quality of the children's friendships.

Moreover, with the notable exception of studies by Stocker and Dunn (1990) and Stocker (1994), most of the work has addressed the association between parent-child and peer relationship which has overlooked the connection between sibling and peer relationships. Interaction between parents of children is often complementary, whereas that between siblings due to smaller difference in age is usually more reciprocal and egalitarian (Dunn, 1983). Thus, the interaction among children theoretically is important for the development of social-cognitive abilities (Piaget, 1932; Sullivan, 1953) and is also likely to include that among siblings as well as among peers. This being so, it would be reasonable to expect similarities across friendship and sibling relationships, particularly since both are relatively dyadic relationships.

Therefore, the present study seeks to explore the correlation between sibling and peer relationship to examine if a correlation does indeed exist between the two. The quality of sibling relationships was measured using The Children's Perceptions of the Quality of Sibling Relationships Scale

(Furman& Buhrmester, 1985). It measures the perceived quality of sibling relationships based on the four components of Warmth/Closeness, Power/ Relative status, Conflict and Rivalry.

Warmth/Closeness refers to the enthusiasm, kindness and affection in a relation. Presence of this enhances the interaction between individuals as it results in positive encounters. This component consists of seven factors:

Intimacy - close familiarity or friendship.

Prosocial Behaviour - behaviour driven by intention to help or altruism.

Companionship - a feeling of fellowship.

Similarity - sharing resemblance.

Admiration by sibling - respect and warm approval received from a sibling.

Admiration of sibling - respect and warm approval towards a sibling.

Affection - feeling of fondness or liking.

Power/Relative status refer to the power dynamics in the relationship. This component is made up the following 4 factors:

Nurturance of sibling - emotional and physical nourishment and care given to sibling.

Dominance of sibling - power and influence over sibling.

Nurturance by sibling - emotional and physical nourishment and care received from sibling.

Dominance by sibling - power and influence exerted by sibling.

Conflict can be defined as a serious disagreement or antagonistic state between two or more individuals. Conflict till a certain point may be seen as healthy or desirable only in the cases where it yields both the parties to actively be considerate of the others' views. Conflict resolutions can serve as important lessons and equip those involved in it with skills to be

utilized in similar situations in the future. However, excess conflict could contribute to the deterioration of a relationship as it increasingly gives rise to unpleasant interactions between individuals. Conflict consists of:

Quarrelling - to have an angry argument/disagreement.

Antagonism – active hostility or opposition.

Competition - the activity or condition of striving to gain or win something by defeating or establishing superiority over others.

Rivalry is the competition for the same objective. In the case of siblings, the objective is more often than not receiving their parents' attention and approval. Sibling rivalry as it is popularly termed entails siblings competing for their parents' attention and acceptance. Whether it deteriorates or enhances the relationship between siblings is something that can't be stated with certainty as its effects tend to vary with different factors. Rivalry consists of the two factors of:

Maternal Partiality - unfair bias in favour of one person or favouritism displayed by the mother.

Paternal Partiality - unfair bias in favour of one person or favouritism displayed by the father.

The peer relationships were measured using the McGill Friendship Questionnaire (Mendelson, M. J. and Aboud, F). The McGill Friendship Questionnaire measures the perceived quality of friendship by taking into consideration the four aspects: Stimulating Companionship, Intimacy, Emotional Security and Self Validation.

Stimulating Companionship is a sense of closeness accompanied by a feeling of interest and enthusiasm brought about by interacting with the individual. It refers to doing things together that arouse enjoyment, amusement and excitement. This is extremely important in any friendship as the pleasure and satisfaction that arises from having a good time with a friend definitely enhances one's perception and value of the friend and the relation itself.

Intimacy refers to sensitivity to the other's needs and states, providing an accepting context in which personal thoughts and feelings and personal information can be openly and honestly expressed. Trust acts as a base for intimacy to occur and is required to further enhance it. Without intimacy and trust, a sense of comfort will not be achieved and thus will hamper the quality of the relation.

Emotional Security refers to the comfort and confidence provided by a friend in novel or threatening situations. This is extremely important as individuals tend to seek comfort and familiarity in novel situations from their friends and close ones. Receiving reassurance and support from friends encourages the individual and makes them feel more confident about their skills and their ability to tackle the different situations that they will face.

Self-Validation refers to perceiving the other as reassuring, agreeing, encouraging, listening and otherwise helpful to maintain one's self-image a competent and worthwhile person. In times of self-doubt, individuals turn to their friends and trust them to discredit the negative thoughts that one has about themselves. The ability to receive this serve as an important factor in the maintenance of friendships as individuals have someone to rely on in their times of need.

It can be seen that both sibling and peer relationships operate on similar dynamics based on the factors that they examine to determine the quality of the interaction between individuals. Research conducted by Brody (1998) suggested 'sibling relationships comprised of a balance of both prosocial and conflicted interactions create experiences that are most likely to nurture children's social, cognitive, and psychosocial development'. Sibling relationship, irrespective of conflicts, rivalry, etc is one of the most long-lasting relationships and tends to impact the development of several aspects such as social skills, cognitive and psychological development to a certain extent.

However, sibling relationships aren't the sole influential relation in an individual's life. (Furman& Buhrmester, 1985) examined the

phenomenon of how children reporting different social networks would provide different social provisions. In accord with Weiss R.S (1974) theory, the research demonstrated that individuals seek specific social provisions in different social relations, with peers being regarded as the greatest source of companionship and receiving high ratings on intimacy. Thus, one's relationship with their peers if close and important to them tends to go a long way. Thus, the quality of these relations may influence the growth and well-being of individuals. Therefore, the study aims to examine if the quality of sibling relationship impacts the quality of peer relationships. In particular, the paper wished to examine whether significant correlations between the four factors of sibling relationship and the four factors of peer relationship. Discovering that the former impacts the latter would mean that efforts made to improve one aspect of a relationship could significantly influence the quality of the other and this in turn would contribute to an improvement in the individual's overall wellbeing.

LITERATURE REVIEW

Sibling relationship is perhaps one of the most long lasting and enduring relationships of an individual's life (Allan 1997, Cicirelli 1989, Lee, Mancini and Maxwell 1990, Voorpostel et al, 2007 and White 2001). Not only is the relationship long-lasting in nature, it also influences several aspects of an individual for a lifetime. Sibling relationships often carry a negative connotation characterized solely by strong dislike, competition, jealousy, unpleasant nature and so on. However, studies have demonstrated that even when sibling rivalry was evident in forms such as verbal teasing, the younger sibling was exposed to rich emotional language from the elder sibling. This contributed significantly to the improvement of social understanding in the younger siblings and they were able to converse almost at the same proficiency as their elder siblings about emotion. (Hughes, 2011)

According to a longitudinal study was carried over 5 years on 140 children, found that siblings had an impact on an individual's early childhood development, even in cases where the relationship was

anything but pleasant. The findings further added that the social understanding among children was facilitated by the interaction with the sibling. (Hughes, 2011)

Therefore, sibling relationships, regardless of their nature and dynamics, exert an influence on the development of a child and determine the social interaction and the quality of varying social relations such as those with peers. To demonstrate, two prospective longitudinal studies conducted with a total of 273 children discovered that nature of interaction among siblings yielded a long lasting and accordant impact on future peer relations. (Roskam, Meunier, Stievenart, 2015) In recent years several studies have linked sibling relationship to various aspects of a person's life. This is because sibling relationship is a source for support, solidarity, companionship and well-being (Allan 1997, Bedford 1995, Connidis and Davies 1990**,** McGloshen and O'Bryant,1988) and also a source of rivalry, conflict and ambivalence. (Allan 1997, Bedford 1998, Conndis 2007)

Each of these factors has been researched in detail in relation to other relationships such as peer relationships. Links between sibling emotional closeness (warmth) and the social skills of children with their peer have been found (Vondra et al, 2001). Lockwood et al (2001) found that warmth in the sibling relationship predicted positive relation in a sample of third through sixth grade.

Also, sibling conflict may provide practise with negotiation, compromise and resolution of conflict. These experiences may help in developing of latter perspective taking abilities (Sang and Nelson, 2017). This may be transferred to other relationships like peer relationships. Due to the fact that individuals with siblings are exposed to a wide range of emotions and an array of situations, the social understanding fostered as a result could help explain the intimacy, they go on to develop with their peers.

Rivalry, in mainstream society, is often viewed as the sole characteristic of a sibling relationship. Although it most definitely isn't the only feature, it is one of the significant aspects of the relationship. One of the

factors that contribute to rivalry is favouritism shown by the parents. One study emphasized on the turbulent nature of sibling relationships and how it fostered negative qualities such as bias and discrimination, more so when favouritism was displayed by the parents. (Hashim and Ahmad, 2016). On the other hand, several studies such as "Toddlers Up" (Hughes, 2011) point out that although rivalry could give rise to behavioural problems and impair the quality of future relationships, lighter magnitude of the same can positively affect a child's early development.

Conflict among siblings can be examined on various dimension such a quarrelling, competition, aggression, antagonism and so on. A study conducted on 4,237 individuals measuring several dimensions of aggression such as physical, verbal and so on established a link between sibling aggression and peer bullying. (Trippetti and Wolke, 2014). Conflict in sibling relationships may result in poor adjustment of behaviour both in the present and future scenarios. Along with that, higher prevalence of conflict among siblings in their childhood has been associated with violent tendencies as adults. Although conflict is often viewed as problematic, it could also provide siblings with the opportunity to develop skills such as conflict resolution. This in turn could facilitate and enhance the quality and nature of their future social alliances. (Howe and Recchia, 2014)

In conclusion, sibling relationships are dynamic and unique, with each evolving over time and differing in their own way. The effect of siblings in an individual's life has been established through the array of studies conducted in this field. Though different researches come out with different findings regarding the equation of a sibling relationship and the effects that it has on other aspects of a child's life, they all seem to agree on the fact that regardless of whether the effects are positive or negative, sibling relationships influence several factors of a child's life such as early development, social skills and so on.

METHODOLOGY

Aim: To find out the impact of the quality of sibling relationship on the quality of peer relationship.

Hypothesis: There will be no correlation between the quality of sibling relationship and the quality of peer relationship.

Predictor Variable: The quality of sibling relationship (Warmth/Closeness, Relative/Status, Power and Rivalry).

Predicted Variable: The quality of peer relationship (Stimulating Companionship, Intimacy, Emotional Security and Self Validation).

Design: Correlational research with survey method used for data collection.

Control:

Equal number of Brother-Brother, Brother-Sister and Sister-Sister pairs (12 each).

6 elder and 6 youngers.

Participants had both parents in common.

Sample: Sample size consisted of thirty-six participants (Average age - 19.5).

Scales:

The Children's Perceptions of the Quality of Sibling Relationships Scale (Furman and Buhrmester, 1985)

McGill Friendship Questionnaire (Mendelson and Aboud)

Data Analysis: A correlation analysis between the four factors of sibling relationship and the four factors of peer relationship was calculated.

DISCUSSION

The sample size consisted of 36 participants who were between the ages of 18 to 25 years. These were divided into three groups of 12 each which

were Brother-Brother, Sister-Sister and Brother-Sister pair. The twelve were further segregated into elder and younger sibling, i.e., six elder and six younger siblings. The average age of the participants was 19.5 years.

The group mean for the 36 participants on the Sibling factor of Warmth and Closeness was 10.28, for Power/Relative Status was 0.916, 9.4006 for Conflict and 1.66 for Rivalry.

Table 1: Group Means of the four Sibling Relationship factors for entire sample

Sample	**Warmth/ Closeness**	**Power/ Relative status**	**Conflict**	**Rivalry**
36	10.28	0.916	9.4006	1.66

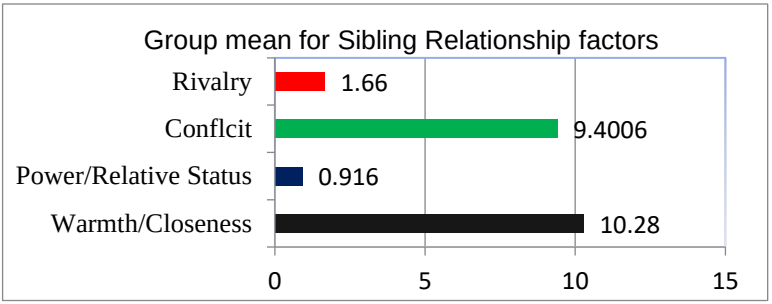
The highest means for Warmth/Closeness and Conflict are in line with past researches where sibling relationships are commonly seen as an intense relationship in which high levels of warmth and conflict co-exist. (Busit and Vermande 2014, Deater-Deckard, Dunn and Lussier, 2002; Noller, 2005)

Several researches have come across sibling typologies where an affect-intense type representing sibling pairs with above average levels of warmth as well as conflict has been found. (Derkman,2011; McGuier et al, 1996; Sheehan et al 2004; Sherman et al 2006; Whiteman and Loken, 2006). The high scores for Warmth/Closeness may be because siblings provide each other opportunities for understanding each other's needs. (Richman and Lanseton, 1988) Closeness, intimacy, high quality of interaction similar needs and interests indicate the reciprocal nature of sibling relationship. (Dunn, 1988)

The high scores in Conflict among siblings may be because of familiarity, easy access, difference in parental treatment and expectations from sibling. (Lockwood et al 2001) The lowest score for Power/ Relative Status is because relationships become more egalitarian as siblings age. (Dunn et al, 2013) The low scores on rivalry are in line with

Minturn and Hitchcock (1963) and Beals and Eason (1993) where the finding on Indian samples was very less rivalry among siblings.

Graph 1: Group Means of the four Sibling Relationship factors for entire sample



Similarly, the mean of all the three groups were calculated separately. The mean for the brother-Brother group was 8.84, 2.42, 9.31 and 1.83 on the factors of Warmth/Closeness, Power/Relative Status, Conflict and Rivalry respectively. For sister-Sister group the mean Warmth/Closeness was 11.50, for Power/Relative Status was 0.33, for Conflict mean was 10.01 and mean for Rivalry was 1.41. The last group Brother-Sister had mean of 10.49, 0.67, 8.88 and 1.75 on factors of Warmth/Closeness, Power/Relative Status, Conflict and Rivalry respectively.

Table 2: Group means of three categories of sibling pairs on the four sibling relationship factors

Sibling Pairs	Warmth/ Closeness	Power/ Relative status	Conflict	Rivalry
Brother- Brother	8.84	2.42	9.31	1.83
Sister-Sister	11.50	0.33	10.01	1.41
Brother-Sister	10.49	0.67	8.88	1.75

Thus, the highest values were for Warmth/Closeness (10.38) followed by Conflict (9.40), Rivalry at (1.66) and the least being Power/Relative Status (0.91). For individual groups, the highest value (11.50) was for Warmth/Closeness and Conflict (10.01) both seen in the sister-sister pair.

One study demonstrated that Sister-Sister relationships are distinctive in nature and that they utilize the relation positively to develop as individuals with its intimate nature being determined by several factors such as upbringing. (Stach, 2007). Moreover, a study stated that Sister-Sister sibling pairs were found to engage in advising one another more than the other pairs and reported being content with the support from siblings. (Tucker, Barber, Eccles, 1997**)** This in turn would explain the high value for Warmth/Closeness.

Table 3: Correlation values between the four sibling and peer relationship factors

Sibling Factors Peer Factors	Warmth/ Closeness	Power/ Relative Status	Conflict	Rivalry
Stimulating Companionship	0.04	0.798**	-0.2239	0.005
Intimacy	-0.35*	0.3010*	-0.008	-0.004
Emotional Security	-0.51**	0.8895**	0.0218	-0.13
Self-Validation	0.29	0.8407**	-0.0081	-0.21

*-.05 level of significance; **- .01 level of significance

A Pearson's product moment correlation coefficient was calculated between the four factors of sibling relationship and four factors of peer relationship. Significant negative correlations were found between Warmth/Closeness in a sibling relationship and Intimacy at a 0.05 level of significance and Emotional Security at a 0.01 level of significance in a peer relationship. This is in line with the research findings of Howe et al (2001) which found that warmth in a sibling relationship was associated with emotional understanding as well as self-disclosure between siblings. Therefore, need for emotional security and intimacy with peers wasn't required in this group, hence the negative correlation.

A significant positive correlation between the factor of Power/Relative Status and the peer factors i.e. Stimulating Companionship, Emotional

Security and Self Validation was found. The correlation values for this were 0.798, 0.8895, and 0.8407 at the 0.01 level of significance respectively for the three peer factors. The value for Intimacy was 0.3010 at the 0.05 level of significance. This may be because as people mature, they want more independence rather than nurturance. If Power/Relative status is high, it indicated more dominance and nurturance from the siblings and hence would seek egalitarian relational elsewhere, which could be found in a way peer relationship. Also, research by Furman et al (1985) indicated that as children increase in age they seek less companionship, intimacy and affection in their siblings. Therefore, they would seek them in a peer relationship.

The research didn't indicate a correlation between the sibling relationship factors of Conflict and Rivalry with the peer relationship factors of Stimulating Companionship, Intimacy, Emotional Security and Self Validation. Rivalry in siblings may arise due to several factors such as gaining the approval of their parents, outperforming the other sibling, etc. One thing common with the motives is that it is linked to the resultant attitude and behaviour of the parents to the child. Since siblings have to share the love and attention of their parents, they may feel the constant need to stand out in order to get noticed. In a peer relationship however, the parties aren't competing with one another for approval from a common authority figure. Thus, the rivalry in their sibling relationship doesn't get reflected or influence aspects of their peer relationship. The notion of siblings being rivals may in turn give rise to conflict among them. Conflict, in addition to fighting for their parent's attention, may also arise due to the need to share their belongings with each other, sharing their personal space, being compared to each other, etc. All these add to the conflict in the relation. In a peer relationship however, the conflict, if any, would tend to be of a different nature (directly linked to aspects of their relationship) rather than on having to share resources. Friendships too involve sharing of resources. The notable difference is that more often than not, the sharing occurs wilfully as compared to a sibling relationship where they are obligated to share simply on the premises that they are related.

Therefore, it can be gathered that the quality of sibling relationship impacts the quality of peer relationship to a certain extent. Particularly, a significant correlation was found between the sibling relationship factor of Power/Relative Status and the peer relationship factors of Stimulating Companionship, Emotional Security and Self validation at a 0.01 level of significance and Intimacy at a 0.05 level of significance. A negative correlation was found between Warmth/Closeness and Intimacy at 0.05 level of significance and Emotional Security at a 0.01 level of significance. Thus, the null hypothesis which stated that there will be no correlation between the quality of sibling relationship on quality of per relationship was rejected.

CONCLUSION

The study indicates that sibling relationship factors, notably Warmth/Closeness and Power/Relative Status share a correlational relationship with the peer relationship factors. Thus, it can be gathered that the quality of sibling relationship does influence the quality of the peer relationship to a certain extent. Qualities present in the sibling relationship weren't sought out for by individuals in their peer relationship. Similarly, qualities absent or minimal in the sibling relationship were sought in peer relationships in the case of this sample. Therefore, the two relations hold and play a significant role in the development of various aspects of an individual. However, these relationships cease to exist in a vacuum. Shortcomings in either can be recognized and active efforts to tackle them could improve the adjustment and overall well-being of an individual to a certain extent.

LIMITATIONS

The sample size was limited.

The study focused on a particular age group (18-25 years) only.

The sample only consisted of individuals from Mumbai thus preventing the generalization of the findings of the study.

FURTHER STUDIES

Research could be conducted on a larger sample and across regions to better understand whether the relationship between quality of sibling relationships and peer relations is significant. This would also mean better chances of generalizing the findings to the target population.

Multiple age groups could be incorporated in to the sample for comparison purposes. This would allow the research to highlight the differences, if any, in the dynamics of siblings and peer relationships in different age groups as they age.

Several factors such as the nature and structure of the family (nuclear, joint, single parenthetic), parenting styles, culture to which the individual's family belongs could also exert an influence on the individual's relationships with their siblings and peers. Thus, further engagement with such areas could aid in the better understanding of the dynamics involved in sibling and peer relationships.

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Chapter 16 - Relationship Satisfaction and Its Link to Adult Attachment and Forgiveness

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Abstract

Relationship Satisfaction as defined by Rusbult et al. (1998) is the positive versus negative effect experienced in a relationship and is influenced by the extent to which a partner fulfills the individual's most important needs. Relationship satisfaction is influenced by many factors. The present study aims to examine the relationship between adult attachment and relationship satisfaction as well as forgiveness and relationship satisfaction. The sample consisted of a group of 89 participants in the age group 18 to 25 years. The Revised Adult Attachment (Collins 1996) Close Relationships Version was used to determine the participants attachment with their partners which included three subscales *close*, *depend*, and *anxious.*

The Couple Satisfaction Index (CSI) was used to determine their relationship satisfaction with their partners. The Transgression-Related Interpersonal Motivation scale-12 Item Form (TRIM-12) (McCullough 1998) was used in determining the interpersonal forgiving in a close relationship and consisted of two subscales- avoidance of the transgressor and revenge. Correlational analysis was carried out. Results revealed a negative correlation between relationship satisfaction and anxious attachment. These findings have important implications in enabling a better understanding of relationships and the factors that influence satisfaction.

Keywords: Adult Attachment, Relationship Satisfaction, Forgiveness, Revenge, Avoidance of Transgressor

INTRODUCTION

There are different factors that contribute to the presence or absence of a couple's relationship satisfaction. However, little is known about the characteristics that each individual brings into their relationship which influence a couple's relationship satisfaction particularly within the Indian context. Past research has shown that adult attachment is one among these factors. Close relationships are essential to health and well-being. The capacity to form intimate relationships with others is considered as an essential developmental task and a principal feature of effective personality development (Bowlby, 1988; Cassidy, 2001; Loubser, 2007). Adult intimate relationships are highly complex phenomena and span manifold levels of analysis including; the person, his/her social, cultural and historical context of their interactions and the systemic interplay among those levels of analysis. (Johnson, 2003; Reis, Clerk and Holmes, 2004).

Bowlby in his attachment theory (1969) suggests that attachment is the deep emotional bond formed between infants and one or more adults; usually a parent or caregiver. This attachment provides a sense of security to children and allows them to explore their environment, returning to the adult during periods of distress. Development of this emotional bond or attachment involves parents providing love, nurturing, trust, safety and respect to their children and sensitively responding to their children's needs. The quality of the early parent-child relationship creates a framework of internalized expectations and beliefs about the self and the self in relation to others. The blueprint of such a framework is long lasting and extended to adult romantic relationships (ISDP, 2004; Feeny, 2008; Simpson and Rholes, 2012; The International Journal of Indian Psychology.) Therefore, attachment systems affect the cognitive, emotional and behavioral responses in romantic relationships (Collins & Read, 1994). This attachment system then influences later social and environmental interactions as well as personal developmental experiences.

Although the ability to forgive transgressions has been linked to overall relationship satisfaction, the mechanisms that mediate this association have not been established. The ability to forgive one's partner may be one of the most important factors in maintaining healthy romantic relationships (Fincham, 2009). Various studies have indicated that forgiveness predicts sustained relationship satisfaction in the face of partner transgressions (Fincham, Hall, & Beach, 2006). Most conceptualizations of forgiveness emphasize a motivational change in which negative response tendencies toward the transgressor (e.g., retaliation, vengeance) decrease (McCullough, Fin- cham, & Tsang, 2003). However, decreased negative motivation alone is likely insufficient for relationship repair when the transgressor is an intimate partner because it implies a return to a state of neutrality rather than positivity toward the partner. Consequently, increased positive motivation (goodwill) toward the transgressor has been postulated as an additional component of forgiveness, especially in close relationships. This "positive" dimension is thought to underlie approach behavior in the face of a partner transgression (e.g., Fincham, 2000) and evidence for the role of this dimension has begun to accumulate (e.g., Fincham & Beach, 2002, 2007; Paleari, Regalia, & Fincham,2009). Thus, forgiveness is theorized to promote not only a reduction in negative responses but also increased goodwill toward the transgressor.

Attachment

Ainsworth (1989) defined adult attachment as an affectional bond that is long-enduring with a seemingly irreplaceable other. Attachment is an important component of adult intimate relationships (Johnson, 2003) and marital commitment, and meeting needs of a felt sense of security is one of the primary reasons for marriage. Human beings naturally seek and maintain contact with significant others and virtually every aspect of human experience is strongly influenced by the quality of attachment bonds (Johnson, Makinen & Millikin, 2001). It is therefore important to understand what constitutes a threat to this attachment bond. Attachment researchers have pointed out that an incident in which one partner

responds or fails to respond at times of urgent need, seems to disproportionately influence the quality of the intimate relationship (Simpson & Rholes, 1994). From Ainsworth's (1978) research three types of attachment styles emerged which are secure, anxious-resistant, and avoidant. These attachment styles later correspond with the adult attachment styles proposed by Hazan & Shaver (1978). These attachment styles can be defined as "Systematic patterns of expectations, needs, emotions, emotion regulation strategies, and social behaviour that result from the interaction of an innate attachment behavioural system" (Shaver & Mikulincer, 2002, p134). The study done by Hazan and Shaver (1987) indicated that the three attachment styles are about as common in adulthood as they are in infancy. They explained a three-category measure of adult attachment style namely Avoidant, Anxious, and Secure. The results of research done by Bartholomew and Horowitz (1991), showed that both self-models and models of others are separate, important dimensions of an adult's orientation to close relationships. Each attachment style has a characteristic way of viewing both the self and the other: *Secure* individuals are characterized by a positive image of the self and positive image of others. Consistently responsive caretaking in childhood is hypothesized to have facilitated the development of both an internalized sense of self-worth and trust that others will generally be available and supportive (Bartholomew, 1997). In the secure style, the self is viewed as basically loveable and others are viewed as generally reliable and responsive. An individual who can count on an attachment figure's responsiveness, support, and protection is free to give full attention to other concerns, such as exploration and/or companionable interaction. Adults with a secure attachment style therefore find it relatively easy to trust others. They can open up emotionally and commit themselves to a long-term intimate relationship. According to Bartholomew (1997), secure adults are high on both autonomy and intimacy, and they are comfortable using others as a source of support when needed. The securely attached adult involves a sense of self worthiness, and the prediction that people are usually accepting and responsive. Secure individuals show a positive self-model

and a positive other- model and this is shown in their independence, trust and lack of jealousy.

Anxious style or preoccupied individuals are characterized by a negative self-model and a positive model of others. According to Bartholomew (1997), inconsistent parenting, particularly if accompanied by messages of parental devotion, may lead children to conclude that they are to blame for any lack of love from caretakers. They question the legitimacy of attachment needs, which renders dependency on others as dangerous and uncertain (Johnson, 1996). This fearful uncertainty explains their excessive vigilance, reassurance seeking, frequent angry protest, and jealousy (Johnson, 2003). They are preoccupied with their attachment needs and actively seek to have those needs fulfilled in their close relationships.

Those with an *Avoidant (dismissive and fearful)* style have learned that in order to feel relatively secure they have to rely heavily on themselves and not openly seek support from a partner. They have learned not to turn to other people as a source of security (Bartholomew, 1997). It can be said that they suppress activation of the attachment system. Research has shown that unlike secure women, insecure/avoidant women failed to seek care (defined as failing to share their concerns with their partners), and in fact withdrew from their partners as they became more anxious. Avoidant attachment is thought to be associated with rules and strategies (learned through interactions with attachment figures) that restrict expression of distress and support seeking (Kobak & Sceery, 1988). More recently, studies by Fraley and Shaver (1997) suggest that avoidant adults experience relatively little distress in relation to attachment - related stressors because they have learned to focus their attention away from these events. Bartholomew suggests that avoidant individuals have very different conclusions about their own self-worth.

In the present study, within adult attachment, the Close dimension refers to the extent to which a person is comfortable with closeness and intimacy. The Depend dimension refers to the extent to which a person feels he/she can depend on others to be available when needed. The

Anxiety dimension refers to the extent to which a person is worried about being rejected or unloved.

The dimensions of attachment proposed by Collins and Read (1990) decompose the classical secure-unsecure categories of attachment, arriving at three continuous scales. A person´s comfort with close and intimate relationships (*close*); the individual's feeling that they can count on others, and that others would be around when in need of them (*depend*); the person's level of fear that others would leave or reject them (*anxiety*; Collins, 1996).

Relationship satisfaction has been defined as an ''adjustment'' (Locke & Wallace, 1959), "functioning" (Honeycutt, 1986) and "well-being" (Acitelli, 1992) and Rusbult et al. (1998, p.359) refer to it as the "positive versus negative affect experienced in a relationship and is influenced by the extent to which a partner fulfils the individual's most important needs". A large body of research supports the proposition that high levels of romantic relationship satisfaction produce well-being (Baumeister & Leary, 1995). According to Mattson, Rogge, Johnson, Davidson, and Fincham (2013), relationship satisfaction is one of the most important variables in romantic relationship research. Romantic relationship satisfaction corresponds to an individual's judgment about the positivity of his/her relationship (Arriaga, 2001; Avivi, Laurenceau, & Carver, 2009). According to De Andrade and Garcia (2012), the construct can be accessed through one-dimensional models (general quality) but also through multidimensional perspectives (specific variables). Specific aspects of romantic relationships such as, for example, communication, commitment, love, intimacy and commitment can contribute with relative influence in assessment of the relationship quality.

Forgiveness is a process (or the result of a process) that involves a change in emotion and attitude regarding an offender. Most scholars view this an intentional and voluntary process, driven by a deliberate decision to forgive. This process results in decreased motivation to retaliate or maintain estrangement from an offender despite their actions and requires letting go of negative emotions toward the offender. Theorists

differ in the extent to which they believe forgiveness also implies replacing the negative emotions with positive attitudes including compassion and benevolence. Some theorists view reconciliation, or the restoration of a relationship, as an integral part of the forgiveness process, and others as independent processes because forgiveness may occur in the absence of reconciliation and reconciliation may occur in the absence of forgiveness. Reductions in revenge and avoidance motivations and an increased ability to wish the offender well are features of forgiveness that can impact upon behavioural intention without obliging reconciliation.

LITERATURE REVIEW

Recent studies suggest that secure attachment and forgiveness share common ground, and that secure attachment even facilitates forgiving. As secure attachment is positively associated with positive emotions, such as hope and self-esteem, and is negatively associated with signs of anger and hostile emotions (Armitage & Harris, 2006; Shorey et al., 2003), it is suggested that secure attachment facilitates forgiving that would allow transition from negative emotions to positive ones (Burnette et al., 2007). Both concepts involve certain structures, such as trust, communication, empathy and arrangement of emotions as well as a series of complex psychological changes focused on oneself and others. For individuals to forgive, they would often need to overcome suspicion, guilt and anger feelings (Enright, 2001). Anxiety of insecurely attached individuals obstructs empathizing with an offender and makes things difficult for them to control negative feelings (Collins & Read, 1994; Gillath et al., 2005). On the contrary, a sense of attachment security reduces the need for self- protection and self-enhancement (Mikulincer & Shaver, 2005), and allows a person to use resources for empathizing with an offender and to control feelings of anger, thereby forgiving more easily (McCullough et al., 1997; Paleari et al., 2005).

Individuals who are securely attached share many of the positive characteristics of disproportionately forgiving people, such as effective self-regulation, empathy, and agreeableness (Macaskill et al., 2002;

McCullough et al., 2001; McCullough et al., 2003). A study using the three-category attachment model recruited 140 Israeli undergraduate participants (Mikulincer, Shaver, & Slav, 2006). Results showed that both major forms of attachment insecurity (anxiety and avoidance) are related to reductions in or distortions of forgiveness.

The association between attachment and relationship satisfaction has been supported in both heterosexual and homosexual relationships (Elizur & Mintzer, 2001 and Collins & Read 1990) research has also given prevailing evidence that factors such as relationship satisfaction, trust and sufficient communication correspond with particular attachment styles. Attachment can also account for relationship dissatisfaction because it arises from attachment worries and insecurities (Mikulincer & Shaver, 2007). Another research study reveals that the duration of a relationship is also influenced by the person's attachment and relationship satisfaction. Hazan & Shaver (1987) found that people who described themselves as anxious or avoidant attachment had shorter relationships (4-6 years) than secure people (10 years) and also had higher marital divorce rates. According to Kirkpatrick & Hazan (1994) anxiously attached couples were more likely to break up and get back together regardless if it was an unhappy relationship.

Forgiveness tends to promote prosocial motivational processes that can lead to relationship repair and the re-emergence of a healthy relationship. In fact, it has been argued that the main function of forgiveness is to help "individuals preserve their valuable relationships" (McCullough 2008, p.116). Researchers have investigated the association between forgiveness- and relationship quality possibly because relationship quality is widely accepted as the common pathway that leads couples to seek help. An association has been documented between both forgiveness and unforgiveness and marital quality (see Fincham, 2010; Fincham et al., 2005), with some indication of a more robust relationship for unforgiveness (Coop Gordon, Hughes, Tomczyk, Dixon & Litzinger, 2009; Paleari et al., 2009). Longitudinal evidence suggests that marital quality predicts later forgiveness and that forgiveness also predicts later

marital satisfaction (Fincham & Beach, 2007; Paleari et al, 2005). Turning to mechanisms that might account for the association, Fincham et al. (2004) suggested that unresolved transgressions may spill over into future conflicts and, in turn, impede their resolution, thereby putting the couple at risk for developing the negative cycle of interaction that characterizes distressed marriages. This is further supported by the finding that forgiveness predicts behavioral responses to partner transgressions (Fincham, 2000).

First, partners in close relationships are more willing to forgive because they are highly motivated to preserve relationships in which they have considerable resources invested and on which they rely for a variety of resources. Second, partners in high-quality relationships have a long-term orientation that might motivate them to overlook hurts in order to maximize the likelihood of preserving the relationship. Third, in high-quality relationships, the interests of oneself and one's partner may become merged. Forgiving a relational offense is hypothesized to contribute to restored relational closeness following the offender's relationship-destructive behavior. Thus, we expect the relationship-constructive motivations toward the offender that are associated with forgiving (low avoidance and low revenge) to be related to higher reported ***closeness*** to the offending relationship partner following an offense. Also, consistent with previous studies (McCullough, Worthington, & Rachal, 1997), we expect forgiving to be related to more positive and less negative behavior toward the offending partner. In addition, forgiving is expected to help restore cooperation between relationship partners after an offense (Komorita et al., 1991). (Interpersonal Forgiving in Close Relationships: II. Theoretical Elaboration and Measurement Journal of Personality and Social Psychology 1998, Vol. 75, No. 6, 1586-1603)

Based on the above literature review the present study predicts the following:

- There is a relationship between Forgiveness and Relationship Satisfaction

- There is a relationship between Adult Attachment and Relationship Satisfaction

METHODOLOGY

Participants - The sample consisted of 89 participants between the age group of 18-25 years from Mumbai. All participants gave their assent prior to data collection.

Measures Attachment - The Revised Adult Attachment Scale (Collins, 1996)- Close Relationships Version was used to determine the participants attachment with their partners. This scale contains three subscales, each composed of six items. The three subscales are *CLOSE*, *DEPEND*, and *ANXIETY*. The *Close* scale measures the extent to which a person is comfortable with closeness and intimacy. The *Depend* scale measures the extent to which a person feels he/she can depend on others to be available when needed. The *Anxiety* subscale measures the extent to which a person is worried about being rejected or unloved. The Participants are rated on a 5-point scale where 1 equals "Not at all characteristic of me" and 5 equals "Very characteristic of me". A person´s comfort with close and intimate relationships (*close*); the individual's feeling that they can count on others, and that others would be around when in need of them (*depend*); the person's level of fear that others would leave or reject them (*anxious*; Collins, 1996).

Relationship Satisfaction - The Couple Satisfaction Index (CSI) was used to determine their relationship satisfaction with their partners. The Couple Satisfaction Index (CSI) is a 32-item scale designed to measure one's satisfaction in a relationship. The scale has a variety of items with different response scales and formats. The authors have also specified that the scale safely be shrunk to either a 16-item format or even a 4-item format depending on a researcher's needs. In this research paper, we have used the 16-item format where each question has a different point scale. Some ranging from "0 - 6" while others ranging from "0-5." (Funk, J. L. & Rogge, R. D. (2007). The CSI was developed with a pool of items from a wide variety of measures, including the

Dyadic Adjustment Scale, Locke-Wallace Marital Adjustment Test, Kansas Marital Satisfaction Scale, Quality of Marriage Index, Relationship Assessment Scale, and Semantic Differential Scale.. CSI scores correlate highly with other measures of relationship satisfaction and discriminate between distressed and nondistressed relationships (Funk & Rogge, 2007).

Forgiveness - The Transgression-Related Interpersonal Motivation Scale-- 12 Item Form (TRIM-12) (McCullough, Bellah, Kilpatrick, & Johnson, 2001; McCullough, Fincham, & Tsang, 2003; McCullough & Hoyt, 2002; McCullough et al., 1998, 1997) was used in determining the interpersonal forgiving in close relationships. It is a 12-item self-report measure of a respondent's level of motivation to forgive an offender. The TRIM Inventory consists of two subscales: one for assessing the extent to which an offended person is motivated to avoid a transgressor (Avoidance) and one for assessing the harm done to the transgressor (Revenge). The TRIM-12 items were rated on a 5- point Likert scale, ranging from 1 being strongly disagree to 5 being strongly agree. The TRIM subscales not only correlate with a variety of relationship, offense, and social-cognitive variables, they have also demonstrated strong relationships to a single-item measure of forgiveness. The test was found to have a high internal consistency (0.85) and moderate test-retest stability (0.5) and evidence of construct validity (McCullough et al.2001, 1998).

Data Analysis - After the descriptive statistics were computed for all variables, Pearson Product Moment correlation coefficient was used to measure the various correlations.

RESULTS

Descriptive Statistics - As shown in the Table 1, 59 female and 30 male participants respectively comprising the sample (n=89) Means and standard deviations for all variables were reported.

Table 1: Mean scores and Standard Deviations of all participants on all variables

Variable	**N**	**Mean**	**Std. Deviation**
Close	89	20.30	4.39
Depend	89	17.20	4.52
Anxious	89	17.08	4.82
Relationship satisfaction	89	67.20	11.57
Avoidance	89	19.52	8.90
Revenge	89	12.35	3.00

For Relationship Satisfaction, the mean score of all participants on the Couples Satisfaction Index was (M = 67.20, SD =11.57). In terms of Attachment, the mean score of all participants on the dimension of ***Close*** was (M =20.30, SD = 4.39) on the dimension of ***Depend*** the mean score was (M = 17.20, SD = 4.52) whereas on the dimension of ***Anxious*** the mean score was (M = 17.08, SD = 4.82). For the variable of Forgiveness, the mean scores of all the participants on the dimension of ***Avoidance of transgressor*** was (M = 19.52, SD = 8.90) whereas the mean scores on the dimension of ***Revenge*** was (M = 12.35, SD = 3.00)

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

Variable	**Female**		**Male**	
	Mean	**SD**	**Mean**	**SD**
Close	20.03	4.52	20.83	4.13
Depend	17.00	4.87	17.60	3.78
Anxious	17.46	4.79	16.33	4.87
Relationship satisfaction	67.53	9.96	66.57	14.40
avoidance	20.02	9.17	18.53	8.39
Revenge	11.98	3.04	13.07	2.83

As illustrated in Table 2, The mean scores of females in this dimension was higher than males, i.e. (M = 67.53, SD=9.96) and (M = 66.57, SD = 14.40) respectively.

The scores for Close attachment was higher for males (M= 20.83, SD= 4.13) than for females (M= 20.03, SD= 4.52) but in case of Anxious attachment it was higher for females (M=17.46, SD= 4.79) than for males (M=16.33, SD= 4.87). The differences were not much, but were even less noticeable in case of Depend attachment with (M=17.00, SD= 4.87) and (M= 17.60, SD= 3.78) for females and males respectively

The mean score of females was higher on the dimension of ***Avoidance***. was higher than males, i.e. (M = 20.02, SD = 9.17) and (M = 18.53, SD = 8.39) respectively. The mean scores of females was lower\on the dimension of ***Revenge*** than males, i.e. (M = 11.98, SD = 3.04) and (M = 13.07, SD = 2.83) respectively. However further inferential statistics were not computed to identify if the differences found were statistically significant.

Analysis of the Relationship between Forgiveness and Relationship Satisfaction

Table 3 : Correlation between Forgiveness and Relationship Satisfaction

		Avoidance	**Revenge**
Relationship Satisfaction	**Pearson Correlation**	-0.059	0.041
	Sig. (2-tailed)	0.582	0.700
	N	89	89

Pearson Product-Moment Correlation Coefficients were computed to examine the relationship between Forgiveness and Relationship Satisfaction Interpretation of the correlation reveals that the relationship between ***Avoidance*** and Relationship Satisfaction ($r_{(87)}$ = -0.059, n.s) and the relationship between ***Revenge*** and Relationship Satisfaction ($r_{(87)}$ = 0.041, n.s) was found to be not significant

Analysis of the Relationship between Attachment and Relationship Satisfaction

Table 4: Correlation between Attachment and Relationship Satisfaction

		close	depend	anxious
Relationship Satisfaction	**Pearson Correlation**	0.06	0.106	-.294*
	Sig. (2-tailed)	0.59	0.322	0.005
	N	89	89	89

* Correlation is significant at the 0.01 level

Pearson Product-Moment Correlation Coefficients were computed to examine the relationship between Attachment and Relationship Satisfaction. Interpretation of the correlation reveals that as predicted, there was a *negative relationship* between ***Anxious Attachment*** and Relationship Satisfaction ($r_{(87)} = -0.294$, $p < 0.01$) which is significant at the 0.01 level. However contrary to expectations, the relationship between ***Close Attachment*** and Relationship Satisfaction ($r_{(87)} = 0.058$, n.s) as well as *the relationship* between ***Depend Attachment*** and Relationship Satisfaction ($r_{(87)} = 0.106$, n.s) was found to be not significant.

No statistically significant correlation indices were found for relationship satisfaction with either close attachment or dependent attachment.

DISCUSSION

The results of the present study provide only some support for the existence of a relationship between Relationship Satisfaction and Attachment but not for Forgiveness and Relationship Satisfaction. The first hypothesis, which states, "There will be a relationship between forgiveness and relationship satisfaction" The relationship between both the variables of forgiveness, i.e. avoidance of the transgressor and revenge with relationship satisfaction was found to be not significant. Both factors of forgiveness in this study were measuring negative aspects of forgiveness, ie. Feeling of revenge and a desire to avoid one's

transgressor. Both acts are not acts of reconstruction but rather acts of either terminating a relationship with the transgressor or seeking retribution. Thus, the terminating tendency, measured by avoidance forgiveness, is not likely to yield any correlation with satisfaction since satisfaction cannot exist in a terminated relationship. Any amount of revenge can only erode satisfaction in a relationship and thus not cause variance in relationship satisfaction. The second hypothesis, which states, "There will be a relationship between adult attachment and relationship satisfaction" here the relationship between close attachment and relationship satisfaction as well as the relationship between dependent attachment and relationship satisfaction was found to be not significant. Therefore, in the present sample, the participant´s comfort with close relationships (*close*) did not have any link with how satisfied the individual felt in their relationship. In addition, whether or not the participants felt that they can count on others, and that others would be around when in need (depend) also did not seem to vary with the satisfaction they felt in their relationship.

However, results did reveal a negative relationship between anxious attachment and relationship satisfaction with a correlation of -0.294 which was significant at 0.01 level. This indicates that a rise in anxious attachment was followed by a decline in relationship satisfaction. The more participants experienced fear that others would leave or reject them the less likely they were to feel satisfied in their relationship.

The results of the study indicate that within this sample any amount of close attachment behavior and dependent attachment would only contribute positively to a relationship. However anxious attachment from participants in a romantic relationship would vary, and most likely deteriorate the quality of a relationship. Thus, relationship satisfaction was linked to how anxious the participant felt about abandonment or rejection. These findings have implications for marital counsellors and therapists who help individuals improve the quality of their relationships.

CONCLUSION

It can be concluded that relationship satisfaction, adult attachment and forgiveness go hand-in-hand. This research study provides some evidence for the assumption that adult attachment and forgiveness are in fact some factors that affect the relationship satisfaction of young adults. This enables us to take the necessary steps required to improve the quality of romantic relationships. It is helpful for marriage /couple/ therapists to address attachment and forgiveness issues and highlight how they are displayed in romantic relationships

Limitations and Recommendations for Further Research

- The results may be interpreted with caution when generalising to the larger population of Indian millennials since the sample size was limited.
- Since the measurement tools were all self-report scales, the participants may have answered them in a socially desirable manner.
- Couples selected for the study were in a relationship ranging from 7 years to just a few months, so their connection with the partner may differ.
- Further research on the correlation between positive forgiveness and relationship satisfaction would bring a lot of insight.
- Couples selected for the study were in a relationship ranging from 7 years to just a few months, this could be studied further as to understand the impact of duration of relationship on the three variables.
- Further research on a different age group could also help gain a better understanding of all three variables.

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SECTION VIII – ENGLISH

Chapter 17- A Comparative Analysis of the Themes of Marginalization and Patriarchal Oppression in the Novels of select African American Women Writers and Dalit Women Writers

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Abstract

By critically analysing memoirs and autobiographies of women authors belonging to Dalit and African American communities, this research paper focuses on bringing awareness about the similar discrimination faced by women of two culturally different marginalized sections.

The women of these communities have been constantly exploited by powerful people on the basis of sex, class, caste and so on. The researchers have selected novels by feminist authors of two culturally diverse backgrounds, who have delineated hardships inflicted upon women of both the communities not only by outsiders belonging to an upper caste and race but also within their own community on account of patriarchy. This paper sheds light on the violent treatment they are subjected to on the whims and fancies of the so-called upper caste/race and the violence engendered by state oppression.

The paper analyzes the two novels by Dalit women novelists which are *Karrukku* by Bama and *The Weave of My Life* by Urmila Pawar and the two novels by African American novelists which are *Sister Outsider* by Audre Lorde and *Bone Black* by Bell Hooks. It is very interesting to note that though the two sets of writers have written in different decades and about different communities yet the similarity of oppressive experience is just the same.

KEY WORDS: Racism, Patriarchy, Casteism, Discrimination, Violence.

INTRODUCTION

Since decades, Africans in the US and Dalits in India have been marginalized on account of their race and caste. They have constantly been subjected to discrimination and exclusion in social, political and economic settings. They have been a victim of cruel and unjust treatment forced upon them by people of outside communities. However, women belonging to these communities have been doubly oppressed, not only by outsiders but also due to patriarchy existing within their own communities. Surprisingly, the oppressors are not only men but also women both within and outside the community. The emergence of literature by those belonging to marginalized groups is a result of the injustice that both these communities have suffered.

"The oppression of women knows no ethnic nor racial boundaries, true, but that does not mean it is identical within those differences. Nor do the reservoirs of our ancient power know those boundaries. To deal with one without alluding to the other is to distort our commonality as well as our differences" (Lorde, 70)

The researchers have selected novels by feminist authors of two culturally diverse backgrounds, and vividly brought out the caste/class and patriarchal exploitations. A comparative analysis of these novels brings out not just the oppression faced by two cultures but also helps to understand their dilemmas as well as inspire others facing the same issues even in the contemporary world. It is equally important to examine how solutions can be found to these age-old tyrannies by re-examining the seminal works of such authors since they have analyzed these issues using the multi-dimensional prong.

Audre Lorde and Bell Hooks are both American feminist writers who have based their works on the civil and social injustices they have faced since childhood. Urmila Pawar is an Indian Dalit writer who writes in Marathi while Bama is a Tamil Dalit writer and both express their frustrations towards their people for perpetuating and justifying the discrimination faced by women in their community. Except for Audre

Lorde's *Sister Outsider*, the other three books, *Bone Black, Karukku* and *The Weave of My Life*, are memoirs. *Sister Outsider* is a collection of essays where Lorde explains the complexities of different marginalized identities while reflecting on her own experiences.

To understand why Africans and Dalits are looked down upon even today, it is important to look at the history of these communities. Africans were uprooted from their homes and country to be transported to the US to work in industries and given the status slaves by whites. Though the books under perusal by African American writers were written in 1984 (Sister Outsider) and 1996 (Bone Black) which is decades after slavery was abolished, the agony expressed by them due to racism is still quite fresh. Blacks were marked as inferior solely because of the color of their skin and their appearance. Even after achieving abolishment of slavery after centuries of protest, racism in the West is rampant even today.

On the contrary, the story of Dalits in India is quite different. Indian society had the caste system ingrained since centuries and was a closed and rigid system. In accordance to the political theory, caste system is the device that was invented by the Brahmins to place themselves in the social hierarchy. Ghurye states that "Caste is the Brahmanic child of the Indo-Aryan culture", cradled in the land of the Ganges and transferred to other parts of the country." (Kapur, Theories of Caste System)

Dalits were not included in the Varna System and were given a 'less than human' status. They were socially degraded and forced to do menial undignified jobs that had no scope of improving their financial status. They were labeled as impure and excluded by all four classes which led to deprivation of basic human needs. Dalit literature emerged in the late nineties where the writers mostly wrote about the early accounts of atrocities they faced. It is interesting to note that though African American literature began early in the 18th century and flourished in 19th century which is a century earlier than Dalit literature, the experiences noted by each of these authors are similar in nature. Their writings are woven around their childhood memories and the authors have

specifically highlighted how inequality is bred right at childhood. The children of both the communities sense their inferiority even before being told about it.

"There were a few games that we played most frequently. Two or three boys would play being Naicker. The rest of them would call them, 'Ayya, Ayya', and pretend to be their pannaiyaal. Those boys would act as if they had a lot of power over us. They'd call out to us, 'Yeppa,Yeppa', humiliate us, and make us do a lot of work." (Bama, 56)

However, the comparative analysis of these novels shows that the world of the Dalit child was filled with greater violence and roughness of behavior than that of the Afro-American child. In fact, what is very apparent is that the Dalit female child is subjected to greater oppression and is made aware of her inferior sex.

"I was the youngest child in my family. Yet I was never indulged. In fact, I was an unwanted child because I was a girl. When I was born, my cousin Govindadada wanted to throw me away onto the dung heap. When I grew older, many would beat me. The largest share of beatings came from Aaye, for bunking school. She seemed to have extra strength while thrashing me" (Pawar, 64)

Dalit children were also abused, oppressed and often hit by authoritative figures like teachers and were often separated from their classmates which led to them experiencing a disenchantment towards educational and other social institutions. A similar sentiment of disillusionment is expressed by Audre Lorde in her work *Sister Outsider*.

"But black women and our children know the fabric of our lives is stitched with violence and with hatred, that there is no rest. We do not deal with it only on the picket lines, or in dark midnight alleys, or in the place where we dare to verbalize our resistance. For us, increasingly, violence weaves through the daily tissues of our living – in the supermarket, in the classroom, in the elevator, in the clinic and the schoolyard, from the plumber, the baker, the bank teller, the

saleswoman, the bus driver, the waitress who does not serve us." (Lorde, 119)

The children of both communities are subjected to a sense of exclusion and worthlessness even without anyone telling them anything. Audre Lorde gives a harrowing and evocative description of how even a young child can be made to feel deviant.

"I clutch my mother's sleeve, her arms full of shopping bags, Christmas-heavy. The wet smell of winter clothes, the train lurching. My mother spots an almost seat, pushes my snowsuited body down. On one side of me a man reading a paper. On the other, a woman in a fur hat staring at me. Her mouth twitches as she stares and then her gaze drop downs, pulling mine with it. Her leather-gloved hand plucks at the line where my new blue snowpants and her sleek fur coat meet. She jerks her coat closer to her. I look. I do not see whatever terrible thing she is seeing on the seat between us – probably a roach. But she communicated her horror to me. It must be something very bad from the way she's looking, so I pull my snowsuit closer to me away from it, too. When I look up the women is still staring at me, her nose holes and eyes huge. And suddenly I realize there is nothing crawling up the seat between us; it is me she doesn't want her coat to touch." (Lorde, 147)

As children belonging to marginalized sections, even before they were introduced to the concept of racism or casteism, they could sense it and lacked a sense of belonging with other children. Violence was well integrated in their childhood but the description by Dalit writers is more hard-hitting. Their children were subjected to greater atrocities and inhumane treatment than Afro-American children. A description by Urmila Pawar in her work, *The Weave of My Life*, stands testimony.

"Some Mahar children also went to school but they had to sit outside in the courtyard. The teacher taught them and examined their slates, from a distance. They would hit the children with stones if they made any mistakes." (Pawar, 17)

Bama also mentions in her book, *Karukku*, how even before she had heard people speak openly about untouchability; she had already seen it, experienced it and was humiliated by it. She recalls an incident where she had noticed a '*Paraya'* elder man holding a small packet by its string so as to not touch it. She mentions how she wanted to shriek with laughter at the thought of the '*vadais'* falling down. She observed the elder walking up to the '*Naicker'* and extending the packet towards him without touching it. She comically recanted this story to her elder brother later that evening. He explained how the '*Naicker'*, belonging to an upper caste, must not touch a '*Paraya'* or anything touched by a '*Paraya'* as they would be polluted. Bama remembers feeling so provoked with anger that she wanted to go and touch those wretched '*vadais'* herself. (Bama, 14-15)

The comparison between the four novels throws up another interesting behavior that is more characteristic of Dalit women as compared to Afro-American women. There is a sharing of experiences amongst the women where they bring out the violence of their husbands towards them and express sympathies with each other and thus forming a communal bond.

"There would be at least one woman among them badly bashed up by her husband. She would walk painfully, somehow managing to drag her aching body along the way. If someone asked her what was wrong, her anger gushed out, 'Let his drinking mouth be burnt off forever. Let his hands rot.' This would be followed by a detailed account of the reason for the beating" (Pawar, 5)

While Urmila Pawar has time and again mentioned the support Dalit women found in each other in her book, it is instilled in Afro-American women to be wary of each other and not trust each other.

"The history of black women for four hundred years in this country that has been presented since when we were brought here as objects and we were taught to suspect ourselves in terms of our humanity, our race as well as our sex. I think it has been one of the tools to keep us separate not only as women but as black people." (Lorde live at UCLA circa)

Another remarkable point in the Dalit novels is that they show aggression towards their husbands by hurling abuses at them only in their absence. These however cannot be seen in the African American novels. On the contrary, Afro-American women are more controlled and passive and though unhappy with their husbands, refrain from aggressively complaining against them.

"'The son of a bitch beats me up without any rhyme or reason. May his face burn!' She started abusing him. Then she saw her husband coming by and, terrified, asked Mother to hide her." (Pawar, 155)

In contrast to Dalit women, assertive behavior can be seen expressed by African American women only towards their children. They become submissive in front of their husbands.

"She could not tell her mother how she became a different person as soon as her husband left the house in the morning, how she became energetic, noisy, silly, funny, fussy, strong, capable, tender, everything she was not when he was around. When he was around, she became silent. She reminded her daughter of a dog sitting, standing obediently until the master, the head of the house, gave her orders to move, to do this or that, to cook his food just so, to make sure the house was clean just so." (Hooks, 98)

All four authors have written about witnessing violence in marital relationships within their community. However, Dalit men, who are themselves, oppressed and poverty-stricken, brutally take out their frustrations on their wives after facing the tyrannies of the upper caste all day. In Afro-American communities, men use violence as a tool for punishment of both women and children but sparingly. In *Bone Black*, Bell Hooks only recalls her father beating up her mother when he suspects her of infidelity. (Hooks, 146-147)

Pawar has written about a similar chilling and horrifying incident in her work *The Weave of My Life*.

"Shantaram for some silly reason had bashed her head with a big stone. She lay unconscious in a pool of blood for a long time. No one demanded

an explanation from him nor came forward to help her. People felt that he was her master and had the right to do anything to her." (Pawar, 154)

However, the violent behavior of men is also connected to alcoholism which is more pervasive in the Dalit women's novels. Whereas, in the Afro-American novels alcohol is consumed on a regular basis but within limits and is also not an excuse for violent behavior towards their family.

"Every house had its own share of drunkards." (Pawar, 5)

On the contrary, Bell Hooks in her work, ***Bone Black***, mentions about how parents taught their children to be responsible drinkers and never drink in excess.

"They let us drink a little. They tell us that we must learn to drink in moderation, properly so that we will not group up to drink in excess." (Hooks, 67)

Women mentioned in these books are quite fatalistic and believe that it's their destiny to belong to a marginalized section and thus suffer. They seem to have made their peace with their conditions of oppression and humiliation. Bama re-quotes what her '*Patti'* told her after a young Bama noticed some Upper Caste people disrespecting her-

"Sometime later, I said to Patti she should not lay herself open to such behavior; it was ugly to see. What Patti said to me in return was this: These people are the maharajas who feed us our rice. Without them, how will we survive? Haven't they been upper caste from generation to generation, and haven't we been lower caste? Can we change this? (Bama, 16-17)

Bama expresses her anger over this incident as well as several others, like when she faces discrimination in her convent but is only able to give words to her frustration later, as an adult.

"Because Dalits have been enslaved for generation upon generation, and have been told again and again of their degradation, they have come to believe that they are degraded, lacking honor and self worth,

untouchable; they have reached a stage where they themselves, voluntarily, hold them apart. This is the worst injustice." (Bama, 28)

There are a few women of the younger generation who seek to liberate themselves through education. As we see Audre Lorde taking a decision to verbalize her opinions and feelings.

"I have come to believe over and over again that what is most important to me must be spoken, made verbal and shared, even at the risk of having it bruised or misunderstood" (Lorde, 40)

However, they find that it is not possible to achieve freedom from their race or caste identities. Even the struggle for liberation from their marginalized identities invites oppression and is seen as a threat to the people around them. Surprisingly, not just outsiders but also their own husbands viewed such quests for freedom with suspicion and were threatened by them. They are criticized for doing either too little or too much.

Urmila Pawar puts across her husband's mixed reactions about her educating herself and how she was often subjected to taunts after she started exploring her identity as a feminist.

"Gradually, it became clear to me that everything that gave me an independent identity – my writing, which was getting published, my education, my participation in public programs – irritated Mr. Pawar no end. Gradually, he became full of resentment [….] on the contrary he would tell me, 'Look at the village woman. The husband's wish is law for her. She does not dare to sit down or get up without his permission. Tell me, in that case, how is she able to run her home well?' [….] He felt that he was losing control over his wife fast and had to establish his authority with an iron hand as to keep her within bounds" (Pawar, 246-247)

Although both sets of writers are feminists themselves, yet they are very vocal about projecting their marginalized identities rather than only struggling for women's liberation. They have said time and again that their oppression due to being women of marginalized communities is way more than any other women of mainstream communities.

Audre Lorde has included an open letter to another radical white feminist, Mary Dale, in her book, where Audre tries to express her concern over the dangers of dismissing the problems faced by non-white women when representing the movement.

"The oppression of women knows no ethnic nor racial boundaries, true, but that does not mean it is identical within those differences. Nor do the reservoirs of our ancient power know these boundaries. To deal with one without even alluding to the other is to distort our commonality as well as our difference. For then beyond sisterhood is still racism." (Lorde, 70)

Both Dalit writers have also expressed similar concerns where they felt that the problems they face, as Dalits and as women, are being seen in exclusion, whereas these identities intersect with each other in reality.

"As a reaction against mainstream Indian feminism that tended to ignore the problems of caste, Dalit women and those who advocate their cause have been making a valid case for Dalit feminism. This standpoint acknowledges both the patriarchal oppression from outside the caste as well as within it." (Shoma Sen)

In her interviews, Bama emphasizes on the term 'Dalit Womanism'. She says that,

"The problems of a Dalit woman are different from that of any woman. All over the world, women are second-class citizens only. But in Indian context, the upper caste woman's problems and the Dalit woman's problems aren't the same. There are certain problems that are the same, as in the experiences of male chauvinism, violence, child bearing/rearing and others. But when it comes to caste, the Dalit women are the Dalits among the Dalits." (Bama)

Audre Lorde has also spoken about how her membership in different marginalized sections intersect with each other and cannot be viewed independently and expressed her views in an essay called '*There is No Hierarchy of Oppression*'.

"Within the lesbian community I am Black, and within the Black community I am a lesbian. Any attack against Black people is a lesbian and gay issue, because I and thousands of other Black women are part of the lesbian community. Any attack against lesbians and gays is a Black issue, because thousands of lesbians and gay men are Black. There is no hierarchy of oppression." (Audre Lorde)

Both Audre Lorde and Bell Hooks have spoken in support of intersectional feminism, a term first coined by Kimberlé Crenshaw, a civil rights advocate.

"Intersectionality is a framework designed to explore the dynamic between co-existing identities (e.g. woman, Black) and connected systems of oppression (e.g., patriarchy, white supremacy). The term was created by Kimberlé Crenshaw and challenges an assumption continuing to undermine the feminist movement – that women are a homogeneous group, equally positioned by structures of power. In a feminist context, it allows for a fully developed understanding of how factors such as race and class shape women's lived experiences, how they interact with gender." (Claire, Wordpress)

In case of Dalit women novelists, their caste has also caused hindrance in voicing out their problems and establishing a social identity. When *Karukku* was first published, Bama was ostracized from her village for portraying their community in a poor light and she was denied entry in her village for seven months. Urmila was also criticized in a similar manner,

"When Urmila Pawar's autobiographical work Aaidan was first published, it sent waves of discomfort in society, among men and women alike. She had faced opposition from male agencies across castes, including her own home — where her book (initially) was not celebrated, but looked down upon." (Maitreya)

Whereas, in case of both the African American authors of these novels, the problems for publishing their books weren't noted to be as high as compared to what the Dalit women had faced.

Both sets of writers have looked after and brought up proud children with a rational school of thought, who abhor the whole concept of racism or casteism and have seen their parents fight against any form of oppression whether within their own community or against those outside the community.

"I simply do not believe that one aspect of myself can possibly profit from the oppression of any other part of my identity. I know that my people cannot possibly profit from the oppression of any other group which seeks the right to peaceful existence. Rather, we diminish ourselves by denying to others what we have shed blood to obtain for our children. And those children need to learn that they do not have to become like each other in order to work together for a future they will all share." (Lorde)

Audre Lorde in her highly inspiring speech made at UCLA circa says,

"We are making history, whether we actively involve ourselves or not. I urge each one of you to examine how you can use whoever you are in the service of what you say, what you believe." (Audre Lorde live at UCLA circa)

Both Dalit women writers and Afro-American women writers' contributions to literature revolve around the hardships they have faced, not just to give voice to their struggles and victories but also in the hope of conveying the irrationality of such systems. It is because of activists like these four that racism/casteism and sexism, as systems of oppression, have been addressed to some extent and there is greater awareness about their plights with the state offering interventions wherever they can. However, even these reformations those are in support of Dalits lead to excluding them further instead of including them in the mainstream society.

The exploitation of Dalits in India is even worse compared to Afro-Americans. At least Afro-Americans were a different ethnic race whereas Dalits belonged to the same ethnic race as the rest of the Indians. Even though the situation of Dalits has drastically improved, many

practices to degrade Dalits continue to exist, more evidently in rural India, despite the abolishment of untouchability and other atrocities against Dalits. To end this battle against poverty and discrimination, education seems to be a powerful remedy for liberation. But for Dalits, extreme poverty and difficult conditions, make getting proper education a huge challenge. However, the few who complete their education use it as a powerful tool to get freedom from the meaningless injustice inflicted upon them.

They may not be called or treated as typical 'untouchables' anymore, but the imposition of social disabilities on them is still high. In every realm of life, they are still perpetuated with a sense of rejection from the past. Going to school may have become easier, but getting a job hasn't. Majority of members in the community are still economically and politically backward. The status of the Dalit women has improved negligibly and they are shamed for standing up against the caste-based and gender-based violence and discrimination. However, despite all of this, today we have come a long way forward when we have many vocal women writers of the Dalit community like Meena Kandasamy, P. Sivakami, Gogu Shyamala etc. and also publishing houses such as Zubaan which has published many books of Dalit women writers. Recently an all-India Dalit writer's conference was held in which many women writers participated. On the other hand, the situation of African Americans has improved considerably as racism is widely detested both socially and by law. It is safe to state that African American women have progressed far more than Dalit women in all professions though the conditions are still not ideal.

These authors and many more like them have helped oppressed women find their identity and challenge their own school of thought. This enables the vicious circle of oppression to break and fosters hopes of a just future. The emancipation of Dalit women still leaves a lot to be desired though there are proactive Indian policies for all Dalits, yet most of such policies miss the target of women empowerment. More and more women writers of both communities, Dalit and Afro-American, are

needed to give blistering pictures of oppression as well as hopeful visions of concrete achievements to their readers. Such writings will empower thousands of down-trodden people and help them move forward to achieve equality in a world dominated by class, caste and gender.

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