JIGYAASA

A Multidisciplinary
Research Initiative of KC College, Mumbai

Volume V; Issue I
2021 - 2022
ISBN 978-93-94138-18-6

Chief Editors
Dr. Sagarika Damle
Dr. Shalini R Sinha

HSNC UNIVERSITY, MUMBAI
KISHINCHAND CHELLARAM COLLEGE

JIGYAASA

A Multidisciplinary
Research Initiative of KC College, Mumbai

ISBN 978-93-94138-18-6

9 789394 138186

SHAILJA PRAKASHAN
57 P-Kunj Vihar-II, Yashoda Nagar, Kanpur-208011
Mob. : 8299046238, 9451022125
E-mail : shailjaprakashan@gmail.com

HSNC UNIVERSITY, MUMBAI
KISHINCHAND CHELLARAM COLLEGE

JIGYAASA

A Multidisciplinary
Research Initiative of KC College, Mumbai

HSNC UNIVERSITY, MUMBAI
KISHINCHAND CHELLARAM COLLEGE

First Edition – 2022

Price – Rs. 300/-

Published by:
Shailja Prakashan
57 P – Kunj Vihar II, Yashoda Nagar,
Kanpur, 208011
Mobile No. 8299046238/9451022125
Email ID: shailjaprakashan@gmail.com

ISBN 978-93-94138-18-6

SHAILJA PRAKASHAN
57 P-Kunj Vihar-II, Yashoda Nagar, Kanpur-208011
Mob. : 8299046238, 9451022125
E-mail : shailjaprakashan@gmail.com

HSNC UNIVERSITY, MUMBAI
KISHINCHAND CHELLARAM COLLEGE

Editorial

'Progress is born out of doubt and inquiry- Robert G. Ingersoll'

The consistent and diligent efforts taken towards the two innovative research-oriented programmes of the college, Jigyaasa - Science Honors Program (SHP) and Certificate Programme for Commerce & Arts (CPCA), have borne fruit with insightful research papers being produced each year. Some of these papers have also been published in international and national level, peer-reviewed journals and acclaimed books.

With the New Education Policy being discussed and debated at many of the Universities across India, several new avenues of multidisciplinary learning have been introduced, including learning the techniques of effective scientific communication, becoming aware of gender nuances, gaining critical knowledge of law, justice and human rights etc. K.C College was much ahead in training its undergraduate students in not only conducting research projects but also in developing critical thinking ability and communicating effectively to the outside world, through the above-mentioned platforms of SHP and CPCA programs. Under the NEP, some of the modules carried out under these umbrellas, can now be upgraded into electives across different disciplines and students can gain credits for the same.

It gives us great pleasure in bringing out Jigyaasa –Volume 5, issue 1, which is a compendium of selected research data generated and analysed by the SHP and CPCA students at K.C College.

The chapters include interesting research projects conducted under the Science and Arts sections from Life Sciences, Biotechnology, Physics and Sociology, that provide insights into topics such as Derogatory Effects of alcohol containing sanitizers on health, A review of Covid 19 and its relation with Human health, Evaluation of Prebiotics and antimicrobial properties of natural extracts, proposing prospective material for Non-Linear Optical Organic-Inorganic applications, Influence of Television and Web series on Career choices of the young

and such. These articles bear testimony to the efforts taken by the research guides and students, at the same time serving as a stepping-stone for the future batches of students who might be inspired to carry forward certain interesting possibilities of their predecessors and discover yet another life lesson. The existence of such compendiums indicates the confidence generated in students who are publishing their research work to the outside world and are ready to defend the outcome in front of the external scholars and scientists as the reviewers. Thus, these research compendiums would act as a guiding light for the future students of KC and other educational institutions who wish to travel the path from Lab to Life.

Editors:

Professor Sagarika Damle – Convener, SHP &

Dr. Shalini R. Sinha – Vice Principal and Convener, CPCA

Foreword

The great philosopher and thinker Socrates said that, "The secret of change is to focus all of your energy, not on fighting the old, but on building the new," and the academic year 2021-2022 saw a tremendous amount of change as all academic and co-curricular activities returned entirely, to offline mode and the UGC proposed the New Education Policy which stipulated a complete overhaul and improvement of the education system in India.

The New Education Policy highlights the importance of research at undergraduate level in building a foundation for students' research endeavours at post-graduate levels. This change is consistent with the ethos at KC College, affiliated to HSNC University, where research activities have been routinely undertaken by undergraduate students for the last decade. The college aims to empower students with the necessary research skills such as, developing a research problem, framing hypotheses, designing experiments, gathering and interpreting data and drawing conclusions through a series of expert-led sessions and workshops under two research-oriented programs- the Jigyaasa-Science Honors Program (SHP) and the Certificate Programme for Commerce and Arts (CPCA).

Through these research activities students are transformed into researchers who are able to think critically, reason logically, broadening their understanding and outlook towards the creation and expansion of knowledge. Participation in these programmes involves exposure to training in communication skills so that students can effectively present their ideas, financial literacy skills, team building skills and advanced computer and software skills. To enable students to have a nuanced understanding of the problems that plague our society, interactive sessions by experts on gender issues as well as environmental protection have been conducted with the goal of all-round development of these students.

For our young researchers at undergraduate level, our Faculty mentors provide guidance and support through their research journey. I

am pleased to witness the fruit of this effort by both students and faculty- a new Volume V Issue 1 of Jigyaasa- a compilation of their research undertakings. This issue contains the research papers from the fields of Life Sciences, Biotechnology, Physics and Sociology.

I congratulate all the contributors for their consistent effort towards these research endeavours.

Dr. Hemlata K. Bagla

Vice Chancellor (Ag), HSNC University, Mumbai.
Principal, K. C. College,
Sr. Dean, Sciences & Director, Niranjan Hiranandani School of Management and Real Estate,
HSNC University, Mumbai

INDEX

SECTION I - LIFE SCIENCES

Chapter 1 - A Pilot Study of Covid -19 and its Relation with Human Health

Ashoi Dantra, Maitri Shah, Dr. Suvarna Sharma*
Department of Life Sciences, Kishinchand Chellaram College,
Churchgate, Mumbai- 400020
Suvarna.sharma@kccollege.edu.in

ABSTRACT

Corona virus disease (COVID-19) is an infectious disease caused by a newly discovered corona virus. Most people infected with the COVID-19 virus will experience mild to moderate respiratory illness and recover without requiring special treatment. Older people, and those with underlying medical problems like Cardiovascular Disease, Diabetes, Chronic Respiratory Disease, and Cancer are more likely to develop serious illness. The study was based on a survey which was conducted globally. Questions were asked on the basis of the regular dietary habits and the physical exercises done in order to prevent COVID -19. The questionnaire was divided into two sections, one based on questions related to the general health of an individual followed by a few specific questions (Section two) based on the recovery rate of the individual from COVID-19. Thus, the second section catered only to those who had contracted the infection. Gender along with diet choices and co-morbidities were flagship with respect to the infection rate. With the help of these questions an estimate of the health measures taken by the individuals during this period, ability of the virus to infect a particular group of people and the severity of infection in a group of people was studied.

Keywords: COVID-19, Gender Bias, Dietary Habits, Co-morbidities

INTRODUCTION

On January 30, 2020 the World Health Organization declared the outbreak of the SARS-CoV-2 a Public Health Emergency of

International Concern and a pandemic on March 11, 2020. The virus causes an acute respiratory infectious disease, called COVID – 19 that spreads dangerously fast through human-to-human contact.[1] The virus has the potential to affect people of all ages, showing severe symptoms in the elderly and people with underlying chronic diseases.[2] Symptoms of COVID – 19 vary from person to person. While some develop very mild symptoms, some suffer from severe symptoms that require

immediate medical attention. Among those who suffer severely are the elderly age groups, African American and Latinos and those with comorbidities like diabetes, asthma, etc. [3] Co-morbidities are perhaps the most fearful set of diseases accompanying a COVID positive patient. A set of symptoms that are indicators of the contracted disease are fever seen in most cases, cough, headache, fatigue, feeling shortness of breath and sore throat.[2] The virus takes between 5 and 12 days to inoculate and show one of the above symptoms. It is believed that most infections are spread in the first two days before the development of symptoms where most people are unaware of their COVID 19 infection.[4] The common recovery period seen is 10-17 days, where the minimum number of days to be symptom free is 10 days. This led to the quarantine period decrease from 14 days to 10 days and finally to 7 days.[5] Nutrition is perhaps the most endearing term to any person that is recovering or has recovered from this disease. It is an all-inclusive term for providing the body what it needs to grow and be healthy.

The food pyramid contains all ideal foods in proportions: carbohydrates, proteins, fat, dairy, fibre, gluten. The food pyramid makes it easier to understand the quantity of the variety of foods to be consumed to maintain best health and drive our bodies to give maximum output.[6] It is not the first time that nutrition and a disease have been connected. Several research papers before 2020 are proof that nutrition and a healthy, well-balanced diets are 'lifeboats' in a pandemic/epidemic catastrophe. [7] However, the impact of COVID – 19 has opened people's eyes to the consequences of having poor dietary choices. More people are now putting emphasis on exercising regularly and reviewing mechanisms for controlling obesity, cardiovascular risks and living a

better suited life.[8] Our study through analysis proposes three hypotheses:

- There exists no relationship between infection and gender of a person.
- There exists no relationship between co-morbidities and infection of a person.
- There exists no relationship between diet vegetarian and non-vegetarian) and the infection of a person.

MATERIALS AND METHODS:

Survey Based: The method used for the project was survey based by selecting a random population. This survey was sent to over 180 people all across the world in order to record the dietary habits and other health issues in different people in the time of COVID-19. The preparation of the form was done by using questions related to the topic COVID-19 and Diet. The first section recorded the day-to-day dietary habits of different people and also gender was taken into consideration wherein the aspects like how does the diet affect the gender was seen. The second section was divided on the basis of if people were infected by COVID-19 or not. The questions asked in this section were based on how many days did one take to recover completely, their symptoms and what were the conditions post recovery. This section recorded the number of individuals suffering from COVID-19 and the condition based on the severity of the symptoms.

Chi-Square Test: Analysis of the data was done using Chi-Square Test. This test helped us to determine if a relationship existed between a parameter, we set for the infection rate.

RESULTS:

From a population of 180 people randomly selected, we found that 106 people selected ‘Female’, 77 people selected ‘male’ and 1 person selected ‘other’ as their gender. Fig. 1. shows the percentile of gender. For further analysis of this section of the group data, ‘other’ was omitted, for ‘1’ other had insignificant contribution to the analysis.

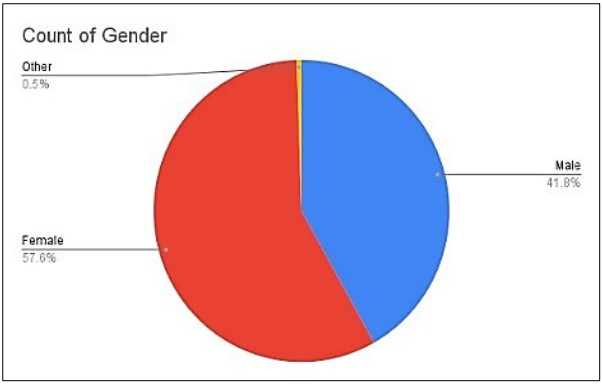


Figure 1: Contribution to the analysis.

Fig. 2 and Fig. 3 show the status of infection of females and males respectively. We see that out 106, 28 were infected by the Corona virus and out of 77 males, 17 were infected.

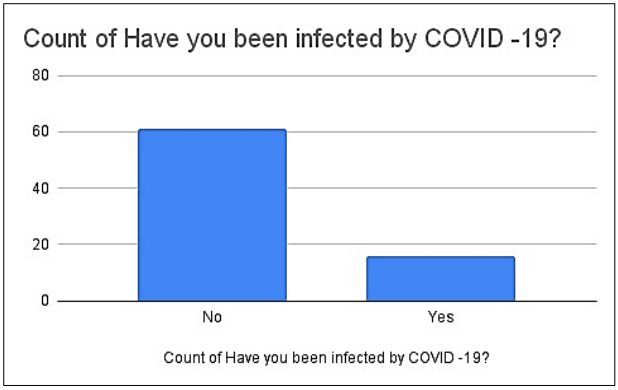


Figure 2: Infection status of females.

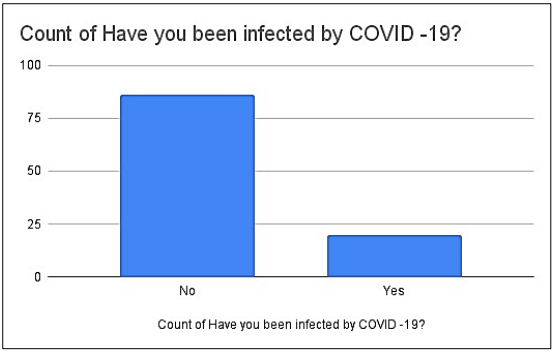


Figure 3: Infection status of males

We see fig. 4 separating the population on the basis of co-morbidities. As per options given in the google form, those with a co-morbidity have registered as a person with an underlying disease. We have segregated the data as people without co-morbidities (151 people) and those with one (33).

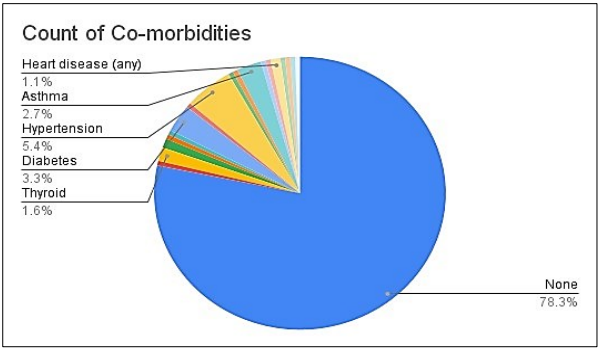


Figure 4: Count of Co-morbidities

Fig. 5 and Fig. 6 Cumulatively show that of 33 people with a co-morbidity, 7 were infected with the virus. Results showed that of 151 people with no co-morbidities, 38 were infected.

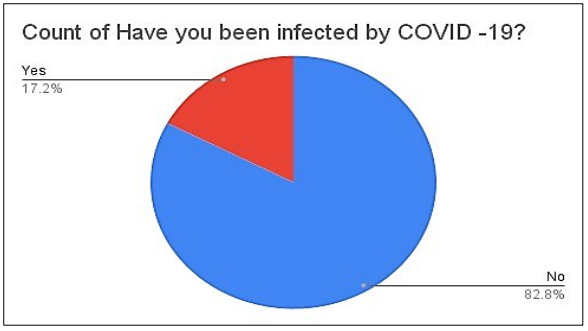


Figure 5: Infection status of people with co-morbidities

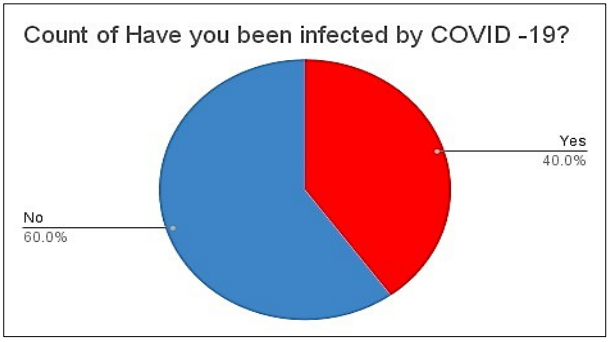


Figure 6: Infection status of people without co-morbidities

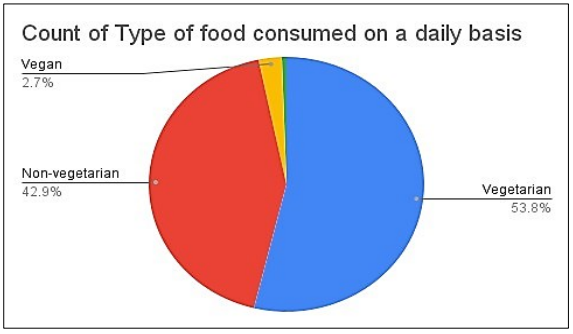


Figure 7: Type of Food Consumed

Forthcoming to diet choices which spanned in the range of vegetarian, non-vegetarian and vegan, we recorded as shown in Fig. 7), 99 individuals to be vegetarian 53.89%), 79 people to be non-vegetarian 42.9%) and 6 people were vegan 2.7%). However, for further analysis, 'vegan' participants were omitted from the study because they delivered insignificant contribution to the analysis.

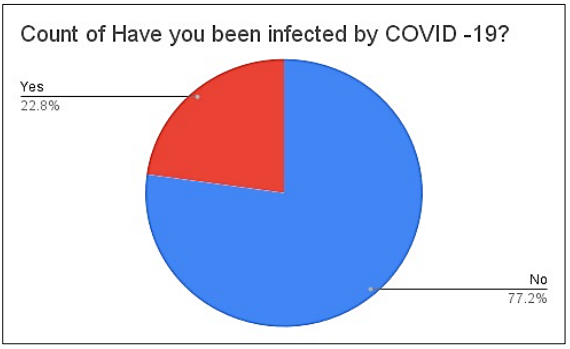


Figure 8: Infection status of non-vegetarians

Further analysis of the data was done by Chi-Square test of correlation in three sums: gender, comorbidities and diet. All degrees of freedom were n-1) = 1° at 5% which gives t_{tab} = 3.84. The following formula was used for all calculations.

$$\chi^2 = \frac{\Sigma(O-E)^2}{E}$$

For all three factors, i.e., gender, presence of co-morbidities and diet choices related to the SARS-CoV-2 infection, we accepted the alternate hypothesis, which claims that there exists a relationship between each of the factors.

Our results, thus states:

- Women are at more risk of contracting COVID-19.
- People with co-morbidities are more risk of COVID-19.
- People consuming vegetarian food are at more risk of a COVID-19 infection.

DISCUSSION:

With the help of this questionnaire method, we were able to interpret as which type of population was more at risk and was affected the highest due to the outbreak of SARS-CoV-2.

According to studies it can be said that the immunity level in women is less than that in male which means that women are prone to COVID-19 more than men. Women, being prone to comorbidities and lessening of Calcium in their bones often fall prey to a weak immune system. A weak immune system serves as the perfect host for the virus, making women susceptible to a disease that is caused by a virus so malicious in nature.

Co-morbidities refers to more than one health related condition present in an individual at one time, it includes Diabetes, Asthma, Tuberculosis, Heart disease, Cancer, Hypertension, HIV, etc. These diseases reduce the immunity of an individual making it easy for the virus to enter the body and cause damage. The best way to fight off this condition is to maintain stable immunity levels. There are a variety of fruits and vegetables one could include in their diet to ensure that their immunity is sustained, and individuals with co-morbidities should remain cautious of their health by following various health protocols.

individuals eating non-vegetarian food were at low risk as they were getting more amount of nutrients from the food as compared to the individuals eating vegetarian food. The diet consumed by the individual affects their immunity which increases the risk of COVID-19. As one says, 'Eat right and healthy and stay healthy'. Doctors suggested people to eat a high protein diet during and after the infection. An average non-vegetarian meal contains adequate amount of protein and iron. Not all vegetarian diets are healthy diets and a study showed a larger number of vegetarian people going in for a bariatric surgery.[9] Vegetarian diets are better in controlling other co-morbidities, but do not have a proof of keeping the deadly Corona virus at bay. A healthy non-vegetarian diet has nine essential amino acids and excellent vitamins like B_{12}, vitamin A, iron, chlorine and selenium which are vital for muscle – liver health.

heme iron from animals is better absorbed in the body than non-heme iron plant based) [10].

CONCLUSION

The study aimed at finding out if there existed a relationship between three factors with infection rate of COVID-19. Through our questionnaire, we received the response of 180 individuals from different backgrounds. With thorough analysis using the Chi-Square test, we proposed hypotheses, of which the alternate hypotheses were accepted. We found a correlation between gender, co-morbidities of an individual and diet choices with respect to infection of COVID-19.

We found, that females were at a higher risk of being infected by COVID-19 over men. In addition to the above, we found a relationship between co-morbidities and the infection. We concluded that an individual with co-morbidities was at a much higher risk of contracting the virus than one without a comorbidity. With respect to diet choices between a vegetarian and a non-vegetarian one, we found that a person with a vegetarian diet was at a risk of contracting the infection over a person with the alternate diet.

Corona virus has impacted each individual worldwide. To the virus, each individual serves as host and those with weakened immune systems serve as their apex victims. COVID-19 has shaken the very ground of science and we are slowly but surely rising from it. Wearing masks, maintaining distance and getting vaccinated is our only hope of eradicating this virus from our lives.

This study was conducted on a population of 180 people. To take this research further, we can conduct a survey on a larger population to understand factors affecting the rate of COVID-19 in depth and discover parameters for the same.

REFERENCES:

1. Li Q, G. X. 2020. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *N Engl J Med.*, 1199-1207.
2. Ludwig, S. &. 2020. Coronaviruses and SARS-CoV-2: A Brief Overview. *Anesthesia and analgesia*, 93-96.
3. Butler, M. J. 2020. The impact of nutrition on COVID-19 susceptibility and long-term consequences. *Brain, behavior, and immunity*, 53-54.
4. Nazario, B. 2020. *Coronavirus Incubation Period*. Retrieved from https://www.webmd.com/lung/coronavirus-incubation-period#2
5. Parul. 2020. *14, 10 or 7 days? Doctors differ on ideal Covid recovery period*. Retrieved from Indian Express Newspaper: https://indianexpress.com/article/cities/chandigarh/14-10-or-7-days-doctors-differ-on-ideal-covid-recovery-period-6584799/
6. Ioan SARAC, M. B. 2020. *Food Pyramid - the Principles of a Balanced Diet.* Retrieved from researchgate: https://www.researchgate.net/ publication/ 339354425 Food Pyramid The Principles of a Balanced Diet/ link/ 5e4d30a0a6fdccd965b0dea8/
7. Haug A, B.-M. J. 2007. A food "lifeboat": food and nutrition considerations in the event of a pandemic or other catastrophe. *Med J Aust*, 11-12.
8. Philip T James, Z. A.-B.-R. 2021. The Role of Nutrition in COVID-19 Susceptibility and Severity of Disease: A Systematic Review. *The Journal of Nutrition*, 1584-1878.
9. Borude, S. 2019. *Which Is a Good Diet—Veg or Non-veg? Faith-Based Vegetarianism for Protection From Obesity—a Myth or Actuality?* Retrieved from Research gate : https://www.researchgate.net/publication/330099524_Which_Is_a_Good_Diet-Veg_or_Non-veg_Faith-

Based_Vegetarianism_for_Protection_From_Obesity-a_Myth_or_Actuality

10. K. Sree Lala Priyadarshini, L. R. 2020. Does Non-Vegetarian Food Increase the Risk of COVID-19? *International Journal of Current Research and Review,* 1221, S-64 - S-62.

Chapter 2 - Comparative Study of Use of Nanoparticles in Transgenic Plant Development

Praniti Pawar, Sanjana Anumalla, Dr. Suvarna Sharma*
Department of Life Sciences, Kishinchand Chellaram College,
Churchgate, Mumbai- 400020
suvarna.sharma@kccollege.edu.in

ABSTRACT

The applications of Nanotechnology in the field of Biology in recent times are commendable. Specifically, the use of nanoparticles as DNA or Gene delivery molecules are an effective therapeutic tool. Nanostructured gene delivery systems can be made of lipids, exosomes, polymers, polypeptides, graphene-family nanomaterials, and inorganic materials, e.g., gold nanoparticles or their combinations. Carbon Nanotubes (CNTs) are nanostructures, allotropes of carbon which are made up of graphene sheets wrapped around it forming cylindrical structures. CNTs have been regarded to have interesting and attractive physical and chemical properties and have been tremendously used in genetic engineering. CNTs are classified into two categories: Single-walled (SWCNTs), and Multi- walled (MWCNTs) structures. CNTs are valuable vectors in various Biomedicine fields such as Gene delivery, Drug delivery, Immunotherapy, Tissue engineering and Biomedical imaging and also, they deliver the DNA without damaging the cells. Based on recent studies, functionalization of CNTs when combined with some other suitable molecules can drastically subside their toxic effects. Having unique properties such as small size, larger surface area is useful in delivering DNA into mammalian cells as well. Modifications in CNTs can make Nucleic acids adhere to them even more efficiently. Also, MWCNTs are crucial in delivery DNA into cytoplasm. Based on other methods, the CNTs- DNA are a preferred choice and the inclination towards dsDNA is used over ssDNA in gene delivery shows effective results. The only downside of CNTs is that they are hydrophobic and are difficult to form an aqueous solution, thus limiting their applicability. This review

will aid you in comprehending useful knowledge related to a general overview of topics related to CNTs.

Keywords: Carbon Nanotubes, Transgenic Plants, Gene delivery, SWCNTs, MWCNTs, Functionalization

INTRODUCTION

Transgenic plant development is an advancement in the fields of plant biotechnology which can solve our problem of limited agricultural produce due to various biological and environmental stresses. The primary reason is to develop a transgenic plant is to obtain a desired trait, introduce a foreign or transgene with desirable characters for better results, to rectify some defective genes, obtaining favourable yield and to generate genetic variability. The desired gene from one plant can make wonders in a preferred plant, improving its quality and quantity. Ever since Transgenic Plants came into the spotlight, it has captivated the researchers in developing and advancing new methods for generating them.

Gene delivery will help in transformation of plants and creation of Genetically Modified plants (GM plants). These plants or crops developed would have perks like increased crop yields, ability to tolerate extreme climates, resistance to pests, improved food quality and increased shelf life. This would prove important with the increase in extreme weather events occurring rapidly and increasing population. For example, using genes from a stress tolerant plant like *Aloe vera*, would help in development of plants or crops which can withstand extreme weather conditions like drought.[1] Some studies have also shown that genetically modified plants are quite useful in the biofuel sector. A few prominent examples of GM plants also include Bt Cotton to combat Bollworm, Golden rice with increased Vitamin A content. Conventional methods which have been used are excellent at gene delivery. CNTs are the new age vectors with immense benefits. To understand and know the role of CNTs in development of transgenic plants, review of research papers in the field was done and the key findings are discussed further.

Conventional Gene Delivery Methods

The most common and preferable conventional techniques to introduce genes into plants cells can be categorized into - Physical methods, Chemical methods, biological methods.

Physical methods

The most favorable one is physical gene delivery techniques are Biolistic particle delivery also known as Gene gun delivery and the other technique include Electroporation (the use of electric field to induce pores into cell membranes. In Biolistic gene delivery they use heavy metals such as tungsten or gold in order to cover or coat the genes which are getting transferred [2]. Then with the help of high-pressure Helium pulses they are targeted into plant cells at certain height. This method is quite inexpensive and easy to perform.

Electroporation, as its name suggests uses electric pulses in order to create several small pores into plant genome. This method was developed in 1982. Some studies have shown it has successfully transferred into plants such as Wheat, Rice and Tobacco.[3] Electroporation has shown some significant results in vivo gene delivery for trans genetic expression, vaccine transfer etc. [4] Although this method is cheap and fast, it has certain shortcomings.

Chemical Methods

Here, they use agents such as Cationic lipids to transfer gene material into plant cells (Cations such as Ca^{+}). A polymer called Polyethylene glycol, which transfers the genetic material directly into protoplasm. When the PEG solution was added to plant membrane, the membrane got soften and it allowed the passage of DNA to enter into plants cells smoothly. Although it's a highly efficient technique but it is restricted to some plants only [3].

Biological Methods

As it was discussed earlier, *Agrobacterium tumefaciens* which is a Gram- negative bacterium is used to transfer genes. [5]

Agrobacterium-mediated transformation is the commonly used method for plant genetic engineering because of its high efficiency. Initially it was concluded that *Agrobacterium* only infects dicotyledonous plants, but it was later established that it can also be used for transformation of monocotyledonous plants such as Rice [3]. The reason why it is so widely used because of its ease of protocol, it is also inexpensive and it has high efficiency.

Carbon Nano Tubes (CNTs) for gene delivery

Over the past few years, research in the field of genetics or biotechnology has primarily focused on nanomaterials and nanotechnology. Especially nanomaterials which are particularly well suited for various medical related applications due to their incomparable properties such as their availability in various sizes, simplistic synthesis, enhanced conductivity and strength, ease of functionalization etc. In addition, CNTs are effectively taken up by many different cell types through various mechanisms. CNTs have also acted as carriers of Anti-cancer molecules, Anti-inflammatory drugs, Drug delivery etc. [6,7].

The major properties of CNTs suitable for application in biotechnology are determined by the combination of their structure, physical and chemical parameters with their nano-size. CNTs are hollow cylindrical tubes usually made up of carbon or graphene sheets with diameters typically measured with nanometers [8]. Resting on these terms, they can be either Single -Walled (SWCNT) or Multi-Walled (MWCNT). CNTs also have unique thermal, electrical and mechanical properties which make them interesting for the development of new materials [9]. Some recent studies have highlighted that CNTs while using on plants have shown some great results.

SWCNT & MWCNT

SWCNTs and MWCNTs have been getting a quite a lot of attention because of their perfect properties in the fields of Genetic and

Biotechnology. Single-walled Carbon Nanotubes are made up of a single layer of Graphene which forms a cylindrical shape. It is seen as two planes under an electron microscope [8]. Multi-walled Carbon Nanotubes are made of multiple layers of Graphene, which form concentric pattern around the smallest nanotube. Making it a little complex than SWCNTs. Some studies revealed that MWCNTs improved the water uptake of plants to salinity [9]. SWNTs can be synthesized to make smallest size [~ 1 nm] which is below the plant size exclusion limit of ~20 nm and provide a large surface area. Therefore, the resulting large surface area to volume ratio is ideal for the fast loading of large quantity of biological cargo. Diameter of SWCNTs is less than 5 nm which allows them to pass through pores of a plant's cell wall [10].

In addition, even the shortening of CNTs length to below 1 μm has been proven useful for increasing the cell penetrating efficiency of the CNTs. Using shortened CNTs is useful for delivering required cargoes into walled plant cells. MWCNTs are less suitable nano-carriers to penetrate through a cellulose rich plant cell wall due to their larger diameter than in SWCNTs.

Functionalization of CNTs

CNTs can be functionalized with proteins, nucleic acids, drugs, polymers and are used to deliver cargo to cells. Covalent functionalization improves the biocompatibility of pristine (chemically unmodified) CNTs [11]. But their physical properties are altered. Non-covalent functionalization of CNTs uses amphiphilic molecules like surfactants and polymers which have different non polar and poly-aromatic regions. Molecules used wrap around CNTs or get adsorb on it. This type of functionalization does not change the special properties of CNTs. For biological applications the substances used for non-covalent functionalization should have properties like being biocompatible and nontoxic, stable attachment [12]. Functionalization of CNTs help in reducing its toxicity to plant and even mammalian cells. Also, it has been shown that functionalized

SWCNTs do not cause cytotoxicity or damage to the tissues when used in appropriate concentrations of <10 mg L−1 [13]. Functionalized CNTs are also better at dispersing in solutions and could be effectively used for biological applications.

Some substances used for functionalization are Poly-3-aminobenzenesulfonic acid (PABS) [14], Fluorescein- polyethylene glycol (Fluor-PEG) [15], Cellulase [16,17], H_2SO_4/HNO_3 mixture [18], Pyrrolidine ring bearing a free amino-terminal Oligoethylene Glycol moiety attached to the Nitrogen atom [19], Aliphatic Amine group [20], Arginine [21], Polyethylenimine [22], Chitosan [23].

Fouad, 2008 incubated *Arabidopsis thaliana* cells with cellulase functionalized CSCNT, and this functionalization led to better entry of the CNT-DNA conjugates into walled plant cells due to the nanoholes created by the cellulase enzyme. This functionalization was done via a Carbodiimide reaction. This cellulase functionalization helps in penetration of CNTs without removal of cell wall as its removal often affects cell viability and division of cells. Cell wall is often removed for better delivery but by this method it could be avoided [10]. Functionalization with Fluorescein-polyethylene glycol (Fluor-PEG) imparted better solubility and fluorescent labelling to the nanotubes. The molecule binds strongly to the SWCNT [15]. CNTs modified with Pyrrolidine ring bearing a free amino-terminal Oligoethylene glycol moiety attached to the Nitrogen atom provided better solubility and were able to interact ionically with the plasmid DNA. These were then able to penetrate the cells without undergoing endocytic cycle and had more gene expression than using DNA alone [19]. Ochoa-Olmos, 2016 noted that pristine MWCNTs used were not able to form conjugates with plasmid, but CNTs modified with Aliphatic Amines show better interaction and were able to form conjugates [20]. Biocompatible Arginine functionalized single-walled Carbon Nanotubes were used for transferring plasmid DNA in Tobacco root cells. Polyarginine acts as a nuclear localization signal which can transfer cargo to the nucleus [21]. Carboxylated SWNTs were modified with Polyethylenimine [PEI], a polymer [13].

Functionalization by PEI helps in neutralizing the negative charge of the DNA and compressing it, as it is a cationic polymer and has a strong positive charge. Also, it protects the DNA by endosomal degradation due to the proton sponge effect [22]. Chitosan coated CNTs are also used for gene delivery as they cross plant membranes, and can enter Chloroplast. They are biodegradable and non-toxic too and protect DNA from Nuclease degradation [23, 24].

DNA binding to CNTs and Gene delivery

DNA and CNTs interact with each other because of the interaction between nucleotides of DNA and CNTs via non-covalent π-stacking. The hydrophilic DNA backbone provides solubility in water. The interaction of CNT- DNA is stable and is due to physical and electronic properties of each nanotube. The models studied show that the DNA wrapped helically around the CNTs and flexibility of DNA led to formation of different structures. This is also understood by a simulation using Microscope Simulator program [25]. Burlaka,2015 claim that the structural change in DNA during formation of conjugation are due to A–B conformation transitions and compared the DNA-CNT interaction as the one in chromosomes during DNA assembly by histones [26]. There was a decrease in the water molecules of the DNA observed, which suggested that the nanotubes displace these water molecules during their interaction with DNA. The nanotubes insert themselves in the major or minor grooves of the DNA they are forming conjugates with [27].

CNTs which are not functionalized, with Double-stranded [dsDNA] and Single-stranded (ssDNA) can even 'self- assemble' or form conjugates by itself without any external aid by hydrophobic and stacking interactions [20].

DNA-SWCNTs are much stable than free DNA. DNA bonded to SWCNTs are protected from Nuclease digestion, provide better delivery. SWCNTs protect the DNA from degradation by enzymes like Nucleases, as the DNA might get embedded in the hollow tubes and it will prevent interaction of DNA with the enzymes. Some hydrophobic

sites of CNTs present even after the functionalization might repel the enzymes away from the DNA- CNT. The structural changes caused by DNA-CNT interaction might make them unrecognizable to the enzymes.

CNTs often do not interact with supercoiled plasmid DNA, but easily with linear DNA. This could be due to the compactness of supercoiled DNA which does not let the nitrogenous bases for interaction. Linear dsDNA binds spontaneously to CNTs due to the partially unwind sections of DNA molecules. CNTs tend to destabilize the double helix of DNA.

Modification of CNTs can make nucleic acids attached to them effectively. Also, these nucleic acids act as functionalizing agent themselves. DNA-MWCNTs are strongly associated with each other as compared to SWCNT. ssDNA-CNT attachment is found to be dependent on the specific DNA sequence [28]. The Process of grafting DNA onto CNT is described by Demirer,2019 as follows:

1. Using dialysis to directly adsorb DNA onto CNTs, the CNTs have to coated with a surfactant like Sodium Dodecyl Sulphate.
2. Using Carboxylated CNTs [COOH-CNT] and PEI, a polymer to graft DNA to CNT by electrostatic grafting [29].

DNA-CNT in gene delivery

CNTs are efficient and are useful for transient DNA-free genome editing of plant and can also deliver multiple biomolecules [30]. CNT-DNA conjugates are useful in delivery of genes, but for which the DNA should be neatly condensed on CNT, must be carried to the cell and be able to detach itself from the CNT when enters the nucleus.

Demirer,2017 observed that localization of ssDNA-SWCNTs in leaf lamina, parenchyma tissue and chloroplast of parenchyma cells was done by imaging leaf tissue cross-section thus proving that these conjugates could pass through the plant Cell wall, Cell membrane and the chloroplast Cell membrane. And even did not damage the plant

cell by their infiltration [31]. Liu, 2009 demonstrated that the SWCNTs/FITC-DNA conjugates were able to penetrate the plant cell wall. Fluorescein isothiocyanate (FITC) is used for fluorescence labelling for indicating their location. *Nicotiana tobacum* L. Bright Yellow (BY-2) cells were used, and after incubation with DNA-SWCNT, show intracellular fluorescence. But the fluorescently labelled DNA was not able to enter the cell, thus proving the requirement of SWCNTs [18]. Cellulase functionalized CSCNT-DNA can deliver DNA in *Arabidopsis thaliana* mesophyll cells, as the plasmid used Green Fluorescent Protein (GFP) gene was observed inside the cells [10]. Demirer,2017 grafted onto GFP plasmids onto SWCNTs and MWCNTs. These conjugates were infiltrated into the leaves of *Arugula* from an abaxial surface with needle less syringe. They were imaged after 72 hrs and GFP expression was found in mesophyll cells of leaf lamina. Where CNTS were not present no GFP expression was seen. This GFP expression is transient as it disappears after 7 days, this shows that genes delivered through CNTs do not enter the plant nuclear genome, which could be beneficial for transgene expression without transgene integration. Protoplasts were incubated with plasmid DNA-CNT solution. GFP expression was observed. Higher concentrations hampered viability and health of cells [31].

MWCNT-DNA can deliver the DNA in the cytoplasm and nucleus as the electrostatic interaction between the conjugates loosen, and let the DNA express inside the nucleus [20]. MWCNTs are useful as they diffuse into the cytoplasm by direct penetration and do not enter the endosomal cycle. This is beneficial as there is no endocytosis and endocytic degradation and MWCNTS can deeply enter in the nucleus and can easily deliver DNA [32].

CNT-DNA is a better option for creating transgenic plants as it is easy, not specific for species, cost-effective and does not let the DNA or the transgene integrate with the original genome of the plants, unlike *Agrobacterium*- mediated transformation. Further even other nucleic acids like dsRNA could be used and delivered in plant cell nucleus for gene activation [33]. And any gene of interest could be used for

transformation of the genome. Plants transformed using SWCNTs would undergo several generations of progeny production before their seeds are cultivated, and therefore the crops grown would be of the generations that have never undergone direct exposure to these nanotubes [13]. In this way they are potential vectors for gene delivery.

DNA solubilizes CNTs

Pristine CNTs are not soluble in water or other aqueous and organic solvents. They are hydrophobic and aggregate in bundles and do not separate singularly when present in an aqueous solution and this changes their unique properties and makes them difficult to use. CNTs align parallel to each other and form bundles due to strong Van der Waals attraction [34]. But functionalization of the CNTs can make them little hydrophilic which in turn can solubilize them so they are easier for application. CNTs could be modified non-covalently by using surfactants and polymers. CNTs could be functionalized with biological substances which will help in dispersing them and will not require excess functionalizing agents. This functionalization can be done by the process of Sonication [35].

Single-stranded DNA (ssDNA) is useful for non-covalent functionalization as it disperses and solubilizes the CNTs. ssDNA is used often, but even double stranded DNA (dsDNA) could be used as it is less costly and could be easily synthesized from natural sources [36]. Ultrasonication helps in the separation of aggregated CNTs and produces individual nanotubes. Ultrasonication is nothing but a technique which uses sound energy to agitate particles [37]. The process should be carried in low temperatures, as heat generated during could denature the biomolecule. Technique of milling is also useful for creation of DNA-CNT conjugates. Sánchez-Pomales,2010 suggests the DNA:CNT ratio of 1:1 or 1:2 for best dispersion [35].

Nakashima, 2003 mentioned that the pi-pi interaction between the sidewall of CNTs and nucleic acid bases of DNA are the possible reason why the nanotubes disperse with the help of DNA. Also, the

partial deformation of the whole structure caused by the process of Ultrasonication might aid to it [38]. Sodium dodecyl Sulphate (SDS), a surfactant and an amphiphilic molecule which is also often used for non-covalent functionalization of CNTs and can disperse or solubilize them works similar to ssDNA, but differs in how it attaches onto the CNT, as it attaches itself irregularly than ssDNA [25]. CNTs will often resist binding to DNA, as they have a strong attraction to cling to each other and form ropes. But still DNA is more effective in dispersion of CNTs than any other polymer due to its chain flexibility and charge of backbone [39].

Applications of CNTs

Carbon Nanotubes have different applications in various fields like Nanoelectronics, Agriculture, Remediation and Biomedical Research. These nanotubes are important in the area of agricultural production, as they can be used as Smart delivery systems, Nano-emulsions, Nano-sensors and Nano-catalysts which may help in acting as vector for delivery of chemicals like fertilizers and pesticides to it required location, and thus being precise and avoiding any wastage [14]. The unique properties of CNTs like small size, large surface area, special electronic structure and good conductance makes them useful for applications like Biosensing and Electrical detection. Carbon Nanotubes as molecular carriers for delivery of proteins and DNA into mammalian cells. These biomolecules could bind to the unique structure of CNTs [40].

Drug delivery is a major application of CNTs as the cavity created by the hollow nanotubes can carry and encapsulate various large molecules like Mellifluence, Fullerenes and DNA to deliver them to the target cells. Functionalized nanotubes are better at this process [41]. One of the applications of CNTs is also Genetic vaccination and Gene therapy. CNTs which are functionalized, positively charged and water soluble can penetrate into cells and can transport plasmid DNA as they form DNA-CNT conjugates. They can be used in place of the conventional viral delivery system for gene transfer [19]. And by non-

covalently functionalizing SWCNTs with PEGylated fluorescein, the fluorescence intensity emitted can be used for detection, imaging and cell sorting in biological applications.

CNTs are extremely useful in Biotransformation as it provides various benefits like faster gene transfer than other vectors, protection of DNA by binding or encapsulating it by any damage which could led to gene mutation. Combination of with physical method causes effective and improved gene transfer [42]. CNTs are good adsorbers with their hollow and layered design and surface chemistry which could be easily modified. They are functionalized to improve or increase their affinity towards specific pollutants, and can be used to adsorb toxic pollutants selectively when used for remediation. They are also quite resistant to heat and changes in pH and therefore could be used in severe conditions for clearing pollutants. Various features of CNTs like Anti-microbial action, customizable pore size, surface chemistry which could be modified easily by functionalization, and electrical conductivity make them an effective material for preparation filters for processing polluted water [43]. SWCNTs and Peptide Nucleic Acid (PNA) which is an uncharged DNA analogue have been combined and hybridized with complementary DNA to use them as probes in biological systems by a sequence specific attachment [44].

DISCUSSION:

Conventional gene delivery methods used like the Biolistic particle delivery are easy and convenient but could rupture or damage target cell organelles. *Agrobacterium*-mediated transformation is highly species specific and expensive. Electroporation can cause cell damage and works for limited species of plants. Hence, a new technique or vector could help in solving these problems.

Carbon Nanotubes are cylindrical molecules that are made up of rolled-up sheets of single-layer carbon atoms (graphene). The two forms of these nanotubes are Single walled Carbon Nanotubes (SWCNT) and Multi walled Carbon Nanotubes (MWCNT), which differ from each other on the basis of the number of Graphene walls

they are made up of. Studies show that MWCNT are less suitable as vectors than SWCNT due to their large diameters. Shortened CNTs are also quite successful in delivering cargoes into walled plant cells. Their small size makes them useful for entering cells. Because of the unique thermal, electrical and mechanical properties, they are used as vectors for Anti-cancer, Anti-inflammatory molecules, Drug delivery and their potential for gene delivery could also be tapped.

Functionalization or modification of nanotubes is the method of adding desired properties to a material by changing its surface chemistry. Surface functionalization of CNT is proven to be important and helpful as it reduces toxicity of the CNTs dramatically and also makes them disperse readily in aqueous solvent as pristine CNTs do not readily dissolve due to strong Van der Waals forces between them. This is helpful for creation of solutions of CNTs which are then easier for biological applications, as CNTs tend to form bundles or aggregates and are difficult to separate into a single nanotube. This also affects the wrapping or coating of DNA, which is used for gene delivery. Many studies also use biocompatible molecules like Chitosan, Cellulase and Arginine for modification, which are biodegradable and non-toxic too, and do not cause any damage to the cells. Being biological in nature solves the issue of using a synthetic molecule while generating transgenic plants. Functionalization with molecules like PEI also facilitate the CNT-DNA to avoid the Endocytic cycle, in turn protecting them from Endosomal degradation and increasing their chances of entering the nucleus. Modification with fluorescent molecules can provide with labelling of these vectors when carrying any cargo and help in localization studies.

When using CNTs as a vector for delivering a gene, it is important to study the interactions between DNA and CNTs. CNTs prove to be a good vector for gene delivery as it protects the DNA from degradation of enzymes which are present in the cell. Studies also show that free DNA is not as stable as DNA combined with CNTs inside a cell. They bind to each other strongly, and these conjugates can be prepared easily with simple techniques. Ultrasonication is a good technique for

preparing these conjugates. Functionalization of CNTs improves their interaction with DNA, but at the same time DNA also acts as a molecule which can functionalize CNT and disperse them in a solution. This polymer is more effective than any other molecule in dispersion of CNTs due to their flexible and charged backbone. But even without any modification, CNTs can self-assemble and form a strong conjugate with DNA.

DNA-CNTs are useful in gene delivery, as many studies showed that these conjugates enter the plant cell membrane, nuclear membranes with ease. They could be localized in the nucleus and cytoplasm. Using CNTs as a vector for delivering DNA with plant cells is beneficial as DNA or the gene delivered does not insert itself into the recipient plant's genome. All these aspects of CNTs explored show that they can help is preparation of transgenic plants with our desired qualities. As we are exploring the role of CNTs in the creation of transgenic plants, CNTs have many other applications as their unique structural features allows them to carry cargoes effectively. They could be prominently used to Deliver drugs to target cells, fertilizers and pesticides in Agriculture without any wastage. As they are good absorbers, they show promising results in Remediation of polluted areas by effective adsorption of pollutants in soil or water.

CONCLUSION:

Effectiveness and feasibility of CNTs as vectors for gene delivery is opening a new age for synthetic vectors and development of transgenic plants. CNTS are potential vectors for gene delivery due to their characteristic structure, affinity towards DNA, multitude of options for modification and ability of penetrating cell membranes with ease. Toxicity of CNTs is often debated, but could be tackled with modification of the nanotubes.

REFERENCES:

1. Sharon, K., & Suvarna, S. (2017). Cloning of HVA22 homolog from Aloe vera and preliminary study of transgenic plant

development. *Int. J. Pure App. Biosci*, *5*(6), 1113-1121.

2. Klein, Ted M., Edward D. Wolf, Ray Wu, and J. C. Sanford. "High-velocity microprojectiles for delivering nucleic acids into living cells." Nature 327, no. 6117 (1987): 70-73.
3. Demirer, Gozde S., and Markita P. Landry. "Delivering genes to plants." Chemical engineering progress 113, no. 4 (2017): 40-45
4. Young, Jennifer L., and David A. Dean. "Electroporation-mediated gene delivery." Advances in genetics 89 (2015): 49-88.
5. Binns, Andrew N. "Agrobacterium-mediated gene delivery and the biology of host range limitations." Physiologia Plantarum 79, no. 1 (1990): 135-139.
6. Karimi, Mahdi, Navid Solati, Amir Ghasemi, Mehrdad Asghari Estiar, Mahshid Hashemkhani, Parnian Kiani, Elmira Mohamed et al. "Carbon nanotubes part II: a remarkable carrier for drug and gene delivery." Expert opinion on drug delivery 12, no. 7 (2015): 1089-1105.
7. He, H., Pham-Huy, L.A., Dramou, P., Xiao, D., Zuo, P. and Pham-Huy, C., (2013). Carbon nanotubes: applications in pharmacy and medicine. BioMed research international, 2013.
8. Del Bonis-O'Donnell, Jackson T., Abraham Beyene, Linda Chio, Gözde Demirer, Darwin Yang, and Markita P. Landry. "Engineering molecular recognition with bio-mimetic polymers on single walled carbon nanotubes." Journal of visualized experiments: JoVE 119 (2017).
9. Martínez-Ballesta, Mª Carmen, Lavinia Zapata, Najla Chalbi, and Micaela Carvajal. "Multiwalled carbon nanotubes enter broccoli cells enhancing growth and water uptake of plants exposed to salinity." Journal of nanobiotechnology 14, no. 1 (2016): 1-14.
10. Fouad, M., Kaji, N., Jabasini, M., Tokeshi, M., & Baba, Y. (2008). Nanotechnology meets plant biotechnology: carbon nanotubes deliver DNA and incorporate into the plant cell structure. In *12th*

international conference on miniaturized systems for chemistry and life sciences, San Diego, California, USA (pp. 12-16)

11. Riley, M., & Vermerris, W. (2017). Recent Advances in Nanomaterials for Gene Delivery—A Review. *Nanomaterials*, *7*, 94.

12. Burlaka, O., Yemets, A., Pirko, Y., & Blume, Y. (2016). Non-covalent Functionalization of Carbon Nanotubes for Efficient Gene Delivery. *Nanophysics, Nanophotonics, Surface Studies, and Applications*, 355-370.

13. Demirer, G. S., Zhang, H., Goh, N. S., González-Grandío, E., & Landry, M. P. (2019). Carbon nanotube– mediated DNA delivery without transgene integration in intact plants. *Nature protocols*, *14*(10), 2954-2971.

14. Cañas, J. E., Long, M., Nations, S., Vadan, R., Dai, L., Luo, M., ... & Olszyk, D. (2008). Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species. *Environmental Toxicology and Chemistry: An International Journal*, *27*(9), 1922-1931.

15. Nakayama-Ratchford, N., Bangsaruntip, S., Sun, X., Welsher, K., & Dai, H. (2007). Noncovalent functionalization of carbon nanotubes by fluorescein− polyethylene glycol: supramolecular conjugates with pH-dependent absorbance and fluorescence. *Journal of the American Chemical Society*, *129*(9), 2448-2449.

16. Serag, M. F., Kaji, N., Tokeshi, M., Bianco, A., & Baba, Y. (2012a). The plant cell uses carbon nanotubes to build tracheary elements. *Integrative Biology*, *4*(2), 127-131.

17. Serag, M. F., Kaji, N., Tokeshi, M., & Baba, Y. (2012b). Introducing carbon nanotubes into living walled plant cells through cellulase-induced nanoholes. *RSC advances*, *2*(2), 398-400.

18. Liu, Q., Chen, B., Wang, Q., Shi, X., Xiao, Z., Lin, J., & Fang, X.

(2009). Carbon nanotubes as molecular transporters for walled plant cells. *Nano letters*, *9*(3), 1007-1010.

19. Pantarotto, D., Singh, R., McCarthy, D., Erhardt, M., Briand, J. P., Prato, M., ... & Bianco, A. (2004). Functionalized carbon nanotubes for plasmid DNA gene delivery. *Angewandte Chemie International Edition*, *43*(39), 5242-5246.

20. Ochoa-Olmos, O. E., León-Domínguez, J. A., Contreras-Torres, F. F., Sanchez-Nieto, S., Basiuk, E. V., & Dinkova, T. D. (2016). Transformation of plant cell suspension cultures with amine-functionalized multi- walled carbon nanotubes. *Journal of Nanoscience and Nanotechnology*, *16*(7), 7461-7471.

21. Golestanipour, A., Nikkhah, M., Aalami, A., & Hosseinkhani, S. (2018). Gene delivery to tobacco root cells with single-walled carbon nanotubes and cell-penetrating fusogenic peptides. *Molecular biotechnology*, *60*(12), 863-878.

22. Ghaghelestany, A. B., Jahanbakhshi, A., & Taghinezhad, E. (2020). Gene transfer to German chamomile (L chamomilla M) using cationic carbon nanotubes. *Scientia Horticulturae*, *263*, 109106.

23. Kwak, S. Y., Lew, T. T. S., Sweeney, C. J., Koman, V. B., Wong, M. H., Bohmert-Tatarev, K., ... & Strano,

M. S. (2019). Chloroplast-selective gene delivery and expression in planta using chitosan-complexed single- walled carbon nanotube carriers. *Nature nanotechnology*, *14*(5), 447-455.

24. Wu, Y., Phillips, J. A., Liu, H., Yang, R., & Tan, W. (2008). Carbon nanotubes protect DNA strands during cellular delivery. *ACS nano*, *2*(10), 2023-2028

25. Campbell, J. F., Tessmer, I., Thorp, H. H., & Erie, D. A. (2008). Atomic force microscopy studies of DNA- wrapped carbon nanotube structure and binding to quantum dots. *Journal of the American Chemical Society*, *130*(32), 10648-10655

26. Burlaka, O. M., Pirko, Y. V., Yemets, A. I., & Blume, Y. B. (2015). Application of carbon nanotubes for plant genetic transformation. *Nanocomposites, Nanophotonics, Nanobiotechnology, and Applications*, 233- 255.

27. Galina, D., Olena, F., Olena, G., Yaroslav, S., Lilia, W., Serena, B., & Zecchina, A. (2010). Nucleic acid interaction and interfaces with single-walled carbon nanotubes. *Carbon Nanotubes*.

28. Singh, R., Pantarotto, D., McCarthy, D., Chaloin, O., Hoebeke, J., Partidos, C. D., & Kostarelos, K. (2005). Binding and condensation of plasmid DNA onto functionalized carbon nanotubes: toward the construction of nanotube-based gene delivery vectors. *Journal of the American Chemical Society*, *127*(12), 4388-4396.

29. Demirer, G. S., Zhang, H., Matos, J. L., Goh, N. S., Cunningham, F. J., Sung, Y., & Landry, M. P. (2019). High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants. *Nature nanotechnology*, *14*(5), 456-464.

30. Wang, P., Lombi, E., Zhao, F. J., & Kopittke, P. M. (2016). Nanotechnology: a new opportunity in plant sciences. *Trends in plant science*, *21*(8), 699-712

31. Demirer, Gozde S., Roger Chang, Huan Zhang, Linda Chio, and Markita P. Landry. "Nanoparticle-guided biomolecule delivery for transgene expression and gene silencing in mature plants." BioRxiv (2017): 179549

32. Serag, M. F., Kaji, N., Gaillard, C., Okamoto, Y., Terasaka, K., Jabasini, M., ... & Baba, Y. (2011). Trafficking and subcellular localization of multiwalled carbon nanotubes in plant cells. *ACS nano*, *5*(1), 493- 499.

33. Serag, M. F., Kaji, N., Habuchi, S., Bianco, A., & Baba, Y. (2013). Nanobiotechnology meets plant cell biology: carbon nanotubes as organelle targeting nanocarriers. *RSC advances*, *3*(15), 4856-4862.

34. Liang, Z., Lao, R., Wang, J., Liu, Y., Wang, L., Huang, Q., ... & Fan, C. (2007). Solubilization of single- walled carbon nanotubes with single-stranded DNA generated from asymmetric PCR. *International journal of molecular sciences*, *8*(7), 705-713.

35. Sánchez-Pomales, G., Pagán-Miranda, C., Santiago-Rodríguez, L., & Cabrera, C. R. (2010). DNA-wrapped carbon nanotubes: from synthesis to applications. In *Carbon nanotubes*. IntechOpen.

36. Xu, Y., Pehrsson, P. E., Chen, L., Zhang, R., & Zhao, W. (2007). Double-stranded DNA single-walled carbon nanotube hybrids for optical hydrogen peroxide and glucose sensing. *The journal of physical chemistry C*, *111*(24), 8638-8643

37. Ontology. (2021). Retrieved July 26, 2021, from Rsc.org website https://www.rsc.org/publishing/journals/prospect/ontology.asp?id=CMO:0001708

38. Nakashima, N., Okuzono, S., Murakami, H., Nakai, T., & Yoshikawa, K. (2003). DNA dissolves single- walled carbon nanotubes in water. *Chemistry Letters*, *32*(5), 456-457.

39. Zheng, M., Jagota, A., Semke, E. D., Diner, B. A., McLean, R. S., Lustig, S. R., & Tassi, N. G. (2003). DNA-assisted dispersion and separation of carbon nanotubes. *Nature materials*, *2*(5), 338-342.

40. Majeed, N., Panigrahi, K. C., Sukla, L. B., John, R., & Panigrahy, M. (2020). Application of carbon nanomaterials in plant biotechnology. *Materials Today: Proceedings*, *30*, 340-345.

41. Husen, A., & Siddiqi, K. S. (2014). Carbon and fullerene nanomaterials in plant system. *Journal of nanobiotechnology*, *12*(1), 1-10.

42. Burlaka, O. M., Ya V. Pirko, A. I. Yemets, and Ya B. Blume. "Plant genetic transformation using carbon nanotubes for DNA delivery." Cytology and genetics 49, no. 6 (2015): 349-357.

43. Liu, X., Wang, M., Zhang, S., & Pan, B. (2013). Application potential of carbon nanotubes in water treatment: a review. *Journal of*

Environmental Sciences, *25*(7), 1263-1280.

44. Williams, K. A., Veenhuizen, P. T., Beatriz, G., Eritja, R., & Dekker, C. (2002). Carbon nanotubes with DNA recognition. *Nature*, *420*(6917), 761-761.

Chapter 3 - The Possible Derogatory Effects Of Sanitizers Containing Various Concentrations Of Alcohol Contents: A Pilot Study

Sonia Joshi, Rhea Nawalkar, Dr. Shalini A Rai*, Dr. Aashu Vajpai*, Mr. Gaurav G Bingi*
Department of Life Sciences, Kishinchand Chellaram College, Churchgate, Mumbai- 400020
shalini.rai@kccollege.edu.in, aashu.vajpai@kccollege.edu.in

ABSTRACT

Awareness and knowledge regarding the 'new normal' due to the onset of the Covid-19 pandemic, is often very vague, incomplete or partially true. Therefore, it is important to understand the repercussions of the newly introduced products in our life, primarily the sanitizers. Since the performed survey reveals that people are either unaware or already facing the side effects of the excessive use of sanitizers, it is important to spread the word of caution so that more people study the alcohol contents of the sanitizers before purchasing them. Contents of sanitizers define, not only the efficacy but also the collateral damage that can be caused due to subsequent overuse. Moderation while using any disinfectant product is a must and sticking to the basic hygiene practices would ensure a higher level of protection against pathogens of any kind.

Keywords: Alcohol, Overuse, Pandemic, Pathogens, Sanitizers

INTRODUCTION:

The first reported case of SARS-CoV2 was found in early December in China (Wuhan), changing the way the hygiene and healthcare sector functions [9]. Since the onset of the SARS-CoV2 pandemic, the usage of sanitizers has gone up exponentially and it has found its way into every home, public place, backpacks and mainly our daily routines. Even prior to the virus outbreak, few people around us had a habit of carrying sanitizers whilst being outdoors. Sanitizer market has seen a 10-fold jump since 2020 [17][2]. Apart from the most preferred formulation i.e.,

the gel, sanitizers are available in various other formulations which include liquid sanitizers, foam sanitizers and spray sanitizers [19]. About 350 new brands have entered the sanitizer market since 2020 in various forms [2]. Sanitizers can also be classified according to its basic raw material into- Alcohol based and alcohol-free sanitizers. CDC recommends alcohol-based sanitizers to combat the SARS-CoV2 pathogen [3]. Alcohol based sanitizers are primarily made using isopropyl alcohol, ethanol (ethyl alcohol), and/or n-propanol. The alcohol-based sanitizers are typically the most popular and the versions that contain 60 to 95% alcohol are most effective. Some versions of alcohol-based sanitizers contain compounds such as Glycerine/glycerol (a trihydric alcohol) [18]. Excessive use of sanitisers can have harmful health effects. This is mainly due to inhalation of fumes of sanitizers [7]. The more destructive element is the alcohol base of these products, fumes from sanitisers are essentially pure alcohol, thus leading to nausea, vomiting and irritation in the nasal tract [7].

Prolonged exposure to these fumes can lead to chemical burns in nasal passages and even addiction to the fumes, which is practiced through huffing of the vapours. This practice is known as alcohol vaping, a simple involuntary action which can turn into an addiction [7]. Depending on the brand of sanitisers, their alcohol percentages vary and therefore certain brands will have varied health effects than other brands. Since people have preferences towards a particular brand of sanitisers, thereby they are more prone to certain effects. A survey on a random population can help us understand the preferences of people and thereby we conclude and extrapolate the results to a bigger population size to get a rough idea, as to how much percentage of the chosen population would be prone to alcohol vaping by prolonged exposure.

METHODOLOGY:

In order to get a brief idea of the preference of sanitizer brands used by people and their views regarding the derogatory effects by overuse of sanitizers, a survey-based questionnaire was circulated by means of a Google form. The questionnaire contains detailed questions regarding

the frequency of use, brands and immediate health effects observed by the individuals if any. The questionnaire was divided into three sections.

Section 1 was basically created to impart awareness regarding the types of sanitizers available, their components and the possible health effects. Section 2 of the questionnaire was a short consent form which consisted of the background information regarding the survey which included the aim of the survey and a short explanation regarding the same and it also asks for the willingness of the participant to be a part of the survey.

Section 3 covered the personal details including name and age group. Books were divided as 15 to 24, 25 to 34, 35 to 44 and so on and capped it at 65. Following the personal details questions for based regarding the frequency of sanitizers used on a daily basis followed by changes in preferences of brand and in general the use of sanitizers before and after the onset of the Covid-19 pandemic. Questions also included any underlying pulmonary disorder and immediate effects of the sanitizer vapours if any. The form concluded with questions regarding effectiveness of sanitizers and whether or not that is awareness regarding the adverse health effects caused by the overuse of sanitizers.

OBSERVATIONS:

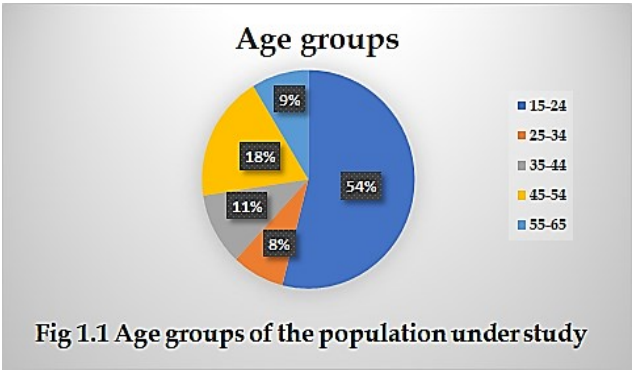


Fig 1.1 Age groups of the population under study

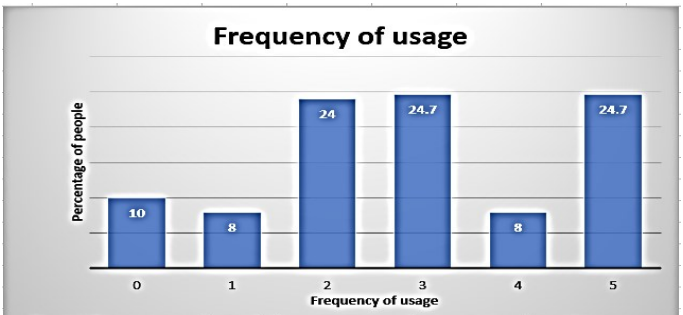


Fig 1.2 Percentage of people having various frequencies of sanitizer usage

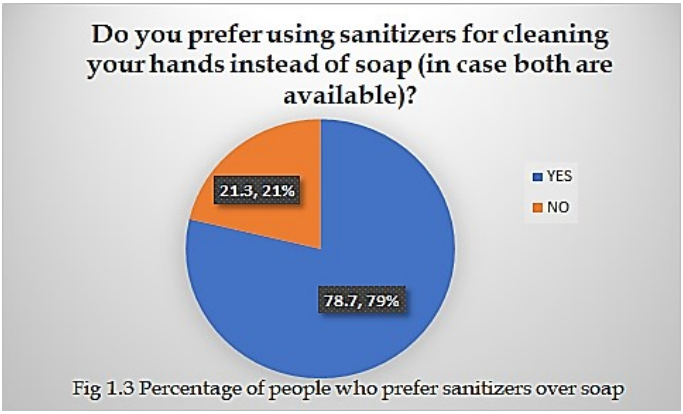


Fig 1.3 Percentage of people who prefer sanitizers over soap

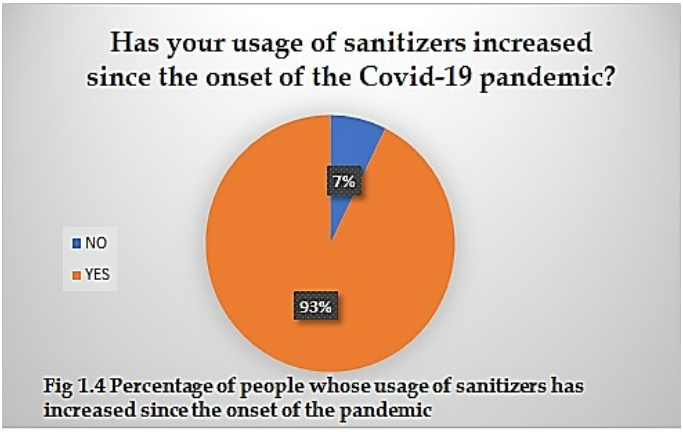


Fig 1.4 Percentage of people whose usage of sanitizers has increased since the onset of the pandemic

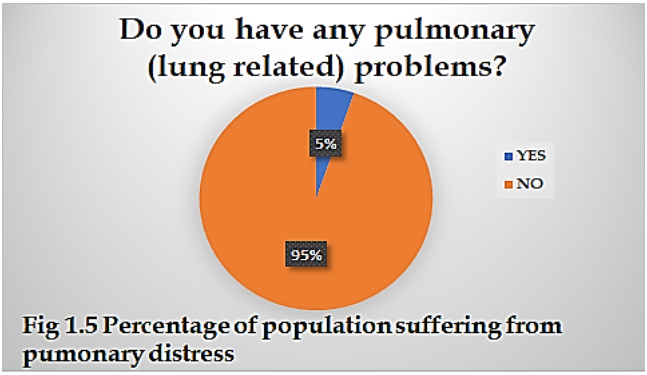


Fig 1.5 Percentage of population suffering from pumonary distress

Fig 1.6 Percentage of people who felt dizzy or disoriented due to vapours of sanitizers

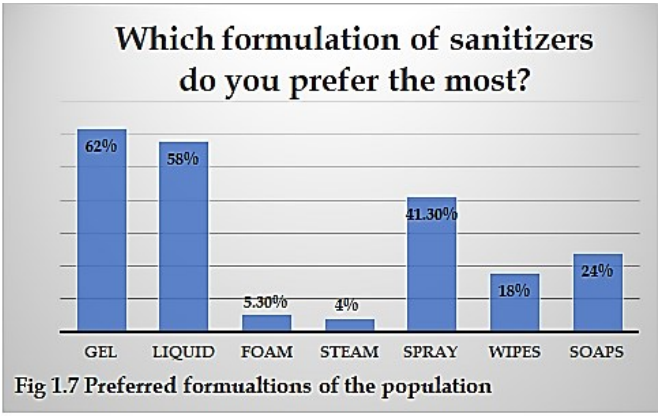


Fig 1.7 Preferred formualtions of the population

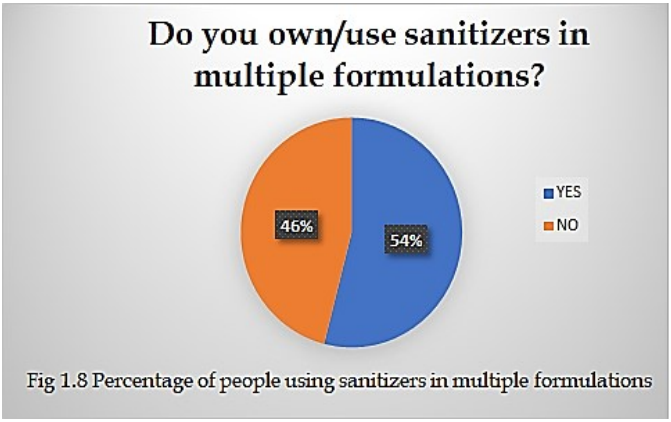


Fig 1.8 Percentage of people using sanitizers in multiple formulations

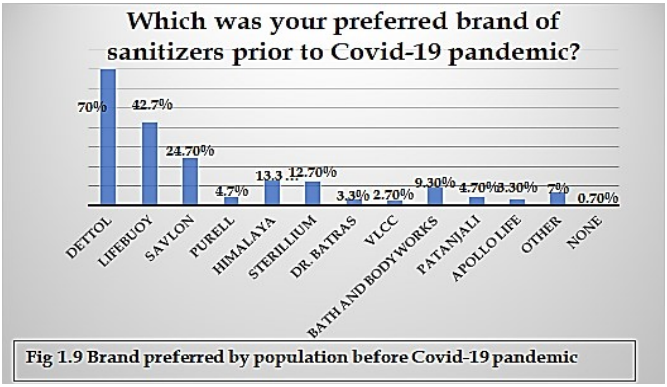


Fig 1.9 Brand preferred by population before Covid-19 pandemic

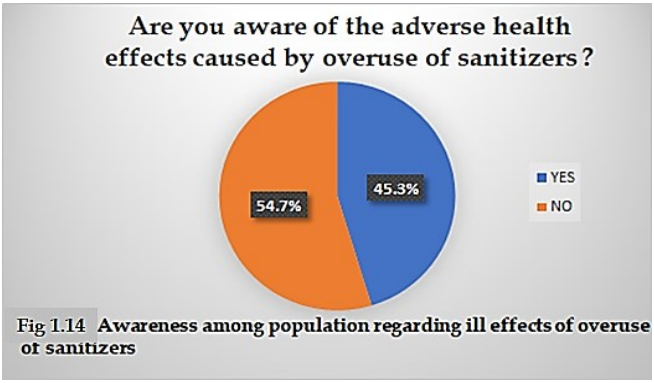


Fig 1.14 Awareness among population regarding ill effects of overuse of sanitizers

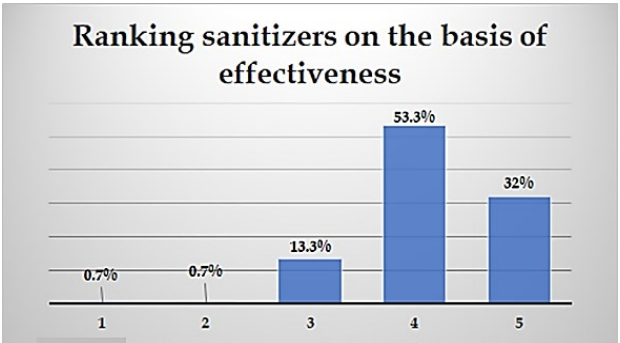


Fig 1.13 Ranking sanitizers on the basis of their effectiveness

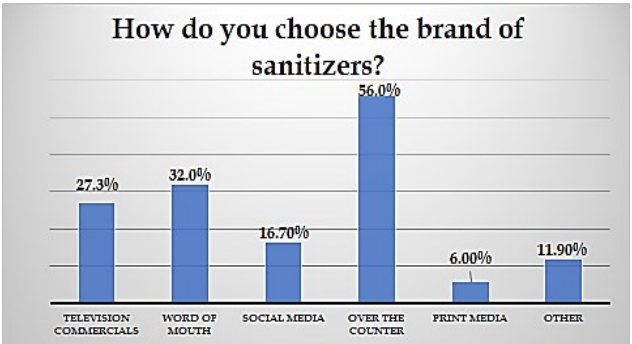


Fig 1.10 Basis of choice of sanitizers among the population

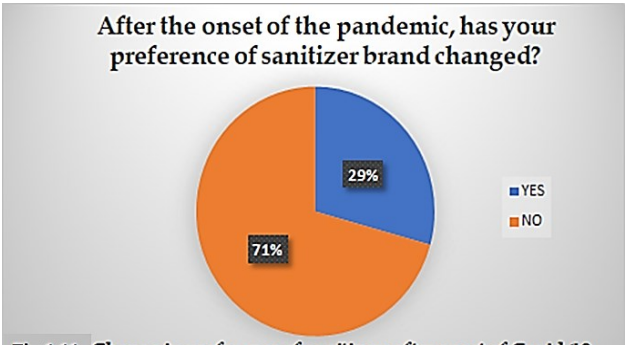


Fig 1.11 Change in preference of sanitizers after onset of Covid-19 pandemic

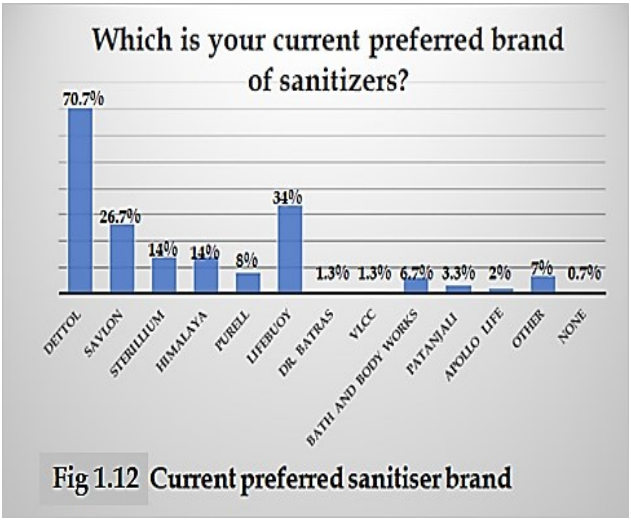


Fig 1.12 Current preferred sanitiser brand

ANALYSIS:

Since the data isn't normally distributed, analysing data by correlation is not possible. Hence Chi-Square Analysis has been applied on the collected data. The null hypothesis set up for the same was that, the population under study have awareness regarding overuse of sanitizers with the alternative hypothesis that people do not have awareness regarding overuse of sanitizers. By application of Chi Square Test, it was found that the null hypothesis can be rejected and the alternative hypothesis is accepted which states that the population under study is not aware regarding the repercussions by overuse of sanitizers. Thus, extrapolating the data, most people of the population are unaware of the harmful effects of overusing sanitizers.

RESULTS:

The participants to the survey belonged to various ages ranging from 15-65 years of age. Over 50% of the participants belonged to the age group of 15-24. The number of participants with sanitizer usage frequency of 3 and 5 times per day tied with each other at 24.7%. A little less than 25% of people admitted to be using sanitizer about 2 times a day. By this data,

we can extrapolate it to the population of India, and conclude that out of 136.64 crore people about 22.5 crore people would use sanitizer 3 and/or 5 times a day. About 21.3% of the population under study prefer to use sanitizers instead of soap and water to clean their hands. So, we can say that about 72.40 crore people in India would prefer sanitizers over traditional soap and water. Approximately 93% people under study claim that their usage of sanitizers has increased since the onset of the Covid-19 pandemic. That amounts to 85.28 crore people in India when extrapolated. Close to 95% people in the population under study didn't suffer from any pulmonary distress.

However, 11.3% of the people reported to feel dizzy and disoriented due to the vapours produced by sanitizers. Gel formulation is the most preferred formulation of the population under study followed by Liquid and spray formulations. About 54% of the people under study own/use sanitizers in multiple formulations, which would be approximately 49.68 crore people in India. Over 100 people from a population size of 150 preferred to use Dettol sanitizers prior to and after the onset of the Covid-19 pandemic. Second on preference is Lifebuoy followed by Savlon and Himalaya. Few other brands like Kaya, Godrej and Anveya. However, few reported to not be using sanitizers at all. Over 50% of the population under study claim to select their sanitizer over the counter. This number accounts to about 69 crore people in India. Word of mouth and Television commercials also show a significant impact in the choice of sanitizer brands.

About 71% of the population under study claim that their choice of sanitizer brands hasn't changed since the onset of the Covid-19 pandemic. About 53% of the people under survey rate the effectiveness of their sanitizers as 4 on a scale of 1-5. About 32% gave a 5-on-5 rating to their sanitizers. Approximately 55% of the population under study are unaware of the harmful effects of overuse of sanitizers. When extrapolated, this would amount to nearly 50.32 crore people in the country of India. Some people mentioned that they experienced headaches, dry skin, rashes, allergies, respiratory distress, flaking and peeling of skin

DISCUSSION:

The pandemic has triggered the growth of the sanitiser market by multi-folds as a result of the increased sales of sanitisers [17][2]. Not only has the usage increased, but also their preference of sanitisers has grown stronger and more definite. The survey conducted, can yield a conclusion that taking into consideration and formulation of certain brands, their sales of sanitisers is higher. Nevertheless, a large portion of the population does prefer brands, other than the most popular ones. One of the major reasons for analysing sanitisers via their brands, is the alcohol content that its formulation contains. A majority showed their preference in using gel sanitisers over liquid ones, however a study conducted conclusively proves that liquid sanitisers are more potent than gel-based formulations. This is because gel-based sanitisers require double the amount of time required by liquid sanitisers in order to show their action [10]. Apart from gel and liquid, there are other formulations available in the market, which include foam, spray, steam and medicated wipes. Most of these formulations are alcohol based.

CDC reports suggest that sanitisers with alcohol percentage of 60-95% have been classified as effective for providing a considerable amount of protection. The FDA being in tandem with WHO standards allows ethanol or isopropyl as potent ingredients for effectiveness of sanitisers [3]. The most common side effects of overuse of sanitisers are skin irritations, dry and broken skin, antibiotic resistance and weakening of the immune system [15]. Some other reported side effects as observed from the survey include dizziness due to fumes and light-headedness. Gas chromatography reflects that major volatile components of sanitisers include methanol, ethanol, n-propanol and iso-propanol [18]. Alcohol vapours have proven to be an effective method for alcohol inhalation study in animal models in controlled concentrations, however in higher concentrations it showed alcohol dependence and withdrawal [13]. Alcohol inhalation is a common phenomenon caused due to vapours released by the alcohol components of sanitisers that can over time lead to severe consequences.

Volatile substances like ethanol and isopropanol produce vapours which can be calculated using vapour pressure of the liquid. According to Raoult's Law, as the concentration of a solution increases, the more is its vapour pressure. Since various companies of sanitisers have different concentrations of alcohol content in their formulations, the number of vapours produced by them would also vary.

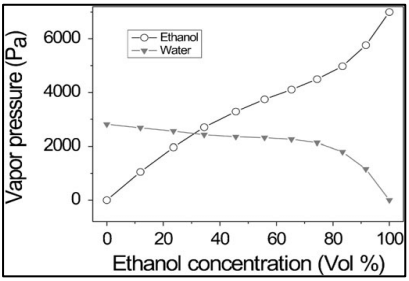


Fig 2.1: Comparison of Vapour pressures of water and ethanol at various concentrations

The above figure explains the phenomenon of Raoult's Law thus forming a bridge between alcohol inhalation and possible effects of the vapours produced by various sanitisers due to different alcohol concentrations. Long term exposure to sanitiser vapours can lead to headaches, nausea and vomiting. The fumes of alcohol can even affect the gastrointestinal tract. Fumes of isopropyl alcohol causes uneasiness in the membranes lining the nasal tract. Its reaction is similar to a chemical burn of the nasal passage. In moderately dangerous cases, constant inhalation may lead to sinusitis, sore throat and excessive headache. However, in worst case scenarios, it may ultimately lead to loss of the ability to smell as well as disruption of mucous membranes. In asthmatic patients, the consequences of repeated alcohol inhalation can cause increased difficulty in breathing [7]. Apart from the unconscious inhalation of these vapours, alcohol inhalation is practiced as a form of addiction known as huffing. The people who fall prey to this show symptoms of agitation, hallucinations, drowsiness and mood changes amongst many symptoms which can be similar to drug addiction [7]. Huffing can have lethal consequences, since sanitisers and other disinfectants have phenol

as their component which can lead to respiratory arrest in extreme conditions [7][4]. The survey reveals that a majority of the population preferred sanitisers manufactured by Dettol which has an average alcohol content of 70-80% which can be classified as one of the highest alcohol percentages in sanitizers [16]. By comparison, the second most preferred brand was Lifebuoy which has an average alcohol concentration of 95% denatured ethyl alcohol with 55% isopropyl alcohol [1]. Depending on the alcohol concentration, more is the concentration more is the susceptibility of the user to come in contact with the vapours produced by the alcohol.

CONCLUSION:

Despite the fact that sanitisers may lead to dangerous consequences in terms of health, they have now become a part of everyone's daily routine since the onset of the pandemic. As a common observation, a small percentage of people prefer sanitisers over washing hands with soap and water. A study reveals that doctors and researchers from the University of Southern California have stated that the use of soap and water should be preferred over sanitisers since it ensures removal of debris and dirt as well as removing pathogens. Therefore, washing hands with soap and water is considered to be more thorough as compared to using sanitisers [1]. However, the flip side of the coin reflects that use of sanitisers in limited proportions and frequencies wouldn't turn out to be extremely harmful. If overused, the internal body health would get affected along with disruption in skin cells and subsequent dry layers [7].

Apart from usage, the components of sanitisers should also be taken into consideration while using. FDA issued a warning against sanitisers containing methanol which can cause blurry vision, headaches, blindness, vomiting and nausea. Also, if accidentally ingested, methanol poisoning can be lethal in children or teenagers who would try to perform substance abuse with it [1]. Since sanitisers have made a way into everyone's lives proved by USA's reports of revenue collection from sanitisers which can amount to US$0.66 per US citizen. There have also been growths seen everywhere in the sanitiser market. Since we cannot

exclude this commodity from our new way of life, we can be careful and aware while buying and using. So, to ensure that sanitisers work in the way that they're actually meant to and not cause any collateral damage, awareness is a must and moderation is the key.

REFERENCES:

1. AAP News (2020), FDA issues warning for hand sanitizers containing methanol, *AAP News & Journals Gateway*, Retrieved from- https://www.aappublications.org/ news/ 2020/ 06/ 23/ hand-sanitizer warning 062320
2. Abid Hussain Barlaskar (2020), How large is the hand sanitizer market?, India, *Afaqs*, Retrieved from- https://www.afaqs.com/ news/mktg/ how large is the hand sanitiser-market
3. Centers for Disease Control and Prevention (2020), *Hand Hygiene Recommendations; Guidance for Healthcare Providers about Hand Hygiene and COVID-19*, Retrieved from- https://www.cdc.gov/ coronavirus/2019-ncov/hcp/hand-hygiene.html
4. David Campbell, (2020) Liquid Hand Sanitizer vs Gel Hand Sanitizer- Which One is Better? *Oiko Times*, Retrieved from- https://oikotimes.com/liquid-hand-sanitizer-vs-gel-hand-sanitizer-which-one-is-better/
5. Dettol Hand Sanitiser Original (2021), *Dettol Co.In* Retrieved from- https://www.dettol.co.in/personal-hygiene/hand-sanitiser/dettol-hand-sanitiser-original-200-ml/?gclid=CjwKCAjw95yJBhAgEiwAmRrutPXowugEfhWYZ9n12ZXePKvPAoOWwhwKR2lEpoaYh_BphvMwQ_JfIBoCmcsQAvD_BwE&gclsrc=aw.ds
6. Ecolab (2018), *Foam Sanitizer Safety Data Sheet*, California (USA), Retrieved from- https://safetydata.ecolab.com/ svc/GetPdf/ ?cntry= US&langid= en-US&sid=908598-04
7. Freedom Detox, (2017) *Huffing Hand Sanitizer- It's Real!*, North Carolina (USA), Retrieved from- https://legacyfreedom.com/ blog/ 2017/ 10/ huffing-hand-sanitizer-its-real/

8. Greenaway, R. E., Ormandy *et al,* (2018). Impact of hand sanitizer format (gel/foam/liquid) and dose amount on its sensory properties and acceptability for improving hand hygiene compliance. *The Journal of hospital infection*, 100 (2), 195–201. https://doi.org/10.1016/j.jhin.2018.07.011

9. Harapan H. *et al*, (2019) Coronavirus disease (COVID-19): A literature review. *J. Infect. Public. Health.*; 13(5): 667–673. https://doi.org/10.1016/j.jiph.2020.03.019

10. Jayshree Bhagat (2014), Lifebuoy Hand Sanitizer Review, *Makeup Review and Beauty Blog*, Retrieved from- https:// www.makeupandbeautyblog.in/ 2014/ 08/ lifebuoy-hand-sanitizer-review.html

11. Jennifer Nied, 2020, The 19 Best Hand Sanitizers of 2021, According To Experts, *Woman's Health Magazine*, Retrieved from- https://www.womenshealthmag.com/health/g31469255/best-hand-sanitizers/

12. Kauvery Hospital (2020), *What you need to know about hand-sanitizers?*, India, Retrieved from- https://kauveryhospital.com/ blog/lifestyle/what-you-need-to-know-about-hand-sanitizers/

13. MacLean *et al*, (2017). Inhalation of Alcohol Vapor: Measurement and Implications. *Alcoholism, clinical and experimental research*, 41(2), 238–250. https://doi.org/10.1111/acer.13291

14. Mahmood, A., Eqan *et al*, (2020). COVID-19 and frequent use of hand sanitizers; human health and environmental hazards by exposure pathways. *The Science of the total environment*, 742, 140561. https://doi.org/10.1016/j.scitotenv.2020.140561

15. Makati Medical Center, (2020*) Harmful Side Effects of Hand Sanitizers and Other Disinfectants*, Retrieved from- https://www.makatimed.net.ph/news-and-exhibits/news/harmful-side-effects-of-hand-sanitizers-and-other-disinfectants

16. Marci Robin (2020), Hand Sanitizer vs Handwashing: Which is Best for Preventing Spread of Germs?, *Allure*, Retrieved from- https://www.allure.com/story/hand-sanitizer-vs-washing-soap-water

17. Press Trust of India (2020), Hand sanitizer makers see 10-folds jump in sales; ramp up production, *The Times of India*, Retrieved from- https://timesofindia.indiatimes.com/business/india-business/hand-sanitizer-makers-see-10-folds-jump-in-sales-ramp-up-production/articleshow/74497994.cms

18. Science unfiltered (2020), Fast Analysis of Alcohol Based Sanitizers By Gas Chromatography, *Phenomenex blog*, Retrieved from- https://phenomenex.blog/2020/03/31/alcohol-based-sanitizers/#

19. Tech-Sci Research (2020), India Hand Sanitizer Market, by product type (Gel, Liquid, Foam and Spray) by End User (Hospital, Households, Restaurants & Hotels, and Others (Schools etc.), by Distribution Channel, by Region, Competition, Forecast & Opportunities 2030, *Research and Markets- The World's Largest Market Research Store*, Retrieved from- https://www.researchandmarkets.com/reports/5010965/india-hand-sanitizer-market-by-product-typ

Chapter 4 - A Comparative Study on the Phytochemical Potential of *Ocimum Basilicum, Ocimum Sanctum* and *Salvia Hispanica* - A Review

Mr. Siddharth Nakhwa, Dr. Tejashree Shanbhag*, Dr. Mayuresh Joshi*
Department of Life Sciences, Kishinchand Chellaram College, Churchgate, Mumbai- 400020
tejashree.shanbhag@kccollege.edu.in, mayuresh.joshi@kccollege.edu.in

ABSTRACT:

Plants are said to be the most important source of medicines. They also possess various bioactive nutrient compounds which are biologically active and are termed "Phytochemicals". The plants studied in this paper for the phytochemical and pharmacological potential are *Ocimum basilicum, Salvia hispanic,* and *Ocimum sanctum.* They belong to the family of Lamiaceae. It is a large family of herbs and shrubs. The various phytochemicals found were Flavonoids, Alkaloids, Terpenoids, Resins, Glycosides, Reducing sugar, Protein, Tannins, Starch, Steroids, Carbohydrates, Phenolics, Saponins, Amino acids, etc. Soxhlet extraction technique was used to obtain extracts from the various plant parts. Treatment of Covid-19 is costly and in few rural areas, proper medical facilities for the same are not adequately available. Extractions obtained from these plants can act as a preventive measure for Covid-19.

Keywords: *Ocimum*, *Salvia*, Lamiaceae, Phytochemical, Soxhlet

INTRODUCTION:

Ocimum basilcium: The plant having the scientific name, *Ocimum basilicum* which is commonly referred to as Sweet Basil around the world belongs to the family Lamiaceae[1]. The family Lamiaceae is known as the mint family of flowering plants consisting of 236 genera and about 7000 species which is largest family of the order Lamiales[2]. The phylogeny of the plant *Ocimum basilicum* is as follows:

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: *Ocimum*
Species: *basilicum* [1]

Ocimum genus contains around 50 to 150 species of herbs and shrubs from the tropical regions of Asia while it is unknown in many countries including Europe, North & South America [4][6]. Sweet basil plants are distributed over Persia and Sindh as well as lower hills of Punjab & Kashmir in India [3]. The plant is broadly grown in the greater part of India, Burma, Cyclone as well as Thailand, Vietnam, China & Srilanka [3][4]. The plant is also distributed in several Mediterranean countries along with Turkey, Iran and Pakistan [3][5]. In various parts of Asia, these seeds are used frequently in beverages such as Sharbat as well as ice desserts such as Faloodeh [6]. Sabja seeds, falooda seeds, tukamaria seeds are some numerous local names given as they act as a major source of dietary fiber and also for aesthetic purposes [6]. The phytochemical constituents that produce definite physiological action in the human body are the important criteria's that determine the medicinal value of the plant [7]. *Ocimum basilicum* has been highly valued for its medicinal properties in the traditional as well as modern medicinal system [8]. Basil seeds have been used in traditional medicine to treat different types of infections and diseases such as colic ulcer, dyspepsia, diarrhoea and many other inflammations [6]. To promote digestion, to stimulate respiratory circulation, and to alleviate mental fatigue and cold symptoms, basil seeds are used as a folklore medicine [8].

***Ocimum sanctum*:** The plant with the botanical name, *Ocimum sanctum* and the scientific name *Ocimum tenuiflorum* commonly referred to as Holy Basil or Sacred Basil belongs to the family Lamiaceae [9]. Lamiaceae is known as the mint family of flowering plants consisting of 236 genera and about 7000 species which is largest family of the order

Lamiales [10]. The phylogeny of the plant *Ocimum tenuiflorum* is as follow:

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: *Ocimum*
Species: *tenuiflorum* [9]

Within Ayurveda *Ocimum tenuiflorum* Linn is described with various names such as "Queen of Herbs", "Elixir of Life", "The incomparable one", "Mother medicine of nature", etc [11]. *Ocimum tenuiflorum* is considered as sacred plant and is worshipped all over India [12]. There are five different morph types that are mainly cultivated in India viz., Rama tulsi, Shyama tulsi, Vana tulsi, Sri tulsi & Krishna tulsi [11][13]. Tulsi is distributed in the entire subcontinent of India and it is cultivated on a commercial scale in various states like West Bengal, Maharashtra, Uttar Pradesh, Madhya Pradesh, Bihar, Jammu, Assam, etc. [14]. Apart from the Indian subcontinent, it is also grown on a large scale in Asia, Africa, Egypt, France, Hungary, Italy, Morocco, and USA [14]

Salvia hispanica: The plant with the scientific name *Salvia hispanica* which is popularly known as Chia all over the world [15]. It is an annual herbaceous plant that belongs to the family Lamiacea[15]. The family Lamiaceae is known as the mint family of flowering plants consisting of 236 genera and about 7000 species which is largest family of the order Lamiales[2]. The phylogeny of the plant *Salvia hispanica* is as follow:

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: *Salvia*
Species: *hispanica* [9]

The genus Salvia consists of approximately 900 species, which have been widely distributed for thousands of years around several regions of the world, including Central & Southern Mexico and Guatemala [16][17]. It is a short-day plant and commercially grown in Australia, Argentina, Colombia, Mexico and Peru [16]. Nowadays, Mexico is recognized as the world's largest chia producer [17]. *Salvia hispanica L.* is mainly grown for its seeds and produces white and purple flowers and it is sensitive to daylight [17]. The word chia is derived from a Spanish word chia which means oily, as it is an oilseed, with a power house of omega-3 fatty acids and α-linolenic acid [15][16]. Diets supplemented with chia have been found to decrease risks from 8 types of cadiovascular diseases, cancers and diabetes [16]. It has been reported that chia diet decreased the tumor weight and metastasis number [16]. There have been a lot of new discoveries regarding the nutritional properties, phytochemicals, and extraction methods regarding chia seeds [17]

METHODOLOGY:

Soxhlet Extraction

The plant part used for the extraction is first dried and then grinded uniformly using a mechanical grinder to make a fine powder [6]. The powder can be serially extracted in various types of solvents such as n-hexane, ethyl acetate, chloroform, ethanol, methanol, petroleum ether, water, etc. using a Soxhlet apparatus. The cycles of these mentioned solvents can be continued till complete extract is obtained [6]. The crude solvent extracts of the plant parts can be dried at room temperature and stored [6].

Soxhlet apparatus mainly consists of three major parts Percolator (boiler and reflux), Thimble & Siphon mechanism [18]. It is a continuous solid/ liquid extraction process which mainly contains the material to be extracted [18]. This material is placed in a thimble which is made of a material that will allow liquid to pass through and will hold the solids [18]. It mostly functions like a filter paper [18]. The thimble containing the dried plant part is placed in the Soxhlet extractor [18]. The required organic solvent is heated and as it boils the vapours rise up and they are

condensed by the condenser [18]. The condensed solvent fills up the thimble and it automatically siphons back down into the container of the required organic solvent [18]. This process repeats over and over again until all the plant material to be extracted from the dried solid in the thimble will be extracted into the required organic solvent [18].

Phytochemical Screening

The filtered crude extract obtained after performing the Soxhlet extraction technique is used for screening of phytochemical qualitative reactions such as Flavonoids, Alkaloids, Terpenoids, Resins, Glycosides, Reducing sugar, Protein, Tannins, Starch, Steroids, Carbohydrates, Phenolics, Saponins, Amino acids [6]. The colour intensity of the precipitate which was formed was used as analytical test controls [6].

Test for carbohydrates (Fehling's test)

About 1ml of Fehling A and Fehling B solution was added to the extract. Then, it was allowed to heat for 30 min and observed for the formation of brick red colour which indicated the presence of carbohydrates.

Test for alkaloids (Wagner's test)

A small amount of extract was taken in a test tube and 3-5 drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100 ml if distilled water) was added.

Formation of reddish-brown precipitate or coloration indicated the presence of alkaloids in the extract.

Test for alkaloids (Mayer's test)

A small amount of extract was taken in a test tube and 1 ml of Mayer's reagent was added. Formation of orange brown precipitate or coloration indicated the presence of alkaloids in the extract.

Test for alkaloids (Dragendroff's test)

A small amount of extract was taken in a test tube and 1 ml of Dragendroff's reagent was added. Formation of orange brown precipitate or coloration indicated the presence of alkaloids in the extract.

Test for saponins (Foam test)

About 0.1 g of sample was mixed with 5 ml of distilled water and allowed to boil. Then the mixture was filtered and 2 drops of olive oil was added in 1 ml of filtrate. The mixture was shaken and formation of emulsion and froth was observed. The 1ml filtrate was diluted by adding distilled water up to 4ml. The mixture was shaken vigorously and observed for a stable froth.

Test for flavonoids

The filtrate was prepared by boiling the mixture of 0.5g of sample and 10ml of ethyl acetate for min. Then, the mixture was filtered and 4ml of filtrate was shaken with 1ml of 1% ammonium chloride solution. Formation of yellow colour in the presence of ammonium solution indicated the presence of flavonoids.

Test for phenols (Ferric chloride test)

About 1ml of extract was mixed with 1ml of distilled water and warmed. To this, 2ml of ferric chloride solution was added. Formation of green or blue colour confirmed the presence of phenols.

Test for phenols (Potassium dichromate test)

About 1ml of extract was mixed with 1ml of distilled water and warmed. To this, 2ml of potassium dichromate solution was added. Formation of red precipitate confirmed the presence of phenols.

Test for phenols (Potassium permanganate test)

About 1ml of extract was mixed with 1ml of distilled water and warmed. To this, 2ml of potassium permanganate solution was added. Decolouration with turbidity confirmed the presence of phenols.

Test for tannins

About 5g of the dried and powdered sample was boiled in 20ml of water in a test tube with the aid of a water bath ad was filtered. Then, few drops of ferric chloride was added. Appearance of brownish green or bluish black coloration indicated the presence of tannins.

Test for terpenoids

The extract was evaporated to dryness and dil. HCl was added to the residue obtained. The mixture was vortexed and filtered through Whatman filter paper no. 1. From this, 1ml of extract was taken and 4 drops of chloroform along with few drops of conc. Sulphuric acid was added from the sides. Reddish brown coloration at the interface indicates the presence of terpenoids.

Test for resins

The extract was evaporated to dryness and dil. HCl was added to the residue obtained. The mixture was vortexed and filtered through Whatman filter paper no. 1. From this, 1ml of boiled extract was taken and 0.5ml of conc. Sulphuric acid solution was added slowly. Formation of reddish-brown coloration indicates the presence of resins.

Test for glycosides

In 1 ml of extract, few drops of distilled water and 0.5ml of 10% NaOH solution is added. Yellow coloration in the solution indicates the presence of glycosides.

Test for reducing sugar

1ml of extract along with 1 ml of DNSA reagent is allowed to boil for a few minutes. Orange coloration in the solution indicates the presence of reducing sugar.

Test for proteins (Biuret test)

In 1 ml extract, 0.5 ml copper sulphate and few drops of NaOH or KOH is added. Blue to violet coloration in the solution indicates the presence of proteins.

Test for proteins (Ninhydrin test)

1 ml of extract along with 1 ml of Ninhydrin reagent are mixed together in a tube. Blue or purple coloration in the solution indicates the presence of proteins.

Test for starch

1 ml of extract along with 1 ml of iodine solution are mixed together in a tube. Formation of blue colour in the solution indicates the presence of starch.

Test for steroids

1 ml of extract along with few drops of conc. Sulphuric acid solution are added in the tube. Formation of red colour in the solution indicates the presence of steroids.

Test for amino acids

1 ml of extract along with few drops of Ninhydrin reagent are added in the tube. Formation of purple or blue colour in the solution indicates the presence of amino acids.

RESULTS AND DISCUSSION:

Ocimum basilicum

Table 1. The phytochemical screening of *Ocimum basilicum* seed in petroleum ether solvent revealed the presence of alkaloids, carbohydrates, flavonoids, tannin and terpenoids [6].

The phytochemical screening of *Ocimum basilicum* revealed the presence of glycoside, gums, mucilage, proteins, amino acids, tannins, phenolic compound, triterpenoids steroids, sterols, saponins, flavones and flavonoids in it [19].

Table 2. The phytochemical screening of *Ocimum sanctum* leaf in aqueous extract revealed the presence of phenol, flavonoid, glycosides, and alkaloids [20]. The phytochemical screening of *Ocimum sanctum* leaf in methanol extract revealed the presence of carbohydrate, phenol, tannin, flavonoid, saponin, glycosides, terpenoid, alkaloids, fixed oils and fatty acids [20]. The phytochemical screening of *Ocimum sanctum* leaf in ethanol extract revealed the presence of carbohydrate, tannin, flavonoid, saponin, glycosides, terpenoid and alkaloids [20]

Phytochemical test	Results
Alkaloids	
Hager's test	+
Wagner's test	-
Carbohydrates	
Fehling's test	+
Molish test	+
Phytosterols	
Libermann Burchard's test	-
Phenols	
Ferric chloride test	-
Flavonoids	
Alkaline reagent test	+
Lead acetate test	
Tannin test	+
Terpenoids test	+
Salkowski test	-

Table 1

Phytochemicals	Aqueous extract	Methanol extract	Ethanol extract
Protein	-	-	-
Carbohydrate	-	+	+
Phenol	+	+	-
Tannin	-	+	+
Flavonoid	+	+	+
Saponin	-	+	+
Glycosides	+	+	+
Steroid	-	-	-
Terpenoid	-	+	+
Alkaloid	+	+	+
Anthraquinone	-	-	-
Fixed oils and fatty acid	-	+	-
Test for lactones	-	-	-

Table 2

Table 3 explains the phytochemical screening of *Ocimum sanctum* leaf in methanol and acetone extract revealed the presence of saponins, phlobatannins, flavonoids, terpenoids, glycosides and steroids [21]. The phytochemical screening of *Ocimum sanctum* leaf in water extract

revealed the presence of tannins, phlobatannins, flavonoids, terpenoids, glycosides and steroids [21].

Chemical constituent	Methanol extract	Acetone extract	Water extract
Tannins	Absent	Absent	Present
Saponins	Present	Present	Absent
Phlobatannins	Present	Present	Present
Flavonoids	Present	Present	Present
Terpeniods	Present	Present	Present
Glycosides	Present	Present	Present
Steroids	Present	Present	Present

Table 3

Table 4. The phytochemical screening of *Ocimum sanctum* in chloroform extract of leaf and stem revealed the absence of all phytochemicals. The phytochemical screening of *Ocimum sanctum* in methanol extract of leaf revealed the presence of flavonoids, amino acids, carbohydrate, proteins, saponins, etc. while the methanol extract of stem revealed the presence of flavonoids, amino acids, proteins, saponins, etc. The phytochemical screening of *Ocimum sanctum* in ethanol extract of leaf revealed the presence of amino acids, proteins, saponins, etc. while the ethanol extract of stem revealed the presence of alkaloids & flavonoids [22].

	Constituents	Chloroform		Methanolic		Ethanolic	
		Leaves	Stem	Leaves	Stem	Leaves	Stem
1.	Alkaloids	-	-	-	-	-	+
2.	Glycosides	-	-	-	-	-	-
3.	Flavanoids	-	-	+	+	-	+
4.	Phenolics	-	-	-	-	-	-
5.	Amino Acids	-	-	+	+	+	-
6.	Carbohydrate	-	-	+	-	-	-
7.	Proteins	-	-	+	+	+	-
8.	Saponins	-	-	+	-	+	-
9.	Determines	-	-	-	-	-	-

Table 4

Salvia hispanica

Test	n-hexane extract	Chloroform extract	Ethyl acetate Extract	Ethanol Extract	Aqueous Extract
Carbohydrates					
Molish test	-	-	-	+	+
Benedict's test	-	-	-	+	+
Fehling's test	-	-	-	+	+
Barfoed test	-	-	-	+	+
Test for pentose sugar	-	-	-	+	+
Test for hexose sugar	-	-	-	-	+
Alkaloids					
Dragondroff's test	-	+	-	+	+
Meyer's test	-	+	+	+	+
Wagner's test	-	+	+	+	+
Hager's test	-	+	-	+	+
Glycosides					
Modified Borntrager's test	-	-	-	+	+
Keller Killiani test	-	-	-	+	+
Legal test	-	-	-	+	+
Sodium picrate test	-	-	-	+	+
Fluorescence test	-	-	-	+	+
Saponins					
Foam test	-	-	-	+	+
Flavonoids					
Vanillin HCl test	-	-	+	+	+
Ammonia test	-	-	-	+	+
Shinoda test	-	-	+	+	+
Phenols					
Ferric chloride test	-	-	+	+	+
Lead acetate test	-	-	+	+	+
Steroids and triterpenoids					
Salkovaski Test	+	-	+	+	-
Libermann Burchard's test	+	-	+	+	-
Fixed oils and fats					
Spot Test	+	+	-	-	-
Saponification test	+	+	-	-	-

Table 5

Table 5. The phytochemical screening of *Salvia hispanica* seed in n-hexane extract revealed the presence of steroids, triterpenoids, fixed oils and fats [15]. The phytochemical screening of *Salvia hispanica* seed in chloroform extract revealed the presence of alkaloids, fixed oils and fats [15]. The phytochemical screening of *Salvia hispanica* seed in ethyl acetate extract revealed the presence of alkaloids, flavonoids, phenols, steroids and triterpenoids [15]. The phytochemical screening of *Salvia hispanica* seed in ethanol extract revealed the presence of carbohydrates, alkaloids, glycosides, saponins, flavonoids, phenols steroids and triterpenoids [15]. The phytochemical screening of *Salvia hispanica* seed

in aqueous extract revealed the presence of carbohydrates, alkaloids, glycosides, saponins, flavonoids and phenols [15].

The phytochemical screening of *Salvia hispanica* in methanol extract revealed the presence of saponins, coumarins anthraquinones, and alkaloid [23].

CONCLUSION:

The phytochemical tests for Flavonoids, Alkaloids, Terpenoids, Resins, Glycosides, reducing sugar, Protein, Tannins, Starch, Steroids, Carbohydrates, Phenolics, Saponins, Amino acids, etc. can be performed with a wide variety of plant extracts prepared in n-hexane, chloroform, ethyl acetate, ethanol, methanol, water, etc. to obtain more accurate qualitative results. The plant extracts too can be obtained from different parts of the plant such as roots, stems, apical region, leaves, seeds, etc. by the principle of Soxhlet extraction. The presence of phytochemicals in the parts of these plant prove that they possess various pharmacological properties

REFERENCES:

1. *Ocimum basilicum* Classification (2018), Search Taxonomy, Arctos. https://arctos.database.museum/ name/ *Ocimum basilicum*
2. Lamiacea (2019), The Editors of Encyclopaedia Britannica, Britannica. https://www.britannica.com/ plant/ Lamiaceae
3. Alia Bilal, Nasreen Jahan, Ajij Ahmed, Saima Naaz Bilal, Shahida Habib, Syeda Hajra (2012), Phytochemical and Pharmacological studies on *Ocimum basilicum Linn* – a review, IJCRR https://www.ijcrr.com/uploads/1538_pdf.pdf
4. Kelvin Bucktowar, Mili Bucktowar, Luchmee Devi Bholoa (2016), A review on sweet basil seeds: *Ocimum basilicum*, World journal of Pharmacy and Pharmaceutical Sciences. https://storage. googleapis.com/ journal uploads/ wjpps/ article issue/ 1480494719.pdf

5. Sara Naji-Tabasi, Seyed Mohammad Ali Razavi (2017), Functional properties and applications of basil seed gum: An overview. https://www.researchgate.net/ publication/ 318598324 Functional properties and applications of basil seed gum - An overview

6. Anudurga Gajendiran, Vidhya Thangaraman, Suji Thangamani, Dhatchayani Ravi and Jayanthi Abraham (2016), Antimicrobial, Antioxidant and Anticancer Screening of *Ocimum basilicum* seeds, Researchgate. https://www.researchgate.net/ publication/ 313692161_Antimicrobial_Antioxidant_and_Anticancer_Screening_Of_Ocimum_Basilicum_Seeds

7. H.C. Srivastava, Pankaj Shukla, Sonia Tripathi and Bramh Shanker (2014), Antioxidant and Antimicrobial activities of sweet basil oils, IJPSR. https://ijpsr.com/bft-article/antioxidant-and-antimicrobial-activities-of-sweet-basil-oils/

8. F. J. Sayyad and Sfurti S. Sakhare (2018), Isolation, Characterization and Evaluation of *Ocimum Basilicum* Seed Mucilage for Tableting Performance, IJPS. https://www.ijpsonline.com/ articles/ isolation-characterization-and-evaluation-of-iocimum-basilicumi-seed-mucilage-for-tableting-performance.pdf

9. *Ocimum sanctum* Classification (2018), Search Taxonomy, Arctos. https://arctos.database.museum/name/Ocimum%20basilicum

10. Lamiacea (2021), The Editors of Encyclopaedia Britannica, Britannica. https://www.britannica.com/plant/Lamiaceae

11. Prakash Khurana (2020), A review on medicinal uses of *Ocimum tenuiflorum Linn* (Tulsi), JMPAS. https://jmpas.com/ admin/ assets/ article_issue/ 1595790976JMPAS_JULY_2020.pdf

12. Arun Dewangan, BP Sahu, Bibekananda Meher (2020), Review on Pharmacological Potential of *Ocimum sanctum* L., AJBM. http://www.advjbiomol.com/index.php/journal/article/view/12

13. P Hanumanthaiah, H Panari, A Chebte, A Haile and G Tefera Belachew (2020), Tulsi (*Ocimum sanctum*) – a myriad medicinal plant, secrets behind the innumerable benefits, AJMAP. https://revues.imist.ma/ index.php/ AJMAP/ article/ view/ 20401/ 11079

14. Yashaswini NP, Vijaymahantesh VP, Singh VP, Kattimani, Rudresh DL and MD Jameel Jhalegar (2019), Effect of integrated nutrient management on growth and yield of tulsi (*Ocimum sanctum L.*) in northern dry zone of Karnataka, Int Journal of Chemical Studies.

15. Akash Sehrawat, Sumitra Singh (2019), Pharmacognostical standardization and preliminary phytochemical explorations on *Salvia hispanica* L. seeds, JDDT. http://jddtonline.info/ index.php/ jddt/ article/ view/ 2375/ 1716

16. Anita Yadav, Dr. Anuja Joshi, Dr. Smita Purohit (2014), In Vitro Morphogenetic Studies and Phytochemical Analysis of *Salvia hispanica* (L.), IIS https://shodhgangotri.inflibnet.ac.in/ jspui/ bitstream/ 123456789/ 5438/1/ synopsis.pdf

17. Maša Knez Hrnˇciˇc , Maja Ivanovski, Darija Cör and Željko Knez (2020), Chia Seeds (*Salvia Hispanica* L.): An Overview—Phytochemical Profile, Isolation Methods, and Application, MDPI. https://www.mdpi.com/1420-3049/25/1/11

18. Soxhlet Extraction (2020), Principle of Soxhlet extraction and Experimental Setup of Soxhlet Extractor, Melbia. https://www.melbia.com/ oleoresins/ principle of soxhlet extraction and experimental setup.html

19. Bihari, G.C., Manaswini, B., Prabhat, J. and Kumar, T.S. (2011) Pharmacognostical and Phytochemical Investigation of Various Tulsi Plants Available in South Eastern Odisha. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2, 605-610.

20. Borah R, Biswas S. P. (2018), Tulsi (*Ocimum sanctum*), excellent source of phytochemicals, IJEAB.

21. L. Srinivas Naik, Perka Shyam, K.Paul Marx, Srinivas Baskari, Prof.Ch.Venkata Ramana Devi (2015), Antimicrobial Activity and Phytochemical Analysis of *Ocimum tenuiflorum* Leaf Extract, PharmaTech. https://www.sphinxsai.com/ 2015/ ph_vol8_no1/ 2/ (88-95)V8N1.pdf

22. Praveen Garg and Rajesh Garg (2019), Phytochemical screening and quantitative estimation of total flavonoids of *Ocimum sanctum* in different solvent extract, Pharma Journal. https://www.thepharmajournal.com/ archives/ 2019/ vol8issue2/ PartA/ 8-1-46-880.pdf

23. Mendoza-Espinoza Jose Alberto, Pea-Miranda Imelda, Aarland Rayn Clarene, Peralta-Gomez Susana, Sierra-Palacios Edgar and Garcia-Ocon Bernarda (2015), Pharmacological and Phytochemical potential study of plants collected in Amecameca, State of Mexico, Mexico, Researchgate. https://www.researchgate.net/publication/ 288991607 Pharmacological and phytochemical potential study of plants collected in Amecameca State of Mexico

Chapter 5 - Nutritional Impact of Spina Bifida: A Review

Nawalkar Rhea, Joshi Sonia, Rai A Shalini*, Vajpai Aashu*, Mr. Bingi Gaurav*
Department of Life Sciences, Kishinchand Chellaram College, Churchgate, Mumbai- 400020
shalini.rai@kccollege.edu.in, aashu.vajpai@kccollege.edu.in

ABSTRACT:

Inadequate nutrition affects the growth and development of the body. Congenital anomalies could be caused due to various etiological factors; nutrition being one of them. Inadequate nutrition could lead to the development of the various congenital anomalies like congenital heart disease, cerebral palsy, neural tube disorders like spina bifida. Periconceptional folic acid deficiency also known as Vitamin B9 deficiency is one of the major causes of improper neural tube closure during development in diseases like spina bifida. Folate metabolism is extremely essential for the formation of the precursors for DNA synthesis and RBC formation. Factors such as lack of availability, lack of awareness, reduced consumption of fortified foods and some antagonists like inorganic arsenic govern the inadequate intake of folate. Since, the discovery of the importance of folate in overall development, nations worldwide have adopted strategies to increase dietary folate consumption through fortification of staples. The present review helps derive a detailed understanding of the relationship between folate and spina bifida.

Keywords: Congenital Anomalies, Folate, Vitamin B9, Fortified Foods, Spina Bifida

INTRODUCTION:

The World Health Organization (WHO) defines 'Health' as a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity [26]. Various factors affect health, some of the being- physical fitness, nutrition, levels of stress and social interaction. When health is affected, it gives rise to a disease or a disorder

in the body. Congenital disorders, commonly known as birth defects, are either prominent at birth or during foetal stage development. They can either be inherited or occur due to the exposure to various substances or injuries during pregnancy [1]. It has been observed that congenital anomalies are seen in 2-3% of live births, *in vivo* or at birth [1, 2]. According to the World Health Organization (WHO) 50% of the causes of these anomalies cannot be linked to a specific cause. They are caused due to genetic, environmental and other risk factors [3].

The common congenital disorders are Spina bifida caused due to folate deficiency, Fetal alcohol syndrome caused due to exposure to alcohol before or during pregnancy, Cerebral palsy, congenital heart disease and many more. Spina bifida is a nutrient deficiency disorder [10]. According to Britannica, 'Nutritional deficiencies may take the form of inadequacies of (1) total caloric intake, (2) protein intake, or (3) certain essential nutrients such as the vitamins and, more rarely, specific amino acids (components of proteins) and fatty acids [27]. Folic acid is extremely essential in foetal development. It is solely responsible for closure of the neural tube during early development. Neural tube closure is one of the key events that occur during the process of neurulation. The neural tube gives rise to the future brain and spinal cord. The improper closing of the same cause defects in brain and spinal cord structure [4]. The governing factor for this process is folic acid which is also known as folate or vitamin B9.

Vitamins and minerals are considered as essential micronutrients that need to be consumed by individuals. There are approximately 30 vitamins that are required for proper functioning. The body is in-capable of synthesizing vitamins and hence they need to be supplemented through an external source [6]. It has been scientifically proven that the deficiency of certain vitamins can lead to various life-threatening diseases. Vitamin A plays an essential role in immunity and defence of the body, reproductive health of men and women and eyesight. According to WHO deficiency of Vitamin A can cause preventable blindness in children and the maternal mortality rate is relatively common for the same. Lack of Vitamin B1 (thiamine) affects the heart,

it plays a key role in the functioning of the nervous system. It is a common cause of Wernicke-Korsakoff syndrome [7].

Present review is an attempt to understand and emphasize the relation between Vitamin B9 (Folic acid) deficiency with congenital disorder-Spina bifida. Vitamin B9 helps the body make red blood cells and produce DNA. Proper brain development and neural functioning is aided by folate. The synthetic form of folate is folic acid, which is found in fortified foods. It is a crucial element in neurulation during foetal development [7,9]. According to the National Institute of Health (NIH) and Centres for Disease Control and Prevention (CDC), women of a reproductive age must consume 4000mcg of folate in addition to a folate-containing diet [2,7,8]. Fortified foods could be an effective preventive measure. The advancement in biotechnology as a whole has contributed to the development of various artificially enriched foods & microbes as a biological source for various biomolecules. These advancements greatly influence the treatment of nutrient related deficiency disorders

Spina Bifida Occulta

Spina bifida occulta is a relatively milder version, it is also called as a hidden spina bifida because it sometimes goes undetected [20]. It is a malformation of one or more vertebrae, usually the lumbosacral vertebrae without a meningeal sac [21,23,24]. It is usually observed in the first month of pregnancy in the mother's womb [22]. According to the National Institute of Neurological Disorders and Stroke and spina bifida associated, approximately 10-20% of people may have spina bifida occulta [21,22]. Even though it is asymptomatic, some symptoms are prominent in early childhood or adulthood [20,21,24]. Symptoms are observed when the defect is extensive -for example, if it involves more than one vertebrae [21]. The clinical presentations of the same are pain and weakness in the back legs, numbness, foot deformity, loss of bowel control, scoliosis, back pain, a small patch of dark hair, Port-wine nevi (deep red-purple macular lesions) and soft fatty deposits [20,21,22,24]. Spina bifida occulta actually does not cause any problems but some versions of them do cause problems. Lipomyelomeningocele and

lipomeningocele are a relatively severe version of occulta, it is like a tethered spinal cord, except it is attached to a benign fatty tumor. Thickened terminale, fatty terminale, Diastematomyelia (split spinal cord), diplomyelia (cord is split in two, usually by a piece of bone or cartilage) and dermal sinus tract formation are other severe forms of spina bifida occulta [20].

Statistical Distribution Studies: Review of Articles

Global statistical data: Population studies reveal that 1 to 2 in 100 babies are born with neural tubes defects causing significant infant morbidities and mortality and are known to have a polygenic origin for occurrence [28]. Environmental and genetic factors in tandem with maternal nutrition play a significant role in causing NTDs. The geographical areas, income, population and race is highly responsible for this. Their distribution varies significantly globally. According to studies conducted by Ibrahim Zaganjor et.al, 75 of the countries were assessed for demonstrating the distribution of population affected by NTDs globally, out of these 40% of the countries were under WHO. South east Asian and African countries data was almost negligible. The overall prevalence estimates greatly between different countries. They conducted their data analysis using the RoB assessment. The data distribution of the most affected regions has been depicted using maps.

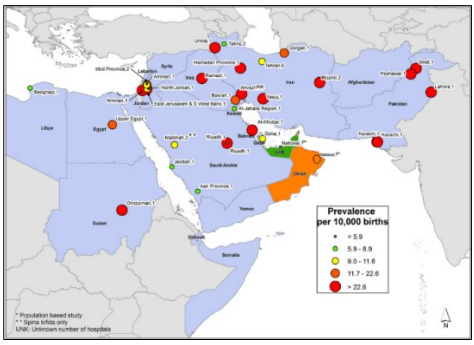


Figure :1 Prevalence of Spina Bifida in Eastern- Mediterranean Region. Image credit

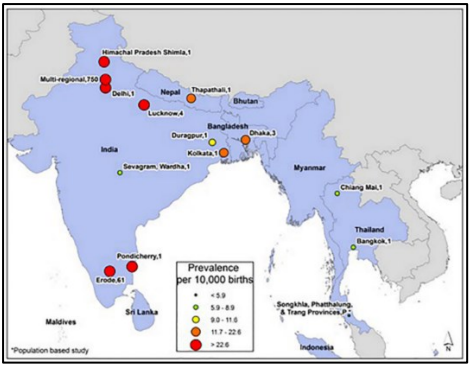
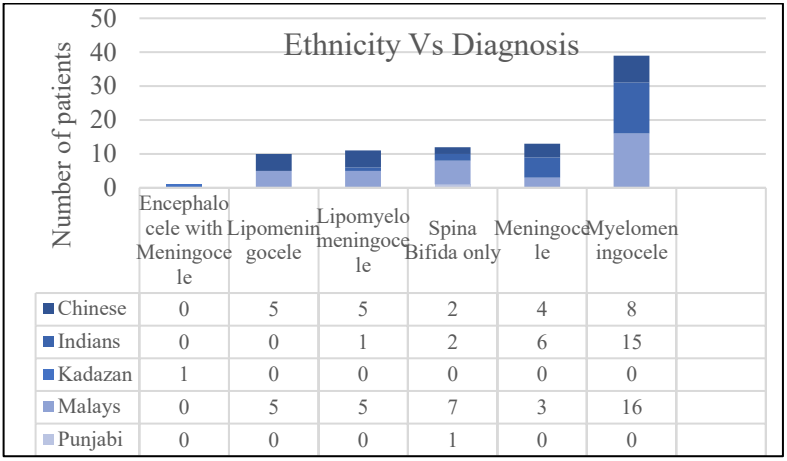


Figure 2: Prevalence of Spina Bifida in Southeast Asia
Image credit-

Malaysian Statistical Data

According to studies conducted by Adibah Sahmat et.al in a single referral centre in Malaysia the statistics were in the range of 0.5–10 per 1,000 live births.



	Encephalocele with Meningocele	Lipomeningocele	Lipomyelomeningocele	Spina Bifida only	Meningocele	Myelomeningocele
Chinese	0	5	5	2	4	8
Indians	0	0	1	2	6	15
Kadazan	1	0	0	0	0	0
Malays	0	5	5	7	3	16
Punjabi	0	0	0	1	0	0

Figure 4: Distribution of Spina Bifida among various ethnicities
Content Credit:
https://www.frontiersin.org/articles/10.3389/fped.2017.00237/full

They collected the patient data based on various factors such as the (1) demographic details on patient's ethnicity, gender, year of birth, birth weight, mother's age, and mode of delivery (spontaneous vaginal delivery/caesarean); (2) details of defects on diagnosis, open or closed lesion, level of lesion, and syndromic or non-syndromic; (3) presence of other conditions associated with spina bifida such as hydrocephalus including any insertion of the ventriculo-peritoneal (VP) shunt; and (4) patients' ambulation and education. They analysed the data using Statistical Program for Social Sciences (SPSS). We have used this data to generate our own graphs. We plotted Ethnicity versus diagnosis and number of patients.

Indian Statistical Data

In accordance with a study conducted by Sunil Rai et.al [29] on the incidence of NTDs in the northern part of India: we confer that the mortality rate is quite high of the patients suffering through it. This is due to the unregistered and untreated cases of NTDs. The prevalence of NTDs in India has shown to range between 0.5-11 per 1000 births.

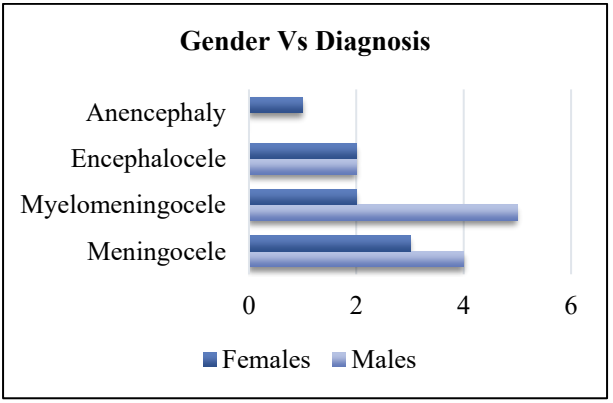


Figure 5: Relationship between gender and type of Spina Bifida
Content Credit:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4974957/

To determine the incidence data was collected from 2 districts of the eastern part of Uttar Pradesh. They adopted the methodology used by Cherian et.al for the population-based study. They did a door-to-door survey to obtain the data. They obtained data of 2540 live births. The estimated crude birth rate was calculated as 24.85 per 1000. They found that 19 babies were born with NTDs out of which 10 died. We used this data to generate our graphs. We plotted gender versus diagnosis; to understand the trends with respect to gender.

Folate Deficiency

Folic acid is the natural form of Vitamin B9 which is a water-soluble micronutrient. It is a collective name for pteroylglutamic acids and their oligo glutamic acid conjugates. Since it is water soluble it is involved in the carbon transfer reactions of amino acid metabolism, in addition to purine and pyrimidine synthesis, and is essential for haematopoiesis and red blood cell production[30]. Folate or Vitamin B9 is found in green leafy vegetables, beans, pulses, peas, lentils, oranges, whole-wheat products, liver, asparagus, beets, broccoli, brussels sprouts, and spinach.

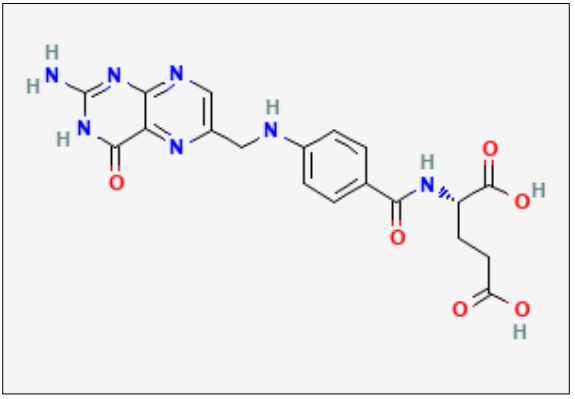


Figure 6: Structure of Folic Acid

Picture Credit- https://pubchem.ncbi.nlm.nih.gov/compound/Folic-acid

However only 50% of that is bioavailable. According to a article written by Hiroko Wantabe et.al, metabolism of nucleic acid precursors and

several amino acids, as well as methylation reactions are critically governed by folate. Epigenetic mechanisms such as DNA methylation plays a key role in diseases such as Alzheimer's.

Folate is strongly associated with homocysteine metabolism. The metabolite 5-methylhydrofolate (5-MTHF) acts as a substrate for methionine synthase which remethylates Hcy, and hence linking the folate cycle with Hcy metabolism. The deficiency of folate has been known to hamper this metabolism, thus affecting the nervous system and causing neuropsychiatry and neurodegenerative disorders. Folate is critical in optimising the pregnancy outcomes. The growth of the foetus and uteroplacental organs require large amounts of folate and it supports the maternal physiological changes. An adequate intake of folate is required before conception and early pregnancy to reduce the risk of congenital malformations of the brain, ultimately causing NTDs.

According to the study conducted by J.Safi et.al, it has been estimated that 50% of CA are responsible for the worldwide infantile mortality rate. They also contribute to severe physical, psychological and social handicaps along with morbidity and distress. Teratology defines the causes of CA as unknown in 50-60% of the cases. The other causes are epigenetic and multifactorial in 20–25% of cases, chromosomal or genetic with a single gene mutation in almost 15% of cases, and epigenetic and acquired under the influence of environmental factors (such as maternal sickness, infections, medications, ionizing radiations, and alcohol) in about 10% of cases [31].

Folate deficiency acts as a teratogen causing NTDs. Folic acid or Vitamin B9 is an essential part of the factors influencing the development of the embryo. In the 1950s, the association of CA and folate deficiency began to be acknowledged in humans when Methotrexate was widely used for abortions. Methotrexate and Aminopterin both are folic acid antagonists and were used for the treatment of psoriasis and certain cancers in pregnant women, which resulted in CA, thus starting to reveal an association. Research into NTDs was further facilitated by the advances in genetics and non mendelian complex diseases, bringing in

perspective the study of metabolism and transport of folate-homocysteine as potential risk factors for spina bifida. Hence, suggesting that folate is an effective preventive measure for spina bifida. During pregnancy, folate requirements accommodate embryonic and foetal development and maternal tissue growth. While folate is actively transported through the placental barrier to the foetus due to higher cord blood folate concentrations relative to maternal blood. Folate concentrations in maternal serum and RBC decline for several reasons such as increased demand, dilution secondary to increased intravascular volume, decreased absorption, increased folate catabolism and inadequate intake. Folate deficiency is known to lead to maternal megaloblastic anaemia, which may be fatal if left untreated. The factors that govern the deficiency of folate could be due to lack of awareness, accessibility to it, adverse effects caused due to other chemicals, poverty etc.

A study conducted by Maitreyi Mazumdar et.al suggested that neural tube defects are caused due to arsenic exposure in several animal models. Arsenic has the capability of crossing the placental barrier, hence influencing the development of the embryo. This study was conducted in Bangladesh, it was observed that the inorganic arsenic levels crossed the WHO guidelines significantly here. For the said study a sample size of 57 cases along with 55 controls were considered. The data was collected through a set of questionnaires emphasizing on the periconceptional folic acid supplementation. The levels of arsenic from the various sources of water that was consumed by the population under study was analyzed using inductively coupled mass spectrometry (ICP-MS). The plasma folate was measured using microbial assay by using *Lactobacillus casei* and was validated using biomarkers. The excessively high levels of inorganic arsenic ($\geq$25ug/L) in the population reduced the efficacy of folate (OR=0.22-1.03). Folate is an essential part of one-carbon metabolism and the generation of methyl groups used in various reactions. On the contrary, inorganic arsenic is known to deplete the supply of methyl donors that are essential for neural tube development, simply because folate has shown to be a vital part of arsenic metabolism.

This study was the first in humans to demonstrate the environmental exposure of arsenic influences the risk of developing myelomeningocele.

DISCUSSION:

Congenital anomalies are influenced by various risk factors. There are different types of congenital anomalies, NTDs being one of the most common. One specific NTD considered by us is spina bifida. Spina bifida is a CA wherein the neural tube fails to close during the 4^{th} week of pregnancy. Spina bifida has varied phenotypes such as myelomeningocele, meningocele, anencephaly and spina bifida occulta. MMC also known as open or aperta is one of the severe types of spinabifida, it results in the Chiari II malformation and other variable symptoms. It is identified by the cyst formation in the lower back of the baby, containing the meninges and spinal cord. On the contrary, Meningocele is similar to MMC but with the absence of the meninges and spinal cord in the cyst. A patient might need a VP shunt to correct hydrocephalus which is a defect that accompanies MMC with a revision in a month. Another type is spina bifida occulta which is also caused as a closed neural tube defect since it is a posterior vertebral defect with the absence of a sac.

Population studies reveal that approximately 1-2 babies per 1000 live births suffer through one of the various forms of spina bifida, increasing infant mortality and morbidity rates globally. Global data studies have helped gain a new perspective towards the distribution of this anomaly, while also revealing the probable causes of the same. This data would help combat this disorder, plan strategies and bring down the mortality rates.

The studies conducted by Ibrahim Zaganjor et.al provided data of 75 of the countries which come under WHO. We have particularly focused on the regions having higher prevalences. The Eastern-Mediterranean region had the highest average prevalence (21.9 per 10,000 births). The average prevalence in the Southeast Asian countries was 15.8 per 10,000 births. From this we confer that the Eastern-Mediterranean regions had the highest prevalence followed by the Southeast Asian countries having

an average of 15.8 per 10,000 births. Out of this, India and Thailand had the highest averages.

A study conducted by Adibah Sahmat et.al suggested that Malaysians and Indians were the highly affected population, thus being affected by MMC and this is supported by the distribution of patients within the different ethnicities and the types. Hence, we can say that there is a possible relationship between the ethnicities and the type of malformation. We can support this by the studies conducted in the Northern part of India. This data also suggests that myelomeningocele is the common phenotype of this disorder.

Another relationship was observed between the gender and the type of spina bifida. Both studies [28,29] concluded that males were more susceptible to Spina bifida as compared to females. It was found that in the Malaysian studies the males contributed to 59% of the patients. Similarly, Figure 5 suggests that males are more susceptible. If we compare both of these observations the ultimate result is that males suffer through MMC widely. The causes for these, lie in a wide spectrum. Both studies also talk about the importance of folate in the prevention of these disorders, suggesting that spina bifida could be classified as a nutritional disorder.

Vitamins and minerals are considered as essential micronutrients that need to be consumed by individuals. There are approximately 30 vitamins that are required for proper functioning. The body is incapable of synthesizing vitamins and hence they need to be supplemented through an external source. Vitamin B9 also known as folic acid in its natural form is essential in overall foetal development. A strong relationship between the two have been derived due to the overlap between folate metabolism and methionine metabolism, which are precursors for DNA synthesis.

Lack of folic acid leads to homocysteinemia and has a negative impact on pregnancy outcomes [12]. The factors that influence the lack of consumption is due to lack of awareness, socio-economic status, no access to folic acid or fortified foods. If we consider the Indian study

conducted so far, the rate of prevalence of cases is higher than economically developed countries; this could be due to unplanned pregnancies, unawareness regarding the benefits of periconceptional folate supplementation, and the absence of such prenatal diagnostic tests mean that parents only become aware of their malformed infant after birth [29]. However, the government has implemented prenatal screening and increased the awareness regarding the benefits of folic acid supplementation [29]. Another study found that high levels of arsenic reduce the efficacy of periconceptional folate as it uses up most of the methyl groups which is essential for further metabolism.

The preventive measures are fairly simple, the consumption of folic acid needs to be increased. The recommended dosage is 400mcg for women who are in the reproductive period[2,3,7,8]. WHO and the FAO also agree that an estimated average requirement of 320mcg of dietary folate equivalents (DFE)/day and a recommended dietary allowance of 400mcg DFE/day for adults [12].

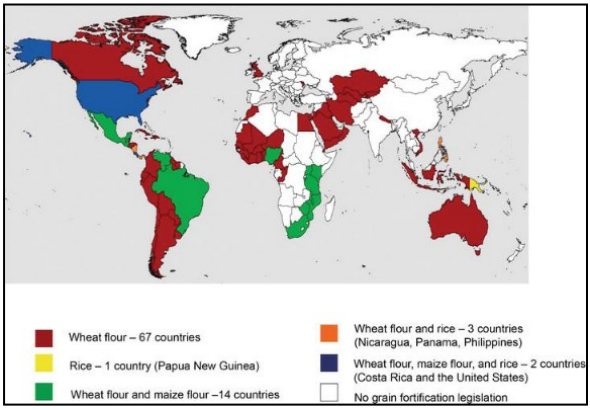


Figure 7: Distribution of Dietary Consumption of Folate Globally
Picture Credit- https://www.intechopen.com/chapters/55722

Figure 7 depicts the use of fortified foods to increase consumption of dietary folic acid. Globally 86 countries have initiated legislation to mandate the fortification of wheat flour alone or in combination with other grains. 140mcg of folic acid is added per 100g of enriched cereal

grain product in the USA while in Canada it is mandatory to fortify white-wheat flour with 150mcg/100g. It has been discovered that USA and Canada after implementation of this strategy have lowered their prevalence rate. In Ireland all bread, yogurts are fortified with folic acid at a level of 120mcg/100g. this has been implemented in several other countries too. The advancement in biotechnology as a whole has contributed to the development of various artificially enriched foods & microbes as a biological source for various biomolecules. These advancements greatly influence the treatment of nutrient related deficiency disorders such as spina bifida.

CONCLUSION:

Spina Bifida can be classified as a nutritional deficiency disorder due to its relationship with the intake of folic acid. The distribution of this disorder is more in the economically underdeveloped countries, due to lack of awareness and due to the reduced consumption of fortified foods. However, some countries have implemented the consumption of fortified foods, which are in tandem with the recommended level of daily folic acid consumption (400mcg).

REFERENCES:

1. Congenital Disorder Definition, Veritas Health, https://www.spine-health.com/glossary/congenital-disorder .Cited on; 12/06/2021
2. Helga V Toriello (2005), Folic Acid and Neural Tube Defects, Genetics in Medicine, Nature. Cited on; 12/06/2021
3. Congenital Anomalies (2020), World Health Organization, https://www.who.int/news-room/fact-sheets/detail/congenital-anomalies Cited on; 12/06/2021
4. Birth Defects, Wikipedia. https://en.wikipedia.org/ wiki/ Birth_defect#Lack_of_nutrients Cited on; 13/06/2021
5. Ranbir Singh,Sunil Munakomi (2021). Embryology, Neural tube. Stats Pearl Publishing LLC. Cited on; 13/06/2021

6. Vitamins and Minerals, Harvard Health Article, Help guide. https://www.helpguide.org/harvard/vitamins-and-minerals.htm# Cited on; 13/06/2021

7. Nutritional Deficiencies (Malnutrition), Healthline. https://www.healthline.com/ health/ malnutrition Cited on; 13/06/2021

8. Folic Acid Helps Prevent Some Birth Defects, Centers for Disease Control and Prevention. https://www.cdc.gov/ ncbddd/ folicacid/ features/ folic acid helps prevent some birth defects.html Cited on; 13/06/2021

9. What to know about Folate, Medical News Today. https://www.medicalnewstoday.com/articles/325310 Cited on; 13/06/2021

10. Andrew J. Copp, N. Scott Adzick et.al (2015), Spina Bifida, PMC Article, NCBI. Cited on; 15/06/2021

11. Mark R Foster, Elizabeth A Moberg-Wolff (2020), Spina Bifida. Medscape. https://emedicine.medscape.com/article/311113-overview#:~:text=International%20occurrence,and%2011.7%20in%20South%20America Cited on; 15/06/2021

12. Hiroko Wantabe et.al (2017), Folic acid and Folate, Intechopen. https://www.intechopen.com/chapters/55722

13. K. M. Laurence (1963), The Natural History Of Spina Bifida Cystica, Bmj Journals, Archives of disease in Childhood. https://adc.bmj.com/content/archdischild/39/203/41.full.pdf Cited on; 15/06/2021

14. Jack M. Fletcher and Timothy J. Brei(2010), Introduction: Spina Bifida—A Multidisciplinary Perspective, Developmental Disabilities Research Reviews. https://onlinelibrary.wiley.com/ doi/ abs/ 10.1002/ ddrr.101 Cited on; 15/06/2021

15. Andrew J. Copp, N. Scott Adzick et.al(2015),Spina Bifida, Nature reviews Disease Primers. https://www.ncbi.nlm.nih.gov/ pmc/ articles/ PMC4898641/ Cited on; 15/06/2021

16. Cristina Brea (2021), Spina Bifida, Stat Pearls. https://www.statpearls.com/ ArticleLibrary/ viewarticle/ 29302 Cited on; 15/06/2021

17. What is Spina Bifida? CDC. https://www.cdc.gov/ ncbddd/ spinabifida/ facts.html Cited on; 15/06/2021

18. Jimmy Ntimbani, Adrian Kelly, Patrick Lekgwara (2019), Interdisciplinary Neurosurgery Volume 19, March 2020, 100502. https://www.sciencedirect.com/ science/ article/ pii/ S2214751919301586 Cited on; 15/06/2021

19. Yvette Brazier (2020), What you need to know about spina bifida, Medical News Today. https://www.medicalnewstoday.com/ articles/ 220424 Cited on; 15/06/2021

20. Spina Bifida Fact Sheet, National Institute of Neurological Disorders and Stroke. https://www.ninds.nih.gov/ Disorders/ Patient Caregiver Education/ Fact Sheets/ Spina Bifida Fact-Sheet types Cited on; 15/06/2021

21. Spina Bifida Occulta, Spina Bifida Association. https://www.spinabifidaassociation.org/ resource/ occulta/ Cited on; 24/06/2021

22. Spina Bifida Occulta, The Spine Hospital, The Neurological Institute of New York. https://www.columbiaspine.org/ condition/ spina bifida occulta/ Cited on; 24/06/2021

23. What to expect with Spina Bifida Occulta, Healthline. https://www.healthline.com/ health/ spina bifida occulta Cited on; 24/06/2021

24. Sipos Tamas-Csab et.al (2019), Study of Spina Bifida Occulta Based on Age, Sex and Localization, Sciendo. https://www.researchgate.net/ publication/ 339257794 Study of

Spina Bifida Occulta Based on Age Sex and Localization Cited on; 24/06/2021

25. Spina Bifida Occulta, Physiopedia. https://www.physio-pedia.com/ Spina Bifida Occulta Cited on; 24/06/2021
26. Adam Felman (2019), Medical News Today. https://www.medicalnewstoday.com/ articles/ 150999
27. Britannica, Nutritional disease. https://www.britannica.com/ science/ human-disease/ Diseases of nutrition#ref525049
28. Ahlia Sekkari, et.al (2016), Describing the Prevalence of Neural Tube Defects Worldwide: A Systematic Literature Review, Plos One.
29. Sunil Rai et.al (2016), High incidence of neural tube defects in Northern part of India, Asian Journal of Neurosurgery. PubChem. https://pubchem.ncbi.nlm.nih.gov/ compound/ Folic-acid
30. J. Safi, et.al (2012), Periconceptional Folate Deficiency and Implications in Neural Tube Defects, Journal of Pregnancy.

Chapter 6 - Changing Trends in Consumer Preference during Covid-19 Pandemic

Nafisa Khan, Mariam Ansari, Amreen Sheikh, Dr. Aashu Vajpai*, Dr. Shalini Rai*, Mr. Gaurav Bingi*
Department of Life Sciences, Kishinchand Chellaram College, Churchgate, Mumbai- 400020
aashu.vajpai@kccollege.edu.in, shalini.rai@kccollege.edu.in,

ABSTRACT

When COVID-19 hit the planet in 2020, causing global lockdowns, it had a wide-ranging impact on humans. In this study, we have studied the changes in the trend during COVID – 19 wrt (i) Natural vs Synthetic Personal Care Products has been always a great topic of discussion between scientific people and non-scientific people. A Personal Care Product is a great range of products largely available in Pharmacy and supermarkets, these are any products which use to maintain or enhance the appearance and non-appearing form of the concerned body part and does not require any prescription from a medical expert.164 people had responded revealing more than half of the respondents have shifted towards Natural Products during the lockdown year 2020. (ii) Home remedies are self-made drugs or tonics with doubtful efficacy that are given without a prescription or professional supervision. They are age-old practices that are thought to be beneficial in treating common health problems. Many drugs might cause adverse reactions. This survey received 78 responses. The majority of the respondents prefer home remedies over pharmaceutical medications for addressing common health conditions during the COVID-19 epidemic. (iii) Multigrain Foods have become quite a huge food and diet trend. Multigrain is made from combining a bunch of whole grains instead of one type, to add more nutrition to our diets. A total of 126 people has responded. The majority of consumers are aware of multigrain products and consider them to be a healthier option. During the COVID-19 pandemic, more than half of the respondents switched to Multigrain for health purposes.

***Keywords**:* COVID-19 pandemic, Natural, Synthetic, Personal Care Products, home remedies, pharmaceutical medicines, common health issues, Multigrain products, and nutrition.

INTRODUCTION:

According to FDA, Personal Care Products refers to a wide range of things found in drug and department stores in the health and beauty section. However, the phrase "Personal Care Product" is not defined by any law. Some of the products usually referred to be Personal Care Products under the legislation include cosmetics. Skin moisturizer, fragrances, lipsticks, fingernail polishes, eye and face makeup preparations, shampoos, permanent waves, hair colors, and deodorants are some examples [1]. A cosmetic can be characterized as natural and certified if the composition contains certified natural raw components. Natural raw materials are vegetable or mineral products that are most commonly produced conventionally and do not always adhere to the organic production requirements [2]. A synthetic is a material that has been created or made by a chemical process and has been chemically changed from a naturally occurring plant, mineral, or animal source [3].

Minor illness is, by definition, any health condition that is not an emergency. Minor illnesses are issues which are not life threatening but require medical attention. They are very common like cough and cold, flatulence, acidity, sore throat, swelling, cuts and wounds, headache, toothache, constipation, diarrhoea, acne, dehydration, etc. These can be treated by consulting a general practitioner who is aware of your medical history. Many people treat minor health problems using home remedies or over the counter medicines. Common health issues can be treated by medical treatment or home remedies. Home remedies a home-made drug or tonic, typically with questionable efficacy, that is given without a prescription or professional supervision. They are ag e old traditions which are considered a boon to treat common health issues like cough and cold, diarrhoea, constipation, wounds, dehydration, acne, etc. Home remedies are also known as Grandma’s pouch. People have used home remedies and herbs for healing since centuries [3] [4] [5].

Multigrain product is prepared by mixing two or more grains together and processed to form a product with special health benefits. Its basic principle lies in the fact that each grain has its own nutritional profile, hence, combining two or more grains may give additional nutrients. Expanding world population and increasing prices of rice, wheat, and corn have led to the expansion of Multigrain food products particularly in developing countries. They are rich in energy source, minerals Vitamins and fiber. There are different opinions and aspects with regard to Consumer. Multigrain flours and other multigrain bakery products are mostly used. Promotions and communication factors play an important role in influencing purchase of Multi grains products. Nutritional benefits are particularly enhanced when different whole grain singly or in combination used in food preparation [6] [7] [8] [9] [10] [11].

Methodology:

The study was carried out by survey analysis. The survey was done using the Google form tool. The survey form was circulated in Maharashtra state. The link to the survey form was used to circulate the form. The sample was randomly selected. Three different Google forms were created.

(i) Every age group's people were included in the study from 13 years to 59 years and above including males and females. A total of 164 participants took part in the survey. The survey form consists of three different sections and all the participants had to fill only 2 sections.

(ii) This survey received 78 responses. Participants ranged in age from 13 to 59 years old and included both males and females. This form was divided into four components.

(iii) A total of 126 people has responded to this form, both male and female. The form consists of 2 sections with detailed questions.

Hypotheses:

(i) COVID – 19 has caused a shift towards Natural Personal Care Products from Synthetic.

(ii) People had preferred home remedies over pharmaceutical medications for addressing common health conditions during the COVID-19 epidemic.

(iii) People prefer using multigrain products as they find it a healthier option during COVID -19 pandemic.

Data Analysis:

Correlation and Chi square test were used for analysis of the data These tests help us to determine relationships and hypothesis testing.

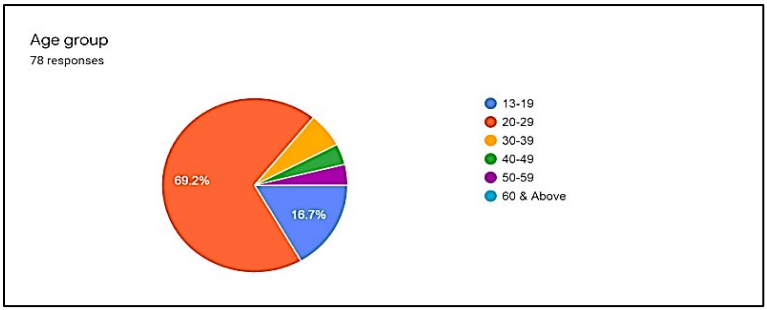
Results: (i):

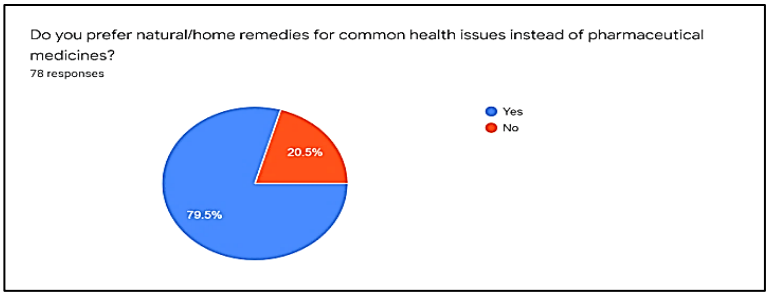
- It was found out that 19.5% respondents use synthetic and 80.5% respondents use Natural Personal Care Product.
- 53.7% of respondents read about the ingredients present in their selected product, while 14.6% of respondents don't read, and 31.7% of respondents sometimes read about the ingredients present. The major reasons to select Natural Products are no side effects and better results.
- The major reason to select Synthetic Products is better results and affordability.
- Aloe vera is the most preferred ingredient in Natural Products followed by Vitamin C and Neem.
- Sulphate and synthetic color are majorly avoided ingredients followed by paraben in both Natural and Synthetic Products using respondents.
- 69.7% of respondents consider Natural Products are better for acne-prone and sensitive skin.
- 71.9% of Synthetic Products using respondents think that Synthetic Products sometimes have side effects.
- 43.2% of respondents had rated the effectiveness of Natural Products as 4 out of 5 and 40.6% of respondents had rated the effectiveness of Synthetic Products as 4 out of 5.
- 13.6% of Natural Product using respondents want to shift to Synthetic Product. 25% of Synthetic Product using respondents want to shift to Natural Product.

- 78.8% of Natural Product using respondents have shifted to Natural Products and home remedies during the lockdown period of the year 2020.

(ii)

- The online survey conducted gathered 78 responses out of which 61 were women and 17 were men. Fig. 1 shows the different age groups of people who participated in the survey.

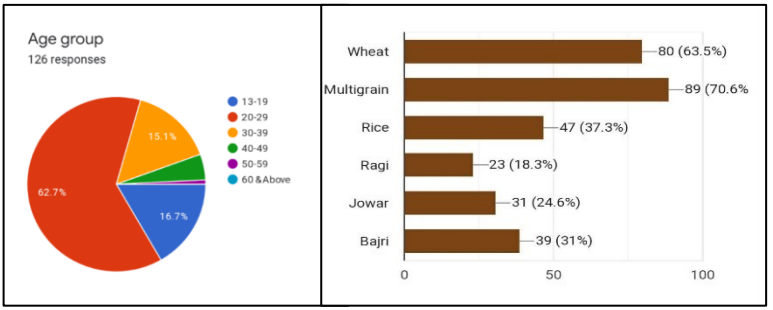




- Majority of the respondents are students (70.5%) followed by 23.1% of Working Professionals and 6.4% of Homemakers. Fig. 2 shows the number of people who chose home remedies and who didn't use them for common ailments during COVID-19.
- The Correlation coefficient obtained was -0.5748 which shows moderate degree of correlation. This indicates there is negative correlation between the Age of the respondents and their preference for home remedies the respondents were using home remedies before the COVID pandemic to treat Common health issues.

Depending on the severity of their health issue, 67.7% of persons sought home/natural therapies.29% of people used home remedies occasionally to treat common health problems, whereas 17.7% always used home remedies to treat common health problems.

(iii) Out of the 126 people who responded, 94.4 percent are aware of the Multigrain products.



- When comparing different grains, 70.6 percent of people believe Multigrain is healthier, whereas 63.5 percent believe Wheat is healthier.
- Out of the total respondents 94.4%people were aware of Multigrain Products.
- 73 % people use locally available wheat flour. While choosing a grain product people mostly look for a healthier option and easily digestible product. People find Wheat and Multigrain a healthier option.
- There is negative correlation between age and change of preference of people. 25.4 percent people consume Multigrain product on daily basis.
- Multigrain flour and Multigrain Bread are the Most preferred Multigrain products by people.61.1% people have shifted towards Multigrain products for health purpose during COVID 19 pandemic.

CONCLUSION:

(i) The study was designed to find out the preference of people using Natural Products and Synthetic Products and the shift towards Natural

Products during the COVID-19 pandemic. The biostatistics test performed shows that there is a strong correlation between qualification and shift toward Natural Personal Care Products during the COVID-19 pandemic. Around half of the respondents only read about the ingredients present in their preferred products. Out of total respondents, 66.5% of respondents have shifted to Natural Products and home remedies during the lockdown period of the COVID-19 pandemic. Also, hypothesis testing shows that there is a shift in preference during the lockdown year of 2020. Henceforth, the hypothesis is proved that there is a shift towards Natural Products during the COVID-19 pandemic.

(ii) The goal of this study was to discover why, during the Covid-19 outbreak, people favored home remedies to pharmaceutical treatments for common health problems. According to the findings of this poll, the majority of overall respondents prefer employing home remedies to cure common health ailments over pharmaceutical medications. Others chose pharmaceutical treatments to treat common health ailments because they are more effective, have a faster recovery rate, and are more easily accessible. As a consequence of this survey, the assumption that individuals prefer home remedies over pharmaceutical drugs to address common health concerns during the pandemic has been acknowledged.

(iii) The survey was conducted to know people's preference towards Multigrain products during COVID 19 pandemic. People's preferences were determined through statistical analysis. Out of the respondents 61.1% people have shifted towards Multigrain products during COVID 19 pandemic for health purpose. People find multigrain products to be a healthier option as they contain more nutritional value. Nowadays Multigrain products have been marketed widely in the market which has also gain people attention and increase in consumption.

REFERENCES:

1. https://www.fda.gov/industry/fda-basics-industry/are-all-personal-care-products-regulated-cosmetics - dated 16-Jun-2022

2. Sustainability, natural and organic cosmetics: consumer, products, efficacy, toxicological and regulatory considerations, January 2015, Bruno Fonseca-Santos, Marcos Antonio Corrêa, Marlus Chorilli

3. Si-Yuan Pan, Gerhard Litscher ,Si-HuaGao, Shu-Feng Zhou, Zhi-Ling Yu, Hou-Qi Chen, Shuo-Feng Zhang, Min-Ke Tang, Jian-Ning Sun, and Kam-Ming Ko . Historical Perspective of Traditional Indigenous Medical Practices: The Current Renaissance and Conservation of Herbal Resources. April 27, 2014

4. Seema Kolhe, Minal Dambhare, Priya Dhankasar, Pallavi Dhole, Ashwathy Nair and Priya Rewatkar. Home Remedies During Covid Pandemic Lockdown. Journal of Research in Medical and Dental Science

5. Passaic, NJ. Minor Illness Treatment. Centre of Adult Medicine and Preventive care (CAMP) https://campmedicine.org/minor-illness-treatment/

6. P. R. Shewry, Department of Plant Sciences, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK, 13 February 2009.

7. Gilberto Igrejas, Gérard Branlard, Wheat quality for improving processing and human health, 1, 2020. https://www.thoughtco.com/ wheat domestication the history 170669

8. ICAR – INDIAN INSTITUTE OF MILLETS RESEARCH (IIMR), June, 2017.

9. Ricepedia - https://ricepedia.org/culture/history-of-rice-cultivation

10. Naomi K Fukagawa et al. J Nutr Sci Vitaminol (Tokyo). 2019. https://pubmed.ncbi.nlm.nih.gov/31619630/

SECTION II - BIOTECHNOLOGY

Chapter 7 - Evaluation of Prebiotic and Antimicrobial Properties of Natural Extracts

Naina Bhanjer, Ms. Prachi Bhatia*, Dr. Sejal Rathod*
Department of Biotechnology, Kishinchand Chellaram College,
Churchgate, Mumbai- 400020
prachi.bhatia@kccollege.edu.in, sejal.rathod@kccollege.edu.in

ABSTRACT:

Prebiotics are compounds in food that induce growth or activity of beneficial microorganisms & are beneficial for host health. The most common example is in the gastrointestinal tract, where prebiotics alter composition of organisms in the gut microbiome. Prebiotics are the non-digestible fibres or carbohydrates that gut bacteria can digest, and prebiotics adds nutritional value & help healthy bacteria grow. Prebiotics are associated with Probiotic, microbes which keep intestine healthy and enhance immune system by fighting with harmful bacteria.

This study centres on evaluation of prebiotic properties for natural, aqueous extracts of Aloe vera (*Aloe barbadensis miller*), Kokum dried (*Garcinia indica*), and Wheatgrass (*Triticum aestivum*) by stimulation of growth, of probiotic organism *Lactobacillus casei* isolated on Sterile Man Rogosa Sharpe Agar and was measured colorimetrically at 545 nm. Antimicrobial and biopreservative study were also performed of these extracts. Hence to evaluate the variable properties of being used in cancer treatment, cosmetics, Food industry, etc.

Presence of Vitamin C in these plants by DCPIP method broadens the idea of Prebiotics applications, like better immune health and wound healing. Probiotic organism is also used in horticulture, in this study it resulted in enhancing the process of germination, *Lactobacillus casei* of 108Cfu/ml was incorporated in soil to enhance germination of fenugreek seed and kokum extract as a prebiotic source. Additionally, an online survey with Questionnaire methodology was conducted on Awareness

and usage about prebiotic and probiotics via Google form. And results were analyzed by graphical method.

Keywords: Prebiotic, *Lactobacillus casei*, Colorimetrically, Biopreservative, Germination.

INTRODUCTION:

Modern society has changed its standard of living and today health is becoming more important as in personal and social value. Consumers today are more aware of the choice of food they consume. Hence, Probiotics and Prebiotics are known for nutritional value and necessary in today's lifestyle. Probiotics are living, non- pathogenic, friendly microorganisms which play beneficial roles in the micro flora compartment of the host [2].

Lactic Acid Bacteria (LAB) are the most important probiotic group of microorganisms especially Lactobacillus spp., Bifidobacterium spp. and Enterococcus spp. Dietary and therapeutic qualities of milk product are determined by probiotic microorganism. Whereas, prebiotics are the fibres utilized by good microbes, functional foods which are to be consumed to maintain health and digestion of an individual.

In 2008, the sixth Meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) outlined "dietary prebiotics" as "selectively fermented ingredient that leads to specific changes within the composition and/or activity of the canal microbiota, so conferring benefit(s) upon host health". The following criteria were used to classify a compound as a prebiotic: (i) it ought to be immune to acidic pH scale of abdomen, cannot be hydrolysed by class enzymes, and conjointly mustn't be absorbed within the GI tract, (ii) it will be fermented by intestine microbiota, and (iii) the growth or activity of the microorganism of intestine will be by stimulated by this compound and this method improves host's health [5].

Higher intake of dietary fibres plays an important role in reducing the risk of cardiovascular disease, regulating weight management and immune function, and shaping microbial. diversity in human

gastrointestinal tract [15]. Probiotic organisms can work more efficiently when enhanced by prebiotics. Benefits of prebiotics for stimulating a healthy intestinal tract are well known. But there are many applications in various segments of food industry, notably dairy, beverage, processed fruit-vegetable, bakery, confectionary, extruded snack, sweetener, infant formula, pet food and livestock industry [15]. Therefore, there are many roles performed by prebiotics in day-to-day life & thus enhances growth by improving immune response.

Multi-drug resistant bacteria are increasing and causing life-threatening infections, especially in hospitals and with patients who are immunocompromised. Hence to develop new agents, natural extracts can be a good option. Also, Biopreservation is an approach for extending shelf life of perishable horticultural commodities against microbial decay using natural or controlled microbiota and/or antimicrobial compounds [1]. In postharvest technology, application of phytochemicals and plant-based material is safe for extending storage/shelf life of fruits and vegetables. Therefore, Aloevera, Kokum and Wheatgrass were targeted as natural sources for prebiotic, antimicrobial, and biopreservatives study.

The nutraceutical properties of Aloe vera have been attributed to a glucomannan known as acemannan. Aloe vera mucilage is reported to be rich in acemannan that is a polysaccharide with a backbone of β-(1→4)-D-mannose residues acetylated at the C-2 and C-3 positions and contains some side chains of galactose and arabinose attached to the C- 6 carbon [6]. It is also rich in Vitamin C and E content. Wheat grass juice (WGJ) is a new vegetative beverage which is obtained from sprout of mature wheat grains (Triticum aestivum). Because of the similarity between hemoglobulin in human blood and chlorophyll molecule of plants, WGJ is called green blood. Vitamin content of WGJ supports blocking disease such as asthma and some allergenic infections [11]. WGJ also includes some flavonoid compounds such as luteolin, apigenin and quercetin. Due to content of this compounds WGJ helps to cure inflammatory bowel diseases in a way [15].

Kokum (Garcinia indica) is underutilized fruits which are known for their therapeutic and enhanced nutritive value and ferment the kokum juice by different yeast and lactic acid bacterial strains. Most of the fruit juices have wholesome therapeutic effect and are consumed as refreshing drinks. Due to of their acid flavor, attractive and appealing color, they are welcome addition to the table. Anthocyanin in this red dried kokum has been predicted to be potential prebiotic and has anti-inflammatory, antioxidant properties [4]. Kokum is also rich in vitamin C that helps slowing the signs of ageing on skin by producing collagen.

In addition, a survey was conducted on awareness, and usage about probiotics and prebiotics. An online Questionnaire methodology of 16 general questions were designed to capture the knowledge of prebiotics from different age groups, gender. It combined multiple choice and multiple selection questions with predefined answers. The survey of this kind contributes to knowledge, usage, awareness of its overall results.

MATERIALS AND METHODS

Isolation of the *Lactobacillus casei*: Isolation of *Lactobacillus casei*, was carried out on Sterile De Man, Rogosa and Sharpe agar (MRS agar) which is a selective media for Lactobacillus species. A loopful of yakult drink was used to streak on the sterile media plate for isolation of *Lactobacillus casei*. In total two Plates were streaked, covered with parafilm tape to avoid contamination. One plate was kept in anaerobic chamber at Room temperature for 48-72 hrs and other plate was incubated at 37ºC for 48-72 hours (As it is facultative anaerobe).

Identification of the isolates: Biochemical tests such as Gram staining, Sugar fermentation test, oxidase test, and catalase test were performed to identify the isolate of both plates as based on Bergey's Manual of Systematic Bacteriology. As well as the isolate at 37ºC was characterized by MALDI- TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectroscopy) (Buchanan and Gibbons, 1974).

Culture medium and Conditions: Following purification, a 0.1 ml culture (adjusted to 0.1 O. D) was inoculated in 100ml of Sterile MRS

broth was incubated at 37°C conditions for 48-72 hrs. Uninoculated Sterile MRS broth was used as a control.

Preparation of plant extracts [Aloe vera (*Aloe barbadensis* miller), Kokum (*Garcinia indica*), and Wheat grass (*Triticum aestivum*)]: All three samples- Aloe vera, Wheatgrass, and kokum (dried) were surface cleansed with D/W or alcohol. 50 grams of each sample was weighed using weighing machine. Extracts were extracted by two methods, aqueous and diluted acid/base pre-treatment (liquid/solid type of extraction)-

By aqueous extraction: Grinding 50 grams of each sample, with 50 ml of D/W each was carried out in an electrical mixer. Further the extracts were filtered with sterile Whatman filter paper No. 1 in sterile ambered bottles. The filtration process was done in sterile conditions. Later, the extracts were stored at 4°C till further use. These extracts were considered as 100% concentration.

By Diluted Acid/base pre-treatment: Grinding each sample, weighing 50 grams, with 50 ml of 0.1M HCl. Neutralization of extract was done by adding dropwise 20 ml of 0.25M NaOH. Further, filtration of extract was done by using Whatman filter paper No.1, in ambered bottles with sterile conditions and were oven dried/air dried for 5-6 hours. And weighed for yield calculation. Extracts were stored at 4ºC until use.

Evaluation of Prebiotic Properties from Aloe vera (*Aloe barbadensis* miller), Kokum (*Garcinia indica*), and Wheat grass (*Triticum aestivum*) extracts: To Evaluate prebiotic property of Aloe vera, Kokum and wheatgrass, colorimetric method by stimulation growth of probiotic organism was performed [7]. Only aqueous extracts were used, as diluted acid/base pre-treatment extracts might not give valid or standard results. The culture suspension of 0.1 O. D and 1% inulin as probiotic control were used. Preparation of evaluating prebiotic property was performed in Sterile Side arm flasks & is described in detail in [Table 1] given below. After the additions as per below [Table 1 below], the absorbance/OD at 545 nm were taken initially (0 minute). Later flasks

were kept in rotary shaker and absorbance/OD at 545 nm was recorded at an interval of half hour i.e., 30 minutes.

Investigating Antimicrobial potential of aqueous and acid/ base extracts of the selected samples: The antimicrobial properties of plant extracts were tested against Gram-positive bacteria [*Bacillus subtilis*, Staphylococcus aureus], and Gram- negative bacteria [*Escherichia coli*, *Salmonella typhi, Shigella*] by agar well diffusion method.

Studying the Bio preservative Potential of Extracts: Aqueous extracts were tested as biopreservatives on two fruits-tomato and pear. To test the shelf life of the fruit and vegetable, control (without any biopreservatives supplement) was maintained with all three extracts in sterile petri dish. Further, the fruits were observed as per their texture and colour, till the fruits extended their shelf life in presence of extracts.

To Enhance the seed germination process: 2-3 Fenugreek seeds were sowed in 100 grams of soil, for each one- Control, Test 1 and Test 2. Only germination factor was considered in this application. Control was maintained. Test 1 plant of Fenugreek seeds was inoculated with 10 ml of 0.1 OD Lactobacillus casei culture, only probiotic organism. L. casei culture was of 108 cfu/ml. Test 2 plant of Fenugreek seeds with 10 ml of kokum extract as Prebiotic source and inoculation of 10ml 0.1 OD Lactobacillus casei culture. Further enhancement in seed germination was noted.

Studying the Presence of vitamin C content in selected Plant extracts: Vitamin C presence was done by 2,6-Dichlorophenol indophenol (DCPIP) titration. 2 ml of 0.3 % DCPIP was added in a test tube and extracts were added- 5 to 6 ml drop by drop till end point was achieved i.e., Blue to colourless.

Conducting Survey on- Awareness & Usage about Probiotics & Prebiotics: This survey was conducted to assess the awareness and usage of probiotics and prebiotics among different age groups. Simple and straight-forward survey method of Questionnaire was created through google forms which included questions about Probiotics and

Prebiotics, their sources, roles and application. This survey was conducted through online platform.

Flask	Positive control	Negative Control	Inulin Contol	Aloevera Extract	Wheatgrass Extract	Kokum Extract	Aloevera + Kokum	Wheatgrass + Kokum
MRS Broth	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml
Inulin(1%)	-	-	2ml	-	-	-	-	-
Aloe vera extract	-	-	-	2 ml	-	-	1 ml	-
Wheatgrass Extract	-	-	-	-	2 ml	-	-	1 ml
Kokum extract	-	-	-	-	-	2 ml	1 ml	1 ml
Saline	2 ml	3 ml	-	-	-	-	-	-
L. casei Culture	1 ml	-	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml

Table 1 Protocol table to test the aqueous extract for prebiotic properties

RESULTS:

Identification of *Lactobacillus casei*: *Lactobacillus casei* is facultative anaerobe bacteria, it was grown under both anaerobic conditions and in incubator at Room Temperature conditions (aerobic). Both plates had identical colony characteristics as described in [Table 2]

Colony	Characters Chart
Size	2-5 micrometers
Color	Creamy white
Shape	Circular
Spore	None
Consistency	Butyrous
Morphology	Short Rods

Table 2: Colony Characteristics

Further Biochemical test- Gram staining, Sugar Fermentation test, Catalase test, and Oxidase test was carried out based on the Bergey's Manual of Systemic Bacteriology, and MALDI-TOF through which we can indicate that the isolated culture is Lactobacillus casei. [Table 3]

Biochemical	Standard	Observed
a) Gram staining	Positive rods	Positive rods
b) Sugar Fermentation:		
-Glucose	Positive	Positive
-Sucrose	Positive	Positive
-Galactose	Positive	Positive
-Maltose	Positive	Positive
-Lactose	Positive	Positive
-Xylose	Variable	Positive
c) Oxidase Test	Negative	Negative
d) Catalase Test	Negative	Negative

Table 3: Biochemical Test

Yield of plant extracts: The yield of each extract and the yield % obtained was calculated.

By aqueous extract: The yield of each extract was calculated by:

Yield (%) = (W2−W1)/W0×100, where, W2 = weight of extract with container, W1 = weight of blank container W0 = weight of initial sample

Wherein, Aloe vera, wheatgrass and Kokum extracts constituted 94.32%, 84.32%, 78.32% respectively.

By Diluted acid/base pre-treatment:

The yield was calculated as - Yield (%) = [Weight of Crude Extract (W)/ Weight of Initial Sample (W) × 100 Where, weight of crude extract (w) = Weight of extract with container ($w2$)- Weight of blank container ($w1$).

With above calculations, *Aloe vera*, Wheat grass, Kokum constituted 68.32%, 60.32%, 56.32% respectively.

Evaluation of Prebiotic Properties from selected plant extracts:

Prebiotic properties were determined calorimetrically based on stimulated growth of Probiotic organism in presence of Aqueous Plant

extracts of Aloe vera, Wheatgrass and Kokum (Acid/Base pretreatment extracts were not used as it might not be suitable to evaluate plant extract for prebiotic property). The flasks were kept at Room temperature and on rotary shaker. Absorbance or Optical Density (OD) at 545nm was determined at interval of 30 minutes. Inulin, which is a prominent prebiotic was maintained as control to compare the results of Plant extracts & mixture of them, and positive, negative as well as blank (saline or D/W) was maintained.

As *Lactobacillus casei* is facultative anaerobe, it can grow in the presence of oxygen or without oxygen. Hence, rotary shaker was used for *Lactobacillus* growth, Prebiotic sources were its supplemented nutrition. Therefore, the below Graphs 1 and 2 analysis, comparing extracts with inulin control at 6.5 hours, OD measured was 0.57 units, all three plant extracts showed almost highest activity. These all three extracts showed better results when compared to control and later can be used for supplementation as Functional foods or nutraceuticals, or beverages. [Table 4 and Graph 1,2 below].

Investigating Antimicrobial potential of selected plant extracts: Antimicrobial activity of both aqueous and acid/base extracts of selected plant samples was investigated. Antibiotic control was maintained to compare the results. By [Table 5 above], Kokum has the highest potential of antimicrobial activity in comparison to other two plant extracts.

Time min	Negative Control	Positive control	Inulin 1% control	Aloe - vera	Wheat grass	Kokum	Aloe vera+ Kokum	Wheat grass+ Kokum
0	0	0.15	0.15	0.15	0.15	0.15	0.15	0.15
30	0	0.2	0.23	0.21	0.2	0.2	0.21	0.2
60	0	0.23	0.26	0.26	0.24	0.27	0.25	0.23
90	0	0.25	0.3	0.3	0.27	0.31	0.28	0.29
120	0	0.26	0.32	0.33	0.3	0.35	0.32	0.32
150	0	0.3	0.34	0.35	0.33	0.4	0.36	0.34
180	0	0.32	0.36	0.38	0.36	0.44	0.4	0.36
210	0	0.34	0.4	0.42	0.4	0.49	0.45	0.41

240	0	0.37	0.43	0.44	0.43	0.52	0.5	0.43
270	0	0.4	0.46	0.49	0.48	0.55	0.54	0.47
300	0	0.42	0.48	0.51	0.51	0.59	0.58	0.54
330	0	0.43	0.5	0.54	0.53	0.63	0.62	0.59
360	0	0.45	0.53	0.57	0.56	0.67	0.65	0.62
390	0	0.5	0.57	0.62	0.61	0.69	0.68	0.65

Table 4 Colorimetric reading at 545nm for prebiotic activity

Studying the Bio preservative Potential of Extracts: Bio-preservatives are the one which does not contain toxic chemical, are safe to use and will prolong the shelf life by keeping fruits, vegetables, meat, etc. the same in texture, colour and taste. Evaluating Bio preservative property of aqueous extracts was studied, Acid/base extracts might be toxic to fruits or vegetables and might not be valid to conclude. Extracts of Aloe Vera, wheatgrass increased the shelf life till four days of the study, and Kokum was observed to increase shelf life till Day eight which shows positive results as efficient bio preservative. Control fruits started to appear blackish and were appeared shrinking from Day 4-5 onwards. Kokum extract was evaluated as better biopreservatives in comparison with the Aloe Vera and Wheatgrass extracts.

To Enhance the seed germination process: Germination of Control fenugreek seeds was observed on sixth day of study, whereas with *Lactobacillus casei* culture, in Test 1 it was observed on fifth day of study, but it was more enhanced when fortified with Kokum extract, and *Lactobacillus casei* culture in Test 2. In Test 2 sample germination was observed on fourth day of the experiment. Therefore, seed germination process was enhanced when fortified with Probiotic and Prebiotic source as nutrition.

Studying the Presence of vitamin C content in Plant extracts: For Vitamin C presence in extracts 2,6-Dichlorophenol indophenol (DCPIP) titration method was used. Vitamin C is a reducing agent which reduces this blue color dye to colourless. In all the six extracts- Aqueous and Acid/Base extracts of all three samples resulted in Vitamin C presence

by reducing DCPIP from blue to colorless and red in case of Kokum extract as it is red in color. Hence, these extracts can be used further in supplements of tablets, powders, juices, cosmetics, etc. as a source of Vitamin C.

Bacterial Species	Aqueous Aloe vera extract	Aqueous Wheatgrass Extract	Aqueous Kokum Extract	Acid/base Kokum Extract
B. subtilis	-	Inconclusive	20 mm	13 mm
S. aureus	In-conclusive	-	21 mm	20 mm
E. coli	12 mm	15 mm	20 mm	20 mm
S. typhi	-	-	15 mm	14 mm
Shigella	-	-	20 mm	20 mm

Table 5: Antimicrobial Activity - Zone size of Inhibition

Survey conducted on- Awareness & Usage about Probiotics & Prebiotics: By graphical analysis of gender-age distribution and overall survey it was evaluated that sample size was 103 responses, 69 were Females and 34 were male respondents. Highest responses for awareness of probiotics and prebiotics, food sources, roles, and their respective applications were under the age category of 18-25 and 26-40 (new youth). But there is still some lack of awareness among 41-50, 51-above age categories. Whereas over all sample size when considered, knew about the key roles and key applications about probiotics and prebiotics.

DISCUSSION:

In the present study, samples used for evaluation prebiotic property were aqueous extracts of Aloe vera (*Aloe barbadensis* miller), Kokum (*Garcinia indica*), and Wheat grass (*Triticum aestivum*). *Lactobacillus casei* culture was inoculated with St. MRS broth with these extracts individually and mixtures of extracts were measured at specific time intervals calorimetrically at 545 nm. Kokum extract and Kokum with aloe vera extract showed the highest prebiotic activity. Previous protocol studies were referred for prebiotic activity [7].

For Aqueous Kokum extract, it exhibited 20 mm zone size for B. subtilis, Shigella spp. and E. coli, 21 mm for S.s aureus and 15 mm for S. typhi. Acid/Base extract of Kokum exhibited 20 mm zone size for S. aureus, Escherichia coli, Shigella 14 mm for S. typhi and 13 mm for B. subtilis. Reported results were similar for [17] studied antimicrobial activity of dried kokum (*Garcinia indica* C). Only aqueous Aloe vera and Wheatgrass exhibited 12 mm, 15 mm Zone of inhibition respectively against Escherichia coli. [18] investigated comparative study for aloe vera juice and gel (leaf) extracts, where better results were obtained at different concentration. [11] Investigated on antimicrobial properties of wheatgrass juice, barley grass juice, hardaliye and boza. Where in Wheatgrass juice showed 11 mm of zone inhibition on EMB agar for E. coli. Aqueous extracts when tested as biopreservatives, Kokum increased the shelf life till Eight days of the study whereas Aloevera and Wheatgrass till four days. Control fruits started to blacken, shrink from third day of the study. [3] Studied the shelf life of Garcinia cambogia fruit for pork, 1% extract was evaluated as efficient biopreservative.

Results obtained for seed germination of fenugreek were culture of *Lactobacillus casei* (Probiotic) and Prebiotic source like Kokum extract enhance the seed germination by two days, germination occurs in 4-5 days whereas normally it occurs in 6 or 7 days. The extracts were found to be rich in Vitamin C or with the presence of Vitamin C by using DCPIP titration, which helps in body regulation, wound healing and these extracts could be used in food industry, cosmetics, symbiotic application, etc. as Prebiotics and Vitamin C rich sources. For survey about probiotics and prebiotics, age group of 18-25 and 26-40 was aware the most, about Prebiotics and Probiotics, Roles, applications and dietary sources. Hence, most of the young or Today's youth knew about Probiotics and Prebiotics whereas the 41 and above age category is getting aware about it.

CONCLUSION:

The current study concluded that natural extracts of Aloe vera (*Aloe barbadensis miller*), Kokum (*Garcinia indica*), and Wheat grass

(*Triticum aestivum*) can be consumed as Prebiotics. The prebiotic activity was compared to inulin-prebiotic control, all the three plant extracts showed better results from the control maintained. Kokum extract and then Kokum + Aloevera were evaluated as the highest prebiotic source amongst the three and their mixtures. Aqueous and acid/base Extracts of Kokum has highest potential of antimicrobial activity in comparison to other two plant extracts. Even as biopreservatives, Kokum increased shelf life of tomato and pear till eighth Day of study, whereas Aloevera and wheatgrass till fourth day of study. Vitamin C presence was also indicated in all plant extracts by DCPIP titration method. For germination enhancement, Probiotic organism and prebiotic extract enhanced the germination of fenugreek seeds. Survey concluded that new youth is aware most about the roles, applications and sources of Prebiotics and Probiotics. Therefore, Kokum extract was evaluated highly fibrous and beneficiary as prebiotic, antimicrobial and biopreservatives agent.

REFERENCES:

1. Ananou S., Maqueda M., Martínez- Bueno1 M and Valdivia E. (2007). Biopreservation, an ecological approach to improve the safety and shelf-life of foods, Communicating Current Research and Educational Topics and Trends in Applied Microbiology, pp. 475-487.

2. Betz, M & Uzueta, A, Rasmussen H, Gregoire M, Vanderwall C, Witowich G (2015). Knowledge, use and perceptions of probiotics and prebiotics in hospital;ised patients. Nutrition & Dietetics, 72(3), pp. 261-66.

3. Bhuvana, K. S., Mandal, P. K., and Pal U.K. (2012), Garcinia cambogia Fruit Extract Enhances the Shelf Life of Pork Fry in Room Temperature, International Journal of Meat Science, 2(2), pp.27-33.

4. Coman, M.M., Oancea, A.M., Verdenelli MC, Cecchini C, Orpianesi C, Cresci A & Silvi S (2018), Polyphenol content and in vitro evaluation of antioxidant, antimicrobial and prebiotic properties of

red fruit extracts, European Food Research and Technology, 244(4), pp.735–745.

5. Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., Berenjian, A., & Ghasemi, Y. (2019). Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications, Foods (Basel, Switzerland), 8(3), p. 92.
6. Gullón B, Gullón P, Tavaria, F., Alonso, J. L., & Pintado, M. (2015), In vitro assessment of the prebiotic potential of Aloe vera mucilage and its impact on the human microbiota. Food Funct. 6(2), pp.525-531.
7. Homayouni A, Ehsani MR, Azizidagger, A., Razavi, S. H., & Yarmand, M.S. (2008), Spectrophotometrically Evaluation of Probiotic Growth in Liquid Media, Asian journal of chemistry, 20(3), pp. 2414-2420
8. Kang A, (2016), Understanding lactobacillus role in agriculture, Research & Reviews: Journal of Ecology and Environmental Sciences,4 (1), pp. 24-25.
9. Kavitha P., Sindhuja D, and Banumathi M (2016) Isolation and Biochemical Characterization of Lactobacillus species Isolated from Dahi, International Journal of Current Microbiology and Applied Sciences, 5(4), pp. 1042-1049.
10. Pathak M. (2013). Germinating Seeds: Source of Probiotics. World Applied Science Journal, 26(2), pp. 224-231.
11. Pehlivanoğlu H, Gündüz HH, Özülkü, G., Demirci, M., & Demirci, M. (2015), An Investigation of Antimicrobial Activity of Wheat Grass Juice, Barley Grass Juice, Hardaliye and Boza, International Interdisciplinary Journal of Scientific Research ISSN, 2(1).
12. Sawangwan T, Wansanit W, Pattani L, Noysang C. (2018) Study of prebiotic properties from edible mushroom extraction, Agriculture and Natural Resources, 52(6), pp. 519-524.

13. Shah S, (2007) Dietary Factors in the Modulation of İnflammatory Bowel Disease Activity, Medscape General Medicine, 9(1), p. 60

14. Silva M, Chibbar R, Walter, J., Goodman, K., Keshteli, A. H., Valcheva, R. S., & Dieleman, L. A. (2018) A110 use use of probiotics, prebiotics and dietary fibre supplements in patients with inflammatory bowel disease, Journal of the Canadian Association of Gastroenterology, 1(2), pp. 167-168

15. Singla, V., & Chakkaravarthi, S. (2017). Applications of prebiotics in food industry: A review. Food science and technology international, 23(8), 649–667.

16. SlavinJ.(2013).Fiber and prebiotics:mechanisms and health benefits. Nutrients, 5(4), pp.1417-1435.

17. Sutar, R. L., Mane, S. P, & Ghosh, J. S. (2012). Antimicrobial activity of extracts of dried kokum (Garcinia indica C). International Food Research Journal, 19(3), 1207-1210.

18. Takon IA, Ikpeme E, Victor, Odey A, Ochegbe M (2015) Comparative study of the antimicrobial properties of aloe vera juice and gel (leaf) extracts against selected clinical isolates, International Journal of Technical Research and Applications, 3(6), pp.108-111

19. Ugbe, F & Ikudayisi-Ugbe, VA & Amusan OT (2017). Determination of Ascorbic Acid Concentration of Some Commercial Fruits Juices Sold in Ugbokolo Benue State, Nigeria. International Annals of Science, 3, Pp.19- 22.

20. Jalal A and Ahmad N, (2019), Aloe vera as a bio-preservative for keeping quality of horticultural products, Research Journal of Food Science and Nutrition, 4(4), pp. 82- 89.

Chapter 8 - Isolation, Characterization and Evaluation of Polycyclic Aromatic Hydrocarbon Degrading Fungi

Sera Fernandes, Sonam Gupta, Ms. Hajra Gupta*,
Dr. Sejal Rathod*
Department of Biotechnology, Kishinchand Chellaram College,
Churchgate, Mumbai- 400020
sejal.rathod@kccollege.edu.in

ABSTRACT:

Motor or engine oil is a lubricant for engines containing 80% of the base oils; these base oils include petrol-based hydrocarbons. Petroleum hydrocarbon contamination is one of the major environmental problems resulting from its large scale uses in transportation, industrial and other sectors. Accidental release and workshop seepage of petroleum products are the key concern of the environment. Fresh engine oil contains polycyclic aromatic hydrocarbons (PAHs). Used engine oil also leads to the generation of PAHs. As an attempt to clean up such hydrocarbons, bioremediation or biodegradation methods are adapted. Bioremediation is a cost effective and eco-friendly treatment for oil contaminated materials by the use of micro-organisms. The present study is an attempt to isolate and find out hydrocarbon degrading fungi from oil and petroleum contaminated regions. Biodegradation potential of soil mycobiota isolated from automobile mechanic workshop in Virar on engine oil was investigated using standard methods. The most capable oil degrading fungi was identified morphologically by wet mount technique as Fusarium sp. The biodegradation of hydrocarbons and oil was determined by using 2 -6 Dichlorophenol- indophenol (DCPIP) assay and gravimetric analysis. The quantitative estimation of engine oil degradation showed rate of degradation as 87% and 89%. This study confirms that isolated Fusarium sp. has the potential exploited in the bio-treatment and removal of hydrocarbons from the polluted soil. Results were recorded to the numerous problems dealing with the petroleum contaminated soil, which can be resolved in favour of human health and natural environment. The present study and their results can give unique

future prospects in the field of bioremediation and biodegradation of petroleum contaminated soil.

Keywords: Bioremediation, Fusarium sp. DCPIP, Petroleum hydrocarbon, Gravimetric analysis.

INTRODUCTION:

Engine oil is a lubricant for engines containing 80% of the base oils, which includes petroleum-based hydrocarbons. Motor oil can have an incredibly damaging effect on the environment, particularly to plants that depend on healthy soil to grow. Petroleum hydrocarbons are released from petroleum industry as major waste product, which when released in environment destroys the natural flora and fauna of the region. Used engine oil leads to generation of more polycyclic aromatic hydrocarbons (PAHs) which too has devastating effect on environment [21]. The term polycyclic aromatic hydrocarbons (PAHs) also known as poly-nuclear aromatic hydrocarbons are a class of organic chemicals consisting of two or more fused aromatic rings and do not contain heteroatom or carry substitutes. PAHs belong to the group of persistent organic pollutants (POPs). These are organic pollutant contaminants that are resistant to degradation, can remain in environment for a long period and have the potential to cause adverse environmental effects. As a pollutant, they are at concern because some compounds have been identified as carcinogenic and mutagenic. PAHs are also present in oil, tar deposits and are even produced as a by-product of fuel burning [9].

The major environmental concern in urban and industrial areas with regard to polycyclic aromatic hydrocarbons is that they possess a relatively low solubility in water, thus affecting the aquatic ecosystem. Toxic effects of used oil on freshwater and marine organisms are significant long-term effects. Used motor- oil dumped on land reduces soil productivity. Improperly disposed used oil ends up in landfills, sewers, backyards, or storm drains where soil, groundwater and drinking water may become contaminated. Treating these contaminated sites using physio-chemical processes prove to be harmful for environment. Bioremediation can be opted for such treatment procedures.

Bioremediation includes use of living organisms like microbes and plants, in the removal of contaminants, pollutants, and toxins from soil, water, and other environments. Mycoremediation involves using fungi to eliminate or remove contaminants or pollutants from the environment. Fungal bioremediation technology is highly efficient and versatile compared to that of protozoan or bacterial type, due to robust growth of fungus, vast hyphal network. Hence, mycoremediation can be an economical, eco-friendly, and effective strategy to combat the ever-increasing problem of soil and water pollution. Robust growth of fungus, vast hyphal network, production of versatile extracellular lignolytic enzymes, high surface area to volume ratio, resistance to heavy metals, adaptability to fluctuating pH and temperature and presence of metal-binding proteins; fungi are an ideal candidate for the remediation of various pollutants [1].

The various fungi genera which have been proven to be effective degraders of engine oil and other PAHs include Aspergillus, Penicillium, Rhizopus, Fusarium, Mucor, etc [18]. Use of these fungi can be preferred in case of oil spills, contamination of land by PAHs and even in treatment of petroleum hydrocarbons which are released as major waste from petroleum industries. The present study aims at exploring efficacy of indigenous fungi as potential bioremediating agents in treating petroleum hydrocarbon contaminated soil and water. Following isolation and identification of a number of engine oil degrading fungi, the extent of degradation of engine oil by the isolates reveals their potentials in alleviating the devastating pollution through bioremediation.

MATERIALS AND METHODS:

Materials and chemicals:

The medium used for enumeration of the heterotrophic fungi was Potato dextrose agar (with engine oil as carbon source), for isolation of engine oil (PAHs) utilizing fungi Potato Dextrose Broth (with engine oil as a carbon source) was used.

Sources of soil sample:

The two contaminated soil samples were collected from two automobile mechanical garages located in Virar, Maharashtra, India; where spillage of engine oil was common over the years. Samples were collected from each site from just 1cm below the soil surface and were transported to a laboratory in polythene bags and were kept in a refrigerator (in order to keep the organisms viable and free from any contamination) before analysis.

Isolation of PAHs degrading fungi using enrichment media:

The collected soil sample was subjected to serial dilution (10-1 -10-5) and then spread plate method was employed. The last three dilutions (10-3-10-5) were plated on PDA medium, to which streptomycin was incorporated, so as to avoid contamination by bacteria. The same soil samples were also subjected to enrichment of PAHs degrading fungi using PDA media. Sugar source was replaced with engine oil in the latter. The soil sample was added to 100ml PDB supplemented with 2%engine oil as a carbon source. [12] Prior to adding engine oil, the media was sterilized by autoclaving at 121^0C for 30 minutes. To this media streptomycin was added to avoid bacterial contamination. Both the flasks were incubated at 28^0C on a rotary shaker at 180 rpm for 7 days. The PDB consisted of engine oil as sole energy source and thus only mycoflora with biodegradation capacity present in the soil were allowed to flourish in the enrichment media. After 7 days of incubation, PDA plates were prepared containing 2ml engine oil and streptomycin. In both the plates 1ml of culture from the incubated flasks were added, and spread plate method was employed. Plates were incubated at room temperature (RT) for 7 days. To observe the growth pattern of the fungi, the colonies were also inoculated on Sabourauds dextrose agar (SDA).

Fungal characterization of fungal isolates: Slide technique was employed for the identification of fungal isolates. The fungal isolates were characterized using cultural and morphological characteristics; the macroscopic and microscopic characteristics were also observed by visual observations.

Colonies from the fungal plate were transferred aseptically to a sterile slide with the help of a sterile forceps. A pure culture of the fungal isolate was flooded with a few drops of lactophenol blue cotton blue stain and covered with a fresh coverslip. Both were thereafter viewed under X10 and X40 objective lens.

Preliminary screening for confirming biodegradation potential of fungal isolates:

MSM 100ml along with 2%, 5%, 10% (v/v) engine oil in Erlenmeyer flasks were used for the biodegradation experiments. The preliminary screening test involved MSM media along with modified DCPIP method to estimate the degradation capacity of fungal isolates. [2]. For this, MSM was sterilized by autoclaving at 1210C for 30 minutes before addition of engine oil. 5 agar plugs from the growing edges of the fungal-colonized PDA with the aid of a sterile cork- borer of 5mm diameter, and inoculated in this degradation media supplemented with 0.4μ/ml of DCPIP and incubated at RT for 14 days in constant shaking conditions. The decolorization of DCPIP from (deep blue to colourless) indicated engine oil degradation capacity of the fungal isolates.

Estimation of petroleum hydrocarbon degradation/gravimetric analysis:

The estimation of biodegradation ability of fungal strains was done and rate of degradation was expressed in percent degradation. The modified MSM which consisted of 5 % engine oil was used to grow fungal isolates. For this, 5 plugs of 5mm diameter from PDA were inoculated in MSM with 5ml of oil and then incubated at 28±10C for 7 days on rotary shaker. Following 7 days cell free culture broth was obtained through filtration and centrifugation. Further the engine oil fraction was extracted from this cell free culture broth with three volumes of toluene using separating funnel. Aqueous and organic layers were formed. The optical density was measured at 420nm by UV visible spectrophotometer. The degradation percentage was calculated using the equation: Degradation %= C0-C/C0 x 100, where C0 and C refer to initial and final concentration respectively [7].

RESULTS:

Isolation of engine oil degrading fungi using enrichment media:

As the soil samples were serially diluted and then plated on sterile PDA medium, a number of fungi colonies irrespective of their ability to degrade engine oil grew on the petriplates. Whereas in case of soil sample subjected to enrichment media (PDB with 2% engine oil) growth of few fungal isolates were observed; only those which had the ability to utilize engine oil. Thus total 2 fungi were isolated by using modified PDA medium (which composed of 2% engine oil as carbon source).

Characterization and identification of fungal isolates:

On PDA plates colonies were fast growing with white to cream cotton mycelium. On SDA the colonies appeared white and red pigment production was observed. Lacto phenol cotton blue staining agent was applied for microscopic identification of fungal isolates. From microscopic observation it was identified as filamentous fungi. Morphological characteristics of strains, including their conidiophores, were compared with those of the known species of fungi. This revealed that the fungi exhibited both macro and microconidia. Microconidia were oval. Macroconidia were oval tapering and septated in 2 cells. Macro-conidia, elongated, gently to strongly curved with somewhat pointed ends. Microconidia which had conidiogenous cells were thicker than vegetative hyphae; which were with terminal or intercalary chlamydospores (thick- walled spore-like swellings of vegetative cells). Thus through microscopic characterization by referring to mycology manual the fungi were identified to be of Fusarium spp.

Preliminary screening for confirming biodegradation potential of fungal isolates by DCPIP assay:

5 discs of agar plugs from PDA plates were inoculated into flasks containing 100 mL of MSM supplemented with 40 µg/100 mL of redox indicator (2,6-DCPIP) and different concentrations of engine oil such as 2%, 5%, 10% (v/v). The cultures were incubated at 28±1°C for 14 days in constant shaking conditions. Control flasks were also prepared which

constituted of MSM media with engine oil. The flasks were observed daily for color change. Change in the color of the inoculated degradation medium from deep blue to colorless indicated the capability of the fungi to degrade engine oil. The mechanism used by fungi to biodegrade engine oil was thus observed by incorporating an electron acceptor, i.e. DCPIP. This result indicated that the two fungi had the capability to degrade crude oil.

Quantitative estimation of engine oil degradation using gravimetric analysis:

To note the oil degradation; after 7 days of incubation in MSM containing 5%of engine oil estimation of oil degradation was done gravimetrically. For this, the cell free culture broth was extracted with three volumes of toluene using separating funnel. The optical density of oil layer of both samples and control were measured as follows at 420nm.

Sr No.	Aliquots	O. D
1	Control	1.76
2	Sample 1	0.22
3	Sample 2	0.19

The rate of degradation or percentage degradation was calculated using the formula: Degradation (%) = (C0-C)/C0×100, where C0 and C refers to initial and final concentration of extracted engine oil, respectively. From above data, the % degradation of sample 1 was found out to be 87% while that of sample 2 was 89%. Thus, both fungi proved to be efficient in petroleum hydrocarbon degradation.

DISCUSSION:

Petroleum based hydrocarbon and polycyclic aromatic hydrocarbons (PAHs) contamination is increasing due to anthropogenic activities. The contamination by PAHs disturbs the environment thus the present study involved isolation of fungi with ability to degrade PAHs which would pave a way for treatment of PAHs contaminated sites. For this fungi were isolated on enrichment media containing engine oil as sole energy source. The media used was modified PDA containing 2% engine oil.

Characterization of fungi was studied; using the stain lacto-phenol-cotton blue. Morphological studies of isolated fungal isolates showed different growth pattern on different media. The morphological and microscopic observation revealed that the isolated fungi were of Fusarium genera.

For analysis of the isolates degrading engine oil screening test was employed. The screening test involved usage of modified MSM containing 2%, 5%, 10% engine oil concentrations. And thus, pure fungus isolates plug from modified PDA was inoculated in this degradation media along with redox indicator. The screening test made use of DCPIP as a redox indicator. The principle of this redox indicator lies on the oxidation of the carbon source (hydrocarbon substrate) in which electrons are transferred to the electron acceptors.

The utilization of substrate can be observed based on the loss of indicator's blue colour. However, the colour changes from blue to colourless occurred before the incubation period. This indicated that the fungal isolate was very effective and efficient in utilizing hydrocarbon present in engine oil as energy source. Further after this qualitative test, a quantitative analysis was done.

The estimation of engine oil degradation ability of fungal isolates was done gravimetrically. Here providing 5% oil was preferred; as fungal isolates proved to be effective hydrocarbon degrader. After 7 days of incubation the rate of degradation was measured. The engine oil fraction of cell free broth was then extracted using three volumes of toluene with the aid of separating funnel. The optical density was then measured at 420nm. Applying the O. D in the formula percent degradation was calculated. The fungal isolate degraded hydrocarbon with 89% efficacy. The present study thus revealed that the fungal species of Fusarium can be used in bioremediation of PAHs contaminated sites.

CONCLUSION:

The study revealed that engine oil degrading indigenous mycobiota could be isolated from soil of automobile workshop contaminated with

engine oil; using modified PDA (with 2% engine oil) as enrichment media. These fungal isolates indicated their ability to degrade engine oil in the degradation media that is, modified MSM (containing 2% engine oil) where, fungi utilized engine oil as sole energy and carbon source. It also showed that fungal isolates would degrade engine oil faster in low pH i.e. acidic conditions. The fungal isolates were grown on different media (PDA and SDA) to study their morphology and note observations like their color and texture. The microscopic characteristics of fungal isolates were recorded and it showed presence of macro and micro-conidia, which are distinct features of fungi belonging to Fusarium genera. Hence fungal isolates were identified to be of Fusarium species.

The preliminary screening test for confirming the engine oil degradation by the fungal isolates was done by DCPIP assay using the Mineral Salt Medium. Through this screening analysis it was indicated that the isolated fungal colonies had the capability in degradation of 2%, 5% and 10% engine oil concentrations. The isolates rapidly utilized the engine oil before the incubation period of 14 days. The quantitative estimation of engine oil degradation was estimated by gravimetric analysis using the MSM media containing 5% engine oil. The estimation of rate of engine oil degradation was then calculated using the formula: Degradation (%) = (C0- C)/C0×100, (here C0 and C refers to initial and final concentration of engine oil respectively) which was found out to be 87% and 89%. This study thereupon revealed about great capability of isolated Fusarium species in rapid degradation of petroleum-based hydrocarbons.

Hence, it revealed that the indigenous fungi of PAHs contaminated soil could spontaneously play a crucial role in restoring a petroleum hydrocarbon-free ecosystem through bioremediation.

REFERENCES:

1. Akhtar, N., & Mannan, M. A. U. (2020). Mycoremediation: Expunging environmental pollutants. Biotechnology Reports, e00452, 2215-017X.

2. Al-Hawash, A. B., Alkooranee, J. T., Abbood, H. A., Zhang, J., Sun, J., Zhang, X., & Ma, F. (2018). Isolation and characterization of two crude oil- degrading fungi strains from Rumaila oil field, Iraq. Biotechnology reports, 17, 104-109.

3. Al-Hawash, A. B., Alkooranee, J. T., Zhang, X., & Ma, F. (2018). Fungal degradation of polycyclic aromatic hydrocarbons. Int J Pure App Biosci, 6(2), 8- 24.

4. Balaji, V., Arulazhagan, P., & Ebenezer, P. (2014). Enzymatic bioremediation of polyaromatic hydrocarbons by fungal consortia enriched from petroleum contaminated soil and oil seeds. Journal of Environmental Biology, 35(3), 521- 529.

5. Chaudhry, S., Luhach, J., Sharma, V., & Sharma, C. (2012). Assessment of diesel degrading potential of fungal isolates from sludge contaminated soil of petroleum refinery, Haryana. Research Journal of Microbiology, 7(3), 182.

6. Chukwura, E. I., Ojiegbu, N. M., & Nwankwegu, A. S. (2016). Hydrocarbon degradation potentials of fungi associated with oil-contaminated soil from selected mechanic workshops in Awka, Anambra State, Nigeria. Frontiers in Environmental Microbiology, 2(6), 38-44.

7. Dhar, K., Dutta, S., & Anwar, M. N. (2014). Biodegradation of petroleum hydrocarbon by indigenous fungi isolated from ship breaking yards of Bangladesh. International Research Journal of Biological Sciences, 3(9), 22-30.

8. Ghosal, D., Ghosh, S., Dutta, T. K., & Ahn, Y. (2016). Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. Frontiers in microbiology, 7, 1369.

9. Igwe, J. C., & Ukaogo, P. O. (2015). Environmental effects of polycyclic aromatic hydrocarbons. Journal of Natural Sciences Research, 5(7), 117- 132.

10. Khan, S. R., Ji, N. K., Kumar, R. N., & Patel, J. G. (2015). In vitro study on assessment of petrol, kerosene and diesel degrading potential of indigenous fungal isolates from different petroleum product effected soils. International Journal of Recent Research and Review, 8(1), 8-15.

11. Romauld, S. I., Venkataraghavan, R., Yuvaraj, D., Devi, V. I., & Hashika, S. (2019). Mycoremediation of Hydrocarbon and its products using Fusarium oxysporum. Research Journal of Pharmacy and Technology, 12(9), 4216-4224.

12. Sandhu, S. S., Shakya, M., Deshmukh, L., Aharwal, R. P., & Kumar, S. (2016). Determination of hydrocarbon degrading potentiality of indigenous fungal isolates. International Journal of Environmental Sciences, 6(6), 1163- 1172.

13. Batelle, C. D. (2000). Mushrooms: Higher Macrofungi to clean up the environment. Environmental Issues, Fall.

14. Cerniglia, C. E. (1984). Microbial metabolism of polycyclic aromatic hydrocarbons. Advances in applied microbiology, 30, 31-71.

15. Cerniglia, C. E. (1992). Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation, 3(2-3), 351-368.

16. Chapelle, F. H.(1995). Bioremediation: Nature's way to a cleaner environment (No. 054-95). US Geological Survey.

17. Chaudhry, S., Luhach, J., Sharma, V., & Sharma, C. (2012). Assessment of diesel degrading potential of fungal isolates from sludge contaminated soil of petroleum refinery, Haryana. Research Journal of Microbiology, 7(3), 182.

18. Chukwura, E. I., Ojiegbu, N. M., & Nwankwegu, A. S. (2016). Hydrocarbon degradation potentials of fungi associated with oil-contaminated soil from selected mechanic workshops in Awka, Anambra State, Nigeria. Frontiers in Environmental Microbiology, 2(6), 38-44.

19. Ghosal, D., Ghosh, S., Dutta, T. K., & Ahn, Y. (2016). Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. Frontiers in microbiology, 7, 1369.

20. Khan, S. R., Ji, N. K., Kumar, R. N., & Patel, J. G. (2015). In vitro study on assessment of petrol, kerosene and diesel degrading potential of indigenous fungal isolates from different petroleum product effected soils. International Journal of Recent Research and Review, 8(1), 8-15.

21. Behera, B. K., Das, A., Sarkar, D. J., Weerathunge, P., Parida, P. K., Das, B. K., ... & Bansal, V. (2018). Polycyclic Aromatic Hydrocarbons (PAHs) in inland aquatic ecosystems: Perils and remedies through biosensors and bioremediation. Environmental pollution, 241, 212-233

Chapter 9 - Action of Antibiotics on *P. Aeruginosa* Biofilm Using Liposome Mediated Delivery System

Reetikesh Patel, Dr. Sejal Rathod*
Department of Biotechnology, Kishinchand Chellaram College,
Churchgate, Mumbai- 400020
sejal.rathod@kccollege.edu.in

ABSTRACT

Biofilms are microbial communities attached to a surface and embedded in an extracellular polymeric substance (EPS) which provides for the protection, stability and nutrients of the various bacterial species indwelling. These communities can build up in a variety of different environments from industrial equipment to medical devices resulting in damage, loss of productivity and diseases. Antibiotic-resistant bacteria have become an increasing burden worldwide. A highly resistant species is *Pseudomonas aeruginosa*, a nosocomial pathogen that produces biofilm that enhances its resistance. This project examined the possibility of using antibiotics enclosed liposome, as a potential treatment for resistant bacteria. In this study, the action of antibiotics and antibiotic enclosed liposomes were carried out using Basic microtiter biofilm dish assay. Optimal concentrations of antibiotics were used at a concentration of 0.01mg/ml for kanamycin, Ampicillin, Cefotaxime. The biofilm was then analyzed to determine if it was inhibited or facilitated by each treatment. The quantitative estimation of biofilm degradation was carried out using a plate reader at 595nm.

The action of liposome containing antibiotics seemed to be high at penetration and inhibition, compared to standard antibiotic therapy. This study shows that liposome mediated delivery system of antibiotics can be clinically applied for the treatment of chronic pulmonary infections in cystic fibrosis patients, and even be used for other such P.aeruginosa infections such as soft tissue infections, joint infections, gastrointestinal infection and a variety of other systemic infections by using anti-biofilm drugs encapsulated in these liposomes for far more efficient pharmacokinetic and pharmacodynamic profiles.

Keywords: Biofilms, Liposome, Antibiotics, *P. aeruginosa*, EPS, Pharmacokinetic, Pharmacodynamic

INTRODUCTION:

Pseudomonas aeruginosa is an opportunistic pathogen responsible for both acute and chronic infections in humans. In particular, its ability to form biofilm, on biotic and abiotic surfaces, makes it particularly resistant to host's immune defense and current antibiotic therapies as well. Innovative antimicrobials material, like hydrogel, silver salts or nanoparticles have been used to cover new generation catheters with promising results. Nevertheless, biofilm produced onto endotracheal tubes (ETT) of ventilated patients plays a relevant role in the onset of ventilation associated pneumonia. P.aeruginosa related biofilms also cause chronic lung infections, specially patients with cystic fibrosis who are prone to these type of infections. A biofilm comprises any syntrophic consortium of microorganisms in which cells stick to each other and often also to a surface [1].

These adherent cells become embedded within a slimy extracellular matrix that is composed of extracellular polymeric substances. Biofilm-associated cells ca be differentiated from their suspended counterparts by generation of an extracellular polymeric substance (EPS) matrix, reduced growth rates, complex process regulated by diverse characteristics of the growth medium, substratum, and cell surface. An established biofilm structure comprises microbial cells and EPS, has a defined architecture, and provides an optimal environment for the exchange of genetic material between cells. Cells may also communicate via quorum sensing, which may in turn affect biofilm processes such as detachment [2]. Biofilm-associated bacteria are less sensitive to antibiotics than free- living(planktonic) cells. Furthermore, with variations in the concentration of antibiotics throughout a biofilm, microbial cells are often exposed to levels below inhibitory concentrations and may develop resistance. This, as well as the irresponsible use of antibiotics, leads to the selection of pathogens that are difficult to eradicate.

For most of the history of microbiology, microorganisms have primarily been characterized as planktonic, freely suspended cells and described on the basis of their growth characteristics in nutritionally rich culture media. Rediscovery of a microbiologic phenomenon, first described by van Leeuwenhoek, that microorganism attach to and grow universally on exposed surfaces led to studies that revealed surface- associated microorganism (biofilms) exhibited a distinct phenotype with respect to gene transcription and growth rate. These biofilm microorganisms have been shown to elicit specific mechanisms for initial attachment to a surface, development of a community structure and ecosystem, and detachment [3].

In laboratory conditions, biofilms have a limited life span and they eventually disassemble in response to self-generated signals and not by any external means. Because much is known about the molecular mechanisms that regulate entry into biofilm formation, the structural components that comprise the extracellular matrix, however the biofilm (EPM) disassembly by external means is still in the question. [4].

We now understand that biofilms are universal, occurring in aquatic and industrial water systems as well as a large number of environments and medical devices relevant for public health. Using tools such as the scanning electron microscope and, more recently, the confocal laser scanning microscope, biofilm researchers now understand that biofilms are not unstructured, homogenous deposits of cells and accumulated slime, but complex communities of surface- associated cells enclosed in a polymer matrix containing open water channels [5]. Further studies have shown that the biofilm phenotype can be described in terms of the genes expressed by biofilm- associated cells. Biofilm-associated microorganisms have been shown to be associated with several human diseases, such as native valve endocarditis and cystic fibrosis, and to colonize a wide variety of medical devices. The exact mechanisms by which biofilm- associated microorganisms elicit disease are poorly understood. Detachment of cells or cell aggregates, production of endotoxin, increased resistance to the host immune system, and

provision of a niche for the generation of resistant organisms are all biofilm processes which could initiate the disease process [6].

Current intervention strategies are designed to prevent initial device colonization, minimize microbial cell attachment to the device, penetrate the biofilm matrix and kill the associated cells, or remove the device from the patient.

MATERIALS AND METHOD:

Origin of culture:

The culture was collected from a private laboratory called National Facility for Biopharmaceuticals (NFB) located at Matunga, Mumbai. It was streaked on Nutrient agar slant under aseptic conditions and was transported to laboratory to keep in refrigerator (in order to keep the organism viable and free from any contamination) before subculture and analysis (Gram staining).

Growth of *P. aeruginosa* in liquid medium:

Liquid cultures of *P. aeruginosa* are commonly used for many applications. For general experiments, including electroporation [7], cultures are grown in LB. When studying effects of particular factors on P. aeruginosa, the bacteria is grown overnight in a minimal medium such as LB. Growth in a minimal medium such as LB was used in this study.

Air-liquid interface coverslip assay:

Air-liquid interface assay was carried out in which after appropriate incubation, the coverslip is rinsed, stained with crystal violet, and visualized by conventional microscopy using an upright microscope (Figure.4) under which the stained EPS formation is clearly visible. If an inverted microscope is not available, a plastic or glass coverslip is placed in a well of a plate such that the air-liquid interface of the culture is in the approximate centre of the coverslip (held at 90°) [8]. After an appropriate incubation, the coverslip is rinsed, stained with 0.1% (w/v)

crystal violet for 10 min, and visualized at the air- liquid interface by conventional microscopy after allowing coverslips to air- dry.

Growing and Analyzing static biofilms.

This experimental system, whose most common format is often referred to as the 96-well plate assay, is a simple high- throughput method used to monitor microbial attachment to an abiotic surface. Cells are grown in microtiter dishes for a desired period of time (48hours), and then the wells are washed to remove planktonic

bacteria. Cells remaining adhered to the wells are subsequently stained with 0.1% (w/v) crystal violet dye that allows visualization of the attachment pattern. This surface-associated dye can also be solubilized using 70% ethanol for semi quantitative assessment of the biofilm formed under a Plate reader or spectrophotometer [9].

Biofilm growth in glass tubes:

To assess the effect of media composition on biofilm growth, *P. aeruginosa* mucoid strain were separately grown in glass tubes in LB media. Cultures were inoculated by adding a loopful of an overnight culture of bacteria into 3 mL of sterile media, and the tubes were incubated statically at either 37 °C for two days. Planktonic growth was documented photographically. The supernatant was then discarded and the adhered cells were rinsed three times with distilled water, and the tubes were patted dry on a paper towel. 3mL of a 0.1% CV solution was added to each tube to stain the adhered biomass and the tubes incubated for 10 min at room temperature. The CV dye was discarded and the tubes were again rinsed three times with distilled water and were patted dry 10]. Tubes were photographed to document the amount of biofilm that was adhered to the glass surface. One mL of 70% ethanol was then added to each tube to release the bound CV dye from the biofilm, and 100 µL of this was transferred to a 96-well plate for quantification at an absorbance of 595 nm (A595) on a plate reader. All of the growth conditions were evaluated in triplicate.

Basic microtiter dish biofilm assay protocol:

Preparing inoculating culture. Subculture overnight cultures of the strain of interest was grown to midlog phase (OD 600~0.5). Distribute 90 µl per well of chosen medium into 96-well microtiter plates. Add 10 µl per well of the culture prepared. Include wells containing media-alone as negative controls. Seal with parafilm/lid and incubate at the desired temperature [11].

Removing planktonic cells. After the desired incubation time (i.e 24hr) gently pour-off the media remaining in the wells containing unattached cells and gently submerge the plate in deionized water. Remove the plate, again pour off contents gently, and repeat wash.

Staining of adherent cells. Add 150 µl 0.1% W/V Crystal violet to each well, and stain for 10 min at room temperature. Gently wash the plate three times. Allow the plate to air-dry. Add 150 µl of optimal concentration of antibiotics (0.01mg/ml) and liposome enclosing antibiotics in the respective wells and incubate at 37ºC for 24hr. Gently was the plate three times. Allow the plate to air- dry.

Measuring dye absorbed by adherent cells and matrix. Add 200 µl 95% ethanol to each well, incubate 10 min at RT. Pipette this solution containing resolubilized dye up and down twice to mix, transfer to a clean 96-well plate, and read absorbance at 595 nm.

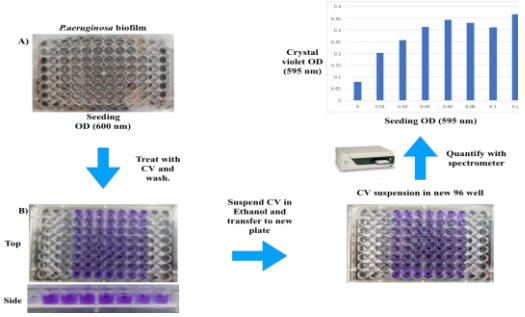


Figure 1: Schematic Representation

Liposome formation:

Appropriate amount of phospholipid powder (L-α Phosphatidylcholine, 30%) is dissolved in chloroform, which is then evaporated using a rotary evaporator. A thin dried film of phospholipid will be obtained at the bottom of round bottom flask. Re-hydration of phospholipid is done using heated Phosphate Buffered Saline pH 7.2 containing Methylene blue for visualization under the conventional microscope under 40X.

RESULTS:

Qualitative analysis

Air-liquid interface assay was carried out in which after appropriate incubation, the coverslip is rinsed, stained with crystal violet, and visualized by conventional microscopy using an upright microscope under which the stained EPS formation is clearly visible.

Liposome formation

Appropriate amount of phospholipid is dissolved in chloroform, which is then evaporated using a rotary evaporator. A thin dried film of phospholipid will be obtained at the bottom of round bottom flask.

Re-hydration of phospholipid is done using heated buffer/saline containing Methylene blue for visualization under the conventional microscope under 40X as seen in (Figure.3)

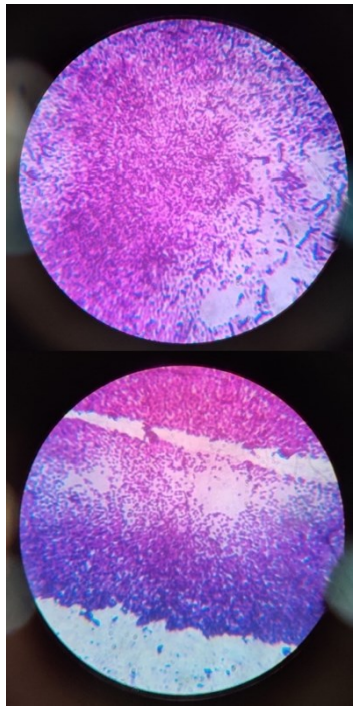


Figure 2: Air Liquid Interface coverslip stained with CV under a conventional microscope showing EPM

Basic microtiter dish biofilm assay protocol.

Crystal violet assay was carried out; This quantification has proven extremely useful as a cell estimate for biofilm growth. The schematic Figure 1; explains a basic biofilm accumulation assay performed in a multi-welled plate. The growth media and planktonic cells are removed from the plate and washed with distilled (DW) water leaving only attached biofilm (Figure1A). A 1% solution of crystal violet in DW water is added and the biofilm incubated with the dye at room temperature for a period of time, typically 10 minutes. After incubation, the dye solution is removed, and the biofilm washed several times with DW water to remove free dye (Figure1B). The decolouring solution is then be added, to a volume greater than or equal to the original culture media volume, and incubated with the biofilm for 10 minutes.

The decolouring solution used was a 90–95% ethanol solution but other decolouring solutions such as pure ethanol or ethanol with acetone or acetic acid are also used as the objective to solubilize the CV. Finally, the CV infused decolouring solution is transferred to a clean 96 well plate with appropriate blanks of decolouring solution to be assessed for absorbance at 595 nm, depending on the instrument's filter availability, with a multi-well plate UV-Vis spectrometer [12]

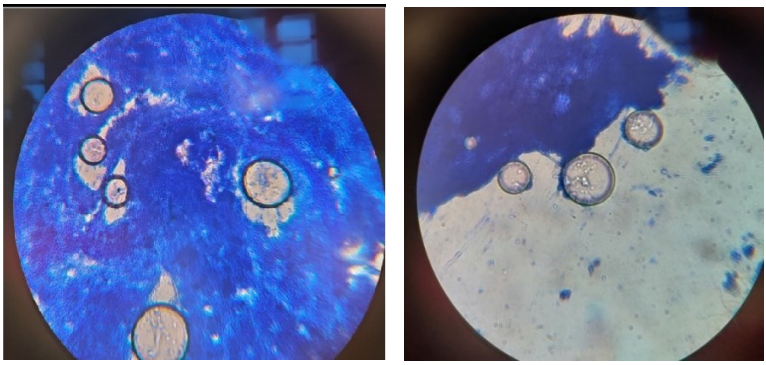


Figure 3: Liposome stained with mb to visualize under40X

Liposome anti-biofilm study was carried out using Basic Microtiter dish biofilm assay protocol, and was conducted within 20 hours after the liposomes were prepared. Two prepared biofilm-coated wells of the 96-well microtiter plate were rinsed twice

in saline to remove planktonic bacteria and then exposed to 200µL of optimal concentrations of antibiotics of 0.01mg/ml for Kanamycin, Ampicillin, and Cefotaxime and liposome containing the above antibiotics respectively, for 24 hours at 37 °C followed by another two washes. In this experiment, saline was used as the negative control (Blank) for the antibiotics and antibiotics enclosed liposomal treatment and the wells that did not contain biofilm were stained as the background. All treatments were carried out in quadruplicate and experiments repeated twice.

At OD (595 nm) reading were obtained using Bio-Rad spectrophotometer/ plate reader for both the plates as seen in Table1 and 2 with respective graphs for obtained data.(Graph 2 and 3)

	Blank	**Positive Control**	**Kanamycin**	**Ampicillin**	**Cefotaxime**
OD (595nm)	0.0412	0.4385	0.195	0.214	0.298

Table 1. Antibiofilm effect using antibiotics against P. aeruginosa biofilm

	Blank	**Positive Control**	**Kanamycin**	**Ampicillin**	**Cefotaxime**
OD (595nm)	0.0413	0.4365	0.086	0.105	0.157

Table 2. Antibiofilm effect using liposome against P. aeruginosa biofilm

DISCUSSION:

The present study systematically investigated the distribution and anti-biofilm effects of the action of antibiotics and antibiotics enclosed liposomes of different sizes on biofilm of *P. aeruginosa.*

The data demonstrated by the spectrophotometer/ plate reader showed that liposome containing antibiotics penetrated better and inhibited *P. aeruginosa* biofilm more efficiently than just antibiotics. Although the liposomes used in this study were of varied sizes which may or may not have influenced penetration. According to the research on the penetration of liposomes and other nanoparticles into *Burkholderia multivorans* and *Pseudomonas aeruginosa* biofilms. also reported that the larger the particle diameter, the more the particles were excluded from the smaller channels within the biofilms. Biofilms have many protective mechanisms including modified microbial gene expression, altered microenvironment of the bacterial colonies (i.e. pH changes and oxygen deficient zones), the local accumulation of enzymes degrading antibiotics but the structural complexity and mechanical stability provided by the matrix undoubtedly restricts drug diffusion and contributes to the antibiotic resistance [13].

The inhibition effect of the liposomes containing antibiotics on biofilms was confirmed by analysing the data demonstrated by spectrophotometer and the decrease in optical density at 595nm before and after its action [14]. It can be hypothesized that after liposomes attach and incorporate themselves into the biofilm structure and microbial membrane, they are able to convert the signalling pathways and disrupt the integrity of the cell membrane. This may play an important role in inhibiting biofilm growth [15].

The main purpose of this study was to investigate the action of antibiotics and antibiotic enclose liposomes on *P. aeruginosa* biofilm. The mucoidal strain of *P. aeruginosa* was grown in minimal medium such as LB and further maintained on NA slant in refrigerator for further use. First the biofilm was grown in glass tubes using Tube adherence assay, in which the cultures were inoculated by adding a loop full of an overnight culture of bacteria into 3 mL of sterile media, and the tubes were incubated statically at 37 °C for two days. Further using Air-Liquid interface in that the glass coverslip is placed in a well of a plate such that the air-liquid interface of the culture is in the approximate centre of the coverslip. After an appropriate incubation, the coverslip is rinsed, stained with crystal

violet, and visualized by conventional microscopy using an upright microscope. Then liposome anti-biofilm study was carried out using Basic Microtiter dish biofilm assay protocol, and was conducted within 20 hours after the liposomes were prepared. Two prepared biofilm-coated wells of the 96-well microtiter plate were rinsed twice in saline to remove planktonic bacteria and then exposed to 200µL of optimal concentrations of antibiotics of 0.01mg/ml for Kanamycin, Ampicillin, and Cefotaxime and liposome containing the above antibiotics respectively, for 24 hours at 37 °C followed by another two washes. In this experiment, saline was used as the negative control (Blank) for the antibiotics and antibiotic enclosed liposomal treatment and the wells that did not contain biofilm were stained as the background. All treatments were carried out in quadruplicate and experiments repeated twice.

Through this project, it was found out that antibiotics encapsulated in these liposomes influenced antibiotics distribution in biofilms and their intrinsic anti-biofilm effect. The inhibition effect of the liposomes containing antibiotics on biofilms was confirmed by analysing the data demonstrated by spectrophotometer (Bio-Rad) and the decrease in optical density at 595nm before and after its action. This study can be clinically applied for the treatment of chronic infections of *P. aeruginosa* by increasing the efficacy of the action of antibiotics on the biofilm.

CONCLUSION:

The nature of biofilm structure and the physiological attributes of biofilm organisms confer an inherent resistance to antimicrobial agents, whether these antimicrobial agents are antibiotics, disinfectants, or germicides. Mechanisms responsible for resistance may be one or more of the following:

- Delayed penetration of the antimicrobial agent through the biofilm matrix.
- Altered growth rate of biofilm organisms.
- Other physiological changes due to the biofilm mode of growth.

Antimicrobial molecules must diffuse through the biofilm matrix in order to inactivate the encased cells. The extracellular polymeric substances constituting this matrix present a diffusional barrier for these molecules by influencing either the rate of transport of the molecules to the biofilm interior or the reaction of the antimicrobial material with the matrix material.

This study showed that using antibiotics encapsulated in these liposomes influenced antibiotics distribution in biofilms and their intrinsic anti-biofilm effect. Even though the different sizes of liposomes and formulating different charged may enhance the penetration, inhibition, and specificity of P. aeruginosa biofilm or any other biofilm producing organisms.

This study can be clinically applied for the treatment of chronic pulmonary infections in cystic fibrosis patients, and even be used for other such *P. aeruginosa* infections such as soft tissue infections, joint infections, gastrointestinal infection and a variety of other systemic infections. Even though these anti-biofilm drugs encapsulated in these liposomes showed increase penetration and inhibition through effective distribution*, P. aeruginosa* biofilms can be expected to enhance their anti-biofilm effectiveness.

Thus, further studies are needed to evaluate whether multi-species biofilm growing on nasal mucosa rather than on glass or plastic surface behave in a similar way as biofilms in in vitro experiments.

It has been reported that in vivo biofilms possess a few structural and component characteristics that differ from most in vitro biofilms. Whether these differences alter biofilms physicochemical properties that determine their interactions with liposomes remains unknown. Biofilms are found in the nasal sinuses of CRS patients which have been mixed with the mucus which can trap nanoparticles by adhesion and/or obstruction. To develop nanoparticles such as liposomes that may penetrate through this mucus barrier and avoid the natural mucus clearance mechanism can be the subject of future studies.

REFERENCES

1. Gupta P, Sarkar A, Sandhu P, Daware A, Das MC, Akhter Y, Bhattacharjee S, Potentiation of antibiotic against *Pseudomonas aeruginosa* biofilm: a study with plumbagin and gentamicin, Department of Molecular Biology and Bioinformatics, Tripura University (A Central University), Suryamaninagar, Tripura, India. J Appl Microbiol. 2017 Jul;123(1).
2. Annette E. LaBauve and Matthew J. Wargo, Growth and Laboratory Maintenance of *Pseudomonas aeruginosa*, Curr Protoc Microbiol. 2012 May;0 6: Unit-6E. 1.
3. Judith H. Merritt, Daniel E. Kadouri, and George A. O'Toole, Growing and Analyzing Static Biofilms, Curr Protoc Microbiol. 2005 Jul; 0 1: Unit-1B.1.
4. Høiby N, Ciofu O, Bjarnsholt T, *Pseudomonas aeruginosa* biofilms in cystic fibrosis, Future Microbiol. 2010 Nov;5(11).
5. George A. O'Toole, Microtiter Dish Biofilm Formation Assay, J Vis Exp. 2011: (47): 2437.
6. Hong Wu, Claus Moser, Heng- Zhuang Wang, Niels Høiby, and Zhi-Jun Song, Strategies for combating bacterial biofilm infections, Int J Oral Sci. 2015 Mar, 7(1):1-7.
7. Rodney M. Donlan, Biofilms: Microbial Life on Surfaces, Emerg Infect Dis. 2002Sep: 8(9): 881-890.
8. Hera Vlamakis, Yunrong Chai, Pascale Beauregard, Richard Losick, and Roberto Kolter, Sticking together: building a biofilm the Bacillus subtilis way, Nat Rev Microbiol. 2013 Mar; 11(3): 157-168.
9. Annette Labauve, Matthew J Wargo, Growth and Laboratory Maintenance of *Pseudomonas aeruginosa*, Current protocols in microbiology Chapter 6:Unit 6E.1. May 2012.

10. Ammar Algburi, Nicole Comito, Dimitri Kashtanov, Leon M. T. Dicks, Michael L. Chikindas, Control of Biofilm Formation: Antibiotics and Beyond, American Society For Microbiology.

11. Rodney M. Donlan, J. William Costerton, Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms, American Society For Microbiology.

12. Victoria E. Wagner, Barbara H. Iglewski, P. aeruginosa Biofilms in CF Infection, Clinical Reviews in Allergy & Immunology, December 2008, Volume 35, Issue 3, pp 124- 134.

13. Dong D, Thomas N, Thierry B, Vreugde S, Prestidge CA, Wormald P-J (2015) Distribution and Inhibition of Liposomes on Staphylococcus aureus and *Pseudomonas aeruginosa* Biofilm PLoS ONE 10(6): e0131806.

Chapter 10 - Isolation of Pseudomonas Species from Soil Sample for Production of Pyoverdine and Evaluation of its Potential as an Antimicrobial Agent

Didhiti Agarwal, Dhevang Rokkala, Shreyas Tawde, Ms. Prachi Bhatia*, Dr. Sejal Rathod*
Department of Biotechnology, Kishinchand Chellaram College, Churchgate, Mumbai- 400020
prachi.bhatia@kccollege.edu.in, sejal.rathod@kccollege.edu.in

ABSTRACT:

Pyoverdine, a fluorescent siderophore that have high- affinity for iron is produced by *Pseudomonas putida* and *Pseudomonas aeruginosa* that synthesizes it under iron-deficient growth conditions. Pseudomonas species are often encountered in diverse ecological habitats alongside being censurable for nosocomial infections spread round the world. Due to these characteristics, there's a growing interest during this microbe for a spread of uses. One such is the production of Pyoverdine, it influences the uptake of iron, along with eliminating the limited iron availability condition.

Pyoverdine is important for *Pseudomonas putida* and *Pseudomonas aeruginosa* to cause acute infections. Intense research and study led to the invention of Pyoverdines being a crucial source of chelating Iron. This study is predicated on Pyoverdine extracted from two different species of Pseudomonas which will act as an antimicrobial agent for various species including Escherichia coli and Staphylococcus aureus. The organism, i.e., *Pseudomonas putida* and *Pseudomonas aeruginosa*, were isolated from soil sample using medias like: Cetrimide media, King's B media. It had been confirmed using primary biochemical tests, alongside species level identification (MALDI - TOF). Isolation was followed by studying the antimicrobial activity of Pyoverdine on different organisms using antibiotics as standard for the same. Results for the tests were obtained, colonies were observed on specific media and zone of inhibition was observed on Muller Hinton plate. Comparative studies were carried out to find which organism used up Pyoverdine or

Pyoverdine - Fe complex without using FeCl3 as sole standard source. Thus, these compounds can synergize with conventional antimicrobials, forming a simpler treatment with serving as a useful gizmo.

Keywords: *Pseudomonas putida*, *Pseudomonas aeruginosa*, Pyoverdine, Antimicrobial, Siderophore

INTRODUCTION:

To date, different sorts of biosensing elements are used effectively for environmental monitoring. Microbial cells seem to be well- suited for this task: they are cheap, adaptable to variable field conditions and provides a measurable response to a broad number of chemicals. Among different pollutants, heavy metals are still a serious problem for the environment. an inexpensive start line for the choice of a bio-recognition element to develop a biosensor for metals might be that of a microorganism that exhibits good mechanisms to deal with metals.[1]

Despite advances in antimicrobial chemotherapy, multi-drug resistant bacteria continue to cause life-threatening infections, especially in hospitals and with immunocompromised patients.

In order to drive infection in these disparate conditions, Pseudomonas utilises a diverse armamentarium of virulence factors, including those involved in colonisation and infection, nutrient acquisition), and various modulators of host response. Pseudomonads were observed early in the history of microbiology. Pseudomonas is a genus of Gram-negative, aerobic gamma proteobacteria, belonging to the family Pseudomonadaceae corroborating a great deal of metabolic diversity, and consequently are able to colonise a wide range of niches. [2]

The best studied species include *Pseudomonas aeruginosa* in its role as an opportunistic human pathogen, the plant Pathogen Pseudomonas syringae, the soil bacterium *Pseudomonas putida* due to their omnipresent rate in water and plant seeds. *Pseudomonas aeruginosa* along with *Pseudomonas putida* are particularly pernicious pathogen [3] as they possess several innate defence mechanisms against antibiotics.

Pseudomonas aeruginosa is one of the main organisms liable for drug-resistant nosocomial infections, and is one among the leading causes of bacteraemia and pneumonia in hospitalised patients. While *Pseudomonas putida* as compared to *Pseudomonas aeruginosa* has low pathogenic potential but developed immune to an outsized number of antimicrobial agents [4]

Later after years of research and intense study novel roles for several key virulence determinants are still being intuited. Pyoverdine, neither old nor new but recent, may be a siderophore produced by Pseudomonads. Pseudobactins, are a crucial group of secondary metabolites produced by microorganisms and plants to facilitate the uptake of iron, [5] which is usually insoluble in most terrestrial environments. They are bacterial products which bind iron and increase the speed of bacterial iron transport comprising a dihydroxyquinoline derivative joined to a type-specific peptide and, usually, an acid or amide. Both the chromophores and therefore the peptide chains are synthesized by nonribosomal peptide synthetases (NRPSs) that enable peptide linkage formation between amino acids that cannot be incorporated through ribosomal synthesis serving as precursor of Pyoverdines and are commonly referred to as ferribactins [6]. Ferribactins of distinct species or maybe strains often differ in their sequence, leading to an outsized sort of Pyoverdines.

Pyoverdine an iron chelating siderophore on the other side behaves as an opportunistic pathogen in terms of infectious diseases. It acts as an antimicrobial agent in various ways, it may also help to form extensive biofilms; along with influencing the ability of certain organisms to germinate and develop biofilms themselves. Pyoverdine was studied to check its ability to displace iron from transferrin as it has high affinity towards iron. In biological and non biological aspects it helps in signalling cascades and transport metals.

Siderophores have received much attention in recent years and are gaining commercial significance due to their potential application in various areas of environmental research, including medicine, agriculture (plant-bacteria synergism and bio-pesticides) and also since they are

safer, and do not cause biomagnification and also provide iron nutrition to the crops thereby promoting plant growth [7] . Therefore, such organisms produce certain useful components that can be put to great use for the uptake of irons as well as development of humans. These substances are usually produced by the organism for their own safety from the predators or for the survival of the fittest.

Therefore, studies have shown that substances with antibacterial, antimicrobial or antifungal activity secreted within the secondary metabolism of microorganisms might be applied within the management of human, animal, and plant diseases [8] and will be more advanced and developed in the near upcoming future.

MATERIALS AND METHOD:

Isolation of Pseudomonas: *Pseudomonas putida* and *Pseudomonas aeruginosa* were isolated from garden soil. Serial dilutions of the sample were carried out and were bulk seeded on Sterile King's B agar plate respectively. After incubation under aerobic conditions at 37°C, appropriate colonies were selected, purified, sub-cultured, stored for further studies and subjected to identification tests. [1,2,9]

Identification of the isolates: The isolates of the selected strains were identified based on the cultural, morphological and biochemical tests such as Gram staining, sugar fermentation, catalase test and oxidase test, as outlined in Bergey's Manual of Systematic Bacteriology. MALDI-TOF was also carried out. [10]

Culture medium and Conditions: Following purification, a 2 ml culture (adjusted to 0.1 O.D) was inoculated in 200ml of Sterile Succinate broth and King's B broth which consisted trace amounts of iron and other metal contaminants and was put on the rotary shaker for 48 hours with a pH ranging between 7.0 -7.3. [11] Uninoculated Sterile Succinate broth and King's B broth were used as controls [12]

Extraction of Pyoverdine: A crude extract was obtained by centrifugation and separation. This served as the sample for further qualitative and quantitative tests[13,8]

Centrifugation: The broths were centrifuged for 5 minutes at 4500rpm. The supernatant was subsequently collected and preserved. It was saturated with 0.02g of NaCl.

Separation and Purification: Supernatant along with 50ml of phenol and chloroform were separated using a separating funnel with treating the organic phase with 200 ml of diethyl ether 20 mins later, two district phases were obtained and the aqueous phase was re- extracted thrice (using the same protocol). [11, 13, 15]. Sample extracts were stored at 4°C and was treated with 5% acidic EDTA Chloroform solution to obtain an iron- free Pyoverdine. The pure Pyoverdine was observed for fluorescence and absorbance was measured.

Siderophore Qualification Tests: The test was carried out using Arnow's method and Tetrazolium test method for catecholates and hydroxymates respectively. [16]

Arnow's method: 1mL of cell free culture filtrate was mixed with 1mL of 0.5 mol L−1 HCl, 1mL of nitrite-molybdate reagent and 1mL of 1mol L−1 NaOH and was incubated for 5 mins at 36°C.

Tetrazolium test method: 1mL of culture filtrate was hydrolysed with 0.5mL of 1N NaOH, and a pich of tetrazolium salt was added. The solution was then mixed with an instant appearance of a deep red colour.

Antimicrobial activity: This activity was carried out on two different organisms that were: Escherichia coli and Staphylococcus aureus thus testing the ability the Pyoverdine for its antimicrobial effect. [17, 18]

Preparation and Plating: 20mL of Sterile MH butts each containing 1 ml culture (adjusted to 0.5 O.D) was aliquoted in sterile petri plates [19]. Using Agar well diffusion method 50 µL of Pyoverdine, Pyoverdine-Fe complex was added with addition of Antibiotics and FeCl3 as standards respectively following incubation at 36°C for 24 hours for zone of inhibition.[20]

RESULTS:

Identification of Pseudomonas species: Gram staining was carried out along with Catalase test, Sugar Fermentation test, Indole test, Methyl Red test, Citrate test, Oxidase test and Voges Proskauer test and, on the basis of the Bergey's Manual of Systemic Bacteriology we can indicate that the isolated culture is Pseudomonas species.

Isolate Characteristics	P. putida	P. aeruginosa
Size	Medium	Small, pin-point
Shape	Round	Round
Colour	Bue-Green	Yellow-Green
Elevation	Convex	Convex
Margin	Entire	Entire
Consistency	Butyrous	Butyrous
Opacity	Translucent	Translucent
Gram - nature	Gram negative	Gram negative
Morphology	Short rods	Short rods

Table 1: Biochemical Tests for Pseudomonas Isolate

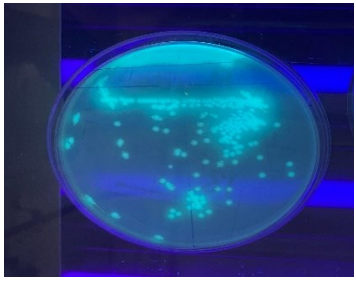


Figure 1: P. putida on Pseudomonas Isolation Media

Biochemical Tests	Standard	Observed
Sugar Utilization		
• Glucose	—	—

• Lactose	—	—
• Mannitol	—	—
• Xylose	—	—
Indole	—	—
Methyl Red	—	—
Voges -	—	—
Proskauer		
Triple Sugar Iron	—	—
Citrate -	+	+
Utilization		
Urease	—	—
Oxidase	+	+
Rapid Catalase	+	+

Table 2. Biochemical Tests for Pseudomonas Isolates

	OD of Pyoverdine			
	P. putida		***P. aeruginosa***	
Wavelength(nm)	**Succinate**	**King's B**	**Succinate**	**King's B**
400nm	0.89	0.5	0.08	0.35
420nm	1.25	0.71	0.12	0.65
470nm	1.11	0.62	0.1	0.5
500nm	1.06	0.56	0.08	0.44
530nm	0.94	0.48	0.07	0.35
620nm	0.76	0.36	0.05	0.17
660nm	0.68	0.32	0.04	0.12
700nm	0.55	0.24	0.02	0.05

Table 3 Absorbance of Fluorescence Quenching Reversal Pyoverdine

DISCUSSION:

In the past recent years, siderophores and Pseudomonas have grabbed pronounced attention and utmost importance in the field of research around the world. Pseudomonas are one of the microbial type of species unveiling their benefit for glycerol utilisation as well as their physiological, metabolic and genetic features; giving us a larger platform for further research on various other components produced by them for industrial as well as clinical use to the world. The current study undertaken not only focuses on production Pyoverdine but it also portrays a great impact in the field of biotechnology today. Soil is considered to be a sample with maximum number of organisms especially Pseudomonas species which are capable of secreting Pyoverdines essential for iron chelation.

Pseudomonas species isolated from soil was was identified up to the species level and was characterised by Gram staining where Gram-negative, short pink rods were seen. Biochemical tests were in accordance with the Bergey's Manual of Systemic Bacteriology hence, on the basis of these results we can safely say that the isolated organism belongs to Pseudomonas species.

Pyoverdines are yellow-green in colour and are found to be of utmost importance for bacterial virulence and thus they were efficiently extracted from Succinate broth and King's B broth using Diethyl ether, Chloroform and Phenol. Purification was done using 5% EDTA Chloroform solution. Separation was based the growth and pigmentation of *Pseudomonas putida* and *Pseudomonas aeruginosa* present in the Succinate medium and King's B medium.

Generally, Pseudomonas species studied produce Hydroxymate type of siderophore. *Pseudomonas putida* and *Pseudomonas aeruginosa* had a strong reaction to tetrazolium test and no reaction to Arnow's method thus this indicates the presence of Hydroxymate type of siderophore in the supernatant of the culture. The antimicrobial tests proved that synthetic media are most accurate and the best for siderophore activity. The reason being that media usually have varied levels of iron

contamination, carbon and energy source and buffering capacity. Siderophore activity was recorded maximum in Succinate broth and minimum in King's B broth.

Pyoverdine from Succinate broth had maxim buffering capacity on account of di-potassium hydrogen phosphate and potassium di- hydrogen phosphate among the ingredients present. Siderophores are very sensitive to high pH and so at higher pH these biomolecules degrade. It is quite frequent that the level of contaminating iron is already high enough to sustain maximum growth in complex culture media.

CONCLUSION:

The choice of microbial hosts for biotechnological applications had long been based on historical tradition rather than on the bacterial platform which meets the desired process criteria in the best possible way. This is mainly because, there are micro-organisms that give out by-products and are extensively investigated and characterized are often more easily manipulated to maximise production, and become useful to mankind. One of such organisms include *Pseudomonas putida* and *Pseudomonas aeruginosa* that produce siderophores namely Pyoverdine.

Pseudomonas species isolated from soil were found to be capable of producing Pyoverdine which was extracted and purified from each of the broths and organism. The Pyoverdine detected belonged to the hydroxymate group. The antimicrobial activity was checked and both the organisms *E. coli* and *S. aureus* used Pyoverdine extracted from the Succinate broth giving a zone diameter of 3.9 cm while King's B broth showed no zone of inhibition. Thus, a synthetic medium will always show a greater activity of siderophore because it is iron free and contaminate free while a non - synthetic medium will contain contamination along with iron and other metals hence the activity will not be enhanced and seen.

Thus, with further studies the Pyoverdine can added in antibiotics and be directly inserted into the bacterial host for further and better

antimicrobial activity. It will reduce costs along with being a good natural source and be a stronger agent.

REFERENCES:

1. Orji Frank Anayo, Ezeanyanso Chika Scholastica, Onyemali Chidi Peter, Ukaegbu Gray Nneji, Ajunwa Obinna and Lawal Oluwabusola Mistura, 2018,
2. The Beneficial Roles of Pseudomonas in Medicine, Industries, and Environment: A Review, DOI: 10.5772/ intechopen.85996.
3. Daniel R. Kirienko, Donghoon Kang, and Natalia V. Kirienko, 2018, Novel Pyoverdine Inhibitors Mitigate *Pseudomonas aeruginosa* Pathogenesis, Front Microbiol. 2018; 9: 3317.
4. Hayder Abdulrahman, Raed Obaid Saleh, 2016, Isolation and Identification of *Pseudomonas aeruginosa* from different sources (soil, wound, urine) and Checking its MIC with various Antibiotics, Helix Vol.4-5: 795-799 (2016).
5. Dhusi, K. Bajpai, A. Ramteke, P. W., 2018, Overcoming antibiotic resistance: Is siderophore Trojan horse conjugation an answer to evolving resistance in microbial pathogens?, Journal of controlled release: official journal of the Controlled Release Society, 269, 63–87.
6. Iain L. Lamont, Lois W. Martin, 2003, Identification and characterization of novel P yoverdine synthesis genes in *Pseudomonas aeruginosa*, Microbiology, volume 149, issue 4.
7. Mary E. Peek, Abhinav Bhatnagar, 1 Nael A. McCarty, 2 and Susu M. Zughaier, 2012, Pyoverdine, the Major Siderophore in *Pseudomonas aeruginosa*, Evades NGAL Recognition, Interdisciplinary Perspectives on Infectious Diseases,volume 2012, article ID 843509.
8. Patrick Abou Raji El Feghali and Tarek Nawas, 2018, Extraction and purification of pyocyanin: a simpler and more reliable method, MOJ Toxicol, Volume 4 Issue 6.

9. Joan L. Slonczewski, Kenyon College, 2015, *Pseudomonas putida*, Microbial Biorealm.

10. Sagar Aryal, 2018, Biochemical Test and Identification of *Pseudomonas aeruginosa*, Microbiology info. Bergey,

11. D. H., Krieg, N. R., & Holt, J. G., (1984), Bergey's manual of systematic bacteriology.

12. J.M. Meyer, 1978, The Fluorescent Pigment of Pseudomonas fluorescens Biosynthesis, Purification and Physicochemical Properties, Journal of general Microbiology(1978), issue: 107, pages: 319-328.

13. Angel R. Cueva, Oanh Pham, Aissata Diaby, Derek Fleming, Kendra P. Rumbaugh, and Gregory E. Fernandes, 2020, Pyoverdine Assay for Rapid and Early Detection of *Pseudomonas aeruginosa* in Burn Wounds, Applied Bio Materials, Volume 3, Issue 8, pages: 4696-5454.

14. Alok Sharma, Bhavdish N. Johri, 2003, Combat of iron-deprivation through a plant growth promoting fluorescent Pseudomonas strain GRP3A in mung bean (Vigna radiata L. Wilzeck), Microbiological. Research. (2003) Volume: 158, Pages: 77–81 .

15. Patrick Abou Raji El Feghali and Tarek Nawas, 2018, Extraction and purification of pyocyanin: a simpler and more reliable method, MOJ Toxicol, Volume 4 Issue 6.

16. Donghoon Kang, Daniel R. Kirienko, Phillip Webster, Alfred L. Fisher, and Natalia V. Kirienko, 2018, Pyoverdine, a siderophore from *Pseudomonas aeruginosa*, translocates into C. elegans, removes iron, and activates a distinct host response, Virulence, volume 9, No. 1, pages: 804-817.

17. Carlos M. H. Ferreira, Ângela Vilas- Boas, Cátia A. Sousa, Helena M. V. M. Soares and Eduardo V. Soares, 2019, Comparison of five bacterial strains producing siderophores with ability to chelate iron

under alkaline conditions, AMB Expr (2019) 9:78 https://doi.org/10.1186/s13568-019-0796-3.

18. Dhusi, K. Bajpai, A. Ramteke, P. W., 2018, Overcoming antibiotic resistance: Is siderophore Trojan horse conjugation an answer to evolving resistance in microbial pathogens?, Journal of controlled release: official journal of the Controlled Release Society, 269, 63–87.

19. Lázaro Molina, Zulema Udaondo, Estrella Duque, Matilde Fernández, Carlos Molina-Santiago, 2014, Antibiotic Resistance Determinants in a *Pseudomonas putida* Strain Isolated from a Hospital. PLoS One, 2014; 9(1): e81604.

20. Silke Peter, Philipp Oberhettinger, Leonard Schuele, Ariane Dinkelacker, Wichard Vogel, Daniela Dörfel, Daniela Bezdan, Stephan Ossowski, Matthias Marschal, Jan Liese & Matthias Willmann, 2017, Genomic characterisation of clinical and environmental *Pseudomonas putida* group strains and determination of their role in the transfer of antimicrobial resistance genes to *Pseudomonas aeruginosa*, BMC Genomics, volume 18, article no. 859, 2017.

21. Qing-Ping Hu and Jian-Guo Xu, 2011, A simple double-layered chrome azurol S agar (SDCASA) plate assay to optimize the production of siderophores by a potential biocontrol agent Bacillus, African Journal of Microbiology Research Vol. 5(25), pp. 4321-4327, 9 November, 2011

SECTION III - PHYSICS

Chapter 11 - Prospective Material for Nonlinear Optical Organic - Inorganic Applications

Krishna Bulchandani, Abbas Dalvi, Dr. Jyotsna Pandey*
Department of Physics, Kishinchand Chellaram College, Churchgate, Mumbai- 400020
Jyotsna.pandey@kccollege.edu.in

ABSTRACT:

The present research paper oversees the various optical properties of the crystals of Potassium dihydrogen phosphate (KDP) and Ammonium dihydrogen phosphate (ADP) doped with L- arginine (amino acid). The AR grade samples of Potassium dihydrogen phosphate (KDP) and Ammonium dihydrogen phosphate (ADP) doped with L-arginine in two different stoichiometric ratios of 2:1:1 and 3:1:1 has been grown. The aqueous solutions of this ratios of the sample kept for slow evaporation at room temperature. The UV-VIS analysis was performed on the samples to determine Absorbance, transmittance, reflectance, and band gap of the sample. The optical band gap value of the sample is evaluated be 5.80eV for 2:1:1 and 5.97eV for 3:1:1eV. The UV-VIS analysis revealed high optical transparency window and the maximum cut-off wavelength found to be around 222.6 nm for 2:1:1 and 214.6 nm for 3:1:1 crystal imperative to evaluate the electronic band structure and optical properties. The grown crystal inherits wide band gap, high optical transparency eminent for optical device fabrication. A range of analysis suggests suitability and potentiality of KDP: ADP: L-Arginine hybrid crystals for various optoelectronic applications.

KEYWORDS: Crystal growth, Non-linear optic materials, Optical properties, UV-VIS analysis

INTRODUCTION:

Nonlinear optical (NLO) crystals with high conversion efficiencies for second harmonic generation are desirable in various applications such as

telecommunication, optoelectronics and laser technology [1]. There have been interesting materials both academically and industrially [1]. KDP is a dielectric material well known for its nonlinear optical and electro properties [2,3]. The KDP offers excellent properties such as resistant to damage by radiation of laser, transparency in wide region of optical spectrum and high non-linear efficiency in addition to reproducible growth to large size [4]. Therefore, it is commonly used in several applications such as laser fusion, electro-optical modulation and frequency conversion [5]. Many studies on the growth and properties of KDP crystals in the presence of impurities have been reported [6-9]. Ammonium dihydrogen phosphate (ADP) is an excellent inorganic nonlinear optical material with different device applications [1]. ADP is widely used as the second, third and fourth harmonic generator of Nd: YAG and Nd: YLF lasers [10]. The crystal is widely used for electro-optical applications such as Q- switch for Nd: YAG, Nd: YLF and Ti-sapphire lasers as well as for acoustic-optical applications [11]. ADP has gained considerable importance in recent years because of its nonlinear, ferroelectric, piezoelectric and electro-optical properties [12]. Organic materials have been of particular interest because the NLO responses in this broad class of materials is microscopic in origin, offering an opportunity to use the theoretical modelling coupled with synthetic flexibility to design and produce novel NLO materials [13-15].

However most organic NLO materials are susceptible to damage because of their poor mechanical and thermal properties [16]. The amino acids are the famous organic materials, play a vital role in the field of nonlinear optical crystal growth. Both organic and inorganic materials have particular merits for application to optical technology [17-18]. If inorganic materials are appropriately combined with organic molecules, the resultant hybrid materials can offer the combined merits of both classes of materials and can acquire individual properties or combinations of properties with practical suitability that exceeds that of conventional parent materials [19- 20]. Therefore, doping KDP crystals with amino acid families has been under extensive investigation in recent times. because of the favourable enhancement of the NLO properties of

the standard KDP crystals [21-22]. The search for effective NLO materials has shown that L-arginine–based crystals such as L- arginine phosphate monohydrate (LAP), which was first reported by Xu et al, exhibit higher nonlinearity, conversion efficiency, and wider transmission range in comparison with KDP.

After this discovery, several amino acid crystals such as L-arginine acetate (LAA), L-arginine chloride monohydrate (LACh), L-arginine hydrochloride monohydrate (LAHCh), L-arginine hydrobromide (LAHBr), L- histidine tetrafluroborate (L-HFB), and L-histidine hydrofluoride dihydrate (LHHF) have been studied [16-22]. Hence new type of NLO materials have been built from organic–inorganic complexes in which the high optical non-linearity of a purely organic compound is combined with the favourable mechanical and thermal properties of inorganic materials. With an intent of discovering new useful hybrid materials for prospective academic and industrial application we have made an attempt to modify KDP crystals and ADP crystals by adding some amino acids. In the present paper KDP and ADP were doped with amino acid L-Arginine. The results and observations are discussed below.

METHODOLOGY:

The AR grade of L-arginine, KDP and ADP were selected for present paper. The pure materials KDP, ADP and L-Arginine were taken in two different ratios which are 2:1:1 and 3:1:1.

For 3:1:1 - KDP:ADP: L-Arginine and for 2:1:1 - KDP:ADP: L-Arginine. All the materials were disintegrated in twofold distilled water in a glass beaker and were blended homogeneously utilizing a magnetic stirrer. for 3 hrs. The pH estimations of both the proportions were determined utilizing pH meter, pH for 3:1:1 example is 5.93 and that for 2:1:1 example is 6.09. The homogeneously mixed solutions were poured in a clean glass beaker for 2 weeks. The grown crystals of 2:1:1 and 3:1:1 ratio have been shown in the Fig (1, a & b) respectively.

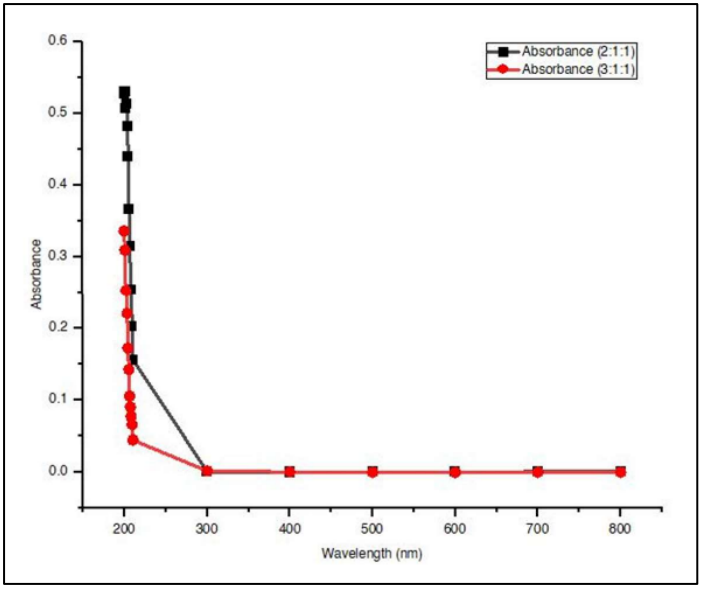


Figure 1 UV-VIS comparative Absorbance spectra between 200 and 800 nm for KDP, ADP doped with L-Arginine in the ratio of 3:1:1crystal and 2:1:1crystal

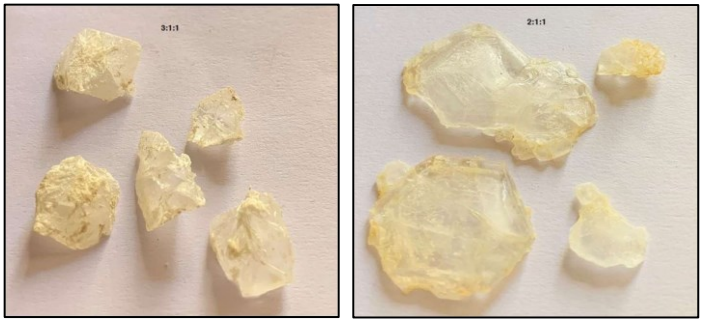


Figure 2 a & b the grown hybrid crystals of KDP:ADP: L-Arginine in the ratio of a) 2:1:1 and b) 3:1:1

RESULT AND DISCUSSION:

UV-VIS spectral analysis

The UV visible spectra of L-Arginine doped with KDP + ADP in different ratios were recorded in the wavelength range of 200-800 nm and it is shown in the Fig (2). The recorded absorbance spectra of KDP, ADP doped with L-arginine in the ratio of 2:1:1 and 3:1:1 is shown in fig (2). The cut- off wavelength is 222.6 nm for 2:1:1, whereas for 3:1:1 sample it is 214.6 nm. It very well may be seen from the spectra that absorbance for both 2:1:1 and 3:1:1 shows reductions as frequency increments and after certain frequency it becomes zero.

This spectral study would be aided in the comprehension of electronic structure and the optical band gap of the grown crystals. This property enables the material to hold good for optoelectronics application and its suitability for second harmonic generation.

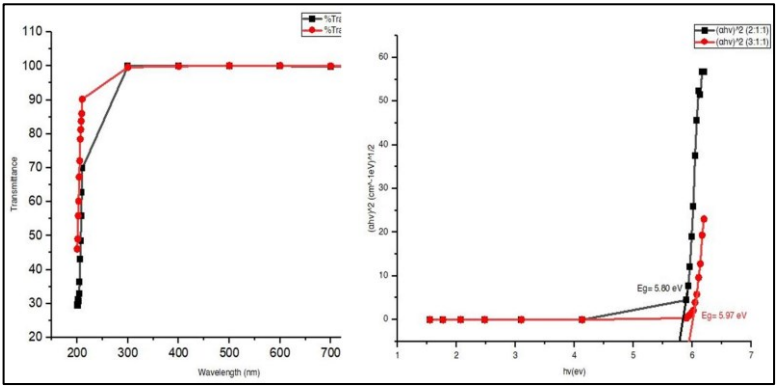


Figure 3 & 4. Comparative Transmittance spectra between 200 and 800 nm for KDP, ADP doped with L-Arginine in the ratio of 3:1:1crystal and 2:1:1crystal. Comparative (αhv) ^2 Vs. hv data between 1 and 7 eV for KDP, ADP doped with L- Arginine in the ratio of 3:1:1crystal and 2:1:1crystal

The least absorption in visible range leads to high transmission as seen in fig (3), this implicates high optical transparency, which is imperative for NLO applications.

Transmittance has been calculated from the relation, %T = antilog (2 – absorbance). Transmittance Vs. wavelength spectrum is shown in the fig (3). From the spectra it is observed that both 2:1:1 and 3:1:1 crystal.

Band-gap Evaluation:

The dependence of optical absorption coefficient with the photon energy helps to study the band structure and the type of transition of electrons [23]. The optical band gap can be obtained by means of a Planck relation, where h is the Planck constant, ν is the wave frequency, and c is the speed of light in vacuum. Band gap energy has been calculated from Tauc's formula where α is the absorption coefficient, hν is photon energy, A is a constant, and m=2 refers to indirect band-to-band transition, and m=0.5 refers to direct band-to-band transition. The plot of (αhν) 2Vs. hν is shown in Fig. (4). To decide the optical band gap energy experimentally, m=1/2 is found as the best fitted value for the linearity of the plot and that indicates indirect band gap transition. The traversing point on energy axis indicates the band gap energy value of 3:1:1 and 2:1:1 crystal.

The optical absorption coefficient α has been evaluated using formula where t=1cm (thickness of the sample) also 5.97eV. This wide bandgap allows us to use materials in high-temperature and power switching applications. The higher band gap, good transmission in overall region, and lower refractive index make the crystal suitable for good optoelectronic applications [25].

CONCLUSION:

Crystals of L-arginine doped with KDP and ADP in two different ratios were grown using slow evaporation technique and comparative Optical studies were performed. The high percentage transmittance in entire visible region is essential for NLO crystals. The variance of Absorbance and Transmittance spectra indicates the crystals poses good optical quality and proposes suitability of grown crystals for optoelectronic

application. The wide bandgap of 5.96eV and 5.98eV of both the crystal suggests crystals are promising material for non-linear optical applications

REFERENCES:

1. Pattanaboonmee, N., P. Ramasamy, & P. Manyum, 2012. Optical, Thermal, Dielectric and Mechanical Studies on Glycine Doped Potassium Dihydrogen Orthophosphate Singles Crystals Grown by SR Method. Procedia Engineering 32, pp. 1019–1025
2. De Yoreo, J. J., Zaitseva, N. P., Woods, B. W., & Rek, Z. U. 1996. Sources of optical distortion in rapidly grown crystals of KH2PO4. Journal of Crystal Growth, 166(1–4), 291–297.
3. Zaitseva, N. P., Rashkovich, L. N., & Bogatyreva, S. V. (1995). Stability of KH2PO4 and K (H, D) 2PO4 solutions at fast crystal growth rates. Journal of Crystal Growth, 148(3), 276-282.
4. N. Zaitseva, & L. Carman, 2001. Rapid growth of KDP-type crystals. Progress in Crystal Growth and Characterization of Materials, 43(1), 1-118.
5. Pv, Dhanaraj & C.K., Mahadevan & Bhagavannarayana, G. & Perumalsamy, Ramasamy & Rajesh, Narayana, 2008, Journal of Crystal Growth, vol. 310, no.24, pp. 5341-46 5341-5346.
6. Wang, B., Fang, C.s., Wang, S.l., Sun, X., Gu, Q.t., Li, Y.p., Xu, X.g., Zhang, J.q., Liu, B., & Mou, X.m., 2006. The effects of Sn4+ ion on the growth habit and optical properties of KDP crystal. Journal of Crystal Growth, 297(2), 352-355.
7. Kannan, V., Bairava Ganesh, R., Sathyalakshmi, R., Rajesh, N. P., & Ramasamy, P., 2006. Influence of La3+ ions on growth and NLO properties of KDP single crystals. Crystal Research and Technology: Journal of Experimental and Industrial Crystallography, 41(7), 678-682.

8. Podder, J., Ramalingom, S., & Kalkura, S. N., 2001. An investigation on the lattice distortion in urea and KCl Doped KDP single crystals by X-ray diffraction studies. Crystal Research and Technology: Journal of Experimental and Industrial Crystallography, 36(6), 549-556.

9. Claude, A., Vaithianathan, V., Ganesh,R. B., Sathyalakshmi, R., & Ramasamy, P., 2006. Crystal Growth of Novel Bimetallic Nikel, Magnesium (Ni", Mg") Potassium Di Hydrogen Phosphate by Solution Growth and their Characterizations. Journal of Applied Sciences, 6(3), 635-638.

10. S. M. Klimentov, S. V Garnov, A. S. Epifanov, A. A Manenkov, 1994, Proceedings of SPIE 342, 2145-2150.

11. Ramirez, R., & Gonzalo, J. A., 1990. Comparative analysis of the antiferroelectric behaviour in C4O4H2 and NH4H2PO4. Solid state communications, 75(6), 481-482.

12. Chemla, D. S. (Ed.)., 2012. Nonlinear Optical Properties of Organic Molecules and Crystals V1 (Vol. 1). Elsevier.

13. Ikeda, H., Sakai, T., & Kawasaki, K., 1991. Nonlinear optical properties of cyanine dyes. Chemical physics letters, 179(5-6), 551-554.

14. Katz, H. E., Singer, K. D., Sohn, J. E., Dirk, C. W., King, L. A., & Gordon, H. M., 1987. Greatly enhanced second-order nonlinear optical susceptibilities in donor- acceptor organic molecules. Journal of the American Chemical Society, 109(21), 6561-6563.

15. Marder, S. R., Kippelen, B., Jen, A. K. Y., & Peyghambarian, N., 1997. Design and synthesis of chromophores and polymers for electro-optic and photorefractive applications. Nature, 388(6645), 845-851.

16. Mazumder, A., Kar, T., & Gupta, S. P. S., 1995. Infrared spectroscopy and thermal studies of as-grown L-arginine phosphate

monohydrate crystals. Japanese journal of applied physics, 34(10R), 5717.

17. Dong, X., Min-Hua, J., & Zhong-Ke, T., 1983. A new phase matchable nonlinear optical crystal—L-arginine phosphate monohydrate (LAP). Acta Chimica Sinica English Edition, 1(2), 230-233.

18. Gulam Mohamed, M., Vimalan, M., Jesudurai, J. G., Madhavan, J., & Sagayaraj, P., 2007. Growth and characterization of pure and doped

19. nonlinear optical l-arginine acetate single crystals. Crystal Research and Technology: Journal of Experimental and Industrial Crystallography, 42(10), 948-954.

20. Kalaiselvi, D., Kumar, R. M., & Jayavel, R., 2008. Single crystal growth and properties of semiorganic nonlinear optical L-arginine hydrochloride monohydrate crystals. Crystal Research and Technology: Journal of Experimental and Industrial Crystallography, 43(8), 851-856.

21. Rashkovich, L. N., & Shekunov, B. Y., 1991. Study of the growth mechanism of L- arginine chloride monohydrate (LACh) crystals. Journal of crystal growth, 112(1), 183-191.

22. Madhavan, J., Aruna, S., Prabha, K., Julius, J. P., Joseph, G. P., Selvakumar, S., & Sagayaraj, P., 2006. Growth and characterization of a novel NLO crystal l- histidine hydrofluoride dihydrate (LHHF). Journal of crystal growth, 293(2), 409-414.

23. Aggarwal, M. D., Choi, J., Wang, W. S., Bhat, K., Lal, R. B., Shields, A. D., ... & Frazier, D. O., 1999. Solution growth of a novel nonlinear optical material: L- histidine tetrafluoroborate. Journal of crystal growth, 204(1-2), 179-182.

24. Tigau, N., Ciupina, V., Prodan, G., Rusu, G. I., Gheorghies, C., & Vasile, E., 2004. Influence of thermal annealing in air on the structural and optical properties of amorphous antimony trisulfide

thin films. Journal of Optoelectronics and Advanced materials, 6(1), 211-217.

25. Qian, G., Dai, B., Luo, M., Yu, D., Zhan, J., Zhang, Z., ... & Wang, Z. Y., 2008. Band gap tunable, donor− acceptor− donor charge-transfer heteroquinoid-based chromophores: near infrared photoluminescence and electroluminescence. Chemistry of Materials, 20(19), 6208-6216.
26. Meena, K., Muthu, K., Meenatchi, V., Rajasekar, M., Bhagavannarayana, G., & Meenakshisundaram, S. P., 2014. Growth, crystalline perfection, spectral, thermal and theoretical studies on imidazolium l-tartrate crystals. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 124, 663-669

SECTION IV – SOCIOLOGY

Chapter 12 - Influence of Television and Web Series on Career Choices of People in Age Group 18 to 21

Paarth Endlaw, Dr. Leena Pujari*
Department of Sociology, Kishinchand Chellaram College, Churchgate, Mumbai- 400020
leena.pujari@kccollege.edu.in

INTRODUCTION

Mass media has been described as "an impersonal vehicle of mass communication such as newspapers, magazines, radio, television, cinema, record music and the Internet" (Bruce and Yearly, 2006, pg. 185). Being a channel of communication and interaction with the masses, it has had a tremendous impact in shaping public narratives. Historically, if the influence of media on society was examined, beginning from MarshallMcLuhan's "GuttenbergGalaxy" and "Electronic Age" (Marcel Denesi, 2019); the impact made by the all-in-one mass media (as a broad amalgamation of the various subparts of media) is paramount in the development of society in all spheres of life. Events as recent as the advent of social media or micro-blogging sites have made tremendous growth and development. The poster child of media has shifted from one medium to another over the years, beginning with books in one of their earliest forms. During the first world war, newspapers were the flag-bearers of media, and by the second world war, the face of media had shifted to the radio, followed by Television (TV). It became a force to reckon with during (and at the end of) the cold war era. Ever since then, television has been the flag bearer of media.

Today, media has grown to such an extent that it not only shapes personal experiences but can also shape larger cultures. An example of the same can be the Vulcan Salute (from Star Trek) in the daily lives of many in the US, or the usage of the phrase 'Lock Kiya Jaye' and 'Kya yeh aapka final answer Hai' from Kaun Banega Crorepati or KBC in regular conversations in India (Roy, 2011). With the internet boom, television

did witness a bust (TV viewership fell). However, that did not fully diminish its influence on society and the culture. To date, TV remains imperative and one of the most impactful mediums to change or shape public opinions.

According to BARC India data (as of 8th September 2022), there are 836 million TV Viewing individuals in India, of which 762 million watch TV weekly. With the population of India at 1.417 billion (World Population Prospects, 2019), this puts the television penetration at approximately 58.99% (as TV viewing individuals) and 53.77% (as individuals watching TV every week). With television having such a large audience, it certainly does have a massive sway on the population and has tremendous potential to influence what has been studied previously (Bandura, A., 1963; Bogatz and Ball, 1971; Brown, W.J., 1990).

The Internet changed the way people perceive information. From an era of too little, the world shifted into information overload. The Internet made everything available at the tip of a finger. The speed of changes in culture, as a result of media, made it perennially dynamic. The temporary nature of the culture nowadays has resulted in viral, trending, and popular being pre-fixed with culture. It resulted in the formulation of previously unimaginable feats such as computer games like space invaders, road rash or counter strike. Additionally, the digital transformation of existing objects also occurred with digital copies of videos (on YouTube), tv shows & movies started streaming (on OTT platforms such as Netflix and Amazon Prime), and millions of books on digital readers (like Kindle).

As mentioned earlier, TV witnessed a drop in its influence (in India, this drop has continued) due to the spread of OTT. However, the influence of TV on popular culture has remained constant parallel to OTT gaining popularity by re-releasing popular series and movies.

Popular series remain substantially more influential with them being broadcast or streamed on various media (like TV and OTT). Their existing influence got boosted by the growth of OTT, especially during

the pandemic (Sontakke, K.S., 2021). There are several examples of the growing popularity of such series at the global level by increased usage of languages like Dothraki (from Game of Thrones) and Klingon (from Star Trek). (Prisco, P., 2019)

Popular series has maintained a strong influence on the population. One of the best examples of this can be Brooklyn Nine-Nine, a police Sit-Com show based on a squad of detectives, their supervising sergeant, and the precinct captain serving in the New York City Police Department or NYPD. While this may sound like a parody of the regular-cop genre, it is much more than that. This show is known for its inclusive and rounded characters that, during the course of the show, tackle real-world issues like racism in everyday life, workplace harassment, discrimination of non-binary genders, etc. It highlights such issues in a humorous (ergo, digestible) yet insightful way that urges the audience to be more inclusive of persons. The show promotes this by showcasing such values during interactions between the characters and how they handle such situations. (Joshi, 2021)

However, there is currently a gap in the literature regarding the influence of television and web series on the career choices of the college-going population. The researcher wondered if the viewing of career-focused shows results in viewers choosing a career of their favourite characters. Additionally, any underlying factors can be responsible & transferable as the key to the individual's decision of choosing their career. To address these points, the researcher surveyed 37+39 students at various colleges in Mumbai, primarily KC College, regarding their television viewing habits, favourite shows/series, and most identifiable characters. The study aimed to determine the possible effects that career-based television shows may have on viewers' decisions in choosing a career. The knowledge from this study allows a better understanding of the effects of series effects on human behaviour.

Literature Review

While there were studies on the influence of media, The trend of studying media for its effects on individuals gained traction in the US following

the assassination of Presidential candidate Robert F. Kennedy during his speech and several other televised incidents. There was a widespread fear (and interpretation) that such televised acts of violence further provoke violent acts.

in the 1960s, Albert Bandura, a social psychologist from Stanford University, conducted a series of studies on role model-based learning by children. During his 1963 study, he (along with his co-author) studied if a virtual role model can influence their behaviour. The researchers found that “subjects who viewed the aggressive human and cartoon models on film exhibited nearly twice as much aggression than did subjects in the control group who were not exposed to the aggressive film content.” (Quoted from Artino Jr., A.R., 2007; Bandura et al., 1963, p. 9) The study showed that people could be influenced by television (and later by other audio-visual modes of communications as well.) Bandura further theorised in his studies and published the social learning theory (2001) refers to "learning that is facilitated through social interactions with other individuals" (APA Dictionary of Psychology). In this context, it shows that people do learn their behaviour from their interactions with others, and these 'others' do not have to be humans; they can also be fictional characters.

In the 1970s, Richard Wollheim theorised about the impact of identification that an individual goes through. While most theorists believed that identification was done primarily with one's parents as a way of internalisation of their values and norms i.e. Socialisation. He extended the concept to all those individuals whom the person wants to model themselves after. Further, he submitted that "In effect what we do when we identify with another is that we write a part for ourselves, based upon the other, in the hope that, when we act it to ourselves, we shall be carried away by the performance." (Quoted from Cohen, J., 2001, p. 247; Wollheim, R., 1974, p. 191) In the context of the study, it shows the extent to which people get influenced by popular characters and change/model themselves based on the characters' personalities.

In the early 2000s, Cynthia Hoffner, a professor and researcher of the effects of media. In her research, she found that a "relationship does exist between occupational aspirations and amount of television watched" (Quoted from Morgan, J., 2017; Hoffner et al., 2006). She additionally suggests that "frequency of viewing and the extrinsic rewards of the favourite character's job, as well as income and education level of the favourite character, are all positive predictors of wishful identification." (Quoted from Morgan, J., 2017; Hoffner & Buchanan, 2005) In this context, it shows that people can get influenced by the characters' occupations from the shows they watch. They may also be influenced by the income and educational level of the characters.

In 2014, the research conducted by Sidneyeve Matrix (2014) from Queen's University found that "binge-watching platforms, such as Netflix, are changing how viewers watch TV and increasing the amount of TV they consume. Teen viewers reported in a Stage of Life study that they learned to set new goals and follow their dreams through watching their favourite shows." (Quoted from Morgan, J., 2017; Matrix, 2014) In the context of the study, the research shows how binge-watching impacts the viewers and how it can shape their goals and dreams.

Research Questions

Based on such review of literature, we pose the following research questions.

- In what ways does series influence a person's career choice?
- Does it help them to make specific career choices?
- How does the influence operate?

Methodology

1. Participants

Participants were recruited using deliberate sampling and snowball sampling by the researcher at various colleges in Mumbai. The research primarily consisted of students from KC College of HSNC University, Mumbai. The final sample of respondents for the study was 77 (N=

38+39), of which 39 participants were part of stage one of the study and 38 participants for the second stage. Of the respondents, 63.63% identified as (Cis-gendered) females, and 36.36% identified as (Cis-gendered) males. The age band of all respondents was between 18 to 21 years.

2. Procedures

In the first phase of the study, the researcher attempted to better understand the media consumption of college students in Mumbai. During this phase, the researcher created an open-ended questionnaire. This included fields such as demographics, "Are you a resident of Mumbai?", "Which locality do you live in?", and "What shows do you watch? (Including TV, OTT & others)". The questionnaire was shared in all student WhatsApp groups of the 2020-23 batch of the BA (Aided) Program of KC College. This phase helped the researcher to identify popular and influential series. Through the questionnaire, the researcher found area-specific data regarding the viewing habits of the SYBA students of KC College. The questionnaire in question was open for three weeks (June 9 to June 24, 2022).

In the second phase of the study, the researcher attempted to understand the influence of characters from popular series on the career choices of the college-going population. From the results of phase one, two series were chosen for research. During this phase, the researcher formulated a close-ended questionnaire that included fields such as "Which character do you identify with the most in The Big Bang Theory?", "Which aspects of the character appeal to you?" and "Which aspects of the character are disliked by you?".

3. Measures

After asking about demographics (including; gender, age, etc.), participants were asked if they watched the specified series (The Big Bang Theory and Suits) with the help of a checkbox to cross-check if they are eligible for the study. For each show that respondents reported having seen, they were then asked several questions about the characters

that they most identify with from both shows, which aspect of the character appealed to them the most as well as what they disliked the most. The researcher identified five variables (i.e. choice of career, educational qualification, appearance & personality, monetary aspect, and work-life balance) and used the same against each previously mentioned question. The study used a Likert Scaling model with a five-point scale that ranged from 5 being the "strongly agree" to 1 being "strongly disagree."

Research Findings and Observations

Phase One:

Popular Series of target audience

Phase one of the study aims at recognising the series which are viewed by the target participant universe. The participants were asked to mention any five of the same that was their Top-of-the-mind recall. The reason behind doing so was to prompt them to choose those series that were influential for them (at an individual level) instead of what was trending at the moment.

List of "Top-of-the-Mind" Recalled Series		
Sr. No.	**Series**	**Frequency**
1	Friends	17
2	The Big Bang Theory	10
3	Brooklyn Nine-Nine	9
4	Suits	9
5	How I Met Your Mother	9

6	Stranger Things	8
7	The Vampire Diaries	5
8	Sherlock	5
9	Money Heist	5
10	The Originals	4
11	Taarak Mehta Ka Oolta Chasma	4
12	Legacies	3
13	Modern Family	3
14	Bridgerton	2
15	Euphoria	2
16	House of Cards	2
17	The Good Doctor	2
18	Grey's Anatomy	2
19	Gossip Girl	2
20	The Office	2

21	The Crown	2
22	Sex Education	2
23	Sarabhai Vs. Sarabhai	2
24	Fear Factor: Khatron Ke Khiladi	2
25	Game of Thrones	2
26	That 70s Show	2
27	Peaky Blinders	2
28	Anupama	2
29	Yeh Rishta Kya Kehlata Hai	2
30	The Fosters	1
31	DC Legends of Tomorrow	1
32	Agents of Shield	1
33	Kim's Convenience	1
34	Haunting Hills	1
35	Designated Survivor	1

36	Arrow	1
37	Young Sheldon	1
38	Reign	1
39	The Circle	1
40	The Last Ship	1
41	12 Monkeys	1
42	What If?	1
43	Breathe	1
44	House MD	1
45	Roadies	1
46	Human	1
47	Drive To Survive	1
48	Heartstopper	1
49	Schitt's Creek	1
50	The Blacklist	1

51	Kota Factory	1
52	Phineas & Ferb	1
53	That's So Raven	1
54	Family Man	1
55	Panchayat	1
56	Special Ops	1
57	YOU	1
58	Grace & Frankie	1
59	Behind Her Eyes	1
60	Daydreamer	1
61	Mr. Wrong	1
62	Full Moon	1
63	Radhakrinsha	1
64	Dr. Stone	1
65	American Horror Story	1

66	Mouse (K-Drama)	1
67	Jujutsu Kaisen	1
68	SHE	1
69	The Winx Club	1
70	WandaVision	1
71	Jane The Virgin	1
72	Virgin River	1
73	Haunting of Hill House	1
74	Breaking Bad	1
75	Midnight Mass	1
76	The Mentalist	1
77	Elite	1
78	Koffee with Karan	1
79	The Summer I Turned Pretty	1
80	Keeping up with the Kardashians	1

Table 1.1: Compiled List of “Top-of-the-Mind” Recalled Series

As a result of the study, Friends was observed to be the most influential series among the participants. Whereas The Big Bang Theory and Brooklynn Nine-Nine nabbed the silver and bronze trophies, respectively.

Based on phase one, the researcher decided to choose two of the top five -most influential series for the next phase of the study. The second (The Big Bang Theory) and fourth (Suits) most influential series were chosen as topics for the next phase using the random selection (chits-in-the-bowl) method.

Phase Two:

Popular Character & their influence

The researcher identified the character's influence as being implicit in nature instead of explicit. The researcher arrived at five variables acting as factors influencing an individual's career choices. These were identified after several conversations with various persons (including their parents, batchmates, and relatives), and were gained from the insights provided by published articles and studies (Agarwala, 2008; Akosah-Twumasi et al, 2018). The persons had either already made their career choices or were currently making the same. The researcher received a total of thirty-eight responses (38), of which three were considered outliers (3) since they did not fulfil the eligibility criteria of the study. A sample of twenty-five had watched The Big Bang Theory, and another twenty-five had watched Suits.

All respondents were asked to include two characters (out of the options of five that were provided) from each series with whom they identified most. For the big bang theory, Leonard (15) and Sheldon (12) were the two most-identified characters; and in the case of suits, Harvey Specter (16) and Mike Ross (16) were the most-identifiable characters for the participants. Following the selection of characters, the respondents were asked to rate the aspects of their chosen characters that they find appealing. This rating was given as per the five variables (as previously identified by the researcher).

In the "choice of profession" variable, the participants were asked to choose the character's influence purely based on their prima facia profession. From this, the researcher observed that in The Big Bang Theory, the character's influence on the participants based on their profession ranged from 32% to 40%. However, in the case of Suits, the influence was much higher at 84%.

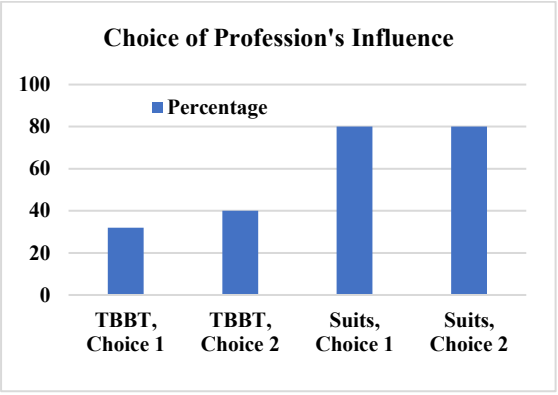


Figure 1.1: Responses to "Choice of Profession" variable being influential from Selected Series "Suits" and "The Big Bang Theory" (TBBT)

The respondents were asked about aspects of the character that they disliked with the same variables. The "choice of the profession" of the chosen characters (with whom the participants resonate) helps in understanding if the characters influence in making a specific career choice. In the case of The Big Bang Theory, influence ranged from 20% to 32%. However, in the case of Suits, the characters' profession influenced the career choice significantly higher proportion of the sample with 68%.

To understand how the influence operates, the research attempts to deep dive into the characters and understand the attributes that are influencing the respondents. Since the focus of the research is on career choice, the researcher examined each of the five underlying factors to understand the implicit influence they yield.

In the variable of "educational qualification," the participants were asked to choose the character's influence purely based on the level of their prima facia educational qualifications. This variable includes the quantity (ex. Sheldon, one of the protagonists from The Big Bang Theory, has 3 PhDs in Physics) or the quality (ex. Pearson Specter Litt, the law firm in Suits, emphasising they only hire from Harvard Law) of educational qualification. From this, the researcher understands that in the case of the first show i.e. The Big Bang Theory, the character's influence on the participants purely based on their educational qualification was 48%. However, in the case of the characters from the second show i.e. Suits, the influence of this variable was significantly higher, ranging from 72% to 80%.

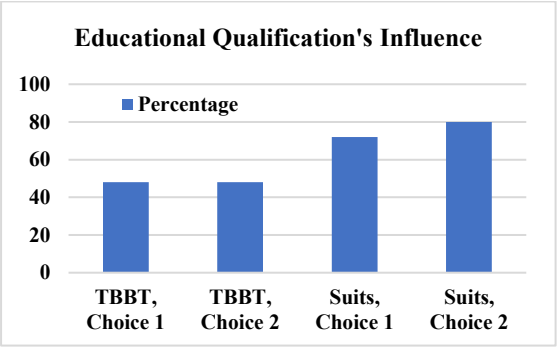


Figure 1.2: Responses to "Education Qualification" variable being influential from Selected Series "Suits" and "The Big Bang Theory" (TBBT).

For the "Appearance and personality" variable, the participants choose the character's influence based on their explicit attributes and behaviours. The extent of this variable ranges from the physical features of characters like Leonard and Penny (from Big Bang Theory) to the mannerisms of Jessica Pearson (from Suits.) In the case of the first show, the influence ranges from 52% to 64% of the sample. Whereas, like most other variables, the characters yield a much higher influence, ranging from 80% to 84%.

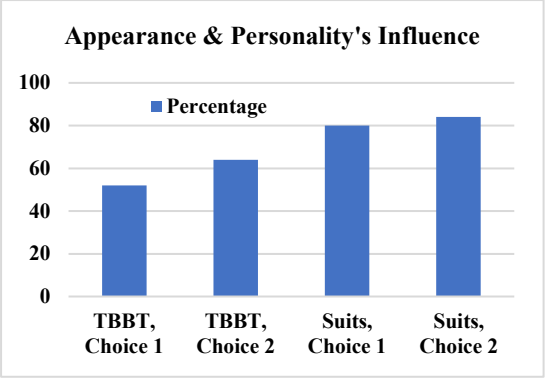


Figure 1.3: Responses to "Appearance & Personality" variable being influential from Selected Series –"Suits" and "The Big Bang Theory" (TBBT)

In the "Monetary aspect" variable, the participants choose the character's influence based on their high salaries and all aspects of high-income consumption that relate to the same. This can either be based on a dollar amount shared in the series, the luxury cars they drive, or any other sign of high income/consumption. In the case of the Big Bang Theory, the influence of this variable ranged from 32% to 40%. Whereas Suits had a significantly higher yield at 76%.

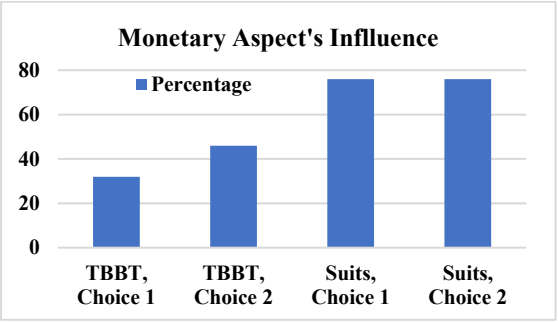


Figure 1.4: Responses to "Monetary Aspect" variable being influential from Selected Series –"Suits" and "The Big Bang Theory" (TBBT)

The only factor where The Big Bang Theory outperformed Suits was the "Work-life balance" variable. There too the difference was not drastically different from each other; with Suits ranging influence from 60% to 64% and The Big Bang Theory's characters' influence ranging from 60% to 76%.

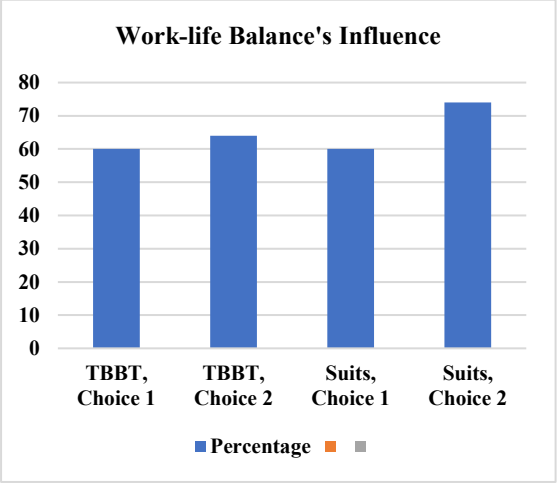


Figure 1.5: Responses to "Work-life Balance" variable being influential from Selected Series –"Suits" and "The Big Bang Theory" (TBBT)

Conclusion

While the impact of media on society remains profound and paramount, there is limited work available regarding the influence of popular series on the career choice of the college-going population, especially in India. This gap in literature needs to be bridged for a more holistic understanding of the impacts of media as well as, for potentially influencing the social behaviour of society at large.

The researcher realises that overt influences of popular series are available in the everyday world - such as people using specific phrases, wearing clothes similar to their beloved characters, or fans channelling a

character's mannerisms. However, such series also influence the individual at an implicit (or underlying) level, which is what this study is trying to decode. Specifically in the context of influencing career choices. In the study, the researcher examines five factors (namely, choice of career, educational qualification, monetary aspect, appearance & personality, and work-life balance) to gain insight into the same. Of these five factors, "Monetary aspect" & "Appearance and personality" featured heavily in the responses given by the respondents, and they appear to be the most influential factors (of the five) for the sample.

The study does not claim that there are no other factors at play here, and neither does it attempt to stake a claim that the above-mentioned are the two most important factors. Instead, the study aims to simply establish that various implicit influences picked up from popular series are acting on an individual which has the potential of shaping their long-term decisions - including their career choice.

In addition to this, of the factors that influence the same, the "Monetary aspect" of the character, and the physical attributes including their mannerisms appear to be yielding a significant influence. These two factors have the potential of playing a big role in shaping the perception of specific careers, and can thus influence their career choice of either accepting or rejecting the same.

- Total Participants: 38
- Outliers: 3
- Remaining in sample: 38 – 3 = 35
    - People who have watched The Big Bang Theory & answered = 25
    - People Who have watched Suits & answered = 25
- Q1. In what ways does TV / Web Series influence the person's career choice

- 1A. Influence of TV / Web Series is determined by choice of characters that APPEAL to the respondent from each of the Web Series i.e., Choice 1 and 2 are the more appealing characters from the Web Series

- All 38 participants were asked to choose characters from the selected series identify with the most. If the participants find the characters to be familiar or identifiable with themselves, they would be individually more appealing or influential to them.
    - 1B. How the character influences the person's choice of character is determined by the respondent response as under.

- 5.1
    - Influenced: 10 / 25 = 40%
    - Not influenced: 15 / 25 = 60%
- 8.1
    - Influenced: 8 / 25 = 32%
    - Not influenced: 17 / 25 = 68%
- 11.1
    - Influenced: 21 / 25 = 84%
    - Not influenced: 4 / 25 = 16%
- 14.1
    - Influenced: 21 / 25 = 84%
    - Not influenced: 4 / 25 = 16%

In the variable of "choice of profession," the participants were asked to choose the character's influence purely based on their prima facia profession. From this, the researcher understands that in case of the first show i.e. The Big Bang Theory, the character's influence on the participants on the basis of their profession ranged from 32% to 40%.

However, in case of the characters from the second show i.e. Suits, the character's influence on the participants solely on the basis of their profession was much higher at 84%.

- Q2. Does it help them to make specific career choices?
- 6.1
    - Helps make career choice: 8 / 25 = 32%
    - Does not help: 17 / 25 = 68%
- 9.1
    - Helps make career choice: 5 / 25 = 20%
    - Does not help: 20 / 35 = 80%
- 12.1
    - Helps make career choice: 17 / 25 = 68%
    - Does not help: 8 / 25 = 32%
- 15.1
    - Helps make career choice: 17 / 25 = 68%
    - Does not help: 8 / 25 = 32%

The study further examined if the choice of profession of the chosen characters (with whom the participants resonate) help the participants in making a specific career choices. From this, the researcher understands that in case of the first show i.e. The Big Bang Theory, the influence of the character's profession in the process of influencing the career choice of the participants ranged from 20% to 32%. However, in case of the characters from the second show i.e. Suits, the character's profession in the process of influencing the career choice of the participants is significantly higher than the first show, at 68%.

- Q3. How does the influence operate?
- 3A To understand how the influence operates, the research attempts to deep dive within the characters and understand the attributes that are influencing the respondents. Since the focus of research is around career choice, we have taken the four key attributes around work choices, which are as follows:
- 3A1. Education level / Qualifications
- 5.2
    - Influenced – 12 / 25 = 48%
    - Not influenced – 13 / 25 = 52%
- 7.2
    - Influenced - 12 / 25 = 48%
    - Not influenced – 13 / 25 = 52%
- 11.2
    - Influenced – 18 / 25 = 72%
    - Not influenced – 7 / 25 = 28%
- 14.2
    - Influenced – 20 / 25 = 80%
    - Not influenced – 5 / 25 = 20%

To understand how the influence operates, the research attempts to deep dive into the characters and understand the attributes that are influencing the respondents. Since the focus of the research is on career choice, the researcher examined each of the five underlying factors to understand the implicit influence they yield.

In the variable of "educational qualification," the participants were asked to choose the character's influence purely based on the level of

their prima facia educational qualifications. This variable includes the quantity (ex. Sheldon from The Big Bang Theory had 3 PhDs) or the quality (ex. Pearson Specter Litt, the law firm in Suits, emphasising they only hire from Harvard Law) of educational qualification. From this, the researcher understands that in the case of the first show i.e. The Big Bang Theory, the character's influence on the participants purely based on their educational qualification was 48%. However, in the case of the characters from the second show i.e., Suits, the influence of this variable was significantly higher, ranging from 72% to 80%

- 3A2. Appearance and Personality
- 5.3
    - Influenced – 13 / 25 = 52%
    - Not influenced – 12 / 25 = 48%
- 7.3
    - Influenced – 16 / 25 = 64%
    - Not influenced – 9 / 25 = 36%
- 11.3
    - Influenced – 21 / 25 = 84%
    - Not influenced – 4 / 25 = 16%
- 14.3
    - Influenced – 20 / 25 = 80%
    - Not influenced – 5 / 25 = 20%

In the case of the "Appearance and personality" variable, the participants choose the character's influence based on their explicit attributes and behaviours. The extent of this variable ranges from the physical features of characters like Leonard and Penny (from Big Bang Theory) to the mannerisms of Jessica Pearson (from Suits.) In the case of the first show,

the influence ranges from 52% to 64% of the sample. Whereas, like most other variables, the characters yield a much higher influence, ranging from 80% to 84%.

- 3A3. Monetary Aspect / Compensation
- 5.4
    - Influenced – 8 / 25 = 32%
    - Not influenced – 17 / 25 = 68%
- 7.4
    - Influenced – 10 / 25 = 40%
    - Not influenced – 15 / 25 = 60%
- 11.4
    - Influenced – 19 / 25 = 76%
    - Not influenced – 6 / 25 = 24%
- 14.4
    - Influenced – 19 / 25 = 76%
    - Not influenced – 6 / 25 = 24%

In the "Monetary aspect" variable, the participants choose the character's influence based on their high salaries and all aspects of high-income consumption that relate to the same. This can either be based on a dollar amount shared in the series, the luxury cars they drive, or any other sign of high income/consumption. In the case of the Big Bang Theory, the influence of this variable ranged from 32% to 40%. Whereas Suits had a significantly higher yield at 76%.

- 3A5. Work – life balance
- 5.5
    - Influenced – 19 / 25 = 76%
    - Not influenced – 6 / 25 = 24%
- 7.5
    - Influenced – 15 / 25 = 60%
    - Not influenced – 10 / 25 = 4-%
- 11.5
    - Influenced – 15 / 25 = 60%
    - Not influenced – 10 / 25 = 40%
- 14.5
    - Influenced – 16 / 25 = 64%
    - Not influenced – 9 / 25 = 36%

The only factor where The Big Bang Theory outperformed Suits was the "Work-life balance" variable. There too the difference was not drastically different from each other; with Suits ranging influence from 60% to 64% and The Big Bang Theory's characters' influence ranging from 60% to 76%.

REFERENCES

1. Agarwala, T. (2008, July 4). Factors influencing career choice of management students in India. Career Development International, 13(4), 362–376. https://doi.org/10.1108/13620430810880844
2. Akosah-Twumasi, P., Emeto, T. I., Lindsay, D., Tsey, K., & Malau-Aduli, B. S. (2018, July 19). A Systematic Review of Factors That Influence Youths Career Choices—the Role of Culture. Frontiers in Education, 3. https://doi.org/10.3389/feduc.2018.00058

3. Bandura, A. (2001, August). Social Cognitive Theory of Mass Communication. Media Psychology, 3(3), 265–299. https://doi.org/10.1207/s1532785xmep0303_03

4. Bogatz, G. A., & Bell, S. (1971, November). The second year of sesame street: A continuing evaluation. Educational Resource Information Center. Retrieved August 10, 2022, from https://files.eric.ed.gov/fulltext/ED122800.pdf

5. Brown, W. J. (1990, January). Prosocial effects of entertainment television in India. Asian Journal of Communication, 1(1), 113–135. https://doi.org/10.1080/01292989009359523

6. Bruce, S., & Yearley, S. (2006, January 26). The SAGE Dictionary of Sociology (First). SAGE Publications Ltd.

7. Cultivation theory. (2015, March 27). Communication Theory. Retrieved January 12, 2022, from https://www.communicationtheory.org/cultivation-theory/

8. Eman Mosharafa. (2015, September 11). All you Need to Know About: The Cultivation Theory. Global Journal of Human-Social Science
Research, 15(8). https://globaljournals.org/GJHSS_Volume15/3-All-you-Need-to-Know.pdf

9. Gibbs, L. (2021, June 21). The big bang theory: 10 characters with the most screen time, ranked. Screen Rant. https://screenrant.com/the-big-bang-theory-characters-most-screen-time-ranked/

10. Harris, R. J., & Sanborn, F. W. (2013, August 29). A cognitive psychology of mass communication (6th ed.). Routledge.

11. Hoffner, C. A., Levine, K. J., Sullivan, Q. E., Crowell, D., Pedrick, L., & Berndt, P. (2006, September). TV Characters at Work. Journal of Career Development, 33(1), 3–18. https://doi.org/10.1177/0894845305282768

12. Hoffner, C., & Buchanan, M. (2005, November). Young Adults' Wishful Identification With Television Characters: The Role of Perceived Similarity and Character Attributes. Media Psychology, 7(4), 325–351. https://doi.org/10.1207/s1532785xmep0704_2

13. How to write a concept note for research. (n.d.). Advance Africa. Retrieved December 15, 2021, from https://www.advance-africa.com/how-to-write-a-concept-note-for-research.html

14. Joshi, P. (2021, October 26). Brooklyn Nine-Nine: Too Good To Be True? Feminism in India. Retrieved October 20, 2022, from https://feminisminindia.com/2021/08/06/brooklyn-nine-nine-propaganda-or-utopia/

15. Kandala, S. (2014, February 7). A Homegrown Comic Con Highlights Growth in Local Comics Market. India Ink. Retrieved October 20, 2022, from https://archive.nytimes.com/india.blogs.nytimes.com/2014/02/07/a-homegrown-comic-con-highlights-growth-in-local-comics-market/

16. Morgan, J. (2014). Cultivating a career: Effects of television binge-watching and character identification on college students' goal occupations. Indiana University Journal of Undergraduate Research, 3, 48–53.

17. Mosharafa, E. (2015). All you need to know about: The cultivation theory. Global Journal of HUMAN-SOCIAL SCIENCE: A (Arts & Humanities - Psychology), XV(VIII), 23–37. https://globaljournals.org/GJHSS_Volume15/3-All-you-Need-to-Know.pdf

18. Pandey, B. M. (2021, February 12). Brooklyn Nine-Nine: The end of a "powerful" and "relatable" show. BBC News. Retrieved October 20, 2022, from https://www.bbc.com/news/newsbeat-56010355

19. Popular Culture Studies Journal. (2022, May 25). Midwest PCA/ACA. Retrieved October 20, 2022, from https://mpcaaca.org/the-popular-culture-studies-journal/

20. Prisco, J. (2019, May 20). The "game of thrones" language that 1.2M people are learning. CNN Style. https://edition.cnn.com/style/article/game-of-thrones-languages-david-j-peterson/index.html

21. Rothwell, J. (2019, August 2). You Are What You Watch? The Social Effects of TV. The New York Times. Retrieved October 20, 2022, from https://www.nytimes.com/2019/07/25/upshot/social-effects-television.html

22. Why You Should Binge Watch Brooklyn Nine-Nine This Weekend. (n.d.). Arc UNSW Student Life. Retrieved October 20, 2022, from https://www.arc.unsw.edu.au/blitz/read/why-you-should-binge-watch-brooklyn-nine-nine-this-weekend

23. https://screenrant.com/the-big-bang-theory-characters-most-screen-time-ranked/

24. Harris, R.J., & Sanborn, F.W. (2014). A Cognitive Psychology of Mass Communication. 116.

LIST OF CONTRIBUTING MENTORS

1. Dr. Suvarna Sharma
2. Dr. Shalini Rai
3. Dr. (Ms.) Aashu Vajpai
4. Mr. Gaurav Bingi
5. Dr. Tejashree Shanbhag
6. Dr. Mayuresh Joshi
7. Ms. Prachi Bhatia
8. Dr. Sejal Rathod
9. Ms. Hajra Gupta
10. Dr. Jyotsna Pandey
11. Dr. Leena Pujari