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Assessment of Biological Embodiment by Foldscope

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ABSTRACT

In developing country like India, teaching science and learning needs huge investment. Resources are limited and hence the Indo-Us project by Dr. Manu Prakash inspired many young minds by developing Foldscope In 2012. Through the production of low-cost tools to community around the world, they aim to break down cost barrier between people & curiosity and excitement of scientific exploration. It can be used as an education tool to make science learning as fun. It brings Microscopy to new place. It is designed to be durable, portable, and make science more accessible to everyone. The present study evaluted the use of foldscope to observe microbes and plant specimen within KME society campus where the observation reveals the presence of beautiful creatures. Like tardigrades, amoeba, Euglena etc along with the plant tissue. Using Foldscope with smartphone documentation qualitative and quantitative were carried out at Department of Biotechnology, GM Momin Women's College, Bhiwandi during 2019-20. Viewing microbes under foldscope was delightful experience that doesn't have to cost a ton of money. It also provides new opportunities to field worker and people who want to explore beauty of nature. The extra ordinary feature of this microscope is that it can attached to Smartphone and use to capture photo and video's in real time which is hard task in other microscope on field and in laboratory at ease.

Keywords: Foldscope, microbes, curiosity, science learning, Indo-Us project.

INTRODUCTION

Invention of microscope changed the perspective of humans towards nature. Most microscopes are rather expensive and, therefore, often beyond the reach of many students and institutions. An affordable and sturdy microscope was developed by Dr. Manu Prakash and his team at Stanford University (USA) in 2012 (Cybulski et al., 2014), which can provide a magnification from 140x to 2000x with submicron resolution. This "Foldscope" microscope is made of paper and uses the principle of "Origami". Although punched only on a flat sheet of paper, it is robust enough for field study and because of its low cost; it can reach in resource-limited conditions (Neikha et al., 2020; Walling et al., 2020).

With the intend of science for all and utility of foldscope, KME society's G.M.Momin Womens college Bhiwandi in the year 2018 received the DBT grand under Indo-US project Department of Biotechnology with the motive of conducting research work, innovative teaching learning, trained and encouraged the student towards science exploration.

Here in this paper the part of work done is highlighted which discuss about the observation of various micro-flora and fauna within the vicinity of college which gives motivation to perform field study and in lab observation of beautiful microscopic world.

METHOD

Foldscupe is ready to use in three easy steps.

- First Involve assembling toldscope unit by Joining together different pieces from flat sheet provided in foldscope kit
- Second focuses on working sample.
- Third capture Image of Object using Smart phone coupled to foldscope.

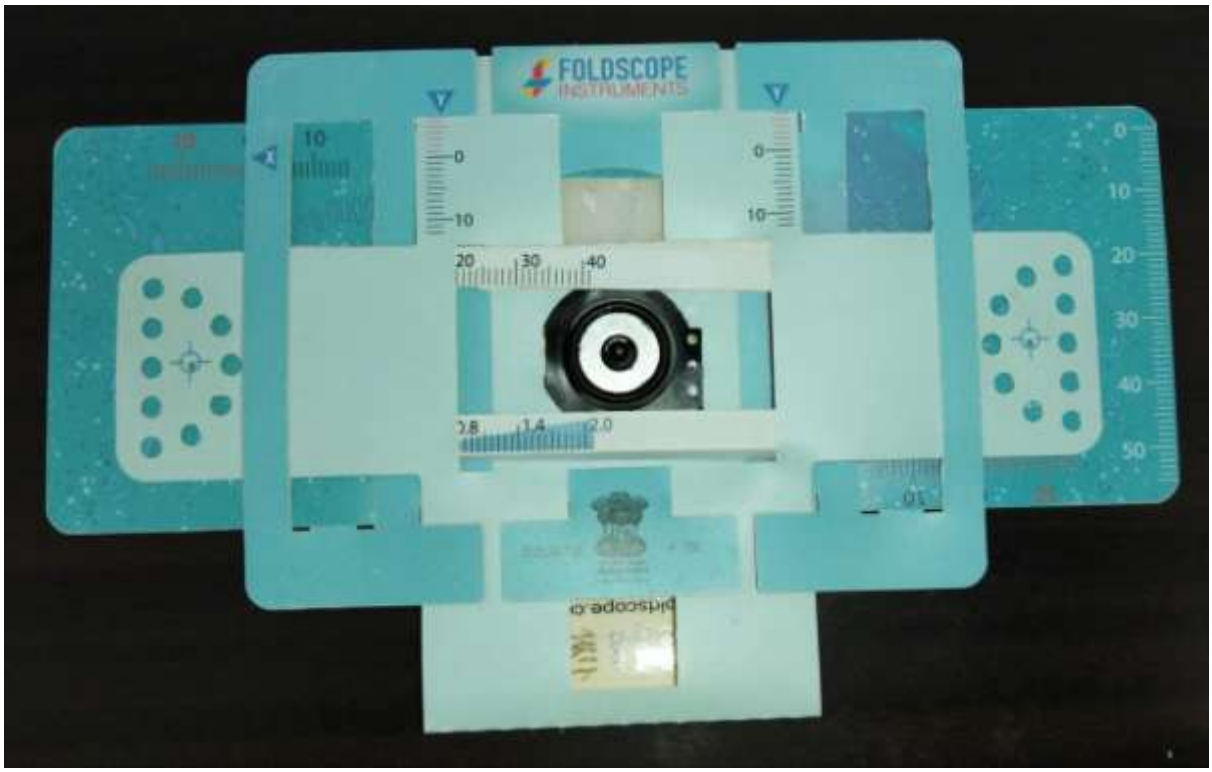

Fig 1: (Assembled Foldscope)

Sample preparation:

Sampling Involves mounting a deep of specimen (water) on slide and covering it with a glass cover slip.

Slide Insertion and Focusing:

The slide is Inserted within slide pocket upside down (towards yellow side). Focusing of mounted object can be adjusted in Simple way by sliding paper platform with thumb and forefinger.

Viewing:

There are three methods to view the samples. First, the sample can be viewed directly through the eyes. Second, it can be viewed through the camera of mobile phones. To view a sample with a phone, attach a magnetic coupler (provided in kit) over the lens of your phone camera- by using either a double-sided ring sticker or with any other tape.Foldscope's lens has a magnification of 140x, and that magnification is multiplied by the zoom feature of the his mobile phone. It is ideal for recording the movements of living specimens by using the video feature of the mobile phone. In the third method, a sample can be projected on a white screen or surface in a dark room. Projection requires a strong light source. A phone's flashlight can also be used. For this, attach a magnetic coupler over the phone's flashlight, and then bring phone's flashlight up to the aperture, on the yellow side of Foldscope. Turn on the flashlight, an image of the specimen will be visible on the screen.

RESULTS

With a keen observation of water sample and plant tissue using foldscope for the study, following images were capture and imaged through smart phone (Redmi note 7 pro).

Table 1: Number of organisms observed during study

Sr No	Sampling Site	Paramacium	Tardigrades
1	KMES Campus	19	07
2	Arif Garden	08	05
3	Samad Nagar Pond	33	25

Fig 2: Tardigrades (Water bear)

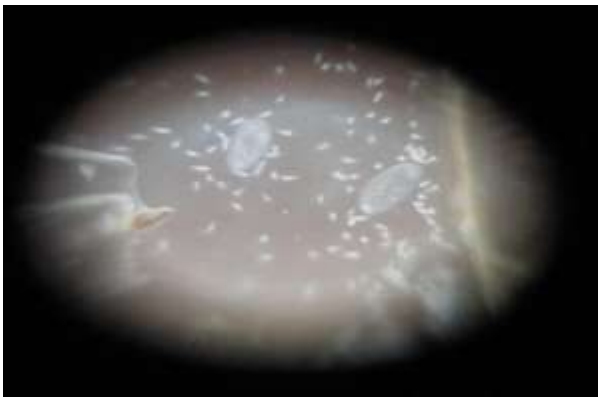

Fig 3: Paramecium

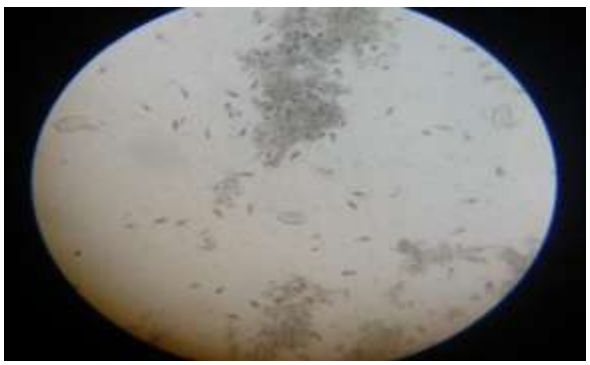

Fig 4: Protozoa

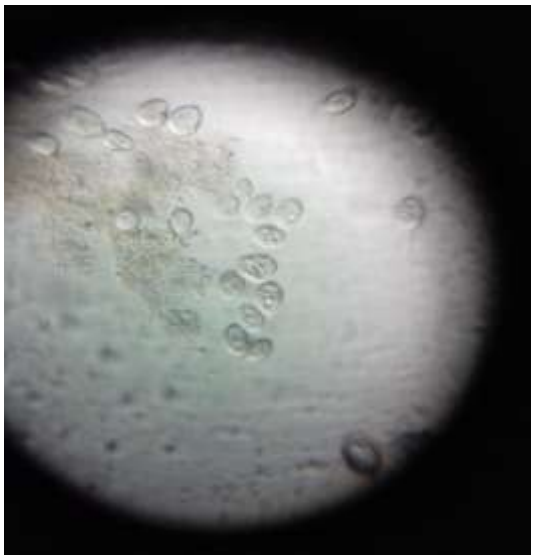

Fig 5: Protozoa

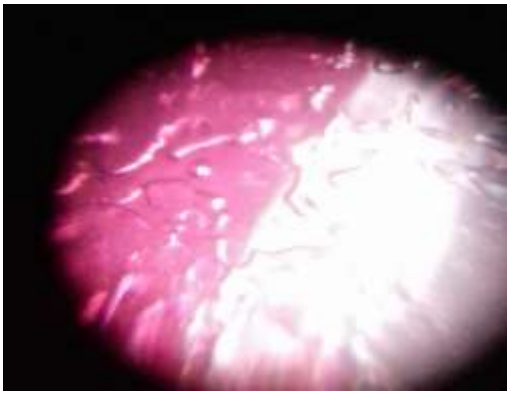

Fig 6: Plant tissue

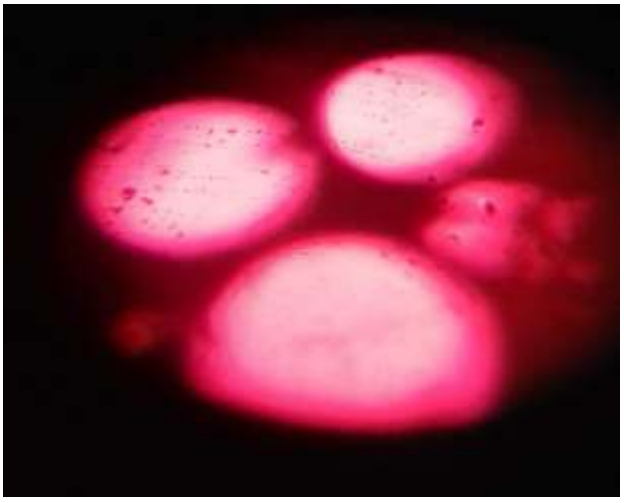

Fig 7: Plant tissue

CONCLUSION

In view of all experiment perform using this paper microscope, it gives us an idea that foldscope can be used as a basic research tool, teaching learning platform and as self learning practices for curious mind. It is also useful to study within classroom with every student having an opportunity to touch, experience, hold.thus laying the foundation for critical thinking. This cheap tool can facilitate practical of biology because of its portability. The growing access to internet across the country can make mobile phone foldscopy a promising technology.

It is a portable microscope with easy accesibility and availability without power and energy requirement and zero maintainanace cost. Foldscope is truly amazing device but it won't replace conventional microscope. Despite its several advantages foldscope has certain limitations. Although a Foldscope is ideal for viewing sub ellular structures,its resolution is still relatively low as compared to the power of compound microscopes. It has a very small focus range so image analysis is difficult.For on-site biological fluid sampling, staining and sample preparation can be major limiting factor.

As foIdscope began with an idea to make science more accessible to everyone. So, Whether you are teacher, scientist, researcher, hiker, nature traveller, photographer and curious Cat, with foldscope in your pocket you can better explore beauty of nature around you.

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Folklore Usage of Meswak (*Salvadora Persica L.*) in Oral Care: A Review

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ABSTRACT

Meswak is being used as a folk medicine for a very long time for various medical condition treatments. The cultural and religious use of meswak for dental hygiene is widely spread in the geographical areas of Asia, Africa, and Middle East because of its wide distribution. The therapeutic aspects of meswak and its major role in plaque control, tooth wear, bleeding gums, and periodontal health as well as its availability and cheap cost factor could also be the important reasons for its usage. Meswak is used for oral hygiene as an alternative to various oral devices. Oral hygiene is the most remarkable factor when itinvolves prevention of oral diseases and oral health. Previous literature reveals that meswak has effective antimicrobial (antibacterial, antifungal, and antiviral) and pharmacological (hypoglycemic, anti-ulcer, and anti-oxidative) activities. The useful effects of meswak with respect to oral hygiene and dental health care because of its pharmacological actions. It is estimated that different natural chemical compounds that are considered good for both oral and dental hygiene are present in meswak according to several researchers. Meswak offers itself as an effective and traditional oral medicine affordable to use as oral hygiene device. The study done in this paper reveal about the usefulness of meswak for oral care.

Key words: Meswak, oral care, antimicrobial activities, pharmacological activities.

INTRODUCTION

Salvadora persica L. belonging to family Salvadoraceae is an well-branched evergreen tree or shrub, 4-6 meter tall having short trunk, white bark,aromatic roots, soft white yellowish wood leaves and smooth green leaves that are glaucous.Salvadora persica, or the Arak tree known in English as the "tooth brush tree" is a large, and somewhat fleshy [1, 10]. In ayurvedic system of medicinesS. persicais reported to own potent activity for dental complaints. Meswak (also called miswak) is a chewing stick prepared from the roots or twigs of S. persica [19]. It is additionally referred to as Meswak tree, for the roots and twinges of this tree are used for teeth improvement since the ancient times. It is one in all the foremost unremarkably used medicative plants for oral hygiene among world Muslim community [34]. The history and therefore the use of meswak as an oral tool are used because of the biological effects of S. persica extracts which are reviewed by many researchers [28].

The use of meswak for oral hygiene includes a long tradition in Middle Eastern and African countries, going back many centuries [11]. Moreover, in the Middle East, the utmost common source of chewing sticks is Arak (Salvadora persica) [5]. Sticks of these plants are chewed usually at one end until they become frayed into a brush likeappearance, which is then used to clean the teeth in a similar manner like a toothbrush. Additionallyin strengthening the gums, it prevents tooth decay, eliminating toothaches and stop further increase in decay that has already set in. It creates fragrancewithin the mouth, eliminates bad odours, improves the sense of taste, and causes the teeth to glow and shine. The other parts of the tree have therapeutic values as corrective, liver tonic, diuretic, analgesic, anthelmintic, astringent, carminative, diuretic, and gastric[24].Moreover, the useful effects of meswak in respect of oral hygiene and dental health are partly because of its mechanical action and pharmacological actions. It's been shown that the utilization of meswak chewing sticks might

contribute to a higher level of gingival recession [17, 18].The study done in this paper reveal about the usefulness of meswak for oral care.

CLASSIFICATION

The term Salvadora, in 1749, was put forward in honour of an apothecary of Barcelona, Juan SalvadoryBosca (1598-1681), Laurent Garcin, botanist, traveller and plant collector. While as persica term indicates Persia and the standard author abbreviation L. is used to indicate Carl Linnaeus (1707–1778), a Swedish botanist and the father of modern taxonomy[23].

Class	: Magnoliopsida
Subclass	: Dilleniidae
Order	: Capparales
Family	: Salvadoraceae
Genus	: Salvadora
Species	: persica

Pharmacology of S. persica

Phytochemical constituents like alkaloids, flavonoids, tannins, phenols, saponins and various other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by several microorganisms, insects and other herbivores[12].The aqueous extracts of S. persica contains important phytoconstituentslike vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins,flavonoids, sterols, salts mostly as chlorides and basic alkaloidswere succeeded to evaluate the chemical composition of S. persica[6-8, 29, 30, 33].

Anti-microbial activity of S. persica

Biological activity of various parts of S. persica recent studies have demonstrated that there is antibacterial, anti-periopathic, anti-fungal and anti-caries properties in aqueous extract of meswak. Studies have also proven oral [14] disinfectant and anti-plaque agents present in meswak.Different antimicrobial activity was performed and an in vitro study showed that the aqueous extract of S. persica had an inhibitory effect on the growth of Candida albicans that may be attributed to its high sulfate content [4].

Antibacterial activities

The meswak exhibited stronger antibacterial activity against the Gram-negative bacteria tested within the study than the Gram-positive bacteria evaluated, as proven by the pronounced differences in inhibition zones associated with the Gram-negative species A. actinomycetemcomitans, P. gingivalis, H. influenzae, and theGram-positive species S. mutansand L. acidophilus. . S. persica roots contain compounds with potent antibacterial activity against the Gram-negative bacteria with some effect against the Gram-positive bacteria [2]. Some studyrecommend that gram-positive bacteria are generally more sensitive to the Salvodora persica extracts than gram-negative might be because of the structure of membrane that the gram-positive bacteria are simpler than gram-negative ones [13].

According to [3] aqueous extract of plant inhibited microorganisms, showing greater activity on Streptococcus species. Methanolic extract was resisted by L. acidophilus and P. aeruginosa. At highest concentration tested (200 mg/ml); the aq. extract of meswak was more efficient than the methanolic extract but were less efficient than the positive control streptomycin and amphotericin B. Study done by[27]reveal that ethanol extract of S. persicashowed more effective than the aqueous extract in inhibiting the S. mutans, L. acidophilus, E. coli, S. aureus, and P. aeruginosa microorganisms.Inhibition zone studied by [33]showedeffective results against Staphylococcus aureus followed by Streptococcus mutans, Lactobacillus acidophilus, E. coli and Pseudomonas aeruginosa respectively. The aqueous extract exhibited antibacterial activity on M. bovisstudy done by[20].

Role of S. persica in dental plaque control

The extract of meswak has found its way into the dentifrices in the recent years as anti-plaque and antigingivitis agents [22]. It is believed that chewing of these stems facilitate salivary secretions which possibly help in oral cleaning and control of plaque [16]. In the endodontic treatment of teeth with necrotic pulps the aq. extract (10%) of S. persica is an effective

antimicrobial agent when utilized clinically as an irrigant [32]. An additional study compared the oral health efficacy of persica mouthwash (containing an extract of S. persica) with that of a placebo. Further the study reveals that the use of persica mouthwash lower carriage rate of cariogenic bacteria and improves gingival health when compared with the pretreatment values [26].

Scientific evaluation of using meswak revealed that it is at least as effective as tooth brushing for reducing plaque and gingivitis and that the antimicrobial effect of S. persica is beneficial for prevention of periodontal disease [35]. A clinical study was carriedusing patients' saliva and measuring the effect of meswak (chewing stick), meswak extract, toothbrush, and normal saline on mutans and lactobacilli by [15]. The results showed that there was a distinct reduction in Strep. mutansamong all groups. After comparison within the group, the reduction in Strep.mutans was significantly greater using meswak in comparison to tooth brushing and there was no significant difference for lactobacilli reduction. The investigators concluded that meswak has an immediate antimicrobial effect. Strep.mutans were more susceptible to meswak antimicrobial activity than lactobacilli [9]. Persica mouthwash significantly lowers the gingival index, plaque index, and bleeding index in case group without any reported side effects according to [25].

CONCLUSION

Oral health has gained increased attention as a considerable public health concern. In several studies, medicinal plant extracts and isolated phytochemical constituents showed highly significant antimicrobial activity. S. persica, commonly called Meswak or Toothbrush tree, is one of the most popular medicinal plants that has proved to be effective in the prevention of tooth decay and mouth infections [31]. From the ancient meswak to the electric toothbrush, oral hygiene practices have come into daily use throughout the world being either mechanical or manual. Meswak offers itself as an effective and affordable oral hygiene device. Many researchers recommend and encourage the use of meswak as an inexpensive and effective oral hygiene tool in areas where it is customary. Its availability, low-cost, simplicity, and use have been extensively studied in regions around the world where meswak can play a significant role in the promotion of oral hygiene. The practice of using meswak regularly proves its major properties of bactericidal effect. S. persica and other related plants are reported to be effective against broad spectrum microbes that are imperative for the development of dental plaque. As a result, current and upcoming public health practitioners and the dental profession should become familiar with the application of meswak within its traditional customs. There is also evidence that meswak is more effective as an oral hygiene tool in buccal than lingual tooth surfaces [21].

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Extraction of Chitosan from Shrimp Shell Waste and its Application in Waste Water Purification

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ABSTRACT

Crustaceans are known to have a large amount of chitosan. Types of crustaceans include shrimp, crab, krill, lobster, prawn, etc. In the present study, shrimp shells were used for chitosan extraction. Chitosan is the deacetylated form of chitin, which is a natural polymer. It is the second most abundant polysaccharide after cellulose. It is widely used in the pharmaceutical industry, the food industry as well as in biological treatments. For this study, we extracted chitosan from shrimp shell waste. The extraction of chitosan starts with demineralization followed by deproteinization and further deacetylated to obtain chitosan. The chitosan yield obtained was 32%. Antioxidant activity and scavenging ability on DPPH of the extracted chitosan were determined. Chitosan has antibacterial activity and the extracted chitosan was used for water treatment and showed an antibacterial effect against Gram-negative bacteria.

Keywords: Antioxidant activity, Chitosan, Scavenging ability on DPPH, Shrimp shell, Water treatment.

INTRODUCTION

Chitosan is a derived form of natural polymer chitin obtained from crustaceans. The main components of crustacean shells are chitin, proteins, calcium, and magnesium carbonate (Aung et.al., 2018). Crustaceans also contain other bioactive components such as carotenoprotein, minerals, lipids as by-products which can be utilized to produce bioactive compounds (Trunget.al., 2012). The chitin content of crustacean shells varies from species to species and it ranged from 7-40% (Tolaimateet.al., 2003). K. Mohan et.al., 2021 reported extraction of 20% of chitin from shrimp shell waste, 21.25% from crab shell waste, 23.75% from squilla shell waste, and 17.50% from lobster shell waste. Due to the presence of a high level of chitin in the shell waste of crustaceans, it has been used widely for manufacturing commercial chitin (Yadav et.al., 2019). Shrimp waste contains diverse high-value products such as carotenoids, chiton and proteins (Ghorbelet.al., 2012). Shrimp and crab shells are considered natural and principal sources of chitin (Daniel et.al., 2016). Shrimp shells contain a large amount of chitin which is used as an essential ingredient in many foods, cosmetic and pharmaceutical industries (Islam et.al., 2016). Chitosan contains a rigid crystalline structure and therefore it is insoluble in water and other common organic solvents (Vanithaet al., 2018). The solubility of the chitosan is due to the presence of free amine groups in the chitosan chain, which gets dissolved in the diluted aqueous acidic solvents (Sarbonet.al., 2015). Chitosan along with its antioxidant properties is a biopolymer of glucosamine derived from chitin (Shiekhet.al., 2018). It is used in water purification also as antibacterial and antiviral in the field of biotechnology, agriculture because it contains biological properties (Vanithaet.al. 2018). It is a natural water coagulant that can decrease turbidity, color and it is also effective for the extraction of organic pollutants, heavy metals, and bacteria from water (Chopra et.al., 2015). It can scavenge free radicals by donating hydrogen ions and it contains two key functional groups like hydroxyl group (OH) and amino group (NH_2) for antioxidant activity (Rajalakshmiet.al. 2013).

Material:

Shrimp shells were collected from Bhiwandi-Thane, Maharashtra, India. The shrimp shells were cleaned by removal of waste from it and further washed with distilled water and kept in the polyethene bags and preserved at 4℃ for further process.

Methods:

A) Extraction of Chitosan

Demineralization: The preserved shrimp shells were kept at room temperature and washed with normal tap water. The shells were placed inside the oven at 60℃ for 24 hours, ground, and further demineralized by treating with 15% of hydrochloric acid (HCl) at 60℃ for 2 hours. The shell sample was filtered using filter paper and the filtrate was washed with water and the demineralized shells were dried in the oven at 60℃ for 24 hours **(***Sarbonet al., 2015***)**.

Deproteinization: The demineralized shrimp shells were treated with 15% sodium hydroxide (NaOH) at 60℃ for 5 hours for the process of deproteinization. The sample was filtered using filter paper and the filtrate was washed with water and the deproteinized shells were dried in the oven at 60℃ for 24 hours (*Sarbonet al., 2015*).

Decolourization: The deproteinized shrimp shells were further decolourised by treating with acetone at room temperature for 2 hours. The sample was filtered using filter paper and the filtrate was washed with water and the decolourised shells were dried in the oven at 60℃ for 24 hours to obtain shrimp chitin (*Sarbonet al., 2015*).

Deacetylation of Chitin: The chitin obtained by the process of decolourization was treated with 60% sodium hydroxide (NaOH) at 100℃ for 2 hours. The chitin was filtered using filter paper and the filtrate was washed with distilled water to recover chitosan. The recovered chitosan was dried at 60℃ for 24 hours in the oven **(***Sarbonet al., 2015).*

B) Characterization of Chitosan

Antioxidant activity of Chitosan: Different concentrations of 2.5 mL of chitosan samples were prepared in the range of 2-10 mg/mL and mixed with 2.5 mL of 200 mM sodium phosphate buffer and 2.5 mL of 1% potassium ferricyanide in 0.2% acetic acid solution. The mixture was incubated for 20 minutes at 50℃. Further 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 3500 rpm for 20 minutes. After centrifugation, 5 mL of supernatant was mixed with an equal amount of distilled water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700nm. Higher absorbance shows higher reducing power ***(****Yen et.al., 2008).*
Scavenging ability on 1, 1-diphenyl-2-picrylhydrazyl radicals: Different concentrations of 4 mL chitosan sample was prepared in the range of 2-10 mg/mL) and mixed with 1 mL of 10 mM methanolic acid solution containing DPPH radicals in 0.2% acetic acid solution. The mixture was mixed and kept in the dark condition for 30 minutes. The absorbance was measured at 517 nm *(Yen et.al., 2008).*

C) Collection of Water Sample

Water samples was collected from Bhadwad, Biotechnology laboratory (B. N. N. College) and Varaladevi lake (Kamatghar) and was named as sample A, sample B, and sample C respectively.

D) Determination of Microorganisms in wastewater

Various culture media like Nutrient Agar, Mac Conkey's Agar, Thiosulphate Citrate Bile (TCBS) Agar, Salt Mannitol Agar, and Salmonella Shigella Agar were ordered from Hi-media, prepared and sterilized as per the manufacturer's instruction. A loopful of wastewater samples was streaked on each medium and plates were incubated at 37℃ for 24 hours to observe the growth of various organisms. Isolated organisms were determined biochemically.

E) Effect of Chitosan on Water Treatment

About 1 gm of extracted chitosan powder was weighed and mixed with 5 mL of 1% acetic acid solution and left to stand for about 30 minutes to dissolve. Further, it was diluted with 100 mL distilled water and stirred for 1 hour at 25℃. Three samples of 100 mL of raw water were taken and treated with extracted chitosan *(Al-Manhelet.al., 2018).*

RESULT AND DISCUSSION

Determination of yield of chitosan

Chitosan content varies in different species of Crustaceans. The crustaceans like sea snails, crabs, woodlice, and barnacles have strong and hard exteriors with a high amount of minerals that lead to low chitin yield compared to other crustaceans

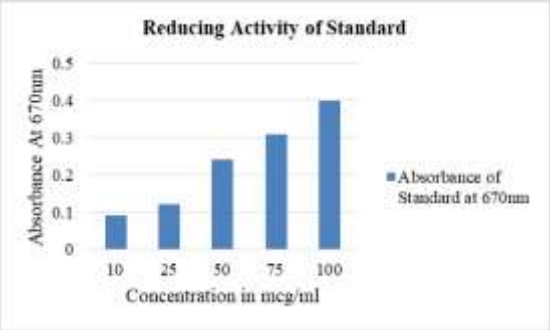
like mollusks, shrimp, and aquatic invertebrates (*Abdouet.al., 2007; Tolaimateet.al., 2003*). The yield of the extracted chitosan from shrimp shell waste was found to be 32%. Vanithaet.al., 2018 reported that a 38% yield of chitosan from the crab shells and this decrease in the yield can be due to extraction methods, amount of removal of acetyl groups from the polymer during the deacetylation process. The yield of chitosan determined by Sarbonet.al., 2014 had 44.57 ± 3.44% yield from the mud crab shells which have been used as an economic source for the production of chitosan on an industrial scale due to the availability and low cost of the source. Al-Manhelet.al., 2018 had obtained a 12.93% yield of chitosan from the shrimp shells.

Reducing Activity of chitosan

By restraining oxidative chain reactions, limitation or inhibition of nutrient oxidation occurs which is called antioxidant activity. Different concentrations of chitosan were checked for their antioxidant activity. The table 1 and graph 1 show the antioxidant activity of standard at different concentrations (10-100 mcg/mL) at the absorbance of 670 nm. The table 2 and graph 2 show the antioxidant activity of Chitosan extracted from Shrimp Shell waste. The reducing power of the extracted chitosan from the shrimp shell waste was found to be 0.40 OD, at a concentration of 10 mg/mL colorimetrically at 670 nm. Sarbonet al., 2014 had obtained reducing the power of chitosan from mud crab shells was 0.23 OD at a concentration of 10 mg/mL at the absorbance of 700 nm. Yen et.al., 2008 extracted chitosan from snow crab shells which found a reducing power of 0.32 OD at a concentration of 10 mg/mL. Thus, chitosan used as an antioxidant, which has a reducing power, can help in the reduction of Fe^{3+} to Fe^{2+} because the amine group (NH_2) present in the composition of chitosan can give hydrogen to the free radical in the reaction. Hydrogen combines with the free radical and thus it breaks the free radical to become stable and less readily available for propagation. This results in Chitosan exhibiting a potential primary antioxidant activity (Sarbonet.al., 2014).

Table 1: Reducing Activity of Standard.

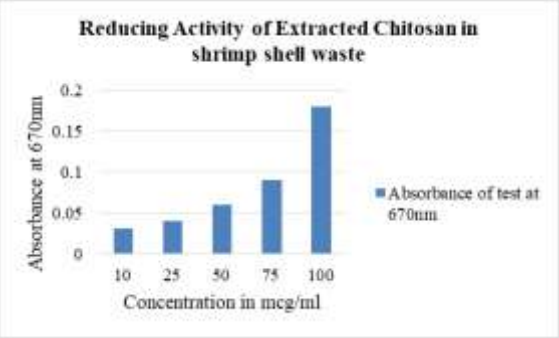
Concentration in mcg/ml	Absorbance of standard at 670nm
10	0.03
25	0.04
50	0.06
75	0.09
100	0.18



Graph 1: Reducing Activity of Standardat different concentrations

Table 2: Reducing Activity of Extracted Chitosan in Shrimp Shell Waste

Concentration in mcg/ml	Absorbance of Test at 670nm
10	0.03
25	0.04
50	0.06
75	0.09
100	0.19



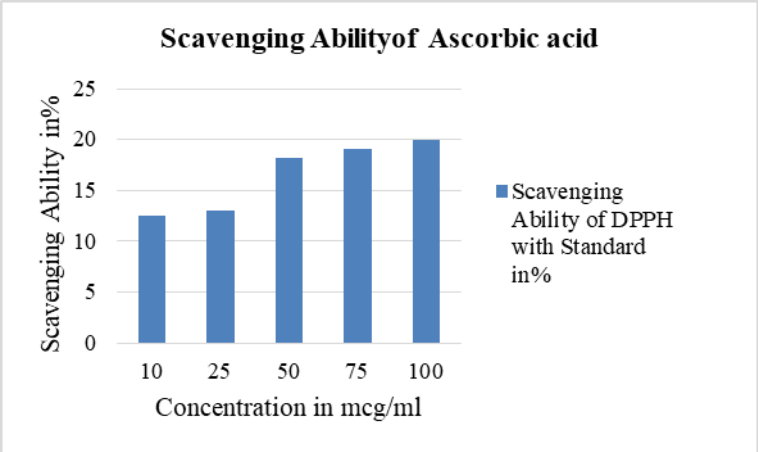
Graph 2: Reducing Activity of Chitosan Extracted in Shrimp Shell at Different Concentration

Scavenging Ability of chitosan on DPPH

Antioxidants can scavenge the free radicals by donating the some of their electrons; hence they inhibit the oxidative chain. DPPH is a common abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. It is a dark-colored crystalline powder composed of stable free radical molecules. DPPH has two major applications, both in laboratory research one is a monitor of chemical reactions involving radicals, most notably it is a common antioxidant assay. The scavenging ability of extracted chitosan was determined colorimetrically at 517 nm. Graph 2 shows the scavenging ability of DPPH of the extracted chitosan from the shrimp shell waste at different concentrations (10- 100mcg/mL) at the absorbance of 517 nm. The scavenging ability on DPPH of the extracted chitosan from the shrimp shell waste was found to be 7.5% at a concentration of 10 mg/mL at the absorbance of 517 nm. Sarbonet.al., 2014 had obtained scavenging activity on DPPH which was 30% at a concentration of 10 mg/mL at the absorbance of 517 nm from the mud crab shell. Yen et.al., **2008** had 46.4% scavenging ability on DPPH from the extracted chitosan of snow crab shells. Hence, an amine group present in the chitosan reacted partially with DPPH to form stable molecules. High scavenging ability shows a reduction of most of the DPPH radical molecules. The DPPH radical scavenging activity was calculated as: DPPH scavenging effect (%) $=A_0-A_1$.
When, A_0 and A_1 are absorbance of a control mixture without antioxidant and a mixture containing antioxidant, respectively.

Table 3: Scavenging Ability of Ascorbic acid.

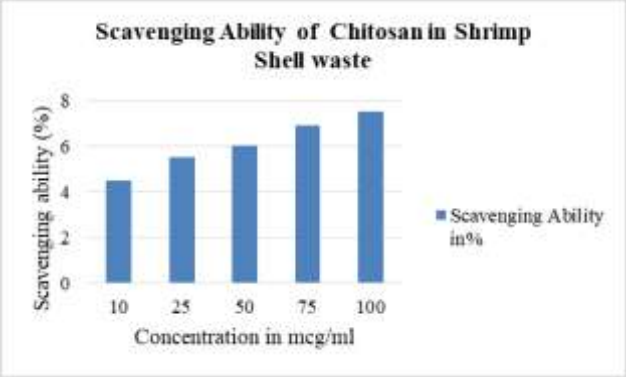
Concentration in mcg/ml	Scavenging Ability in%
10	12.50
25	13.04
50	18.18
75	19.04
100	20.00



Graph 3: Scavenging Ability of Ascorbic acid at different Concentrations

Table 4: Scavenging Ability of Chitosan in Shrimp Shell waste

Concentration in mcg /ml	Scavenging Ability (%)
10	4.5
25	5.5
50	6.0
75	6.9
100	7.5



Graph 4:Scavenging Ability on DPPH of the extracted Chitosan from shrimp shell waste at different Concentrations

Effect of chitosan on water treatment

The three raw water samples was tested prior before being treated with extracted chitosan and Table no.1 represents the growth of the organisms in the water sample.

Table 1 Growth of the organisms in the water sample

Sr. No	Agar Medium	Sample A	Sample B	Sample C
1.	Thiosulphate Citrate Bile Salt Agar	-	+	+
2.	Mac Conkey's Agar	-	+	-
3.	Salmonella Shigella Agar	-	-	-
4.	Salt Mannitol Agar	-	+	+
5.	Nutrient Agar	+	+	+

Key: (+) Growth; (-) No Growth

The raw water sample before treatment with chitosan showed the growth of microbes on Thiosulphate Citrate Bile Salt (TCBS) agar, Salt Mannitol agar, Mac Conkey's agar, and Nutrient agar. There was no growth observed on Salmonella Shigella agar. The identification of the isolated organisms was done by various biochemical tests. Based on morphological characteristics and biochemical results, organisms were concluded as E.coli, Vibrio species, and Staphylococcusaureus. After using chitosan no growth was observed on any agar and these show the antibacterial activity of chitosan on water treatment. **Al-Manhelet al., 2018** observed similar and concluded that chitosan has antibacterial activity against Gram-negative bacteria as compared to the Gram-positive bacteria. Chitosan inhibits the bacteria between the amine group (NH^+) of the chitosan and phosphoryl group of phospholipid found in bacteria.

Statistical Analysis: The data are presented at the mean +/- SD, Followed by Unpaired t-test were performed. The two tailed p-value equals to 0.228, by conventional criteria this difference is considered to statistically significant.

CONCLUSION

Chitosan is derived from chitin obtained from crustaceans like shrimp, crab, lobsters, etc. Chitin is the second most abundant natural polymer after cellulose obtained from crustaceans. For this study chitosan was extracted from dried

shrimp shells. About 20 grams of dried shrimp shells yield 32% of chitosan. The results of this work demonstrate that the extracted chitosan exhibits potent antioxidant activity whereas scavenging ability is found less. Thus, it can be concluded that extracted chitosan from shrimp shell waste is a good antioxidant. Chitosan inhibits Gram-negative bacteria present in wastewater as Chitosan has an amine group present in it. Hence, it can be used for wastewater treatment for the removal of dispersed particulates as well as dissolved pollutants. Chitosan can also be used as a biological treatment as a biological dressing of wounds and also for the large-scale purification of water reservoirs.

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Conflicts of interest: The authors declare no conflict of interest.

Authors' contribution: The corresponding author, Malika Ahuja initiated the project idea and guided the project followed by reviewing the manuscript. Areeba Ansari and AkshayAnumanla carried out the research work, concluded the results, and developed the manuscript.

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Evaluation of Antioxidant and Antibacterial activity, Phenol and Proline content of Terminalia catappa

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ABSTRACT

Plants perform vital role for the prosperity of mankind. They have been used for medicinal purposes long before prehistoric period. Terminalia catappa leaves have been known as a folk medicine for the treatment of various diseases. In the present work various parts of T. catappa like Green leaf (GL), Green and Red leaf (GRL), Red leaf (RL), Red epicarp (RE), Green epicarp (GE), Red fruit kernel (RK) and Green fruit kernel (GK) were evaluated for their antibacterial and antioxidant potential. Also Proline and Phenolic content of all these parts was determined. Methanol, acetone, and aqueous extracts of T. catappa leaf, epicarp and kernel were evaluated for antibacterial activity by using agar well diffusion method. Methanolic extracts showed best antibacterial activity. Quantitative determination of Phenol, Proline and Antioxidant activity in leaves, epicarp and kernel of T. catappa was carried out using spectrophotometric method. Catechol, Ascorbic acid and Proline were used as standard for calibration of the Phenols, Antioxidant activity, and Proline content respectively. The leaf (GL, RL) contained maximum amount of Phenols as compared to epicarp and kernel. The antioxidant activity of RK and GK was quite low compared to that of other parts. There was no significant variation in Proline content of leaf, epicarp and kernel. Leaves of T. catappa showed high antioxidant and antibacterial activity, high phenols and proline content as compared to others parts.

Keywords: Antibacterial activity, antioxidant activity, phenol, proline.

INTRODUCTION

In many parts of world especially in rural areas folkloric medicine has been practiced for many centuries due to availability and low cost [1]. Medicinal plants have the potential to synthesize a wide variety of chemical compounds that play a vital role in primary health care system [2]. Currently, application of natural products with therapeutic properties is increasing worldwide. Pathogenic microorganisms are the important agents for many diseases [3]. Potential herbal extracts portray as a bridging agent for novel bioactive molecules. The diversification of medicinal plants makes them a treasure for obtaining novel compounds which can be used in medicinal system as drugs or pilot molecules for invention of new drugs [4].

Terminalia catappa Linn. (Combretaceae) belonging to Southeast Asia [3] is a large deciduous tree [5]. T. catappa (Tropical almond) is a large, spreading tree now distributed throughout the tropics in coastal environments. It can tolerate strong winds and moderately high salinity in the root zone. It grows principally in freely drained, well aerated; sandy soils [6]. The obovate leaves of this plant turn pink-red to red-yellow before falling. Some of the pigments responsible for these changes are violanxanthin, lutein and zeaxanthin. In addition to these pigments leaves also contain violeoxanthin, epoxidelutein and cryptoxabthine. Fruits are produced from about 3 years of age, and the nutritious, tasty seed kernels may be eaten immediately after extraction [6].

Phenolic compounds are aromatic compounds that result from the secondary metabolism of plants [7]. They are one of the most broadly occurring groups of phytochemicals that exhibit antiallergenic, antimicrobial, antiartherogenic, antithrombotic, anti-inflammatory, and vasodilatory and cardioprotective effects [8, 9]. Many phenolic compounds have the calibre to function as antioxidants by scavenging free radicals involved in oxidative processes [10]. Phenolic compounds are always present in the form of glycosides in plant and are rarely present in the free form [11, 12].

It has been reported by earlier researchers that T. catappa leaves were found to possess good antioxidant activity, reducing power and inhibitors of peroxidation [13, 14]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, alzheimer's disease and cancer [15]. Antioxidants such as carotenoids, glutathione, as well as antioxidant enzymes like peroxidase, superoxide dismutase and catalase has major role in protection of plant cell under stress conditions [16]. Plants are affected by several kinds of stress which results in accumulation of glycerol, sorbitol, proline, etc. [17, 18]. The leaves, fruit kernel and epicarp of T. catappa undergo colour changes from green to red during the entire life cycle. In this study, changes in antioxidant and antibacterial activity, phenol and proline content was measured during this period of visible change.

MATERIALS AND METHODS

Plant Collection

Both the seeds and leaves of the plant were collected in month of October from the Botanical garden of G. M. Momin Women's College. The plant was authenticated in Blatter Herbarium of St. Xavier's College, Mumbai. The plant specimen matches with the Blatter Herbarium specimen no.16063 of H. Santapau and was identified as Terminalia catappa.

Different parts of the plants used for various investigations are as follows:

GL	:	Green Leaf
GRL	:	Green and Red Leaf
RL	:	Red Leaf
RE	:	Red Epicarp
GE	:	Green Epicarp
RK	:	Red Kernel
GK	:	Green Kernel

Antibacterial Activity

Extract preparation for Antibacterial activity

Aqueous Extraction

Aqueous extract of plant material was made by dissolving 1.5 gm of plant material in 25 mL of D/W by boiling for 2 h and then cooled and filtered with muslin cloth. This filtrate was used for further experiments.

Acetone Extraction

Both seeds and leaves of T. catappa were washed and air dried. They were ground to fine powder using mixer grinder and powdered materials were maintained at room temperature and protected from light. Powdered seeds and leaves (0.25gm) with 12.5 mL of acetone were extracted for 24 h in mechanical shaker at room temperature. Extracts were filtered with filter paper (Whatman No.1) and were stored at 4^{o}C [19]. It was then evaporated at room temperature and redissolved in water (1.5 mL) and analyzed for antimicrobial activity.

Methanol Extraction

The fruits and seeds of T. catappa were collected, washed and air dried. They were ground to fine powder using mixer grinder and powdered materials were maintained at room temperature and protected from light. A fine dried powder of each sample (3g) was extracted using 50 mL of methanol in mechanical shaker at room temperature for 60 min and kept overnight. The extracts were filtered through Whatman No. 1 paper and evaporated to dryness at room temperature. All of the samples were redissolved in distilled water (8 mL) and analyzed for antimicrobial potency.

Organism used

Escherichia coli (E. coli) belonging to family Enterobacteriaceae was used as the test organism.

Agar well diffusion method

The aqueous extract, acetone extract and methanol extracts were used for antibacterial activity. The antibacterial activity was evaluated at a concentration of 50µl/well. Antibacterial activity was performed by agar well diffusion [20]. Luria Bertani Agar was the media used to study the antibacterial susceptibility. The media and test bacterial culture (E. coli) were poured into petri dishes. The sample (50 µl) was impregnated in to a well of diameter 0.5 cm followed by incubation of plates at 37°C for 48h. The experiment was performed under aseptic conditions and susceptibility was determined by measuring the zone of inhibition. The experiment was performed in triplicates.

Extraction and Detection of Antioxidant Compound

Extract Preparation

The fruits and seeds of T. catappa were collected, washed and air dried. They were ground to fine powder using mixer grinder and powdered materials were maintained at room temperature and protected from light. A fine dried powder of each sample (3 g) was extracted using 50 mL of methanol in mechanical shaker at room temperature for 60 min and kept overnight. The extracts were filtered through Whatman No. 1 paper and evaporated to dryness at room temperature. All of the samples were redissolved in distilled water (8 mL) and analyzed for their contents in terms of antioxidant activity.

Determination of Antioxidant activity

The method described by Prieto et al. in 1999 [21] was adapted to measure the antioxidant capacity of T. catappa extracts. In brief, to a known aliquot of sample solution (0.4 mL) taken in a vial, 4 mL of the reagent solution (0.6 M sulphuric acid, 4M ammonium molybdate) was added and incubated in a water bath at 95^{o}C for 90 min. Absorbance was measured at 695 nm. Calibration curve was prepared by using standard solution of ascorbic acid (5-100 ug mL^{-1}) and the antioxidant activity was expressed as mg AAEAC (Ascorbic acid equivalent antioxidant capacities) per gram extract [22].

Extraction and Detection of Phenols

Extract Preparation

Both seeds and leaves of T. catappa were air dried and used for further experiments. Accurately weighed powder (0.5gm) of sample was ground with mortar and pestle in the measured volume of solvents (10 mL) of 80 ethanol:20 water. Filtered extract was used further.

Determination of Phenols

The total phenol content of each plant extract was estimated by method described by Malik & Singh [23]. Aliquots of extracts (0.01 mL) were taken and final volume (3mL) was made with distilled water. Then 0.5 mL folin-ciocalteau reagent (1:1 with water) and 2 mL Sodium carbonate (20%) were added sequentially in each tube. Blue colour was developed because phenols undergo complex redox reaction with phosphomolibdic acid in folin-ciocalteau reagent in alkaline medium which resulted in blue coloured complex, molybdenum blue. The test solution was warmed for 1 min, cooled and absorbance was measured at 650nm against the reagent used as a blank. Calibration plot was generated using catechol. The concentration of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol\g of dry material by method given by Khatiwora et al. [24].

Extraction and detection of Proline

Extract Preparation

Both seeds and leaves of T. catappa were extracted (0.5 gm) by homogenizing in 10% of 3% aqueous sulphosalicyclic acid. The filtrate was used for measuring proline content.

Determination of Proline

The total proline content of each plant extract was estimated by method described by Bates et al. [25]. Each sample (2 mL) was taken in a test tube and 2 mL of glacial acetic acid and 2 mL of acid ninhydrin was added. The mixture was then kept on boiling water bath for 1 h. The reaction was then terminated by placing the tube in ice bath. After that 4 mL toluene was added and mixture was thoroughly shaken for 20-30 s. The absorbance of red colour intensity was measured at 520 nm versus the toluene blank. A standard curve was prepared by running a series of standard with pure proline [25].

Statistical Analysis:

All assays were carried out in triplicates and results are presented as Mean $\pm$ SD.

RESULTS AND DISCUSSION

In the present study the parts of T. catappa extracts in methanol, acetone and water were investigated for their antibacterial potential against E. coli strain. The antibacterial activity of methanolic extract was maximum in Green leaf (13 mm) as compared to Red leaf (6 mm) and Green & Red leaf (11.33 mm). No significant difference was found in Green Epicarp (9.5 mm) and Red Epicarp (9.5 mm) (Fig.1a). In acetone extract maximum activity was found in leaf parts i.e. Green & Red leaf (10.33 mm), Red leaf (9.75 mm), and Green leaf (9.6 mm) respectively. In case of Epicarp and kernel there was no activity found in Acetone extract but in Methanolic extract antibacterial activity was found in Epicarp (Fig.1b). In aqueous extract the zone of inhibition was maximum in Red leaf (14 mm) as compared to Green leaf (8.5 mm) and Green & Red leaf (10 mm). Antibacterial activity was maximum in Green Epicarp (10.5 mm) compared to Red Epicarp (9.83 mm). In Kernel there was no activity was found (Fig.1c).

The best antibacterial activity was shown by the methanolic extract in maximum plant parts. The need of the hour is to find new antimicrobials because the microorganisms are getting resistant to the existing antibiotics [26, 27]. Utilization of herbal medicine in treatment of health ailments is gaining popularity in all over the world [28]. Instances of multiple drug resistance in human pathogens have developed due to indiscriminate use of commercial antimicrobial drugs. In addition to this problem, antibiotics are sometimes associated with adverse effect on host including hypersensitivity, immune suppression and allergic reactions [1]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infections obtained from various sources such as medicinal plant [29, 30].

The results of present study signify the potential of T. catappa leaf as a source of therapeutic agents which provides leads in the ongoing search for antimicrobial botanicals and it also suggest that methanol is the best solvent to extract the active compound. However, the extracts are needed to be checked against some human pathogenic organisms also.

Antioxidant compound is associated with the total phenol content [31]. The plant extracts with higher level of total phenolic and flavonoids also exhibit greater free radical scavenging activity [31, 32]. Antioxidants are the substances that provide protection against oxidative damage [33]. In the present study antioxidant activity was evaluated for T. catappa leaves and parts of seeds (Epicarp and Kernel) (Fig. 2).

The Antioxidant activity of T. catappa was found maximum in Green Epicarp (1.13 mg AAEAC/g extract) followed by Green leaf (1.11 mg AAEAC/g extract), Red Epicarp (1.09 mg AAEAC/g extract), Green & Red leaf (1.07 mg AAEAC/g extract), Red leaf (1.00 mg AAEAC/g extract), Red Kernel (0.65 mg AAEAC/g extract) and Green Kernel (0.42 mg AAEAC/g extract).

Polyphenols present in plants, fruits and vegetables are an important source of natural antioxidants as they act as reducing agents, hydrogen donors, single oxygen quenchers and potential metal chelators [19, 25]. The level of Phenol in different parts of the plant extract is shown in (Fig. 3). The result indicated that the total phenol content of various extract had significant variation. Analysis of the phenolic contents revealed that the Green leaf (GL) contained the maximum phenolic content (23.8 mg catechol equivalent/ g extract) followed by Red leaf (18.5 mg catechol equivalent/ g extract), Green Epicarp (3.23 mg catechol equivalent/ g extract) and Red & green leaf (1.538 mg catechol equivalent/ g extract). There was no Phenolic content found in Red Epicarp (RE), Red Kernel (RK) and Green Kernel (GK). The result indicates that the leaf of T. catappa consist of the maximum phenolic compounds as compared to the other parts of the plants.

Earlier research indicates that T. catappa leaf is rich in polyphenolic compounds. Consumption of fruits, vegetable and plants rich in polyphenols is associated with the reduced risk of certain Cancer, Cardiovascular diseases, Atherosclerosis, Diabetes and Alzheimer's diseases [34, 35, 36]. These properties may be due to its phenolic content. However in the

present study a comparative account is made between different plant parts which suggest that red leaf is also rich in phenolic content. Results show that GL has highest phenolic content and antioxidant activity. Several investigations of antioxidant activity of plant extract have confirmed a high linear correlation between the values of phenol concentration and antioxidant activity.

Proline has also been estimated in the present study. Total proline content in the examined extracts ranged from 0.72 mg proline/ g extract to 0.83 mg proline/ g extract. There was no significant difference in the proline content measured in any of the plant part (Fig. 4). The exogenous proline has been reported to protect plants under stress. Proline, Antioxidant activity and phenol get accumulated under various abiotic stresses (Heat, Cold, Drought, Moisture and Salinity).

Thus here there is a strong correlation found between the presence of secondary metabolites of the plant like proline, antioxidant compound and phenol with the antimicrobial activity tested. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results so it can be recommended for pharmaceutical importance subjected to further tests.

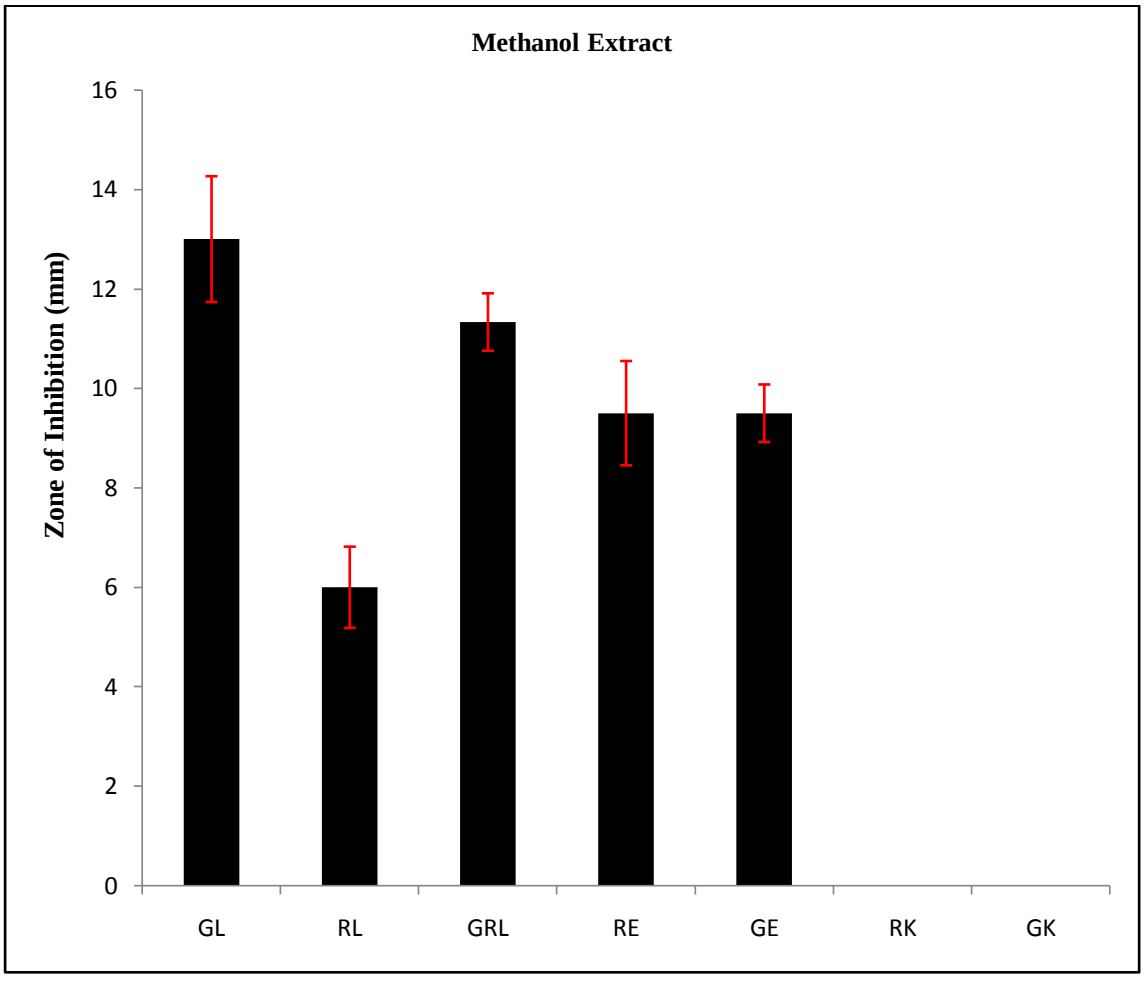

Fig-1a: Antibacterial Activity of Methanol Extract of T. catappa

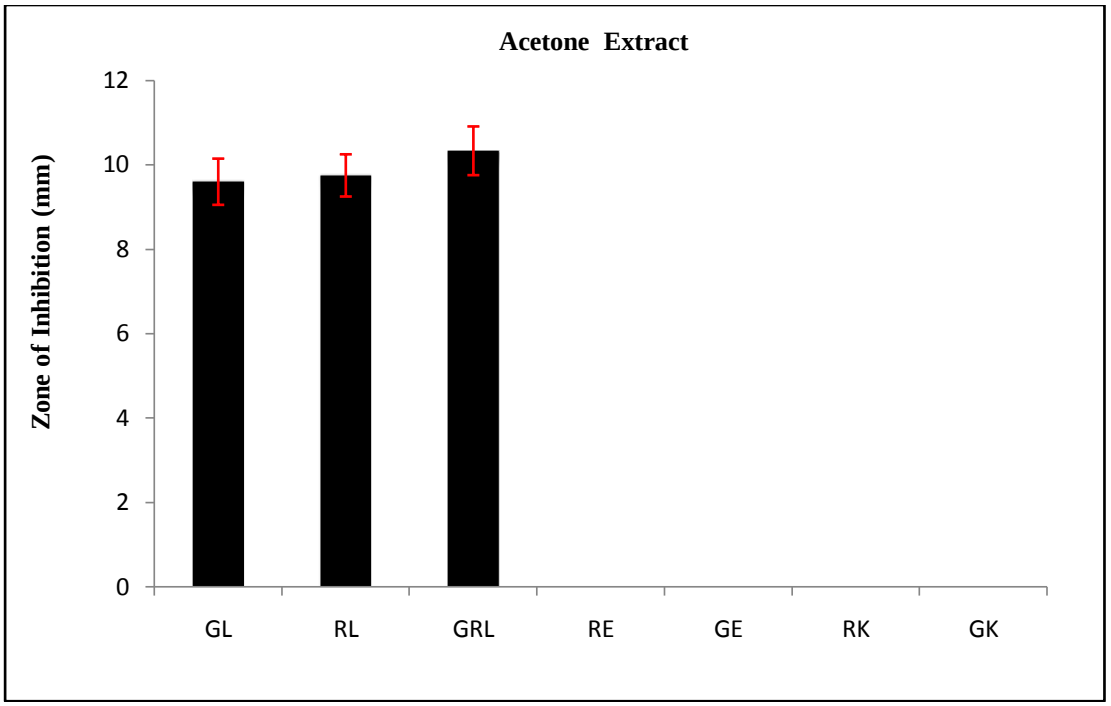

Fig-1b: Antibacterial Activity of Acetone Extract of T. catappa

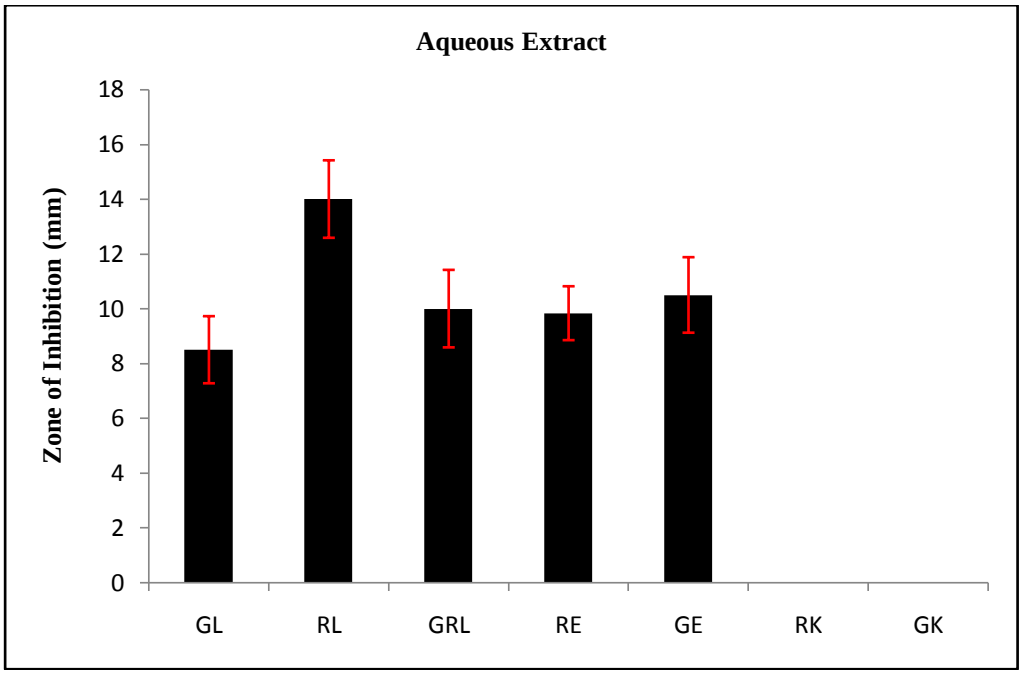

Fig-1c: Antibacterial Activity of Aqueous Extract of T. catappa

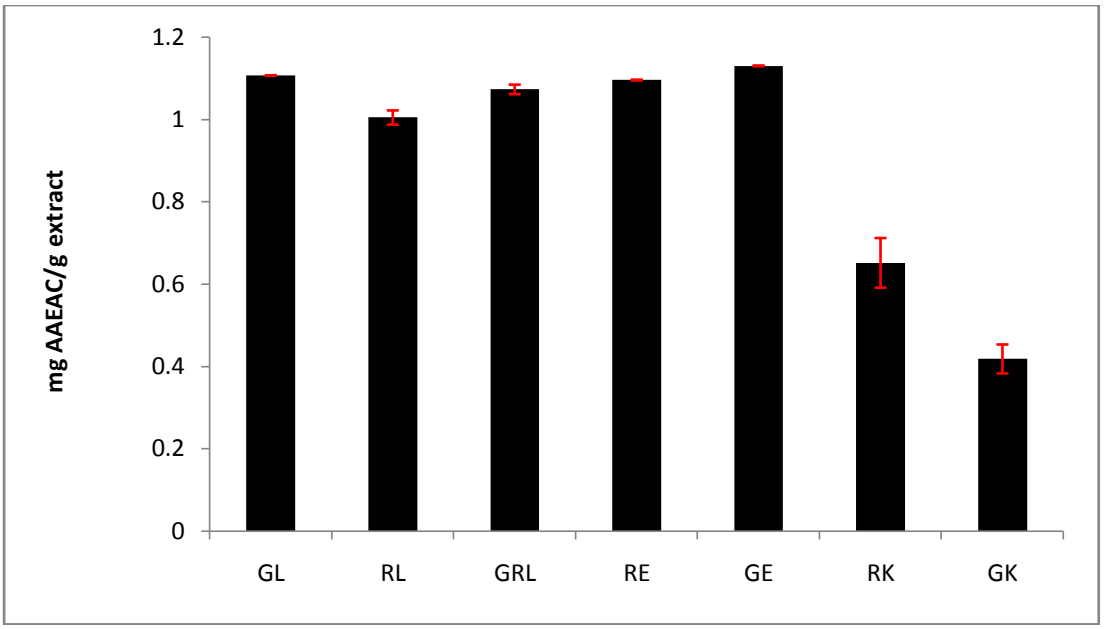

Fig 2: Determination of Antioxidant activity in various parts of T. catappa

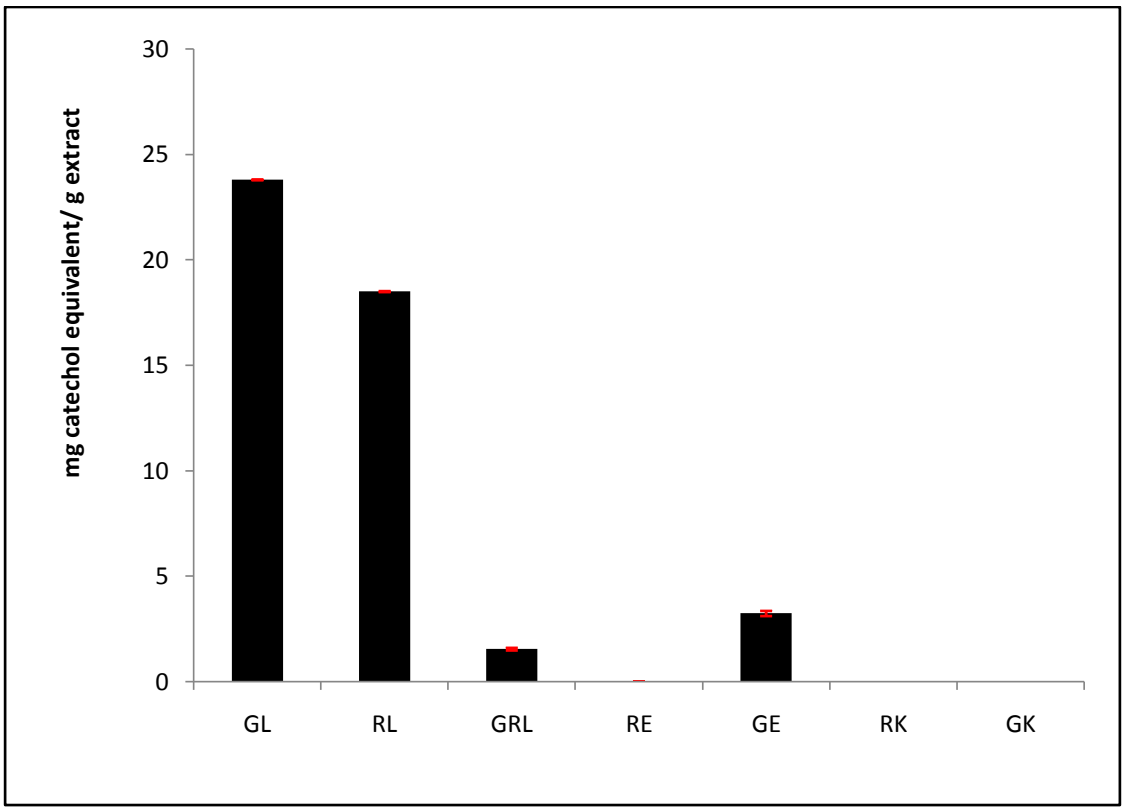

Fig 3: Determination of Phenol contents in various parts of T. catappa

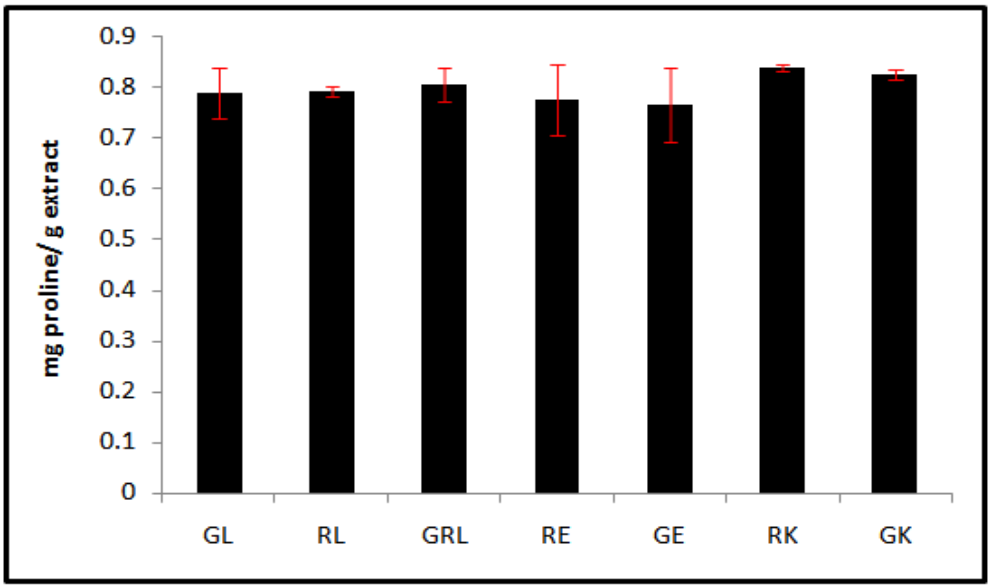

Fig 4: Determination of Proline contents in various parts of T. catappa

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Space Industry and COVID-19: An Insight into Their Shared Relation

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ABSTRACT

Space and its technologies have always been used primarily for beyond-earth explorations. Secondarily, society has benefitted from space technologies concerning communications, navigations, earth observations, disaster response. Additionally, during COVID-19, space-based technologies have helped bring about innovative solutions to global health by tracking the spread of the pandemic, mapping hotspots, and providing location-based services. Today, we are all aware that the virus has adversely affected funding and human resources for the space industry. This, in turn, has led to the delay of many pre-scheduled space missions. The present article, thus, aims to lay down the negative and positive implications of the COVID-19 on the space sector and, at the same time, highlight the innovative uses of space-based technologies in different societal aspects and in combating the impact of the pandemic.

Keywords: COVID-19 impact, Global health, Spinoff products, Telemedicine, Space policy, Space-related recommendations.

INTRODUCTION

The year-long pandemic and its repercussions have had a tremendous impact on the space industry. This can be understood from the fact that the pandemic resulted in the postponement of scheduled space missions and a decrease in private financing for space-related activities. Furthermore, if one were to understand the extent of the impact of COVID-19 on the space industry, a bibliometric outlook (Fig. 1) would come in handy.

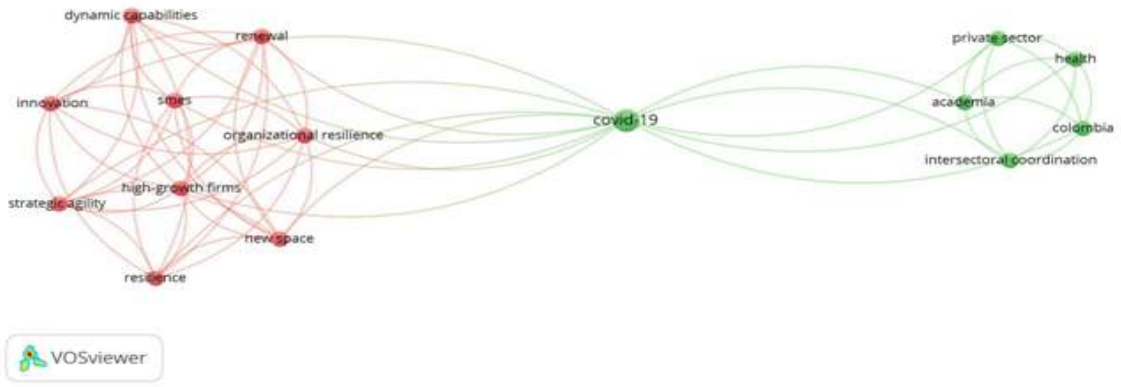

Figure 1: Bibliometric outlook on the impact of COVID-19 on the space industry

The bibliometric study revealed that COVID-19 mostly affected smaller and medium-sized enterprises (SMEs), high-growth firms, academia, and the health sector. Additionally, the pandemic served a heavy blow to organizational resilience, innovation, strategic agility, inter-sectoral coordination in terms of values. Therefore, the authors of the present article aim to highlight the effect of COVID-19 on the space industry, with special reference to the bibliometric mind-map generated above.

ROLE OF SPACE SECTOR IN SOCIETY

Science and technology emerged as a way to understand the world we live in, so; it has been researched for the sake of societal good from its very beginning [1]. The United Nations general assembly declared 2022 as the 'International Year of Basic Sciences for Sustainable Development,' thus emphasizing the need to increase 'evidence-based decision making' while tackling immediate global challenges [2]. These would also include developing technologies based on potential human needs. Therefore, public policies should be framed as per the available evidence-based knowledge integrated with science and technology. This will help support decision-making, provide safety and security to the public, bring about innovation and economic prosperity, respond to urgent global needs [1].

Space and its technologies are highly trans-, inter-, and intra-disciplinary aspects of science and technology, which started around half a century ago. Today, it benefits us in understanding our world better and helps us develop applications for societal good. Some of the key areas where space technologies have been implemented in our day to day life include:

A) Space technology as an inspiration for all human endeavors:

Space has inspired humans for ages by feeding the insatiable curiosity of humans. As a result, today, space is not just limited to mathematics, physics, engineering, or xeno-sciences but has extended its boundaries to chemical, life sciences, etc. Currently, space boosts the world economy and has provided a unified view of the world, thus aiding world peace. It has also opened up the new possibility of space tourism and has our defense and security initiatives. Therefore, in a nutshell, space technology has provided humanity with a long-term vision and a possibility of a limitless future [1].

B) Earth observation as a way to better understand and mitigate global challenges:

Earth observation collects data regarding Earth's physical, chemical, biological systems. Data received from these observations and their analysis helps us: [1, 3, 4, 5]

- Take immediate actions in natural or anthropological disasters, thereby reducing life loss;
- Better manage our energy resources;
- In predicting, reducing, and adaptation to climate change;
- Reducing desertification and promoting sustainable agriculture (through 'Precision Agriculture');
- In the management and conservation of terrestrial and oceanic resources;
- In monitoring and conservation of biodiversity;
- In managing our water resources and gaining a fundamental understanding of the water cycle;
- In weather forecasting, disaster warning and reduce the loss of lives;
- In detecting urban heat islands which are comparatively warmer than its surrounding rural areas due to anthropological activities; and.
- In monitoring deforestation, reforestation, and assessing environmental risks.

C) Space and its technologies in day to day life:

Solar panels, generation of power, storage of energy, managing waste, miniaturization, advanced robotics, and computing are all the results of space and its technologies [1]. Space-based technologies like satellite phones help communicate with remote areas, in high seas, with aircraft, in broadcasting (via the geostationary satellites), and navigations [1]. Furthermore, services provided by satellite communications are cost-effective, available globally, reliable, scalable, and provide versatile effectivity [1].

D) Developmental goals in rural and remote areas and in fighting poverty:

Space technologies have enabled education and health facilities in rural and remote areas where proper infrastructural facilities are absent [1]. In this, remote sensing has helped in poverty mapping based on nutrition status (a valid, reliable,

objective, and feasible poverty indicator) at the community level. This helps in achieving the goals of poverty alleviation initiatives [1,6].

E) Space-based technology in medical treatment, health services, and medicine and epidemiology:

These include [1]:

a) Avoiding loss of bone;
b) Asthma and cancer treatment;
c) Ultrasound immunology;
d) Surgery of the eye ;
e) Support in the purification of water;
f) Development of vaccine;
g) Growing protein crystals of high quality;
h) Tele-Medicine;
i) Tele-Epidemiology; and
j) Heart monitors

F) Space and its technologies as a driver for ' International cooperation and action against global challenges':

The International Space Station (ISS) is a true symbol of international cooperation. It is the most peaceful and collaborative project ever initiated by humans [7]. Thus, space provides humanity with a unified worldview and helps international peace-keeping. This, in turn, leads to the advancement of international collaboration and preparedness on potential issues and threats to humanity such as asteroid strikes, space debris, and environmental degradation [1].

G) Spinoff products from space exploration:

These are products or effects derived from technological developments in areas of space sciences which later proved to be useful in other (non-space) sectors. Some of which are listed below [1]:

a) Fuel-cell engine;
b) Euro-bot wet model;
c) IGAR: Image-Guided Autonomous Robot (helping breast cancer patients);
d) Biosensor to test for pathogens;
e) Algae-derived ingredients;
f) Water mapping technology;
g) Magnetic fluids for speakers; and
h) Chlorophyll detectors (as plant stress detectors).

IMPACT OF COVID-19 ON SPACE SECTOR

All of humanity has been affected by the COVID-19 pandemic mostly negatively, and the space sector is no exception.

The negative impacts:

a) The COVID-19 pandemic has greatly cut down on the funding of the space sector. Small and medium-sized startups and companies faced many problems as the investors didn't provide funding due to uncertainty in future events. Remote working and lack of workforce in premises due to quarantine measures decreased the demand for many space services. Moreover, companies went bankrupt; contracts remained unfulfilled; as a result, even market giants suffered great losses [8].

b) Many pre-scheduled significant space launches got delayed or even canceled. There was also a cut down in the operational space missions [8].

c) Concerns were raised regarding the interaction between public and private sectors during remote work. There were also frequent occurrences of legal issues related to mitigating contract liability in the case of defaulters [8].

On the other hand, the pandemic also had some positive implications on the space sector, which are as follows:

The positive implications:

a) Space-based technologies and the data collected from them began to be used increasingly in societal problems and to combat the pandemic [9, 10, 11].

b) Space, which was earlier meant primarily for exploration purposes, now became a source of exploitation by bringing a more commercial approach to space-related activities. Private space entrepreneurship was promoted, bringing about a low-cost approach to space innovations [12].

c) Telemedicine (a space-based technology) was implemented in India in the public health sector. This milestone could not be achieved before the pandemic owing to concerns regarding its practice (mainly lack of guidelines and ambiguity) [13, 14].

KEY RECOMMENDATIONS

Space agencies and public organizations have acted rapidly to ensure the continuity of space activities. Still, more specific steps would be needed for SMEs to sustain the diverse space ecosystem. Policymakers may consider the following:

a) Helping out SMEs in their crisis response and making such processes easy [15].
b) Framing eligibility criteria for support and procurement initiatives to enable public and private funding (e.g., advance payments, keeping the facilities open, liaising with local and regional powers is essential) [15].
c) Enhancing the visibility of new and existing government long-term space-based initiatives and their funding schemes[15].
d) Building incubation centers for businesses and product trials and demonstration schemes, thereby meeting the needs of SMEs and entrepreneurs (e.g., promoting reduced or no procurement fee to access trial facilities) [15].
e) Keeping track of everyone's work and requirement of high-quality data about the space industry base to make informed decisions on space policies [15].
f) Facilitating the companies to reassure their investors to provide funding and retain required skilled staff [15].

ROLE OF SPACE-BASED TECHNOLOGIES IN COMBATING COVID-19

When the COVID-19 pandemic struck the world, it changed almost everybody's way of living due to quarantine restrictions. Nevertheless, professionals from every field of the study tried their best to mitigate problems faced by people during these challenging times. It was then that the healthcare sector and space stakeholders recognized the impact of space-based technologies on medical services. Some of the major events in this regard included:

- In June 2020, NASA, ESA, and JAXA collaborated to form the COVID-19 earth observation dashboard, which helped monitor the pandemic worldwide [10].
- ISRO personalized its geo-portal into a national level portal named 'Bhuvan-COVID-19' tracks the spread of the virus; provides statistics on deceased cases; map containment and buffer zones; and initiate control at the field level. It also enables easy navigation, which helped 'Anna Unavagam' (an initiative to provide food for the needy) to provide food in places of need based on data provided by the geo-portal. An electronic device named Monal-2020 enabled by the use of 'Bhuvan' geo-portal helped in around-the-clock monitoring and providing essential and medical services to home-isolated COVID-19 patients based on the geo-location services provided by 'Bhuvan' [9].
- Tele-epidemiology is defined as "using space technology with remote sensing to study incidence, distribution, and control of infectious diseases and other factors relating to human health." It has proved to be useful for clinicians and associates for zoonotic diseases. Tele-epidemiology with geographic information science technology can improve our understanding of COVID-19 and its control through monitoring, sharing of data, digital tracing of contact, and looking into the risk factors and forecasting of infectious diseases [10].
- Telemedicine can be defined as "the delivery of health care services, where distance is a critical factor, by all health care professionals using information and communication technologies for the exchange of valid information for the diagnosis; treatment; prevention of disease and injuries; research and for the continuing education of health care providers, all in the interests of advancing the health of individuals and their communities" [14]. Telemedicine has

played a crucial role during the covid times in providing medical services and providing remote monitoring of home isolated COVID-19 patients. The space sector has provided healthcare services via telemedicine to astronauts for a long time. Telemedicine can help provide medical and healthcare services in rural and remote areas, especially in low and middle-income countries with poor medical infrastructure in rural and remote areas [10]. Thus, telemedicine can make quality healthcare services accessible to all only if measures are taken concerning non-disclosure of patients' privacy and improving internet infrastructure [13].

- The BIO-MONITOR was initially developed for the Canadian Space Agency to store and forward physiological data from unobtrusive body-worn sensors. This wearable technology offered around-the-clock monitoring of vital body parameters, which were critical when treating an active COVID-19 patient. Such devices could also be used to monitor the health of healthcare personnel who are tirelessly working during the pandemic [10].
- The point-of-care ultrasound and the ESA's Biological Light Fieldable Laboratory for Emergencies boosted biomedical capabilities. The latter is a lightweight, mobile autonomous laboratory deployed in emergencies [10].
- The space sector has considerable experience managing astronaut self-isolation and confinement to small spaces for long periods. It thus can contribute substantially to global dialog on managing mental health, stressors, and self-isolation during pandemics. NASA has provided numerous resources and support for managing the effects of isolation during the COVID-19 pandemic [10].

Therefore, the pandemic provided a major opportunity to implement space-based technologies for societal good in the field of Earth observations (Remote Sensing, High-resolution Satellite imagery, Global Information Science Technology-GIS, Global Positioning System Technology- GPS), tele-epidemiology, telemedicine, and space spinoff products [9,10,11,13,14].

CONCLUSION

The pandemic has shattered our lives for over a couple of years now. However, this, in turn, has also opened doors to new possibilities. Possibilities (such as remote work or telesurgery) that we would never have dared to venture into. Yet, given the recent losses that we have suffered, it would be advisable to further deal deep down into the effects of the pandemic on the space industry through systemic reviews and meta-analysis. If done so, only then can we be fully prepared to take on future crises with bold steps.

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CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

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Response of Different Media on Growth and Sporulation of Alternaria Alternata Causing Fruit Rot of Pomegranate

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ABSTRACT

Alternaria alternata (Fr.) Keissl is one of the most serious post-harvest diseases throughout the World. It causes severe post-harvest losses of perishable due to A. Alternata ranging up to 10 to 50 percentages. In this view, a total of fifteen isolates of A. alternata were isolated from infested pomegranates in the various locality of Maharashtra. Infected pomegranates were rinsed with sterile distilled water up to 5 to 6 times then cut into smaller pieces and placed aseptically at equidistant onto sterile culture media augmented with streptomycin (20µg/ml) in petriplate. Potato dextrose agar, Czapek Dox Agar,Corn Meal Agar and Martin Rose Bengal agar was undertaken to ascertain the requirement of different culture media on growth and sporulation of A. alternata. The culture plate was incubated at 22 ± 2ºC for seven days. Growing mycelium was transferred to slants and pure culture of the pathogen was obtained. Potato dextrose agar medium (90.00 mm) and Corn meal agar (75.33 mm) show maximum growth and sporulation of A. alternata whereas Martins Rose Bengal agar (48.17 mm) and Czapek Dox Agar (51.67 mm) shows minimum growth and sporulation.

Keywords: Pomegranates, A. alternata, culture media.

INTRODUCTION

Fruit rot of pomegranate caused by Alternaria alternata (Fr.) Keissl is a filamentous heterothallic septate mycelium, an ascomycetes comprising great variability in the mycelia growth and sporulation. Conidial germination of A. alternata is induced by different physical and chemical signals including the presence of quality nutrients (Apet and et al., 2014). Conidia of A. alternata are typically nutrient-dependent, they do not readily germinate in sterile water and they usually require an exogenous input of nutrients for germination. In addition, it has been proposed that nutrient dependent of phytopathogenic fungi may use germination stimulating compounds from a host plant as an alternative chemical when nutrient concentrations are too low for conidial germination and diverse carbon sources are effective at low concentrations (10 µg/ml) to induce sporulation in A.alternata (Filonow, 2002, Dahiwale et al., 2009 and 2012). Rich media such as malt extract induced rapid germination and early germ tube branching. The mechanism of nutrient sensing by A. alternata is unknown (Forsberg and Ljungdahl, 2001). The current study has illustrated the effect of such several culture media on the growth and sporulation of A. alternate.

MATERIALS AND METHODS

The infected pomegranate samples were brought from the field to the laboratory and used for the isolation study. The infected samples were surface sterilized with 0.1% $HgCl_2$solutions for two minute with gentle agitation. Samples were rinsed with sterile distilled water up to 5 to 6 times then cut into smaller pieces and placed aseptically at equidistant onto sterile PDA augmented with streptomycin (20µg/ml) in petriplate. Plate was incubated at 22 ± 2ºC for seven days, examined daily for the growth of the organism. Pure culture of the pathogen was obtained and the pathogen was purified using a PDA medium. Growing mycelium was transferred to slants. The identification was carried out by macroscopic and microscopic observation which confirmed the A. alternata (Samson et al., 1984). A. alternata was obtained and maintained at 4OC in the PDA medium tilted in sterile tubes and used for further study wherever necessary. The response of different media on growth and sporulation of A. alternata was used for detailed studies. Potato Dextrose Agar Medium (Hawksworth et al.,1983), Czapek's Dox Agar (Onions et al., 1981), Corn meal Agar (Baron and Finegold, 1990), Martin Rose Bengal Agar (Dahiwale and

Suryawanshi, 2012) were used for growing A. alternata. Potato dextrose agar medium (PDA) showing maximum growth and sporulation of A. alternata and this medium is used for further study.

RESULTS AND DISCUSSION

A. alternata were grown on four different media viz. PDA, CDA, CMA and MRBA in order to find the best medium suitable for the growth of pathogens. Total four media were taken for isolation and purification of A. alternaria. Media were prepared using standard techniques. In case of pure culture of the A. alternaria was obtained onto sterile PDA, CDA, CMA and MRBA medium. Corn meal agar (CMA) (75.33 mm) and Potato dextrose agar medium (PDA) (90.00 mm) shows maximum growth and sporulation in seven days where as Martin Rose Bengal agar (MRBA) (48.17 mm) and czapek dox agar (CDA) (51.67 mm) shows minimum growth and sporulaton. (Fig 1 and Table 1). The A. alternaria was purified using PDA medium. Plate was incubated at 22 ± 2ºC for seven days. Growing mycelium was transferred to slants. The identification was carried out by macroscopic and microscopic observation which confirmed the A. alternaria (Samson et al., 1984). Similar result reported by Reddy and Gupta, (1981) the growth and sporulation of Alternaria helianthi while Mohanthy et al., (1981) reported Richards B medium shows maximum growth of A.alternata. Morphological characteristics of the cultures on potato carrot agar and V8 agar media after seven days exhibited typical characteristics of Alternaria mali (Simmons, 1999). PDA, Oatmeal agar, Czapek Dox, Richards, Walkman's agar medium, water agar and Martins Rose Bengal Agar media were also studied by Hubballi (2010), maximum growth was observed on PDA.

Table 1: Effect of different media on germination and sporulation of Alternaria alternate

Sr.No.	Medium	Colony diam (mm)	Sporulation
1	Potato dextrose agar	90.00	++++
2	Czapek Dox Agar	51.67	++
3	Corn Meal Agar	75.33	+++
4	Martins Rose Bengal Agar	48.17	+

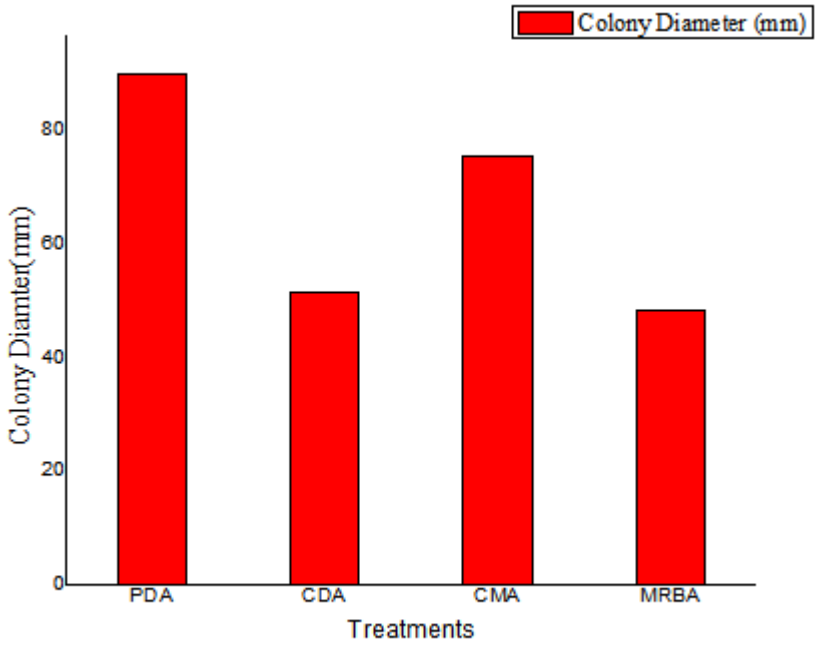

Fig 1: Effect of various media on radial growth of *A. alternata* infecting Pomegranate (graph)

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Nutritional Quality of Fruits during Pandemic Covid-19

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ABSTRACT

Fruits are universally promoted as healthy. Everyone eats different kinds of fruits daily by thinking that it contains a lot of vitamins and minerals. But do they really contain lots of vitamins and minerals today also. The research on fruits has been done a long time ago when there were no adulterants and today as we all know adulterants are used to enlarge the size of the fruit and to make it look fresh even its stale from inside so by using certain techniques, we can find that how much proteins and minerals we can get from the fruit which have been adulterated and how much the adulterants used are affecting our body.

Keywords: Fruits, Vitamins, Minerals, Affecting Adulterants.

INTRODUCTION

Currently Covid-19 pandemic is a leading challenge all over the world. To prevent virus infection, it is critical to achieve and maintain a healthy nutritional state. Optimal nutrition and dietary nutritional requirements have an impact on immune system, strengthening immune system is the only long-term method to live in the current environment [1]. There is no proof found that dietary supplement can cure the immune system except Vit C, which is one of the best ways to improve immune system. An adequate intake of Protein, and vitamins A, B_{12}, B_6, C, and E is essential for the maintenance of immune function [2]. COVID-19 has provided a new set of obstacles for anyone trying to maintain a healthy diet in the current situation. Which increase the demand of fruits leads to the higher consumption and Adulteration in Fruits [3]. Fruits are consumed in every form in order to support the different biochemical and physiological activities of our body. Most of the times, these Fruits are prone to Adulterated [4].

Common Adulterant used:

Adulteration of food items began a couple of decades ago and this practice is increasing day by day. Fruits are adulterated with calcium carbide, ethephon, formalin, injections of colors and sweeteners [5]. i.e. Apples are sold in the market at 120-150 Rs/kg. From 1 kg of fresh apple, how much fresh juice can we obtain. If we realize this then why do we expect to get about 200 ml of any fruit juice with 20 Rs. only. Again, we need to change our attitudes and expectations.[6]Consumption of adulterated food items may cause asthma, sore throat, larynx constriction, bronchitis, skin infections, allergic reactions, diarrhea, hematuria, circulatory failure, numbness, dizziness, kidney failure, stomach cancer, liver cancer, nervous disorders and many other diseases [7].

The objective of this work is to determine the nutrients content of some common fruits available in markets.

Materials and methods:

Samples of fresh fruits were purchased from a local market located in city. All the samples were thoroughly cleaned with distilled water to remove adhering contaminants. All reagents used were of analytical grade.

Sample preparation:

100 g of each sample was cut into small pieces, blended together with 50 mL of distilled water using an electric blender, and then filtered. The filtratewastransferredintoa500mL volumetric flask and the flask was filled up to the mark with distilled water. [8]

The Bradford Method: The Bradford assay was conducted according to the method described by Bradford for protein estimation.

Determination of Vitamin C by titrimetric: the extract 20 mL of the sample solution was pipetted into a 250 mL conical flask. 150 mL of distilled water was added into the flask followed by 1 mL of starch indicator solution. The sample was then titrated with the 0.005M iodine solution until a dark blue-black color was persisted due to the starch-iodine complex. Titration was repeated until three titers are obtained that agree within 0.1 ml.

Determination of Calcium by titrimetric: A 25.0 mL sample of each digest was pipetted into a beaker and 1M NaOH solution was added to adjust the pH to 12-13. Two drops of solo chrome dark blue were then added and immediately titrated against a 0.01M EDTA solution to the blue end-point.

RESULT AND DISCUSSION

Nutritional Values of Some Fruits

Nutrient	Orange	Guava	Banana	Musk melon	Dragon fruit
Protein					
Normal value	1.3 gm	2.55 gm	1.09gm	0.84 gm	1.2 gm
Conventional	1.0 gm	2.55 gm	0.89gm	0.64 gm	1.02 gm
Sugar					
Normal value	12 gm	8.92 gm	22.84gm	12gm	13gm
Conventional	15 gm	10 gm	28.84gm	12gm	11 gm
Vitamin C					
Normal value	92 gm	228.3mg	8.7mg	40.56 mg	4.5 gm
Conventional	85 gm	189.7mg	4.6mg	20.56 mg	2.9gm
Calcium					
Normal value	5 gm	18 mg	1 gm	-	53 gm
Conventional	5 gm	10 mg	0.5 gm	-	18 gm
Potassium					
Normal value	3gm	378mg	258mg	331.00 mg	286.47 mg
Conventional	5gm	417mg	358mg	531.96 mg	399.5 mg

The prepared fruit samples are analyzed for Protein, Vitamin C, Sugar, Calcium, potassium suspension converted to mg/100g of daily value. Each value is the mean value of triplicate analysis. In analyzed fruit, Protein ranged from 0.84 to 2.55gm/100g, potassium from 258 to 5000mg/100g and Calcium from 5 to 53gm/100g, of the sample [7]. The top fruit in the rank order of higher protein guava 2.55gm/100gramhigher sugar in Banana 28.84gm/100gram, higher vitamin C content in Orange 85gm/100gm as compared to musk melon and dragon fruit which are slightly low as compared to daily value demand [8] One of the major reason of fruits lost it nutritional content is no proper cultivation or handling and adulterated to achieve fast growth of fruits cheaply available in market. It cannot full fill the dietary requirement of individual as well as may cause an acute or chronic disease due to the adulteration

CONCLUSION

It's been found that fruits have many benefits but because of adulteration we are unable to get the nutrition. Organic fruits are very costly for everyone to afford them and hence people look upon for conventional fruits. So, are we are looking forward to healthier and happier India. The answer is hidden somewhere within our minds and hearts.

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Impact of Mutation on Metal Resistance in Pseudomonas Spp.

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ABSTRACT

The toxic heavy metal pollution of the environment is a serious environmental concern. The bacterial resistance to heavy metals is of practical significance and can be useful for remediation of heavy metal polluted sites. In this research, the impact of mutation in the metal resistant bacteria isolated from wastewater was investigated. The Polluted water sample was collected from Waldhuni River near Ulhasnagar, District Thane, Maharashtra, India. Pseudomonas spp. were isolated from contaminated wastewater using cetrimide agar medium and confirmed on the basis of morphological, cultural and biochemical characteristics. Pseudomonas spp. showing tolerance to the metals (0.5mM) were selected and Minimum inhibitory concentrations for copper (Cu) and zinc (Zn) were determined. 5 different Pseudomonas spp. showing the highest tolerance to the metals were exposed to Sublethal concentrations of the two mutagenic agents, acridine orange and ethidium bromide and the minimum inhibitory concentration values for copper and zinc metal ions were again determined for the mutant strains. Two Pseudomonas spp. showed the prominent increase in minimum inhibitory concentrations value. Thus, the results concluded the effectiveness of the artificially mutated bacteria as an appropriate solution for the treatment of contaminated wastewater containing heavy metals.

Keywords: Bioremediation, Mutation, Pollution, Waste water.

INTRODUCTION

Water pollution due to heavy metals is a global problem. Indiscriminate disposal of wastes from industries, housing areas, agricultural fills is one of the major causes of gross pollution of many rivers in India and in many other countries. (Moore et al., 1998). There are microscopic organisms in the environment like bacteria that have ability to grow and absorb effluents containing heavy metals (Lovely and Coates, 1997). The heavy metal resistant bacteria are capable of bioaccumulation of high concentrations of metals like Ag, Cu, Pb or Cd can play an important role in clean up or bioremediation of the effluents from heavy metal industries (*Shakibaieet al., 1999; Wood and Wang, 1985*). Humans are exposed to heavy metals since long back, but rapid industrial development and urbanization have augmented the issue.

Therefore, bioremediation of heavy metals is very important for environmental health. Resistance to heavy metals is observed in a wide variety of bacteria, especially in Gram negative bacteria, such as Pseudomonas, Alcaligenes, Ralstonia and Burkholderia (Lovely, D. R., 1993; Diels et al., 1999). Bacteria of the genus Pseudomonas are well-studied and are of great interest not only because of their high resistance to heavy metals and other toxic substances but also due to their simple nutritional requirements and rapid growth on standard laboratory media. Hifelieet al., (1984) used Pseudomonas stutzeriisolated from silver mines for the removal of heavy metals from industrial wastes.

The mutagenic agents like acridine orange and ethidium bromide have ability of intercalating with DNA and thus cause frameshift mutation. Induced mutations in resistant strains have been reported to increase the tolerance limit and thus enhance the potential of such strains in the management of metal contaminated sites. Future research on induced mutations would also be important in the functional genomics of stable bacterial strains for bioremediation studies. Thus, the objective of this research was to study the impact of the mutation on metal resistance in Pseudomonas spp. for bioremediation of metal contaminated sites.

MATERIALS AND METHODS

A. Sample collection

Water sample was collected from the polluted Waldhuni river near Ulhasnagar, District Thane, Maharashtra, India.

B. Determination of concentrations of Cu and Zn in the polluted water

The pH of the collected sample was determined by using pH paper. For measurement of concentrations of Cu and Zn, 300 mL of polluted water was filtered using blotting papers. The concentration of the heavy metals in the effluents was then determined by atomic absorption spectrophotometer attached to a graphite analyzer.

C. Isolation of Pseudomonas SPP from wastewater

For isolation of Pseudomonas spp, the collected water sample was streak inoculated on the selective media, cetrimide agar and incubated at 35°C for 24 h. The plates were observed for bluish green colonies and isolated colonies were further confirmed by the biochemical tests (*shakibaieet al., 1999*).

D. Determination of MIC

All the selected colonies were then studied for their tolerance towards heavy metals. The minimum inhibitory concentration (MIC) of heavy metals for isolated strains was determined by broth dilution technique (*Xin Cai et al., 2006*). For MIC experiment all the selected organisms were grown for 8 h in 20 mL sterile Muller Hinton broth separately and 0.1 mL log phase (*108cells/mL*) cultures were inoculated into serially diluted Muller-Hinton broth containing 0.5, 1.0, 5.0, 10, and 20 mM concentrations of copper and zinc. The sensitivity of the isolates was determined by observing growth in the form of turbidity after 24-48 h at 35°C.

E. Induction of mutation

The ability of the organisms to grow in higher concentrations of copper and zinc was augmented by exposing the isolates to different mutagenic agents like acridine orange and ethidium bromide. For induction of the mutation, the Gradient plate technique (GPM) was used. For GPM, 40 mg of mutagenic agents were dissolved in 100 mL D/W and mixed properly. To 10 mL of melted nutrient agar medium, 1 mL mutagenic agent solution was added and immediately plated into Petri plates and kept in a slanting position. After solidification of the medium, 10 mL Nutrient agar was poured onto the medium and allowed to solidify, by this method a gradient concentration of mutagenic agent was created. 0.1 mL of the bacterial culture was then spread into the medium and incubated for 24 h at 35°C. The colonies showing growth at the highest gradient concentration of the medium were selected and further studied for their tolerance to heavy metals by broth dilution technique.

RESULTS

The pH of the collected wastewater was found to be 5.2 indicating the acidic nature of the water. The concentration of Cu and Zn in the collected water was 50.525 ppm and 20.130 ppm respectively. The cetrimide agar is a selective medium for Pseudomonas which shows bluish green growth. 14 morphologically distinct colonies were observed on cetrimide agar and were found to be of Gram negative motile, aerobic non spore forming short rods. Further, all the isolates were spot inoculated on Muller-Hinton agar containing 0.5mM Cu and Zn separately. Only 5 Pseudomonas spp showed growth in presence of 0.5mM Cu and Zn hence were selected for further study. Pseudomonas spp were confirmed on the basis of their oxidase activity and ability to ferment glucose and mannitol by aerobic fermentation. The sensitivity of 5 different Pseudomonas strains isolated from polluted water was determined by the broth dilution technique (Table 1). Strains 3 and 4 showed the highest MIC value for Cu and Zn and showed growth up to 1.0 mM and 2.0 mM concentration respectively while the remaining strains showed growth till 0.5 mM concentration of Cu and 1:0 mM of Zn. After subjecting to the mutating agent acridine orange, all the strains showed an increase in their tolerance towards both the heavy metals (Table 2). Strains 3 and 4 showed a prominent increase in MIC value and both the strains showed growth up to 5mM for Cu and 15mM for Zn with $P<0.5$, while ethidium bromide did exert an increase in the MIC values but comparatively less than acridine orange (Table 3).

Table 1: The MIC values of Cu and Zn for the isolated Pseudomonas strain

Isolate No.	Growth in presence of Cu concentrations (mM)					Growth in presence of Zn concentrations (mM)				
	0.5	1:0	1:5	2:0	2:5	0.5	1:0	1:5	2:0	2:5
Isolate 1	+	_	_	_	_	+	+	_	_	_
Isolate 2	+	_	_	_	_	+	+	_	_	_
Isolate 3	+	+	_	_	_	+	+	+	+	_
Isolate 4	+	+	_	_	_	+	+	+	+	_
Isolate 5	+	_	_	_	_	+	+	_	_	_

Table 2: The MIC values of Cu and Zn for the isolated Pseudomonas strain after exposure to acridine orange

Isolate No.	Growth in presence of Cu concentrations (mM)					Growth in presence of Zn concentrations (mM)				
	2.5	5	10	15	20	2.5	5	10	15	20
Isolate 1	+	_	_	_	_	+	+	_	_	_
Isolate 2	+	_	_	_	_	+	+	_	_	_
Isolate 3	+	+	_	_	_	+	+	+	+	_
Isolate 4	+	+	_	_	_	+	+	+	+	_
Isolate 5	+	_	_	_	_	+	+	_	_	_

+ = growth, - = no growth

Table 3: The MIC values of Cu and Zn for the isolated Pseudomonas strain after exposure to ethidium bromide

Isolate No.	Growth in presence of Cu concentrations (mM)					Growth in presence of Zn concentrations (mM)				
	2.5	5	10	15	20	2.5	5	10	15	20
Isolate 1	+	_	_	_	_	+	_	_	_	_
Isolate 2	+	_	_	_	_	+	_	_	_	_

Isolate 3	+	–	–	–	–	+	+	–	–	–
Isolate 4	+	–	–	–	–	+	+	–	–	–
Isolate 5	+	–	–	–	–	+	–	–	–	–

+ = growth, - = no growth

DISCUSSION

In the present study, the impact of the mutational enhancement technique with respect to tolerance to heavy metals was studied in Pseudomonas spp. The polluted water sample was collected and 14 Pseudomonas strains were isolated. Pseudomonas strains were the predominant bacteria which could tolerate high concentrations of Cu and Zn. The isolated strains were subjected to different concentration of Cu and Zn, some could grow up to 2.5 mM Cu and 5 mM of Zn. Similar results have been reported by Shakibaieet al., (2008).

To enhance the accumulation of heavy metal ions, isolated bacterial cells were exposed to two mutagenic agents, acridine orange and ethidium bromide. These agents are intercalating dyes and are capable to bind to the DNA of bacteria and induce frame shift mutation, therefore they are strong mutagenic agents. The mutagenic agent, acridine orange had a profound effect on the ability of isolates to grow at very high concentrations of Cu (10 mM) and Zn (20 mM) compared to ethidium bromide.

A genetically engineered Acinetobacter baummannii has been reported to remove 2.5 mg/g biomass of Ag from effluents of the film industry (*Shakibaieet al., 1999*). Xin Cai et al., (2006) studied the tolerance and biosorption of copper and zinc by Pseudomonas. putida CZ1 isolated from metal-polluted soil and reported removal of about 8.72% of Cu and 9.8% of Zn during the active growth cycle.

CONCLUSION

The data presented in this paper indicated the use of mutation for the enhancement of metal resistance in bacteria. Such mutant bacterial with increased metal tolerance would considerably enhance the bioremediation of heavy metals from effluents of the factories and improve the disposal problems of the waste with little expense.

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Isolation of Microbes from Marine Water and Screening for Potent Microbefor Dye Degradation by Immobilization

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ABSTRACT

Colors have been dazzling humanity since ages and ended up being perfectly key unit of individuals advancing groundbreaking circumstance. Due to speedy extension in people's plan resolved assortment demands, there is massive development in material ventures which has shown a tremendous use of produced complex regular tones as the Coloring material. A part of these tones, their precursors, or their biotransformation things like sweet-smelling amines, have been exhibited to be mutagenic and malignant growth causing and consequently this implies why their removal is a hot conversation as of quite recently. Wide creative work has focused in on regular procedures as an eco-obliging choice for remediation of shadings. Most assessments on azo shading biodegradation have focused in on infinitesimal life forms and developments, in which microorganisms were by and large used for azo tones decolorization in view of their high development, wide transport and strong adaptability. This study was finished with a place of disengagement of microorganisms from marine water since marine water environment is an inconceivably mentioning situation for perseverance and accepting minute life forms from this environment creates life with thrashing survival skills,and couple of physically productive properties as well, as there is some incredible in each life coming from this environment, this study focused on division and depiction of organisms prepared for defiling azo shadings and degradation of azo tones by the compelling microbial cell mass in a collaboration called as immobilization.

Key Words: Azo Dyes, Microbes, Dye degradation, Immobilization, Marine Water.

INTRODUCTION

The material business is a giant water client and conveys huge volumes of ruined water. The material business everything considered experiences issues in party squander water release limits, especially as to isolated solids, ionic salt, pH, COD, hiding and critical metal. Treatment of concealing degraded squander water set liberated from the material and other concealing stuff associations is basic to forestall pollution of soil, surface and ground water. Planned colors and colorants are dynamic consistently utilized these days by paper, material, food, greatness care things, and prescription associations. Among these, material undertakings are the best client of tones and shades, tending to 80 % of full-scale creation (Jyoti Kumar Thakur et al., 2014). They are comprehensively used in the material, paper, food, calfskin, magnificence care items and medication organizations (*Telke et al., 2008*). Less than ideal arrival of material tone spouting containing azo tones and their metabolites in watery organic frameworks is beautifully unpleasant and prompts an abatement in sunshine entrance, which in this manner reduces photosynthetic activity, separated oxygen concentration, and water quality, and hurtfully affected maritime verdure, making outrageous normal issues all over the planet (*Vandevivere et al., 1998*). Additionally, azo shadings in like manner have an ominous impact similar to hard and fast total organic carbon (TOC), biological oxygen demand (BOD) and chemical oxygen demand (COD) (*Saratale et al., 2009b*). This insight has incited the suggestion that anaerobic/oxygen consuming structures might be convincing in achieving the all-out biodegradation of azo shadings. Furthermore, bacterial decolourization is regularly faster appeared differently in relation to fungi with respect to the decolourization and mineralization of azo colors (Banat et al., 1996). Since different bacterial species including Bacillus, Pseudomonas, Enterobacter, Halobacterium, and Aeromonas have been to decolorized and detoxify a wide extent of azo tones took a gander at of phenylamine, benzenediazonium chloride or phenol.(Telkeet al., 2008; Mendes t al.,2011; Feng et al.,2012).

Presentedwork here, based on screening and disengagement of shading spoiling microorganisms from marine environment since creatures of this environment have novel bioactive compound that they produce as a result of strain threw via ocean environment.

MATERIALS & METHOD

Sample Collection:

The water sample was taken at Rangaon Beach in Vasai. Samples were collected in sterilised BOD bottles, transported to the lab in an ice-cube-filled box, and processed within hours.

Enrichment and Isolation of organism:

The marine microorganisms in the acquired water sample were increased in Sterile Zobell Marine (ZM) Broth. The cells were then dispersed plate grown on Sterile ZM Agar Plates with an inoculum size of 0.1mL. In addition, pure cultures of the various colonies collected were maintained on the same medium.

Morphological characterization:

Characterization of colonies Gram staining, sugar fermentation, and biochemical analysis according to Bargey's protocol were used to identify probable bacterium isolates. The colony's cultural properties, such as margin, size, form, colony type, and nature (mucous, rough, smooth, translucent, etc.) were noticed.

Screening of dye degrading bacteria:

In 100ml dw, 0.5gm Congo red was made. The dye was inoculated with the bacterial strains that had been obtained. For the degrading activity, it was incubated for two days. O.D. was measured after two days.

Preparation intracellular materials:

The bacterial isolate that showed highest dye degrading activity following primary screening was centrifuged for 3 hours at 3000rpm, with the supernatant containing intracellular components recovered and the pellet discarded.

Immobilization of Cellular components:

To carry out the process of immobilization of the cells, the 4% sodium alginate solution and 6% calcium chloride solution was prepared and then autoclaved to make them sterile. Then 5ml of cell supernatant was added to the 25 ml 4% Na Agsolution aseptically and stirred well. For the beads, drew this solution in 10ml of syringe and added it drop wise manner to 100ml of 6% $CaCl_2$ solution. The beads were kept in $CaCl_2$ solution for 24 hrs. The alginate will be cross linked by calcium ions. After 24 hrs. of incubation the beads were taken out of the $CaCl_2$ solution and washed with distilled water several time. For the activation of beads activation media used was glucose at 15% concentration. After washing of beads was done, the beads were transferred to the activation media for 24 hrs. The cells were immobilized using a 4 percent sodium alginate solution and a 6 percent calcium chloride solution that had been prepared and autoclaved to make them sterile. The cell supernatant was then added aseptically to the 25 ml 4 percent NaAg solution and well mixed. For the beads, draw this solution into a 10ml syringe and drop it into a 100ml 6 percent CaCl2 solution drop by drop. For 24 hours, the beads were immersed in CaCl2 solution. Calcium ions will crosslink the alginate molecules. After 24 hours of incubation, the beads were removed from the CaCl2 solution and washed numerous times with distilled water. Glucose at a concentration of 15% was employed as the activation medium for the beads, after a thorough cleaning beads were transferred to this activation media.

Treatment of Synthetic Dyes:

The colours were made by combining 1 gramme, 0.5 gramme, and 0.1 gramme of each dye (Congo red, Crystal violet) in 100 milliliters of distilled water. The beads were then incubated with the appropriate colors and concentrations (1 percent, 0.5 percent, 0.1 percent). It was subsequently incubated for 7 days, with the O.D. being monitored every day.

Decolorization Study:

Percent decolorization ability was used to represent the degree of decolorization capacity. At A_{660}, and A_{400} nm, the reduction in absorbance was measured. The activity of decolorization was computed using the formula,

$$\% \text{ Decolourization} = \frac{\text{Initial OD - Final OD x 100}}{\text{Initial OD}}$$

RESULTS & DICUSSION

Isolates:(Figure1& 2)

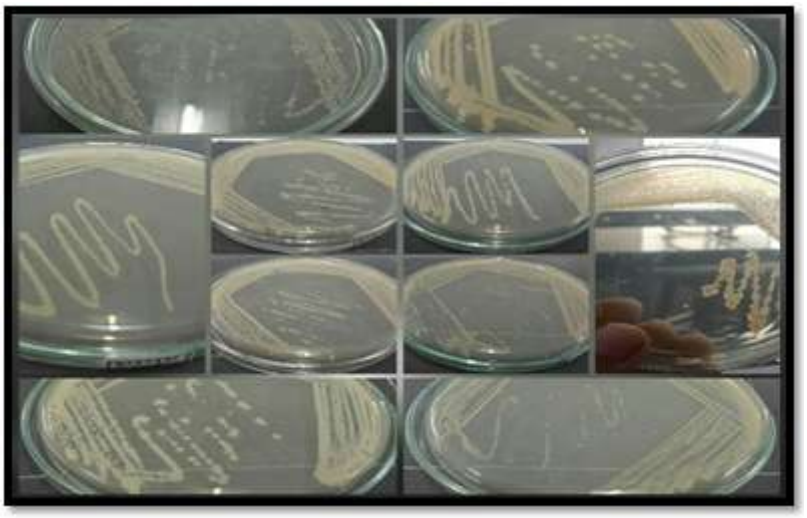


Figure 1 Marine Isolates

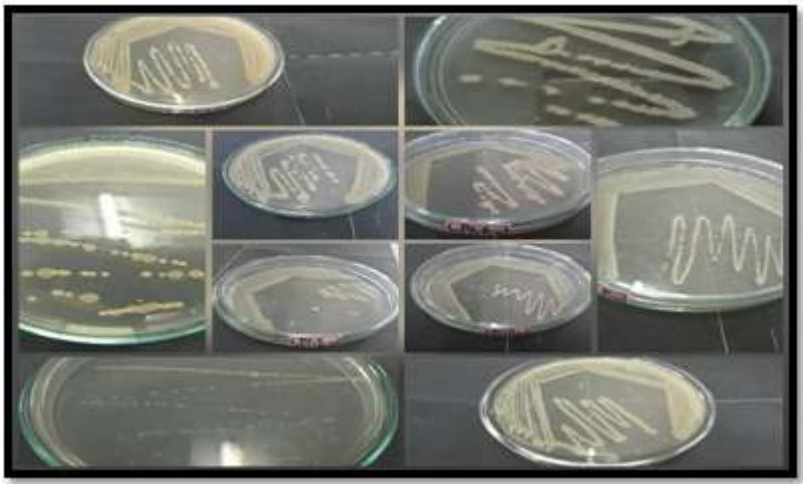


Figure 2 Marine Isolates

Colony Characteristics: (Table 1)

Table 1 Colony Characteristics

Sr. No.	Orgm.	Size (mm)	Shape	Margin	Elevation	Colour	Opacity	Gram Nature
1	PC1	5	Irregular	Entire	Concave	Cream	Opaque	+ve cocci
2	PC2	3	Circular	Irregular	Flat	Off White	Opaque	-ve rod
3	PC3	4	Circular	Undulated	Raised	Cream	Opaque	-ve cocci
4	PC4	3	Irregular	Entire	Raised	Cream	Opaque	-ve rod
5	PC5	3	Circular	Irregular	Concave	Cream	Opaque	-ve rod
6	PC6	2	Circular	Entire	Concave	Cream	Opaque	+ve rod
7	PC7	3	Irregular	Irregular	Raised	Cream	Opaque	-ve rod
8	PC8	2	Circular	Irregular	Concave	Cream	Opaque	-ve rod
9	PC9	2	Circular	Irregular	Concave	Cream	Opaque	-ve rod
10	PC10	2	Irregular	Irregular	Flat	OffWhite	Opaque	-vecb
11	PC11	3	Circular	Irregular	Concave	Cream	Opaque	-ve rod
12	PC12	4	Irregular	Irregular	Concave	Cream	Opaque	-ve rod
13	PC13	2	Circular	Entire	Concave	Cream	Opaque	+verod
14	PC14	4	Circular	Irregular	Concave	Cream	Opaque	-verod
15	PC15	4	Circular	Irregular	Raised	Cream	Opaque	-ve rod
16	PC16	3	Circular	Entire	Raised	Cream	Translucent	+ rod
17	PC17	4	Circular	Entire	Concave	Cream	Translucent	+verod
18	PC18	2	Circular	Irregular	Raised	Cream	Opaque	+verod
19	PC19	3	Circular	Irregular	Flat	Cream	Opaque	-vecocci

20	PC20	2	Circular	Entire	Raised	Cream	Opaque	-verod

Gram's Staining:(Figure 3)

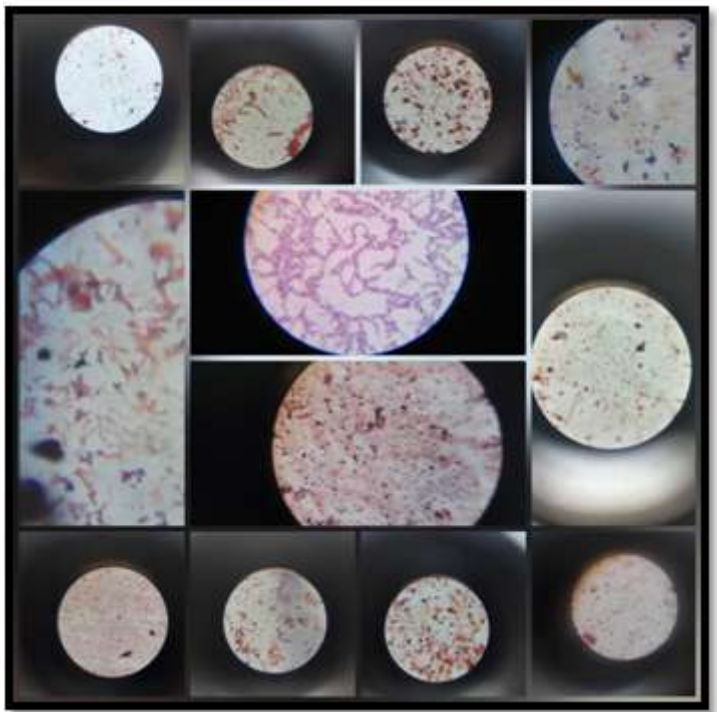


Figure 3 Gram's Staining of bacterial cells

Biochemical Tests: (Table 2)

Table 2 Biochemical Characterization of Isolates

Organism	Indole	Methyl Red	Voges-Proskauer	Citrate	TSI	Catalase	MSB
PC1	-	++	±	-	k/A/-/-	++	+
PC2	-	-	+	-	k/A/-/-	±	±
PC3	-	++	+	±	k/A/-/-	±	±
PC4	-	++	+	-	k/A/-/-	++	+
PC5	-	-	+	-	k/A/-/-	++	±
PC6	-	-	+	+	k/A/-/-	++	+
PC7	-	++	+	+	k/k/-/-	++	+

PC8	-	++	±	-	k/k/-/-	++	+
PC9	-	++	+	-	k/k/-/-	++	+
PC10	-	±	+	-	k/k/-/-	++	-
PC11	-	+	+	-	A/A/-/-	++	+
PC12	-	+	+	-	A/A/-/-	±	±
PC13	-	+	±	-	A/A/-/-	+	+
PC14	-	++	+	-	k/A/-/-	++	±
PC15	-	++	±	-	A/A/-/-	++	-
PC16	-	+	+	-	k/A/-/-	±	±
PC17	-	±	+	-	k/A/-/-	++	++
PC18	-	±	+	-	k/k/-/-	+	-
PC19	-	++	+	-	k/A/-/-	++	±
PC20	-	++	+	-	k/k/-/-	±	±

KEY:
±: Positive/Negative
- : Negative
+: Moderately Positive
++: Strongly Positive
k/k: Alkaline Slant/Butt
A/A: Acidic Slant/Butt
-/-: No gas/No H_2S

Citrate Utilization Test: (Figure 4)

Fig 4

Figure 5 Immobilization of PC18 Cell Suspension inNaAg-CaCl2 Beads

Primary Screening for Dye Degrading Bacteria:
13 of the 20 isolates showed some dye degrading activity, with PC18 exhibiting the greatest, i.e., 25% degradation activity, and was then integrated for immobilization and subjected to several dyes of varying concentrations.
Immobilization and Dye Degradation Activity by PC18 Isolate:

Figure 6 Control Beads From day 1 to Day 7 Remained Unchanged

Figure 7 Day1 of Dye Inoculation of Congo Red

Figure 8 Day 1 of Dye Incubation of Crystal Violet

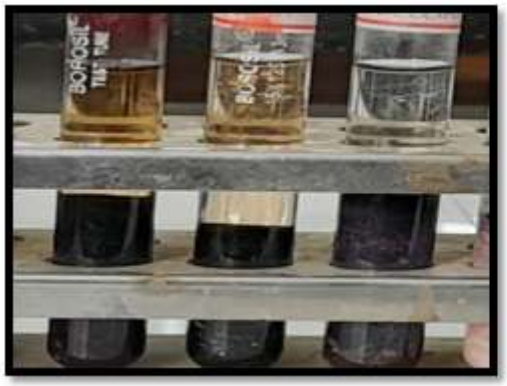


Figure 9 day 7 of Dye degradation by Congo Red

Figure 10 Day 7 of Dye Degradation by Crystal Violet

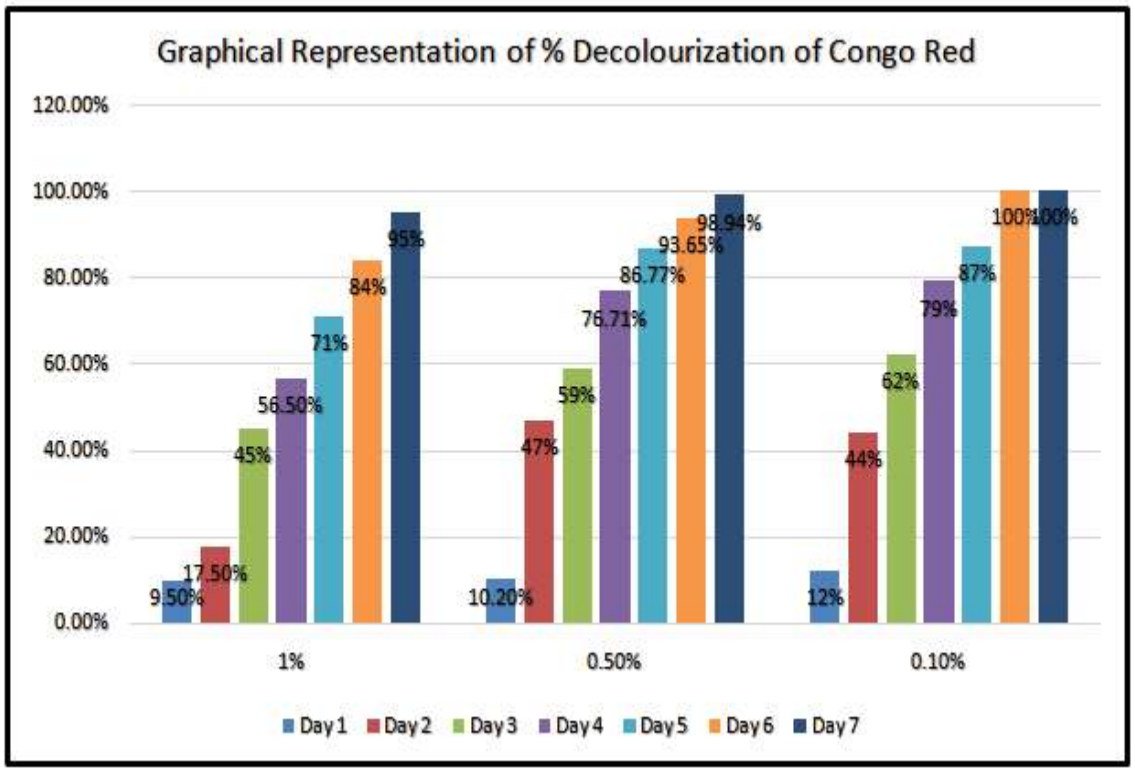
Table 3 Day wise O.D of PC 18 + Dyes containing tubes

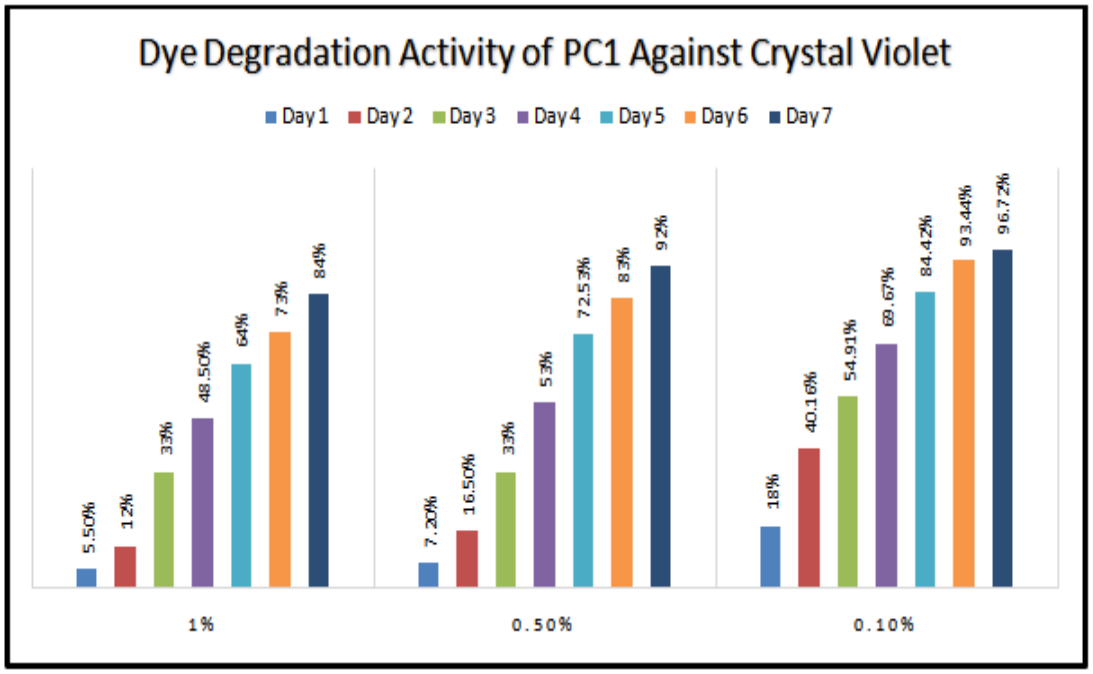
Dyes	Days						
	1	2	3	4	5	6	7
Congo Red (660nm)							
1%	1.81	1.65	1.10	0.87	0.58	0.32	0.09
0.5%	1.69	1.00	0.76	0.44	0.25	0.12	0.02
0.1%	0.88	0.56	0.38	0.21	0.13	0.00	0.00
Crystal							

Violet (400nm)							
1%	1.89	1.76	1.34	1.03	0.72	0.54	0.32
0.5%	1.79	1.61	1.28	0.89	0.53	0.31	0.15
0.1%	1.00	0.73	0.55	0.37	0.19	0.08	0.04

Table 4 Day wise % Decolorization

Dyes	**Days (% Decolourization)**						
	1	2	3	4	5	6	7
Congo Red 1%	9.5%	17.5%	45%	56.5%	71%	84%	95%
0.5%	10.2%	47%	59%	76.71%	86.77%	93.65%	98.94%
0.1%	12%	44%	62%	79%	87%	100%	100%
Crystal Violet 1%	5.5%	12%	33%	48.5%	64%	73%	84%%
0.5%	7.2%	16.5%	33%	53%	72.53%	83%	92%
0.1%	18%	40.16%	54.91%	69.67%	84.42%	93.44%	96.72%





DISCUSSION

Azo colors are the biggest gathering of colors. Various assortments of azo colors are widely utilized in the material, paper, food, beauty care products and drug ventures. They are the biggest and most adaptable class of color, yet have underlying properties that are not normally eliminated from water by ordinary waste water framework. Azo colors are intended to oppose compound and microbial assaults and to be steady in light and washing. The expulsion of azo colors from profluent is significant because of their mutagenicity and cancer-causing nature along with their extraordinary shading. Both physicochemical and natural strategies for the expulsion of colors have been researched broadly. The separation of good color decolorizing species requires screening, and these disengaged strains ought to have capacity to corrupt and detoxify material colors (Silveira et al., 2009). The current review was centered around decolourization of material azo colors and biodegradation of material color emanating by utilizing microbes separated from marine water test. Biodegradation of monetarily accessible material colors Congo red, Crystal Violet, was contemplated against PC18 which has been disengaged from the marine water by spread plate strategy and % decolourization was displayed in the figures and Tables going with the outcomes. Twenty unique microbes were secluded from the material color defiled water. In view of primer test, plating on particular media and biochemical test, the strong color debasing microbes with greatest decolorization movement can be accepted that is from Bacillus species for additional explanation of the species and variety of segregates explicitly PC18 Identification utilizing progressed sub-atomic devices like 16S rRNA Sequencing and, bioinformatics is under review.

Sriram et al., 2013 separated three unique bacterial, for example, Bacillus subtilis, E.coli, Pseudomonas fluorescens for the corruption study. Saranraj et al., 2010 segregated 5 unique bacterial from the material color profluent and distinguished as Bacillus subtilis, Proteus mirabilis, Pseudomonas fluorescens, and Staphylococcus aureus. In view of starter tests, plating on particular media and biochemical tests, they were distinguished as Bacillus subtilis, Pseudomonas fluorescens, and E. coli. The reduction in the decolorization productivity apparently was diminished with expansion in the grouping of the colors. Comparable perceptions have been recorded before for decolourization of Turquoise Blue color by Bhoomi Joshi et al., 2013.

Lately, the utilization of immobilized cell has been getting expanded consideration in the field of wastewater decolorization since this technique not just works on detachment and recuperation of immobilized microbes and the limiting specialist yet in addition makes the application reusable, which diminishes the general expense. All in all, immobilized cells are more lenient to neighborhood bothers like changes in temperature, pH and presence of inhibitor compounds. It has been expressed that sodium alginate is an appropriate network material since it is non-harmful and the strategy utilized for its gelation is gentle towards the microorganisms.The outer carbon source utilized for this study was glucose. The decision of glucose was conscious. During decrease of azo colors, it is by and large detailed that the presence of promptly accessible substrates that go about as electron benefactors for azo security decrease is crucial. a few investigations have announced the

utilization of glucose as an optimal wellspring of carbon and energy for Decolouration of azo colors. In this concentrate on the glucose fixation utilized was 15% of glucose and most extreme decolorization was acquired for Congo Red with 95% decoloration in 1%, 98.94% in 0.5%, and 100 percent decolorization was seen in 0.1% as contrasted and Crystal violet whose % decoloration for 1%, 0.5% and 0.1% are 84%, 92%, 96% individually.sss.

CONCLUSION

In ends, the material, coloring and completing industry utilize wide assortment of dyestuffs because of the quick changes in the client's requests. Accordingly, by the utilization of the above confine economical biodegradation of the hurtful azo colors used by the color, material, paper ink and so forth ventures can be conceivable. These techniques are eco-accommodating as well as industrially reasonable in any event, for the limited scale ventures. An intensive examination, thinking about of specific boundaries, for example, streamlining of the color focus for the disengages as well with respect to the color to be corrupted, impact of physicochemical boundaries on debasement and so on the loose scale is important to give unequivocal proof to the value of these confine in supporting color corruption ability. Further sub-atomic review on their enzymatic property and debasement cycle could uncover them as a significant material color degrader. Hence, by this current it is inferred that the bacterial disconnects like Bacillus sp., can utilized as a decent microbial hotspot for treatment.

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Assessment of Microbial Load and Drug Resistant Staphylococcus Aureus Contamination on the Train Handles of Western Railways in Mumbai

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ABSTRACT

We aimed to study the bacterial load on handles of local trains' of western railways in Mumbai city. We mainly focused on the recovery of skin opportunistic pathogen Staphylococcus aureus from 20 swab samples, to study their virulence factors and antibiotic sensitivity profile. Besides the high bacterial load on studied samples, 17 gram positive and catalase positive Staphylococcus sp. were obtained. Among these, 8 isolates were identified as S. aureus by cultural, morphological and biochemical studies. All these isolates showed virulent characteristics. The Antibiotic Sensitivity Testing (AST) showed7 out of 8 S. aureus strains to be Multi Drug Resistant (MDR). Interestingly, a common resistance pattern was observed among these isolates where all 7 MDR S. aureus strains were resistant to penicillin G, 3rd generation cephalosporin (*i.e., cefoxitin and/ or cefotaxime*), ciprofloxacin and erythromycin. Overall, our study indicated poor hygienic conditions in local train compartments of western railways in Mumbai.

Keywords: AST, Fomites, S. aureus, Train handle, virulence.

INTRODUCTION

Micro-organisms are present everywhere. Although their presence does not generally concern human activities, sometimes the contamination of common objects by pathogens may turn into potential health risks. These objects are defined as fomites and typically transfer infectious agents by indirect contact from hand to common surfaces like door knobs, switches and handles, and thus from one person to another in a community [1]. The concealed and oblivious nature of pathogens combined with turning of fomites into reservoirs, thus leads to exponential spread of infections. To make matters worse, the infectious agent may have acquired antimicrobial resistance that may further aid in the spread of these resistance genes in the community.

Several studies have indicated smooth surfaces to be better reservoirs of infectious agents as compared to rough surfaces that absorb or trap the infectious agents, thereby limiting its spread through simple touch. The epidemiological studies have indicated high risk exposure to infectious agents in child-care, domestic environment, public transport and sports facilities, through fomites. However, they are particularly associated with hospital acquired infections through equipment like stethoscopes, IV drip tubes, catheters and life support systems [2].

The contamination of fomites is affected by several factors like moisture, frequency of use, unhygienic practices and frequency of exposure. The survival of pathogens on fomites is also affected by their specific virulent characteristic, bio film formation as well as its initial load (bio burden) during contamination. The presence of specific substrate components, acting as artificial niche, also favours microbial contact and growth [3]. The occurrence of infections in humans on contact with fomites under above mentioned ideal conditions ultimately depends on the immuno-competence, personal hygiene and overall health of the person in contact. Previous studies have reported the survival of viruses like HBV, HIV, CMV and HSV from few hours up to a week on fomites. Other viruses like astrovirus, polio and rotavirus can survive for over 2 months. Many infection- causing gram negative as well as gram positive bacteria may also survive for months and spore-forming bacteria may remain dormant for years, on fomites [4].

Some documented reports suggest ready transmission of gram positive bacteria, specifically S. aureus, through fomites as compared to viruses and gram negative bacteria. This is most probably due to its ubiquitous nature and occurrence as a part of normal flora of skin. Besides, they are also known to persist longer at low humidity levels and develop resistance to penicillin and other antibiotics. Although S. aureus is not always pathogenic, it is a common cause of skin infections (i.e., cellulitis, folliculitis, carbuncles, scalded skin syndrome, impetigo, boils), wound infections, abscesses and food poisoning. Less often they are also associated with respiratory infections (i.e., sinusitis, pneumonia), meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia, and sepsis [5]. It is commonly spread through infected towels, sheets, clothing, athletic and medical equipments. The above being said, the drug resistantStaphylococcus aureus infections, can spread easily in the healthcare environment, and become increasingly difficult to treat. More recently, they are also emerging as a community-associated infectious agent by means of transportation sources [5].

With the increase in sophisticated transportation system, we have witnessed global pandemics of communicable diseases caused by familiar or completely new strain of microbes. This is a reflection of the faster movement of pathogenic microbes and the scenario may become more severe if the pathogens are drug resistant. With this understanding, the much contemplated convenience of Mumbai suburban railways helping over 7.5 million passengers [6] travel throughout the city per day may not be so convenient after all. Instead, the unhygienic and humid conditions inside trains provide the perfect environment for incubation of pathogenic microbes. Propelled by popularity of local train services as well as poor hygiene conditions, in Mumbai city, the current study was carried out with an objective to assess the bacterial load on local train handles, identify pathogenic S. aureus strains and characterize them on the basis of virulent factors, bio film forming ability and Antimicrobial Susceptible Testing (AST).

MATERIALS AND METHODS

Media and chemicals

All the nutrient media and chemicals used in the current study were procured from Himedia, and discs for AST were purchased Pathoteq Biological Laboratories.

Sample collection

Local train handles of the western railways in Mumbai city were selected as sampling sites. Approximately $10cm^2$ area of a train handle was swabbed using a sterile cotton swab moistened with phosphate buffered saline (PBS). These samples were transported to the laboratory in sterile tubes and processed immediately for determination of bacterial load.

Determination of bacterial load

The samples collected above were grown on common nutrient media (Nutrient Agar, NA), and a selective and differential media (Salt Mannitol Agar, SMA) to determine the bacterial load and identify pathogenic S. aureus strains respectively. Initially, the samples were serially diluted using PBS up to 10^{-6} dilutions and 0.1ml volume of the last three dilutions (i.e., 10^{-4}, 10^{-5} and 10^{-6}) was spread on sterile NA using a glass spreader. Same volume of undiluted sample was spread on sterile SMA. These plates were incubated at 37°C for 48h and results were reported as cfu/cm^2. All the obtained isolates were maintained on NA slants and refrigerated until further use.

Identification of the isolates

Well isolated colonies from the selective and differential medium (i.e., SMA) were selected and their colony characteristics were studied. The isolates were identified on the basis of cultural, morphological and biochemical characteristics [7].

Detection of bio film formation

All the pathogenic isolates detected on SMA were screened for bio film production by tube method. It is a quantitative method for bio film detection. To carry out this process, the cultures were inoculated in 4ml Brain and Heart Infusion (BHI) broth containing 1% glucose and incubated at 37°C for 24h. After incubation the tubes were decanted, washed with PBS (pH7.2) and dried. The tubes were then stained with 4mL of 0.1% crystal violet for 20min. After staining, the tubes were decanted, washed with saline and 4 mL of ethanol was added to each tube. The intensity of blue colour in tubes is directly proportional to the amount of biofilm formed on the sides of the tubes. Hence it was determined spectrophotometrically at 570nm [8].

Determination of antimicrobial resistance profile of the isolates

The obtained isolates were subjected to AST by using Kirby Bauer method following CLSI guidelines [9]. To carry out AST, the cultures were spread on sterile Mueller Hinton agar plates and four antibiotic disks were placed at suitable distance. After overnight incubation, the plates were checked for zone of inhibition and the resistance pattern was recorded. The antibiotics used in our study were Ciprofloxacin (Ci, 5 µg), Chloramphenicol (Ch, 30 µg), Cefotaxime (Ce, 30 µg), Ampicillin/Sulbactam (AS, 20 µg), Tetracycline (T, 30 µg), Ceftizoxime (Cz, 30 µg), Amikacin (Am, 30 µg), Gentamicin (Ge, 10 µg), Ofloxacin(O, 5µg), Gatifloxacin (Ga, 10 µg), Cotrimoxazole (Co, 25 µg), Cefoxitin (Cf, 30 µg), Penicillin G (P, 10 units), Nitrofurantoin (N, 300 µg) and Erythromycin (E, 15 µg).

RESULT AND DISCUSSION

Determination of bacterial load

Figure 1 represents the bacterial load on train handle samples collected in our study. From the observations on NA plates and as per our expectations, it was clearly noticed that the train handles were heavily contaminated and harboured upto 8.2×10^5 cfu/ cm^2 bacteria.This can be attributed to the popularity and over-crowdedness of trains, frequent skin contact with train handles, absence of routine cleaning practices and lack of consciousness among passengers. Apparently, the poor hygienic conditions of trains, thus, can be concluded without further assessments.

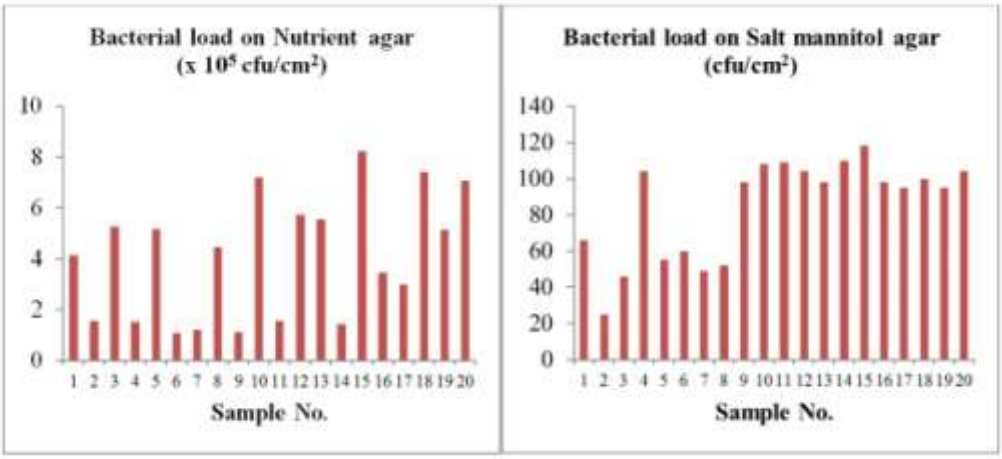

Figure 1: Bacterial load on train handle samples

Previously, considerable number of studies has been published describing hand-touch surfaces in buses, trains, mobile phones, door knobs, and computer keyboards as potential sources of spread of infectious agents [10, 11]. These studies reported presence of Escherichia coli, Vibrio cholerae, multi drug resistant Staphylococcus aureus and even Mycobacterium tuberculosison these surfaces. However, to the best of our knowledge, this is the first systematic study to report the occurrence of Staphylococcus strains on local train handles of western railways in Mumbai city.Although, the transportation system is indispensable to fulfil the needs of every individual, recently, it has drawn great attention from public health researchers as a means of spread of infectious agents at a community level. The advantages of efficient transportation including speed and connectivity, ironically puts people at risk for highly contagious and infectious diseases. More frequently than realised, it can lead to epidemics of communicable diseases, and as observed recently with the spread of Covid-19, it can lead to a serious global pandemic as well.

A similar study was carried out in Guangzhou metro system, China that reported extremely high bacterial load in all 320 samples collected in their study. In their study, 75.6% strains were identified as Staphylococcussp. [12]. The surfaces of public transport vehicles in Kathmandu showed bacterial load up to $2.5x10^5$ cfu/cm^2 [13] whereasthe handles of trams, metro and buses in Turkey showeda bacterial load of upto $6.2x10^6$ cfu/ ml [14].Among other sources, a recent study reported heavy contamination of S. aureus strains (from 50% up to 62.5%) on weight ball, cable driven curl bar, weight plates and treadmill handles, in gym [15].

Identification and characterization of the isolate

Table 1 represents the morphological and biochemical characteristics of test cultures isolated in our study. Given the increased skin contact with fomites in public transport, we mainly focused on the identification of S.aureus, since it is one of the main causes of skin infections. Out of the 20 samples collected in our study, 17 distinct isolates were obtained and confirmed to be Staphylococcus species. The catalase test was used to differentiate between morphologically similar, but catalase negative, Enterococcus and Streptococcus from catalase positive Staphylococcus species [16]. The ability to ferment mannitol was further tested to identify cultures that may most probably be S.aureus

strains.On the basis of the cultural characteristics on SMA, together with the observed biochemical tests, 8 isolates were confirmed to be S.aureus strains.All these isolates showed positive coagulase, haemolysis and lecithinase test confirming their pathogenic nature.

The contamination of fomites with pathogenic strains of S.aureus raises considerable public health concern. This is mainly because it is a normal flora of skin and thus possesses naturally acquired ability to colonize the skin. The pathogenic nature of S.aureus can hence aid in increasing the spread and severity of infections.In the last 10 years, the drug resistant and clinically significant MRSA is increasingly being reported to occur in community settings from its initial hospital settings [2]. An earlier study carried out in Japan, reported isolation of 2.3% MRSA strains from public transport facilities in Tokyo and Niigata cities [17]. In another study, 25.9% isolates were found to be S. aureus and another 31.4% were identified as MRSA from samples isolated from public transport facilities in Kathmandu, Nepal [13].

Table 1: Biochemical characteristics of Staphylococcus sp.

Sample	Catalase	Mannitol Fermentation		Glucose Fermentation		Nitrate Reduction	Salt Tolerance	Urease	Coagulase	Haemolysis on blood agar	Lecithinase	Gram Nature and morphology
		Anaerobic	Aerobic	Anaerobic	Aerobic							
1	+	-	-	-	-	+	+	+	-	-	-	Gram positive cocci in clusters
2	+	+	+	+	+	+	+	+	-	+	+	
3	+	+	+	+	+	+	+	+	-	+	+	
4	+	+	+	+	+	+	+	+	+	+	+	
5	+	+	+	+	+	+	+	+	+	+	+	
6	+	+	+	+	+	+	+	+	+	+	+	
7	+	-	-	-	-	+	+	-	-	+	-	
8	+	-	-	-	-	-	+	+	-	-	-	
9	+	+	+	+	+	+	+	+	+	+	+	
10	+	-	-	+	+	+	+	+	-	+	-	
11	+	-	-	+	+	-	+	-	-	+	-	
12	+	-	-	+	+	+	+	+	-	+	-	Gram positive cocci in tetrads
13	+	+	+	+	+	+	+	+	+	+	+	Gram positive cocci in clusters
14	+	-	-	+	+	-	+	-	-	+	-	
15	+	-	-	+	+	-	+	+	-	-	-	
16	+	-	-	+	+	-	+	-	-	+	-	
17	+	+	+	+	+	+	+	+	+	+	+	

Detection of bio film formation and determination of antimicrobial resistance profile of test isolates

Apart from the virulent factors like production of coagulase and lecithinase, and blood haemolysis, all pathogenic S. aureus strains tested positive for bio film formation by Tube method. These isolates showed a visible film lining the walls and the bottom of the test tube. Bio films are microbial communities characterized by close assembly of cells that are irreversibly attached to a substratum or to each other. The study of bio film forming ability of a pathogenic strain is extremely important because the environment in bio films offer several selective and proliferative advantages to cells allowing them to evolve notoriously. Few of these advantages include restricted penetration of antibiotics through bio films, availability of nutrients and decreased growth rate [18]. Although micro-titre plate is shown to be much more sensitive in detection of bio films, they require sophisticated instruments to read the plates and document the results [19]. The tube method, on the other hand, is a simple and sensitive technique that can be carried out without the help of expert technicians, making it a reliable method for detection of biofilm formation.

Table 2 represents the antibiotic resistance profile of pathogenic bio film forming S. aureus strains isolated in our study. The antimicrobial resistance profile was checked against 15 common antibiotics used for treatment of infections caused by S. aureus. Only one isolate showed sensitivity towards all these antibiotics. The remaining 7 isolates were found to be resistant to 3 or more antibiotics used in our study, thus they were characterised as MDR strains. Careful observation of AST results indicated an interesting finding of common resistance pattern among these isolates. All 7 MDR S. aureus strains were resistant to penicillin G, 3rd generation cephalosporin (i.e., cefoxitin and/ or cefotaxime), ciprofloxacin and erythromycin. Moreover, six isolates showed resistance to ampicillin sulbactam. Resistance to

ciprofloxacin was observed in 3 isolates. All isolates showed sensitivity towards chloramphenicol and nitrofurantoin antibiotics that target the protein synthesis and ribosomal proteins respectively. Relatively higher sensitivity towards other antibiotics like gentamycin (5/8), gatifloxacin (5/8), amikacin (4/5) and cotrimoxazole (6/8) was also observed.A similar study reported isolation of 242 Staphylococcus sp. from metro system in Guangzhou, China. Among these isolates, 79.75% MDR strains were identified. These strains showed resistance towards penicillin (94.21%), erythromycin (88.84%), rifampicin (64.46%), trimethoprim (45.04%), clindamycin (40.91%), gentamicin (31.40%), moxifloxicin (13.64%), tobramycin (12.40%), cefoxitin (10.74%), linezolid (2.89%) and teicoplanin (2.48%) [12]. In another study, 35 MDR strains were identified out of the 40 S. aureus cultures isolated from public buses in Kerala, India. Eighteen of the MDR strains were resistant to oxacillin and cefoxitin and had MIC value of ≥ 4µg/ml. Among the MRSA strains resistance was observed towards clindamycin and linezolid (22.7%), amikacin (44%) and netilmycin (61.1%) [20].

Table 2: Antibiotic resistance profile of S. aureus strains isolated from train handles

Isolate No.	Sensitive	Intermediate	Resistant
2	Ch, AS, T, Cz, Am, Ge, O, Ga, Co, N		Ce, Ci, Cf, P, E
3	Ci, Ch, T, Cz, Am, Ge, O, Ga, Co	N	Ce, Cf, AS, P, E
4	Ci, Ch, T, Am, Ge, O, Ga, Co, N		Ce, Cf, AS, Cz, P, E
5	Ch, Ge, Co, N	Ga	Ce, Ci, Cf, AS, T, Cz, Am, O, P, E
6	Ch, N		Ce, Ci, Cf, AS, T, Cz, Am, Ge, O, Ga, Co, P, E
9	Ch, N	Ci	Ce, Cf, AS, T, Cz, Am, Ge, O, Ga, Co, P, E
13	Ce, Ci, Ch, Cf, AS, T, Cz, Am, Ge, O, Ga, Co, P, E, N		
17	Ci, Ch, O, Ga, Co, N	T	Ce, Cf, AS, Cz, Am, Ge, P, E

Key: Ciprofloxacin (Ci, 5mcg), Chloramphenicol (Ch, 30mcg), Cefotaxime (Ce, 30mcg), Ampicillin/Sulbactam (AS, 20mcg), Tetracycline (T, 30mcg), Ceftizoxime (Cz, 30mcg), Amikacin (Am, 30mcg), Gentamycin (Ge, 10mcg), Ofloxacin(O, 5mcg), Gatifloxacin (Ga, 10mcg), Cotrimoxazole (Co, 25mcg), Cefoxitin (Cf, 30mcg), Penicillin G (P, 10 units), Nitrofurantoin (N, 300mcg) and Erythromycin (E, 15mcg).

CONCLUSION

The number of MDR pathogenic microbes is increasing globally. However, till now it was believed that the infections caused by these microbes are hospital acquired, and maintenance of hygienic practices at personal and home level may suitably prevent serious infections. In the current study, the observed potential of all pathogenic S. aureus strains to form bio films is epidemiologically concerning. The high moisture levels, continuous contact, sweating and unhygienic practices may collectively contribute to the formation of rich microbial bio films on train handles, similar to those observed in bathrooms and kitchens. Our study reports important findings relevant to the ineffective hygienic conditions prevalent in the local trains of western railways of Mumbai, clearly suggesting otherwise. The common day to day objects may be potential sources of infectious agents, and hence we may unknowingly we may get exposed to pathogens causing serious infections. In order to prevent possible health hazards an interdisciplinary research approach integrating public health, microbiology as well as architectural understanding, needs to be undertaken to prevent colonization of pathogens on common surfaces.

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Eco-Friendly and Effective Ways of Removal of Heavy Metals using Immobilized Cells

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ABSTRACT

Heavy metals in effluents are toxic as well as carcinogenic to living organisms. Chemical and physical methods reviewed for the removal of heavy metal shave several disadvantages. Immobilization of whole cells has gained importance in the area of waste water treatment, as they are simple, economical and effective.
For this, waste water samples from two different places were collected in Thane district and checked for various chemical parameters. In this study, efficacy of bio sorption in removal of heavy metals (Chromium, Nickel and Cadmium) was tested using heavy metal resistant microorganisms immobilized on sand. The bio sorption efficiency was determined by chemical estimation methods.
Immobilization by sand was very efficient in removal of the heavy metals (80-90%) compared to free cells and sand alone. It also improved various parameters of waste water. Thus, it proved that cells immobilised on sand can be an effective and eco-friendly method for removing heavy metals.

Keywords: heavy metals, immobilisation, sand, waste water.

INTRODUCTION

Heavy metal ions, such as cadmium, lead and mercury, are highly toxic to living organisms.[1] Most of the heavy metals discharged into wastewater are found to be toxic and carcinogenic and causes a serious threat to the human health. The release of large quantities of hazardous materials into the natural environment has resulted in a number of environmental problems and due to their non-biodegradability and persistence, can accumulate in the environment elements such as food chain, and thus, may pose a significant danger to human health. To avoid health hazards, it is essential to remove these toxic heavy metals from waste water before its disposal.[2]Environmental pollution particularly from heavy metals and minerals in the wastewater is the most serious problem in India. [3]

A number of efficient methods have been reviewed for the removal of heavy metals such as chemical precipitation, ion exchange, reverse osmosis, electro-dialysis, ultra-filtration, nano-filtration, coagulation, flocculation, floatation, etc. however these methods have several disadvantages such as high reagent requirement, unpredictable metal ion removal, generation of toxic sludge etc. Adsorption process being very simple, economical, effective and versatile has become the most preferred methods for removal of toxic contaminants from wastewater.[4]

A vast array of biological materials, especially bacteria, algae, yeasts and fungi have received increasing attention for heavy metal removal and recovery due to their good performance, low cost and large available quantities.[5]Heavy metal resistant microbes might be present in heavy metal contaminated sites.[6]Recently, immobilised whole cell has been regarded as an alternative method of enzyme immobilization, since it is a tedious and time consuming process.[7]The present study aims at using isolated heavy metal resistant microorganisms in their immobilised form for removal of heavy metals from waste water samples.

MATERIALS AND METHODS

Collection of waste water samples:

Waste water samples were collected from two different locations in Thane District, namely Ambernath and Ulhasnagar. Samples were collected in sterile plastic bottles and the bottles were transported immediately to the laboratory with appropriate care and stored at 4^{o}C till further processing of samples. The samples were then processed as per protocol.

Isolation and preservation of pre-existing isolated heavy metal resistant microorganisms:

Pre-existing heavy metal resistant microorganism, Gram positive rods (with MIC of 500ppm for Cadmium and more than 1000ppm for Nickel and Chromium) from Department of Microbiology, R. K. Talreja College were isolated on St. Nutrient agar, following incubation for 24-72 hours. Further the cultures were maintained on St. Nutrient agar slants and subcultured after every 4 weeks.

Analysis of waste water:

The analysis of waste water was performed before and after treatment with immobilised cells for various parameters like nitrate, nitrite, sulphate, phosphate content, BOD and COD as per standard protocols.[8]

Chemical estimation of individual heavy metals:

The waste water sample was checked for the presence of different heavy metals: chromium, [9]nickel[10] and cadmium[11]qualitatively and quantitatively by diphenyl carbazide method, dimethylgly oxime method and alizarin red S solution method respectively.

Bio sorption efficiency of various heavy metals using microorganisms immobilised on sand:

An inoculum of heavy metal resistant organism, 1ml (O.D. 0.1) was added to defined amount of St. Nutrient broth and incubated for 24 hours under shaker conditions. To this, sterile dried sand (32.5g) was added and further incubated for 24 hrs on shaker. The broth was then decanted and 100ml of stock of heavy metal salt solution was added to it and kept on shaker. Different flasks were maintained for the heavy metals, Cr, Ni and Cd. All the flasks were incubated at R.T. on shaker for 4 days. The samples were withdrawn at regular intervals, diluted and its heavy metal ion concentration was determined by chemical estimation methods. Two controls, free sand and free cells, were also maintained. The stock solutions of heavy metal salts were used at following concentrations: $K_2 Cr_2 O_7$ (700ppm), $NiCl_2$ (700ppm) and $CdCl_2$ (300ppm).

Bio sorption percentage was calculated as-

$$\text{Percentage Bio sorption (\%)} = \frac{\text{initial - final metal concentration}}{\text{initial metal concentration}} \text{x } 100$$

RESULTS AND DISCUSSIONS

Bio sorption efficiency of various heavy metals using microorganisms immobilized on sand:

The bio sorption efficiency for various metals were assessed using immobilised cells on sand and using free sand and free cells as control.

Bio sorption of Chromium, Nickel and Cadmium by immobilized cells using sand:

The percentage removal of heavy metals, by cells immobilized on sand, increased with an increase in incubation period from 0 hour to 96 hours as shown in Table 1. The bio sorption efficiency of Cr, Ni and Cd was 84.8 %, 93.26% and 95.5% by the end of 96 hour. With increase in the incubation period no increase in bio sorption was seen in both the controls, free cells and free sand.

Bio sorption of Nickel and Cadmium was much faster compared to Chromium, as around 85-86% of Ni and Cd were removed within the first 24 hours. By the end of 96 hours, efficiency of removal of Cadmium by immobilised cells was the highest (36.7%) followed by Nickel (31.73%) and Chromium (15.2%) compared to control.

Table 1: Bio sorption of Chromium, Nickel and Cadmium by immobilized cells on sand

Time (hours)	Percentage of heavy metals removed								
	Chromium			Nickel			Cadmium		
	A	B	C	A	B	C	A	B	C
0	0	0	0	0	0	0	0	0	0
24	69.6	69.6	69.6	86.53	49.61	49.61	85.3	58.8	71.2
48	78.7	69.6	69.6	86.53	49.61	49.61	85.3	58.8	71.2
72	84.8	69.6	69.6	93.26	61.53	61.53	90.6	58.8	71.2
96	84.8	69.6	69.6	93.26	61.53	61.53	95.5	58.8	71.2

A-Immobilised cells, B- Free cells and C- Free sand

Gram-positive bacteria accumulate much higher concentrations of heavy metals on their cell walls than that of metals Gram-negative bacteria **(Nanda M.et al., 2019)**. Studies have reported that bacteria which are multi-heavy metal resistant have greater MIC values as compared to bacteria showing resistance to a single heavy metal**(Goyal Pet al., 2020).**

Elahi and Rahman, 2018 have reported that B. aerius S1 and B. iodinum S2 were capable of removing upto 99% Cr^{6+} from tannery effluent after 6 days of incubation.

Ilunga Kamika and Maggy NB Momba (2013) reported that living Pseudomonas putida had the highest removal rates of heavy metals Ni-51% in 5 days. **Hany Hussein and et al (2004)** also reported that maximum removal of Ni(II) was in the range between 35 to 88% by Pseudomonas species in 20 mins.

K. Mathivaan et al, 2014 study showed that strain TT-10 belonging to Pseudomonas species showed 99% biosorption of cadmium in 10 days. **Hany Hussein et al (2004)** also reported that maximum removal of Cd (II) was in the range between 35 to 88% by Pseudomonas species in 20 mins.

Analysis of waste water:

The chemical analysis of waste water was performed before and after treatment with immobilised cells.[8] The analysis was done for various parameters like nitrate, nitrite, sulphate, phosphate content, BOD and COD as per standard protocols.The results were as shown in Table No. 2.

Table 2: Percentage removal of Nitrate, Nitrite, sulphate, phosphate, BOD&COD from Ambernath & Ulhasnagar wastewater effluent

ParameterTests	Ambernath	Ulhasnagar
	% Reduction	% Reduction
Nitrate(ppm)	35.71	58.8
Nitrite(ppm)	41.66	71.4
Sulphate(ppm)	46.8	50
Phosphate(ppm)	35.6	18.36
BOD (mg/L)	50	50
COD(mg/L)	7.69	7.69
Crcontent(ppm)	88.5	87.8
Nicontent(ppm)	91.75	80.8

Cdcontent(ppm)	95.3	94.3

On treatment with immobilized cells, the nitrate, nitrite and sulphate content were reduced effectively upto the permissible limits from both the waste waters. As compared to others, the phosphate reduction was less; it was around 18-35%. Also, BOD of both the waste waters were reduced by 50%, while COD reduction wasn't much significant. The heavy metals Cr, Ni and Cd were bio sorped from both the waste waters by 80-95%.

Adhoni S A et al. (2018) reported similar results as immobilized algal cells biosorped most nitrates such that the nitrate content was almost negligible after 30 days. According to J. Abarnadevi, M. Anu, M. Bharani (2013) the level of BOD was recorded maximum as 340 mg/l initially. It was reduced nearly 65% in the effluent treated with Aspergillus when compared to control.

CONCLUSIONS

Heavy metal pollution is one the most important environmental problems in marine, terrestrial, and freshwater areas. In the present study, bio sorption efficiency of heavy metals resistant isolate was determined. Bio sorption of heavy metals like Chromium, Nickel & Cadmium was performed by immobilizing heavy metal resistant organisms on sand. Rate of removal of heavy metals was very high in first 24 hours. As contact time increased, bio sorption capacity of immobilized cells also increased. Treatment with immobilised cells also improved the chemical parameters of waste water along with its stabilization. The maximum heavy metals were bio sorped after 96 hours. Thus, use of heavy metal resistant organisms immobilised on sand proved to be highly effective & eco-friendly method for bio sorption of heavy metals and stabilization of waste water.

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Evaluation of Toxicity of Bleaching Agents on Tilapia Fish

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ABSTRACT

Bleaching agent is used in various industries to lighten a substance. They are majorly used in textile processing, paper whitening, and pulp bleaching as well as for home laundering. Effluents from textile industries contain different bleaching agents and these are directly released into water bodies. They in turn show adverse effect on the ecosystem and cause damage to the fish in general. Present study was done to evaluate the LC_{50} for Sodium Percarbonate, Sodium Hypochlorite and Sodium Hydrosulphite at different concentration ranging from -10mg/lit to 50 mg/lit on fresh water fish Tilapia Mozambique. The lethal concentration was in the order- Sodium Hydrosulphite > Sodium Per carbonate > Sodium Hypochlorite. These agents also affected the behavioral pattern of the fish.

Keywords: LC_{50}, Bleaching agent, Sodium Percarbonate, Sodium hypochlorite, Sodium Hydrosulphite, Tilapia.

INTRODUCTION

Textile and clothing industry globally is one of the largest and oldest industries of economic importance (*Gereffi, 2002*). In the textile industry the final product is obtained after a series of procedures which includes; sizing, desizing, scouring, bleaching, mercerizing, dyeing and printing (*Dey and Islam, 2015; Saini, 2017*).During this processes large amount of wastewater is generated which are generally released as effluents in the nearby water bodies, and acts as sink for toxic chemicals (*Karthikeyan et al., 2006; Adewoye et al., 2005; Roopadevi and Somashekar, 2012*). They are of major causes of concern due to their toxicity, persistency and accumulation both in environment and the flora and fauna (*Nabi Bidhendi et al. 2007, Vinodhini et al., 2009*).

Fish act as bioindicators as they are highly sensitive to changes in the aquatic environment (*Siroka & Drastichova, 2004*). Pollutants cause behavioral changes in fishes (Little and Finger, 1990; Michael . Barry, 2012) and release of effluents can also results in mass mortality of fishes (Das, 2003).

LC_{50} is the concentration of a substance that is lethal to 50 percent of the organisms in a toxicity test. 96h LC_{50} tests is conducted to measure the susceptibility and survival potential of organisms to a particular toxicant (Sadat Sadeghi and Peery, 2018). The present study aims to investigate the LC_{50} of bleaching agents Sodium Percarbonate, Sodium Hypochlorite, Sodium Hydrosulphite on Tilapia fish and also study changes in behavior pattern.

MATERIALS AND METHODS

Fish Collection and Acclimatization

Tilapia Mozambique irrespective of sex but of similar size was procured from Fish Seed Hatchery, Aarey colony Mumbai, Maharashtra. Fishes were acclimatized in the laboratory in dechlorinated water for a week prior to experiments. Acclimatization was done at 25 ± 2^{o}C under a constant 12:12h light: dark photoperiod. Acclimatized fish were fed with commercial diet twice a day.

Experimental Design

Bleaching agents, Sodium Per carbonate, Sodium Hypochlorite and Sodium Hydrosulphite of analytical grade were used for preparation of stock solutions. Fishes were exposed separately to different concentrations (Ranging from 10mg/lit to 50

mg/lit at increments of 5mg/l) of bleaching agents. Experiment was done by grouping 14 fish and exposed to 96h in glass tanks. Test medium was not renewed during the assay period and no food was given. Mortality was recorded at 24, 48, 72 and 96h of exposure and dead fish were removed immediately from the test media.

STATISTICAL ANALYSIS

Finney's method of probit analysis was used to calculate the 96-hr LC_{50} with SPSS Statistical Software.

RESULTS AND DISCUSSION

Physical and behavioral changes during the study period are shown in Figure 1. Pollutants can lead to change in schooling behavior, cause hyperactivity resulting in erratic swimming, seizures and loss of buoyancy in fishes (Madhu,2019).Significant change was seen in the experimental fishes exposed to higher concentrations of bleaching agent. They showed discomfort within few minutes of exposure, which included erratic swimming, rapid movement, loss of equilibrium with increased opercular movement and change in body color when compared to control (Figure1). Change of behavior in C.carpio was also reported when exposed to mercury chloride (Masudet al', 2005). Body was slimy due to mucus secretion from the epithelium of gills when fishes were exposed to bleaching agents. Similar results was observed in C.carpio when they were exposed to pesticide chlorpyriphos (Hallap and David, 2009).

Figure 1. Photographs showing physical and behavioral changes- (A) Loss of equilibrium. (B) Change in body colour. (C)Increased opercular movement

The LC_{50} value for Sodium Percarbonate, Sodium Hypochlorite and Sodium Hydrosulphite was calculated using Finney's method of probit analysis and SPSS Statistical Software at 96 hours of exposure time and is shown in Table 2 (A,B,C).

Table 2: (A) LC50 value of Tilapia Mozambique exposed to different concentrations of Sodium Percarbonate for 96 hours

Sr. No.	Concentration of SodiumPercarbonate (mg/l)	No. ofFishes Exposed	No. of Fishes died at 96hr	log concentration	Probit Kill%	Percentage mortality
1	05	14	0	0.69897	0	0
2	10	14	0	1	0	0
3	15	14	2	1.176091	3.92	14
4	20	14	2	1.30103	3.92	14
5	25	14	5	1.39794	4.04	36%
6	30	14	6	1.477121	4.8	43
7	35	14	6	1.544068	4.8	43
8	40	14	7	1.60206	5	50
9	45	14	10	1.653213	5.55	71
10	50	14	14	1.69897	8.09	100

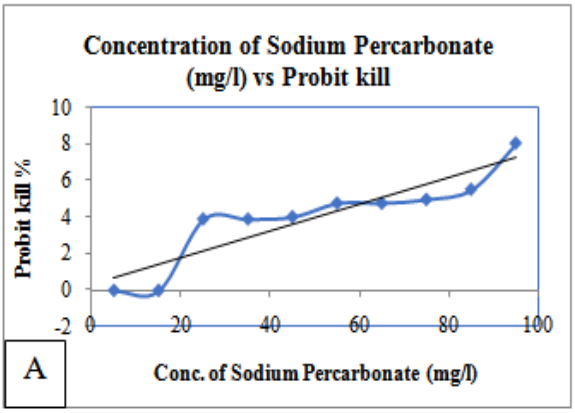
Table 2: (B) LC50 value of Tilapia Mozambique exposed to different concentrations of Sodium Hypochlorite for 96 hours

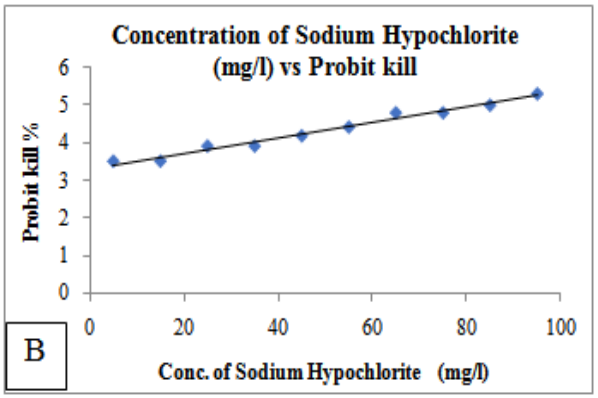
Sr. No.	Concentration of Sodiumhypochlorite (mg/l)	No. of Fishes Exposed	No. of Fishes died at 96hr	log concentration	Probit Kill%	Percentage mortality
1	05	14	01	0.698970004	3.52	7
2	10	14	01	1	3.52	7
3	15	14	02	1.176091259	3.92	14
4	20	14	02	1.301029996	3.92	14
5	25	14	03	1.397940009	4.19	21
6	30	14	04	1.477121255	4.42	28
7	35	14	06	1.544068044	4.8	42
8	40	14	06	1.602059991	4.8	42
9	45	14	07	1.653212514	5	50
10	50	14	09	1.698970004	5.3	64

Table 2: (C) LC50 value of Tilapia Mozambique exposed to different concentrations of Sodium Hydrosulphite for 96 hours

Sr. No.	Concentration of Sodium Hydrosulphite (mg/l)	No. of Fishes Exposed	No. of Fishes died at 96hr	log concentration	Probit Kill%	Percentage mortality
1	05	14	01	0.69897	3.52	7
2	10	14	03	1	4.19	21
3	15	14	04	1.176091	4.42	28
4	20	14	06	1.30103	4.8	42
5	25	14	07	1.39794	5	50
6	30	14	10	1.477121	5.52	70
7	35	14	12	1.544068	6.04	85
8	40	14	13	1.60206	6.41	92
9	45	14	14	1.653213	8.09	100
10	50	14	14	1.69897	8.09	100

The probit line graph with toxicity data and probit kill is shown in Figure 2.





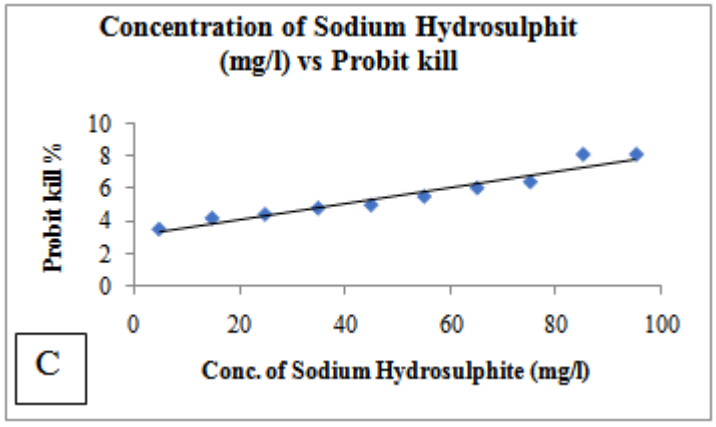
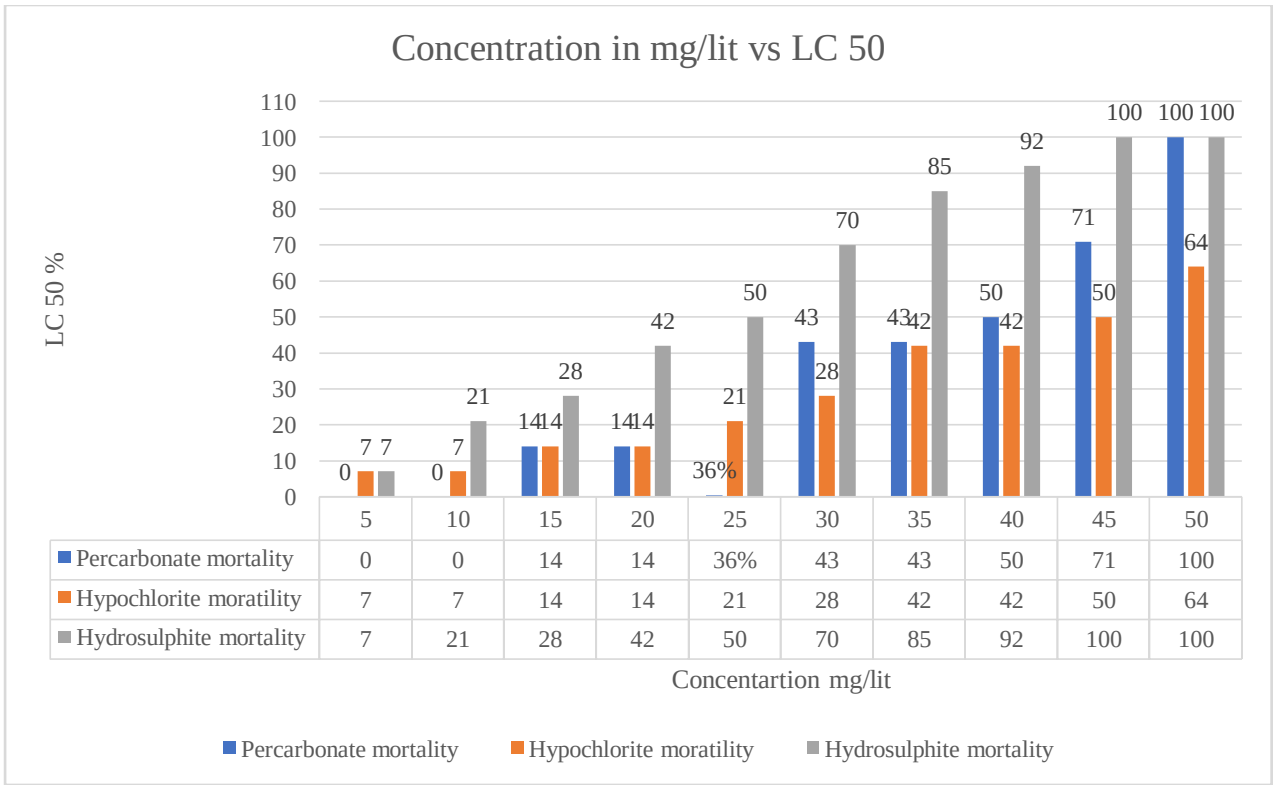


Figure 2: Probit line graph showing concentration of toxicant and probit kill – (A) Sodium Per carbonate. (B) Sodium Hypochlorite. (C) Sodium Hydrosulphite

A comparative median LC_{50} value against percent kill is shown in Figure 3. The 96 hr LC50 value for Sodium Percarbonate, Sodium Hypochlorite and Sodium Hydrosulphite was found to be 40 mg/L, 45mg/L and 25mg/L respectively.



	5	10	15	20	25	30	35	40	45	50
Percarbonate mortality	0	0	14	14	36%	43	43	50	71	100
Hypochlorite moratility	7	7	14	14	21	28	42	42	50	64
Hydrosulphite mortality	7	21	28	42	50	70	85	92	100	100

Figure 3: Comparative data of concentration of bleaching agents against Percent kill and median LC50 value

Toxicity testing is done to determine if a chemical has the potential to be toxic to organisms when present in the ecosystem. The present study was done to compare the toxicity of Sodium Percarbonate, Sodium Hypochlorite and Sodium Hydrosulphite in an aquatic ecosystem.The results indicated a positive relationship between the mortality and concentration levels of toxicant; when the concentration of toxicant increased it resulted in increase in mortality rate(Witeska, 2003). Figure 3 also shows that rate of mortality for any fixed time increased with increase in concentration and for a particular concentration with increase in exposure time. A comparative median LC_{50} value and percentage kill is shown in Figure 3. The 96 hr LC_{50} value for Sodium Percarbonate, Sodium Hypochlorite and Sodium Hydrosulphite was found to be 40 mg/L,

45mg/L and 25mg/L respectively. Lower the LD_{50} value the more toxic the chemical (Karasu and Koksal, 2005). When compared to the other two bleaching agent's Sodium Hydrosulphite has a lower LC 50 and hence it is more toxic. This is due to high solubility in water and can change the chemical properties of water and result in decreased oxygen. 96-hr LC_{50} for fish, Leuciscus idus was shown to be 62.3 mg/l, for Daphnia magna, 48-hr EC_{50} was 98.3 mg/l (OECD 2004). Higher LC_{50} values are less toxic as greater concentrations are required to produce 50% mortality (Basha and Rani, 2003) The LC_{50} for sodium Hypochlorite was high and was less toxic. The toxicity of bleaching agents was found in the following order of Sodium Hydrosulphite>Sodium Per carbonate>Sodium Hypochlorite. However, it was seen that as the concentration was increased, the mortality time decreased showing a negative relation.

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Thrust Area: Bioremediation
Optimisation of Factors Affecting Biodegradation of Hexavalent Chromium by Arthrobacter, Isolated From Soil and Sewage Samples

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ABSTRACT

Arthrobacter are commonly found in soil and sewage environments because of their nutritional versatility. They have the capability to degrade a wide array of organic and inorganic pollutants. Hexavalent chromium,a toxic and carcinogenic pollutant, is widespread throughout the environment because of its use in dyes, pigments, refractory material, leather tanning and electroplating. Several species of Arthrobacter can not only grow in the presence of hexavalent chromium, they can also reduce it to trivalent chromium; it's less toxic form. The Arthrobacter isolates A11 and A40 were found to be potential biodegradors of hexavalent Chromium. The optimum conditions of biodegradation of Chromium by these isolates, identified as Arthrobacters, by 16SrRNA sequencing, were determined. The two categories of factors used were nutritional and physicochemical. Glucose, lactose, sucrose, sodium nitrite, casein hydrolysate and peptone were employed to check their effect on degradation. The effect of temperature, pH, incubation time, inoculum density and volume, aeration and salinity, on degradation was also studied. Both these isolates showed maximum degradation of Chromium in presence of lactose and casein, room temperature, pH 7, 48 hours of incubation, static conditions, inoculum density of 1(optical density), inoculum volume of 3% and salt concentration of 0.5% in the medium.

Key words: chromium, pollutants, physicochemical, salinity, toxic.

INTRODUCTION

Hexavalent chromium, a toxic pollutant is widely spread in the environment. It is used in tanning of leather, dyes, pigments, refractory material, and electroplating. From the two forms of chromium, hexavalent chromium is hundred times more toxic than trivalent chromium because of its oxidation state. It is also much more soluble in water and thus seeps into ground water very easily. Very few organisms can grow in the presence of hexavalent chromium. Arthrobacter can not only grow in the presence of hexavalent chromium, it can also reduce it its less toxic form of trivalent chromium [1]. Thus, Arthrobacter can be used for bioremediation of chromium contaminated niches.

The choice and successful implementation of bioremediation technologies on contaminated areas depends upon the characteristics of the contaminated site, mechanism and a complex system of many factors that affect biodegradation processes. It is extremely important to consider and understand those limiting factors [2, 3].

If the substrate/nutrient in the surroundings of the microorganism is novel, presence of easily degradable carbon source in the medium initiates metabolism and simultaneously co-metabolize the novel substrate/pollutant. It may also affect the degradation [2, 4]. Nitrogen sources are reported to hasten the rate of degradation to a certain extent [5].

The solubility, the bioavailability of the molecules and degradation of compounds increases with temperature. The degradation activity is affected at low temperature due to change in optimum temperature for functioning of enzymes [6]. Chromium degradation/removal is better at acidic pH. Many sites contaminated with pollutants are not at the optimal pH.The degradation percent of the compound increases with time due to initial catabolite repression and the time taken by the microbe to synthesize enzymes necessary for degradation. However, with increasing time percent degradation may decrease due to accumulation of toxic products affecting the viability of microbes or due to saturation of active sites of

enzymes [7].For successful biodegradation this microbe's number should not be lower than 10^3 microbes per gram of soil and should have high catabolic activity. On one hand a smaller number of cells will slow the rate of degradation, but on other hand denser culture may not be available because of cell packing [8]. Less volume inoculum and more dense culture may not serve the purpose because the increase in number of cells will again take a lag phase to multiply [9]. Dissolved molecular oxygen is required for respiration of the microbe and for degradation pathways. At sufficient depths and in deep water sediments, degradation of pollutants can turn anaerobic when the oxygen supply is depleted. Oxygen has been shown to be the rate limiting for the activity of such organisms [10]. Microorganisms are typically well adapted to cope with the wide range of salinities common to the world's ocean. However, increasing salinity in aquatic environments has had a negative impact on the biodegradation of various pollutants of soil and water ecosystems [11, 12].

Two categories of limiting factors studied in the current work were nutritional and physicochemical factors and their effect on biodegradation by two identified Arthrobacter isolates. Glucose, lactose, sucrose, sodium nitrite, casein hydrolysate and peptone were employed to check their effect on degradation. The effect of temperature, pH, incubation time, inoculum density and volume, aeration and salinity, on degradation was also studied.

MATERIALS AND METHODS

The method used for chromium estimation i.e; diphenyl carbazide method, remains same throughout the study**.** All experiments were carried out in triplicates. Positive and negative controls were kept wherever applicable. Appropriate dilutions were carried out as required.

Materials

Standard Chromium estimation

Standard solution of $K_2Cr_2O_7$ (100 mcg/ml), Orthophosphoric acid and Diphenyl carbazide reagents.
Sterile test tubes and pipettes, Centrifuge and Colorimeter.

NUTRITIONAL FACTORS

Culture suspensions of identified Arthrobacter isolates A11 and A40.

i. Effect of carbon sources

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$ and100 mgs (1 gm/lit) of 3 selected carbon sources in 3 different flasks-2 sets

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$ and no carbon source -3 flasks as controls
Carbon sources used - glucose, lactose and sucrose.

ii. Effect of Nitrogen sources

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml. of $K_2Cr_2O_7$ and 100 mgs (1 mg /ml) of 3 selected nitrogen source in 3 different flasks -2 sets.

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml. of $K_2Cr_2O_7$ without nitrogensource ـ3 flasks as controls
Nitrogen sources used - sodium nitrite, casein hydrolysate and peptone.

2. PHYSICOCHEMICAL FACTORS

Effect of temperature

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml. of $K_2Cr_2O_7$- 5 flasks for 5 different temperatures (4, RT, 37, 45 and 55^0C) – 2 sets and 1 set of flasks as control for each temperature.

Effect of pH

Sterile 100ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$-5 flasks with different pH (5, 6, 7, 8 and 9) – 2 sets and 1 set of flasks as control for each pH.

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml. of $K_2Cr_2O_7$.. 2 flasks plus 1 flask as control.

Effect of Incubation time

Sterile 100ml Mineral salt broth media containing 100 mcg/ml. of $K_2Cr_2O_7$.. 2 flasks plus 1 flask as control.

Effect of inoculum density

Culture suspensions of identified Arthrobacter isolates A11 and A40 with 5 different inoculumdensities 0·6, 0·7, 0·8, 0·9 and 1.0 (at 660 nm).
Sterile 100 ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$.. 5 flasks for 5 different inoculum densities –2 sets and 1 flask as control .

Effect of inoculum volume

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$.. 5 flasks for5 different inoculum volumes of cultures–2 sets and 1flask as control.
Sterile 100 ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$..2 flasks-2 sets and 2 flasks as control .

Effect of aeration

Sterile 100ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$..2 flasks-2 sets and 2 flasks as control,Shaker.

Effect of salt concentrations

Sterile 100 ml Mineral salt broth medium containing 100 mcg/ml of $K_2Cr_2O_7$ .5 flasks with 5 different salt concentrations of 0.5, 1, 1.5, 2, 3 and 4 %– 2 sets and 1 set of flasks as controls.

Method

Degradation assay for chromium. Hundred ml of sterile Mineral salt liquid medium containing $K_2Cr_2O_7$ and different carbon/nitrogen sources were taken in different flasks and inoculated with 2 ml of Arthrobacter identified isolates A11 and A40 (0·5 @ 660 nm). A similar set up was done for both the isolates. A control flask without any carbon /nitrogen sources was also kept. They were all incubated at **RT-room temperature** for 48 **hrs-hours**. Control flask without any culture was also included. A similar set up was employed for physicochemical conditions as mentioned above.

Aliquots were removed from medium at end of 24 and 48 hrs and hexavalent chromium estimation was done by diphenyl carbazide method. They were diluted as and when required. In acid solution, diphenyl carbazide and hexavalent chromium form a soluble red-violet product that absorbs light at 540 nm which is read colorimetrically. A set of standards was run by using potassium dichromate standards ranging from 5-100 mcg/ml [13, 14]. Standard graph was plotted and the unknown values were determined from the graph. Degradation Percent was calculated by the standard formula.

RESULTS AND DISCUSSION

Standard Chromium estimation

Standard hexavalent Chromium estimation by was carried out by diphenylcarbazide method and the values were obtained from standard graph depicted in figure 1.

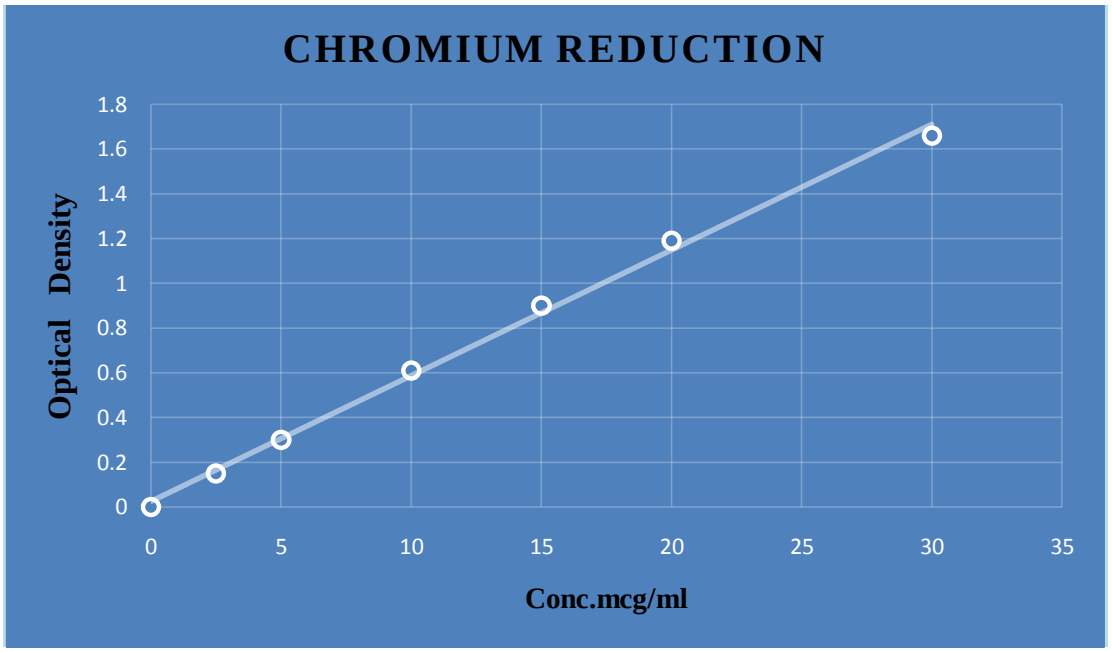

Figure:1 Standard hexavalent chromium reduction graph

1. Nutritional Factors

The results of effect of Carbon and Nitrogen sources on biodegradation by Arthrobacter isolates A11 and A40are tabulated in **tables 1and 2.**

i. Effect of carbon sources

Table:1 Degradation of Chromium in presence of different carbon sources, by Arthrobacterisolates A11 and A40

Isolate/Carbon source Hrs-hours	**Glucose**		**Lactose**		**Sucrose**	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
A11	27.5 %	53.6%	**30.6%**	**60.1%**	5%	7%
A40	58.73%	58%	**59.7%**	**63.2%**	4%	7.3%

ii. Effect of Nitrogen sources

Table:2Degradation of Chromium in presence of different nitrogen sources at 24 and 48 hrs, by Arthrobacterisolates A11 and A40.

Isolate/Carbon source	**Peptone**		**Casein hydrolysate**		**Sodium nitrite**	
	24 hrs	48 hrs	**24 hrs**	**48 hrs**	24 hrs	48 hrs
A11	22.5 %	51.6 %	**28.6 %**	**61.2 %**	4 %	7.4 %
A40	41.73 %	43.7 %	**55.7 %**	**60.2 %**	5 %	6.3 %

2. PHYSICOCHEMICAL FACTORS

The results of effect of temperature, pH, incubation time, inoculum density and volume, aeration and salinity, on degradation by Arthrobacter isolates A11 and A40are tabulated in **tables 3 to 9.**

i. Effect of temperature

Table:3 Degradation % of Chromium at different temperatures by byArthrobacterisolates A11 and A40at24 and 48 hrs

Isolate/Temp	4^0C		RT		37^0C		45^0C		55^0C	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
A11	1%	3%	**29.5 %**	**63.2 %**	26.1 %	55%	22.5 %	51.6 %	22%	50%
A40	1%	3%	**45.7 %**	**62.9 %**	41.7 %	54%	40%	43.7 %	36%	40%

ii. Effect of pH

Table:4 Degradation % of Chromium at different pH by Arthrobacter isolates A11 and A40 at 24 and 48 hrs

Isolate/pH	5		6		7		8		9	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
A11	0%	0%	12.5 %	30.75 %	**30.5 %**	**49.97 %**	35%	38.1 %	32.5 %	33.8 %
A40	5%	0.5%	7.5%	23.12 %	**67.5 %**	**72.37 %**	47%	49.3 %	27%	29.8 %

iii.Effect of Incubation time

Table:5 Degradation % of Chromium by byArthrobacterisolates A11 and A40,at 24 and 48 hrs

Isolate / Incubation time	24 hrs	48 hrs
A11	27.5%	**53.6%**
A40	58.73%	**58%**

iv. Effect of inoculum density

Table:6 Degradation % of Chromium using different inoculum densities by Arthrobacter isolates A11 and A40, at 24 and 48 hrs

Isolate/ Inoculum density	0.6		0.7		0.8		0.9		1	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
A11	12%	14.7 %	17%	20.4 %	20%	22%	29%	48%	**32.1 %**	**51.7%**
A40	19.6%	21.4 %	25.9 %	27%	50%	51%	66.4 %	70.11 %	**64.5 %**	**74.7%**

v. Effect of inoculum volume

Table:7 % degradation of Chromium using different inoculum volumes by Arthrobacter isolates A11 and A40, at 24 and 48 hrs

Isolate/Inoculum volume - %	1		2		3		4		5	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs

A11	17.6 %	19%	28.9 %	32.6 %	**35.5 %**	**52.97 %**	26.9 %	25%	10.6 %	10%
A40	20%	25%	60.1 %	64.5 %	**69.5 %**	**75.37 %**	58.1 %	50%	13%	11%

vi. Effect of aeration

Table:8 Degradation % of Chromium under Static and Shaker conditions by Arthrobacter isolates A11 and A40, at 24 and 48 hrs

Isolate / Aeration condition	**Shaker**		**Static**	
	24 hrs	48 hrs	24 hrs	48 hrs
A11	27.5%	**53.6%**	27.5%	**53.6%**
A40	58.73%	**58%**	58.73%	**58%**

vii. Effect of salinity

Table:9 Degradation % of Chromium using different salt concentrations in medium, by Arthrobacter isolates A11 and A40, at 24 and 48 hrs

Isolate/Salt concentration%	**0.5**		**1**		**2**		**4**		**6**	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
A11	**67.9%**	**77.2%**	30%	40%	14%	14.1 %	9%	9.3 %	0%	3.6%
A40	**41.5%**	**49.9%**	19%	31.2 %	8%	8.2%	7%	7.4 %	0%	5.5%

CONCLUSION

Arthrobacter a common occurant in soil and sewage habitats, can biodegrade multitude of pollutants including hexavalent Chromium. The modern times need to develop microbial inocula for bioremediation of such contaminated niches. It is necessary to know the degrading abilities of native microbial populations [15]. The use of microorganisms in bioremediation requires the optimization of parameters used in cultivating the organism.If the optimum conditions for degradation are provided to microbe, biodegradation can be achieved efficiently. A widely used approach to bioremediation involves biostimulation, thatcan be achieved through changes in pH, moisture, aeration, or additions of electron donors, electron acceptors or nutrients. Bioremediation can also be achieved through bioaugmentation [16]. Biostimulation and bioaugmentation, are not mutually exclusive. Hence optimum nutritional and physicochemical conditions were determined that can be employed for chromium degradation by Arthrobacter isolates. Maximum degradation of chromium was found to be in presence of lactose and casein hydrolysate as carbon and nitrogen source respectively, in the medium, by Arthrobacter isolates A11 and A40. Theycould carry out maximum degradation of chromium at room temperature, pH 7 in 48 hrs, at an inoculum density of 1, inoculum volume of 3%, static condition and a salt concentration of 0.5%in the medium.The isolates appear to be not fastidious and due to their all-pervasive presence in soil, can beexplored to remediate subsurface pollution and environmental clean-up by bioremediation. An important challenge that remains to be overcome for applying itpractically, is to develop strategicbioremediation that are feasible technically and economically [15].

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The Capability of Soil Enzymes *(Bio Stimulants)* in Sustainable Crop Production

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ABSTRACT

Soil quality is a key factor for the growth of crop plants and the deciding factor for the availability of plant nutrients. Sustainable crop production is the current focus of agricultural research across the globe. The use of chemical fertilizers since the green revolution has enhanced food grain production to fulfill the requirement of a huge population.
The soil enzymes (*biostimulants*) are the mediators of organic matter decomposition and soil nutrient transformations. The total enzyme activity of the soil has been found to stabilize soil organic matter through humification, enhance decomposition, increase the availability of plant nutrients, and regularize nutrient cycling. Their patterns of activity concerning environmental factors and management practices help to design sustainable management practices. Thus, knowledge of soil enzymes is essential to design and evaluate new sustainable crop management practices.
The objective of this study was to compare the Physico-chemical characteristics and it was found that the soil physicochemical parameters had a greater impact on enzyme activity. It was suggested that the use of biostimulant formulations is a promising technique in crop production.

Key Words: Soil enzyme, Nutrient cycling, Sustainable crop, green revolution.

INTRODUCTION

Soil enzymes increase the reaction rate at which plant residues decompose and release plant-available nutrients. Sources of soil enzymes include living and dead microbes, plant roots and residues, and soil animals. Enzymes stabilized in the soil matrix accumulate or form complexes with organic matter (humus), clay, and humus-clay complexes, but are no longer associated with viable cells. It is thought that 40 to 60% of enzyme activity can come from stabilized enzymes, so activity does not necessarily correlate highly with microbial biomass or respiration (Kompała - Bąba, A., 2021). Therefore, enzyme activity is the cumulative effect of long-term microbial activity and activity of the viable population at sampling (Dick, R. P. 1994).

Soil enzymes have varying optimum pH and temperature values at which they function most effectively. For example, the activity of phosphatase, arylsulfatase, and amidase involved in phosphorus, sulfur, and nitrogen cycling, respectively, are strongly correlated to variations in soil pH (Sherene, T.,2017). Since enzyme structure and substrate binding can be altered by heat and extreme cold temperature, enzyme activity decreases above and below the optimum temperature. The activity of many enzymes often correlates with soil moisture content, as well. Drought may suppress enzyme activity. It can be said that soil enzymes can be a corresponding ecological indicator for the requirements already mentioned. Soil enzymes can reveal ecosystem perturbations, as they are sensitive to management practices, and have been used as indicators of biogeochemical cycles, organic matter (OM) degradation, and soil remediation. Thus, they can represent soil quality, especially in combination with other physical or chemical properties. Many studies have been published on the use of soil enzyme activities as ecological indicators of soils affected by contamination, such as toxic trace elements (TTEs), stress conditions, and management practices (Lee, S. H.2020). Soil enzymes can be used as biological indicators for diagnosing soil quality because of their stability and sensitivity; they can well indicate whether the biochemical reactions in the soil to which soil enzymes are involved are correctly performed.

MATERIAL AND METHODS

Collection soil samples

Samples were collected from the fields of the Neral, Badlapur, and Ambarnath regions. Random soil sampling was done and samples were collected in polythene bags. Soil samples were harvested at low temperatures till further use.

Physicochemical analysis of soil samples:

Physicochemical characterization of soil samples was carried out which includes physicochemical parameters such as pH, organic content, salini4ty, etc. (Walkely and Black, 1934).

Enrichment of soil samples:

Collected soil samples were inoculated in St. Luria Bertani medium and flasks were enriched at RT on a shaker for 48-72 hrs.

Isolation of soil bacteria

Enriched soil samples were streak inoculated on Sterile Luria Bertani agar and incubated at RT for 24-48 hrs. Well, isolated colonies were checked for their colony characteristics. Further isolates were purified and preserved on a slant for future use.

Screening of enzyme producers:

Extracellular enzyme activities were checked by the spot inoculation method on different media.

Catalase activity:

Catalase test was performed by taking 3-4drops of hydrogen peroxide (H2O2) was added to 48 h old bacterial colony which is grown on Luria Bertani medium. The effervescence indicated catalase activity.

Caseinase (protease) activity:

The qualitative assay for protease production was performed on sterile skim milk agar plates. Isolates were spot inoculated and followed by incubation at a t30C and zone of clearance around the colony indicating the enzymatic degradation of protease (Chaiharn 2008).

Amylase (starch hydrolysis) activity:

The Bacterial Isolates Were Spot Inoculated On Starch Agar medium plates and incubated at 30°C for 48h. At the end of the incubation period, the plates were flooded with iodine solution, kept for a minute, and then poured off. Iodine reacts with starch to form a blue color compound. This blue color fades rapidly. Hence the colorless zone surrounding colonies indicates the colorless production of amylase.

Cellulase activity:

Cellulase production was determined by using the method (Miller G.L 1959). M9 agar medium with yeast extract plates was inoculated with individual bacterial isolates and incubated for 3-5 d at 28C. Bacterial growth surrounded by clear halos was considered a positive indication of cellulase production.

Urease activity:

Urea degrading enzymes were screened and were expressed as µg NH4 + released g-1 dry soil h-1 at 37 ºC, were assayed spectrophotometrically by the indophenol blue method described by Guan (1986).

Determination of plant growth-promoting activity of selected isolates

PGP traits characterization will include the study of phosphate solubilization test described by Gaur (1990), Production of ammonia by Nessler reagent (Cappuccino and Sherman, 1992), Production of HCN by Lorck method(1948), IAA

production Salkowsky's method (Brick et al.1991) and Siderophore production ability by CAS method (Schwyn and Neilands, 1987).

Sustainability of enzyme producers at various physical conditions

The sustainability of enzyme producers was examined at different environmental conditions like temperature, pH, and different salinity.

Application of biostimulant producers as a plant growth promoter

Plant growth promotion activity was observed by pot assay. In the pot, an assay-prepared bioinoculant was added to the soil. Application of inoculants will be done along with the control set. Plant characterization will be done after the specified growth of the plant.

RESULT AND DISCUSSION

Sampling and characterization of soil

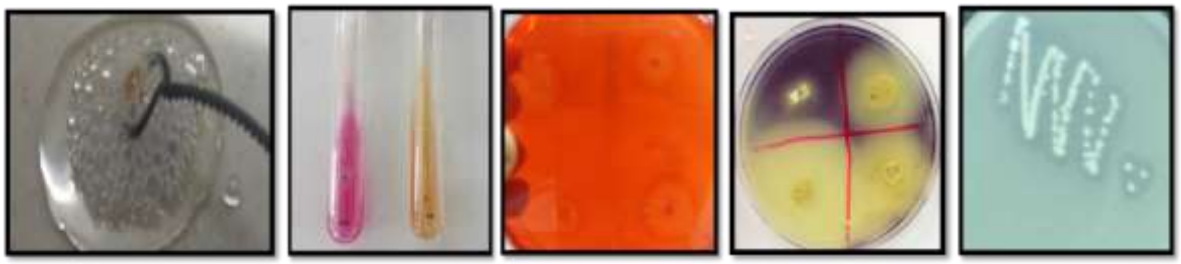
Six Soil samples were collected and immediately checked for their physicochemical characterization. Results of physicochemical characterization are mentioned in table no.1. All samples show pH range from slightly acidic to slightly alkaline, Organic matter ranging from 10-31% and salinity is ranging from 0.2 to 5%.sample no. 5 shows higher salinity than others.

Table 1: Physiochemical characterization of collected soil samples

Soil Samples	pH	Organic matter (%)	salinity
S-1	6.1	23	3
S-2	5.3	31	4.1
S-3	5.3	19	2.5
S-4	6.4	20	2.2
S-5	7.2	28	3.4
S-6	7.4	10	4.1

Screening of enzyme producers

Six soil samples were collected and 56 soil isolates were screened out on their colony and morphological characteristics. All 56 isolates were screened for soil enzyme production ability. Results of screening are shown in Figure no.1.



(a) (b) (c) (d) (e)

Figure 1: Enzyme activity (a) Catalase activity, (b) Urease activity, (c) Cellulase activity (d) Amylase activity (e) Casease activity

Out of 56 isolates 12 isolates shows good enzyme activity which was further screened for quantitative assay. Quantitative enzyme assay of all the isolates shown in Figure No. 2. Isolate No. 3,4,5,6,8,10,11 and 12 shows potential enzyme activity.

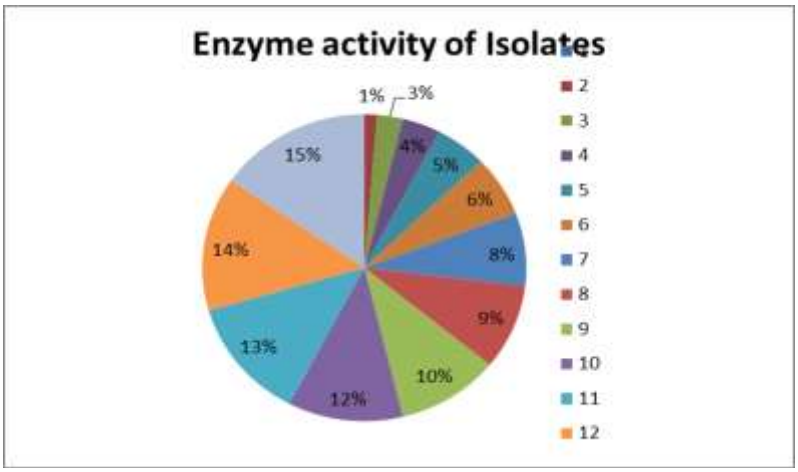


Figure 2: Enzyme activity of selected isolates

8 isolates showed maximum enzyme activity, further screened for plant growth-promoting ability.

Plant growth-promoting activity of isolates

It was observed that 5 isolates show potential plant growth-promoting activity which includes IAA production, ammonia production, Siderophore production, Catalase activity, and HCN production ability. Results of plant growth promotion are shown in Figure 3.

(a) (b) (c) (d) (e)

Figure 3: Plant growth promotion activity: (a) IAA production (b) Ammonia production (c) HCN production (d) Siderophore production (e) Phosphate Solubilization

Sustainability of enzyme producers at various physical conditions

Out of 5 isolates two isolates were good enzyme producers at various environmental conditions such as pH, Temperature and at different salt concentrations.

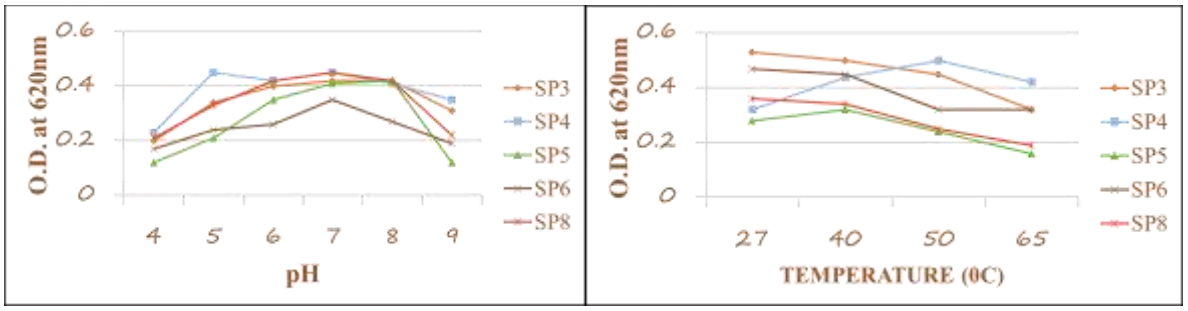


Figure 3: Growth of isolates at different pH **Figure 4: Growth of isolates at different temperature**

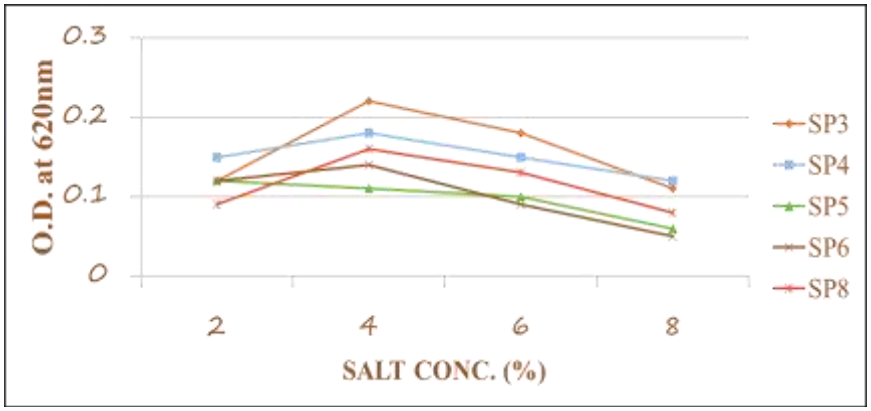


Figure 4: Growth of isolates at a different salt concentration

Application of biostimulant producers as a plant growth promoter

S 3 and S 4 shows potential PGP activity as well as sustainability at a broad range of physical conditions. Hence, both the isolates are characterized morphologically as well as biochemically. It was observed that S - 11 belongs to Bacillus spp. and S - 12 belongs to Pseudomonas spp.

Bacillus sp. and Pseudomonas sp. were used for pot assay to check their influence on plant Vignaradiata. Combination of Bacillus sp. And Pseudomonas sp. Shows better results than individual sp. Results of pot assay are shown in Table no.2.

Table no.2: Effect of biostimulant producers on plant root growth, shoot growth, and biomass by Pot assay

Strain	Root length (cm)	No.of root branches	Shoot length (cm)	No. of shoot branches	Biomass (gm)
Control	5.0	5	12	1	0.158
Bacillus sp	5.3	7	12.5	2	0.345
Pseudomonas sp.	5.2	9	14.7	4	0.564
Bacillus and Pseudomonas	6.1	12	15.4	4	0.840

CONCLUSION

This study showed that the application of the biostimulant improved the plant morphology and growth. Our results also showed differences in the response of the individual and the combined effect of biostimulant producers on plant growth. Biostimulants offer an opportunity to circumvent the excessive use of chemical fertilizers, which lead to a deterioration of

the structure and biological composition of soils and thus contribute to environmental protection. In summary, we conclude that biostimulant treatments can help to preserve soil health and ecosystems, and enhance plant growth, which contributes to improved plant development and productivity. The smart strategies of soil amendments with biostimulants can support sustainable agriculture and contribute to environmental protection. In future work, we would like to study the effect of biostimulants on the crop under stressed conditions.

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Evaluation of Phytochemical, Antioxidant Anti-inflammatory and Antimicrobial Activity of Phoenix dactylifera

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ABSTRACT

Natural products have been an integral part of the ancient medicine systems. Various studies have reported the nutritional benefits of P. dactylifera. However, limited studies have been done on phytochemical, antimicrobial, antioxidant, and anti-inflammatory activity of P. dactylifera. Therefore, the objective of the present study was to examine phytochemical composition, antioxidant and anti-inflammatory activity, and investigate antimicrobial activity of P. dactylifera extracts against some gram-positive, gram-negative bacteria, and fungal species. Two different solvents (aqueous and methanol) were used for extraction. The phytochemical screening showed that the P. dactylifera extract contains a mixture of phytochemicals such as flavonoids, phenolics, and alkaloids. The results of antioxidant study showed that extract of P. dactylifera and its constituents could be easily accessible source of natural antioxidants and possible food supplements in pharmaceutical industry. It was found that P. dactylifera has dose-dependent RBC membrane stabilization and can be used for anti-inflammatory action. The P. dactylifera was also found to be a potential antimicrobial agent.

Keywords: Anti-inflammatory, Antimicrobial, Antioxidant, P. dactylifera, Phytochemical.

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. According to World Health Organization (WHO), around 80% of the world's population relies mainly on traditional medicine for their primary health care. This is due to the availability, low cost, and no side effects of herbal medicines [1]. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates, and chemical entities for synthetic drugs [2]. Since ancient times, in various cultures worldwide, inflammatory disorders and related diseases have been treated with plants or plant-derived formulations as plants have the ability to synthesize a wide variety of secondary metabolites. It motivates the researchers for finding new potential of such plants with variety of pharmacological activities Date palm tree belongs to Arecaceae family (Angiosperms, monocotyledon) consists of about 200 genera and more than 2,500 species. Phoenix is one of the genera with approximately 14 species native to the tropical or subtropical regions of southern Asia or Africa [3]. The name of the species dactylifera means "finger-bearing," which refers to the fruit clusters produced by this plant. Dactylifera is a grouping of the Greek word dactylus, means "finger," and the Latin word ferous means "bearing" [4]. Date fruit (Phoenix dactilyfera) is consumed as a staple food and an important component of the diet in the Middle East region. This fruit is considered highly nutritional because of its rich sugar content in the form of fructose and glucose, dietary fiber, vitamins, and minerals. [5]. Chemical constituents and biological activity of date seeds have been reported by [6]. Date palm seed is one of the rich sources of polyphenols and flavonoids [7]. Date palm seed has been extensively investigated for pharmacological activities such as immuno-stimulant [8], antidiabetic [9], and antioxidant [7], [10]. Although various studies have reported nutritional, chemical, and pharmacological activities on date seed, limited studies have been done on phytochemical, antimicrobial, antioxidant, and anti-inflammatory activity of P. dactylifera fruit. Therefore, this study aims to examine phytochemical composition, antioxidant and anti-inflammatory activity, and investigate the antimicrobial activity of P. dactylifera extracts against some gram-positive, gram-negative bacteria, and fungal species.

MATERIAL AND METHODS

Sample Collection

Dates (P. dactylifera) were procured from a market at Bhiwandi (Maharashtra), India. The selected dates were such that they were uniform in size, free from physical damage and injuries by insects and fungal infection. Dates were pitted to remove seeds. The sample was washed, cut (uniform thickness), and dried at 65˚C. Then it was ground into a fine powder and used for the analysis.

Extraction of Fruit Material

The fine powder of date fruit was extracted using both polar and non-polar solvent. Distilled water was used for aqueous extraction. The aqueous extraction was done by taking 10 grams of fruit powder and mixing it with 250 mL of distilled water in a beaker. The mixture was heated on a hot plate at 30-40 ºC and mixed with continuous stirring for 20 minutes. Then, the extract was filtered using Whatman filter paper and concentrated using water bath. Soxhlet method was used for methanol extraction as described by [11] with slight modification. 10 gram of powdered fruit was packed in a thimble which was placed into an extractor and then extracted with 250 mL of methanol. The extraction process was carried out until the solvent in the siphon tube of Soxhlet apparatus became colorless. After that, the extract was heated in hot water bath at 35 ºC until all the solvent evaporated. The dried fruit extracts were kept in refrigerator at 2-8 ºC for their future use.

Phytochemical Screening

The phytochemical analysis of both aqueous and methanolic extract of P. dactylifera was done by standard methods as described by [12].

Test for Carbohydrates

A quantity of 0.1 g each of the extracts was shaken vigorously with water and then filtered. To the aqueous filtrate, few drops of Molisch reagent were added, followed by vigorous shaking. 1 mL of concentrated sulphuric acid was carefully added to form a layer below the aqueous solution. A violet ring appeared at the interphase of the test tube, indicating the presence of carbohydrates.

Test for Steroids

The amount of 0.5 g of extract was dissolved in 10 mL anhydrous chloroform and filtered. The filtrate was mixed with 1 mL of acetic anhydride followed by the addition of 1 mL of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration as indicative of steroids.

Test for Flavonoids

0.2 g of extracts was heated with 10 mL of ethyl acetate in boiling water for 3 min. The mixture was filtered consecutively, and the filtrates were used for the flavonoid test. 4 mL of the filtrates was shaken with 1 mL of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed at the ammonia layer, which turned colorless when 2 drops of diluted hydrochloric acid were added to the solution. This result indicated the presence of flavonoids.

Test for Alkaloids

0.5 g of extracts was boiled with 5 mL of 2% HCl on a steam bath for 10 min and filtered. To 5 mL of the filtrate, 2 mL of dilute ammonia solution was added. After that, 5 mL of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. Draggendorff's reagent was added to the extract. The formation of reddish-brown precipitate was regarded as positive for the presence of alkaloids.

Test for Saponins

0.5 g of extract was added 5 mL of distilled water in a test tube. The solution was shaken vigorously and observed for a stable, persistent froth. Formation of foam indicated the presence of saponins.

Test for Tannins

About 0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green colouration.

Test for Cardiac Glycosides

0.5 g of extract diluted to 5 mL in water and added 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1 mL of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides.

Test for anthraquinones

0.5 g of extract was placed in a dry test tube, and 20 mL of chloroform was added. This was heated in steam bath for 5 min. The extract was filtered while hot and allowed to cool. To the filtrate, 10% ammonia solution was added with an equal volume of filtrate. This was shaken, and the upper aqueous layer was observed for bright pink colouration as indicative of the presence of anthraquinones.

Antioxidant Assay

The antioxidant activities of P. dactylifera powder were measured using three different methods, namely (i) DPPH(2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging assay, (ii) Hydrogen peroxide scavenging (H_2O_2) assay, and (iii) Reducing power method (RP).

DPPH Scavenging Assay

The free radical scavenging potential of both methanolic and aqueous extracts were determined according to the procedure of [13] with some modifications. An aliquot of 200 µL of sample solution of various concentrations (100–500 µg/mL) were mixed with 2mL of methanolic solution of DPPH (0.5mM). The reaction mixture was incubated at 37°C for 1 h in the dark. The free radical scavenging potential of the extracts were expressed as the disappearance of the initial purple color. The absorbance of the reaction mixture was recorded at 517 nm using UV–Visible spectrophotometer. Ascorbic acid was used as the positive control. DPPH scavenging capacity was calculated by using the following formula:

Hydrogen peroxide Scavenging Assay

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of [14]. Aliquot of 0.1 mL of extracts (100–500 µg/mL) was transferred into the eppendorf tubes, and their volume was made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H_2O_2 solution (2 mM). The reaction mixture was vortexed, and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

Reducing Power Assay

The reducing power was determined according to the [15] method with some modifications. Aliquot of 0.2 mL of various concentrations of the extracts (100–500 µg/mL) were mixed separately with 0.5 mL of phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of 1% potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 min. After cooling at room temperature, 0.5 mL of 10% trichloroacetic acid was added, followed by centrifugation at 3,000 rpm for 10 min. Supernatant (0.5 mL) was collected and mixed with 0.5 mL of distilled water. Ferric chloride (0.1 mL of 0.1%) was added, and the mixture was left at room temperature for 10 min. The absorbance was measured at 700 nm. Ascorbic acid was used as positive control.

Anti-inflammatory Activity

The anti-inflammatory activity of aqueous and methanolic extract of P. dactylifera was estimated by membrane stabilization method. Acetylsalicylic acid available in the commercial name of Ecosprin ʀ-75 was used as a source of acetylsalicylic acid. The blood was collected from a healthy human volunteer who had not taken any $NSAID_S$ for 2 weeks prior to the experiment and collected in heparinized vacutainer. The blood was washed three times with 0.9% saline and centrifuged simultaneously for 10 minutes at 3000 rpm. The packed cells were washed with 0.9% saline, and a 40% v/v suspension made using isotonic

phosphate buffer composed of 154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4 used as Stock erythrocyte or RBC suspension. Membrane stabilization test was done according to the method described [16] with slight modifications. The test sample consisted of stock erythrocyte (RBC) suspension 0.030 mL mixed with 5mL of hypotonic solution (154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4) containing P. dactylifera extract ranging from concentration 100-500 µg/mL. The control sample consisted of 0.030 mL RBC suspension mixed with hypotonic buffered solution alone. The standard drug acetylsalicylic was treated similarly to test at 100-500 µg/mL concentrations. The mixtures were incubated at 10 minutes at room temperature, centrifuged for 10 minutes at 3000 rpm, and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated by the following equation.

Where;
A_1=Absorbance of hypotonic buffered solution alone
A_2= Absorbance of test /standard sample in hypotonic solution.

Antimicrobial Activity

The agar well diffusion method was used for the antimicrobial test. The strains used for the present study were Escherichia coli, Staphyloccocus aureus, and Candida albicans. Mueller Hilton agar and dextrose medium were prepared according to manufacturer's specification. The media were autoclaved and dispensed into sterile petri-dishes and allowed to solidify. Inoculum of each isolate was streaked on the agar plate. Four wells of 6mm each were made in each plate using a sterile cork borer. The wells were filled with 0.1 mL of different concentrations (500 µg/mL, 1000 µg/mL) of the extract with the aid of sterile pipettes per well. Diameters of zones of inhibition were measured after 24 hours of incubation at 37°C.

RESULTS AND DISCUSSION

Phytochemical Screening

Phoenix dactylifea (date) fruit powder was extracted with methanol and water and analyzed for their phytochemical content. Preliminary phytochemical analysis of both extracts (aqueous and methanol) are shown in Table. 1. Screening of phytochemicals of P. dactylifea showed the availability of natural compounds such as alkaloid, flavonoid, anthraquinone, carbohydrates, saponin, steroid, and tannin by both the aqueous and methanol extracts.

Table.1. Preliminary phytochemical screening of P. dactylifea

Phytochemical Test	Ethanol Extract	Aqueous Extract
Carbohydrate	+	+
Flavonoids	+	+
Saponin	+	+
Alkaloid	+	+
Cardiac Glycosides	+	+
Steroid	+	+
Anthraquinone	+	+
Tannins	+	+

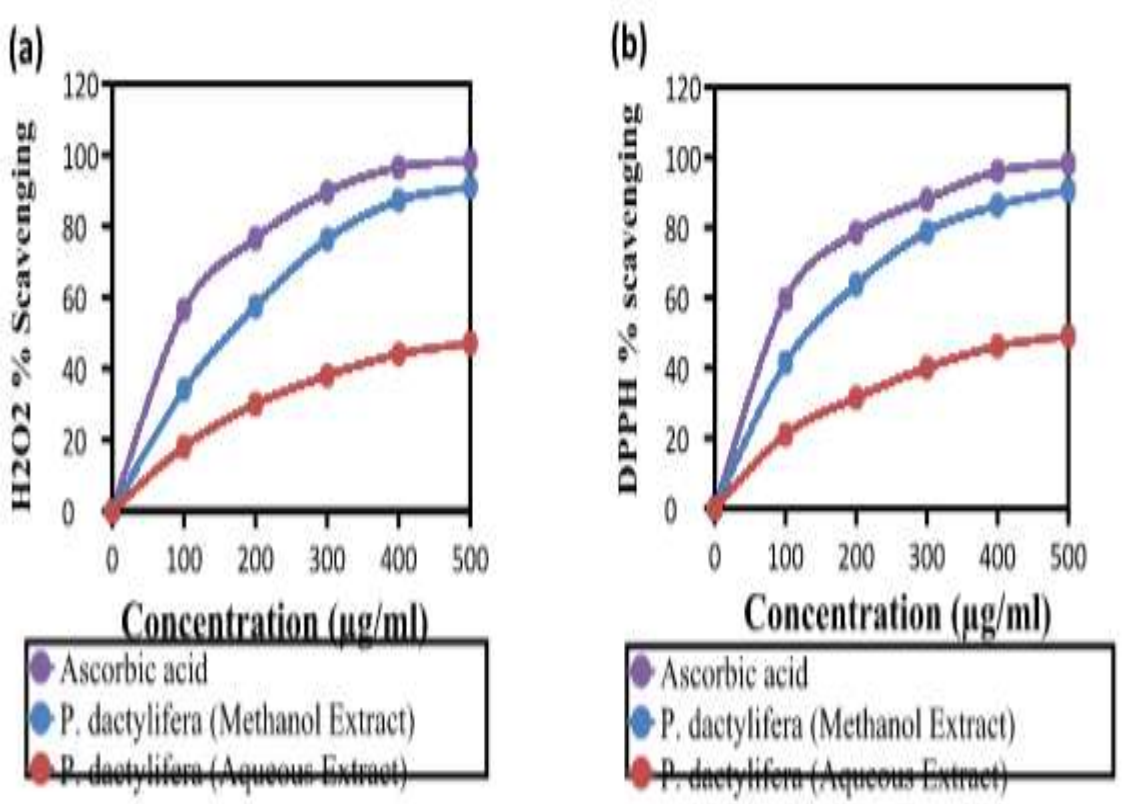
Antioxidant Assay

Three different methods and modifications have been used to measure antioxidant activity. A single method is not enough to assay the whole antioxidant activity; therefore, application and combination of several tests recommended providing a better description. The scavenging activity of both extracts were expressed as % inhibition and was compared with standard antioxidant as ascorbic acid as shown in Figure. 1. As shown in Figure. 1, concentration of both extract samples increases the % inhibition as the concentration of sample increases. It indicates the hydrogen donating ability of the sample antioxidants such as phenolic compounds. Among the three-antioxidant activity assay, the methanol extract of P. dactylifea showed more

% inhibition than that of aqueous extract. Antioxidant activity is recognized due to the wide range of phenolic compounds present in dates, including p-coumaric, ferulic, sinapic acids, flavonoids, and procyanidins [17].

Anti-inflammatory Activity

Anti-inflammatory activity of P. dactylifea was analyzed by HRBC (Human Red Blood Cells) membrane stabilization assay. Study was conducted for both the methanolic and aqueous extract at concentration range of 100-500 µg/mL. In this assay, acetylsalicylic acid was considered standard. As shown in Figure. 2, with increase in the concentration inhibition percentage of hemolysis also increases. The results were comparable with the reference drug, which was also subjected to the same treatment. These results showed the effective bioactivity of P. dactylifea for the pharmaceutical usage as anti-inflammatory drugs. For the methanolic extract highest inhibition percentage of hemolysis of 49% was observed at 500 µg/mL concentration, while for the same concentration of aqueous extract highest inhibition percentage of hemolysis was 23%. Acetylsalicylic acid showed maximum inhibition tendency of hemolysis 87.2% at a 500 µg/mL concentration. The lysosomal enzymes released during inflammation generate different disorders. The extracellular activity of these enzymes is responsible for acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or stabilizing the membrane. Since RBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity-induced RBC membrane lysis is taken as a measure of anti-inflammatory activity of drug. Compounds with membrane-stabilizing properties are well known for their ability to interfere with the early phase of inflammatory reactions, namely the prevention of the release of phospholipases that trigger the formation of inflammatory mediators. The extract of this plant demonstrates significant membrane-stabilizing properties, which suggests that extract of P. dactylifea may offer some beneficial effects in the management of inflammatory conditions.



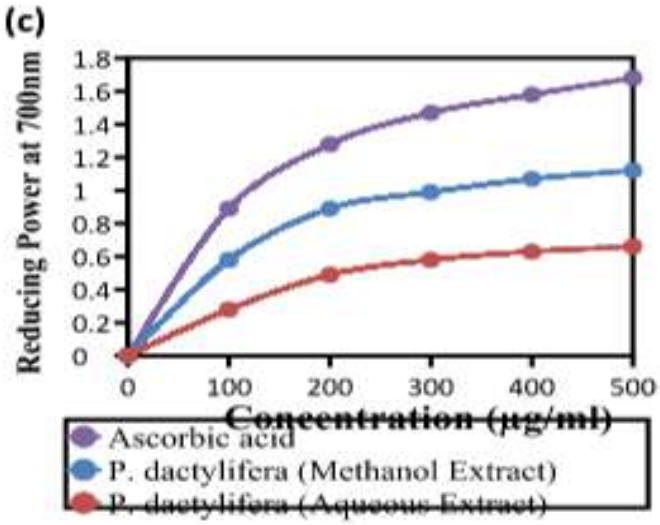


Figure. 1. Antioxidant activities of P. dactylifea extracts: (a) DPPH free radical scavenging activity; (b) hydrogen peroxide scavenging activity; (c) reducing power

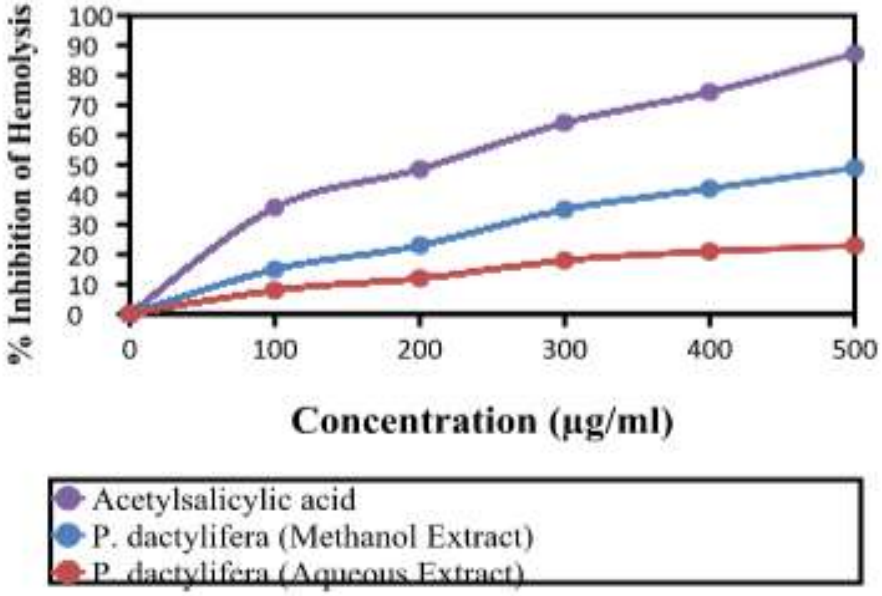


Figure. 2. Anti-inflammatory activities of P. dactylifea extracts

Antimicrobial Activity

The results of the antimicrobial activity of P. dactylifea are shown in Table. 2. From the result, highest zone of inhibition was observed in methanolic extract for E. coli. As shown in Table. 2, P. dactylifea extracts contain significant antibacterial activity against gram-negative bacteria like Escherichia coli. It was also effective against gram-positive bacteria like Staphylococcus aureus and fungal species like Candida albicans. In the present study, P. dactylifera extract has antibacterial effect against E. coli, indicating that this extract can be used for treating enteric diseases.

Table. 2. Antimicrobial activities of P. dactylifea extracts

Culture	Concentration (µg/mL)	Zone of inhibition (in mm)	
		Ethanol Extract	Aqueous Extract
E. coli	500	18	12
	1000	20	16
S.aureus	500	16	10
	1000	19	17
C.albicans	500	13	8
	1000	19	15

CONCLUSION

Our present investigation concluded that P. dactylifera is a medicinal plant with enormous biological activities. This is due to the potential constituents like flavonoids, terpenoids, alkaloids, saponins, and steroids. P. dactylifera demonstrated high phytochemical content and are potent antioxidants and could be used as a supplement to prevent oxidative stress. Phytochemical analysis proved the availability of natural chemical constituents. The anti-inflammatory assays carried out on the fruit extracts indicate that the plant is an excellent source of anti-inflammatory drugs. The P. dactylifera was also found to be a potential antimicrobial agent. Dates fruits in the control of disease create optimism towards the novel therapeutic strategy. Keeping all information in hand as antioxidant, anti-inflammatory, and antimicrobial, further research based on clinical trial is required to authenticate the exact mechanism of P. dactylifera and their constituents in disease prevention.

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