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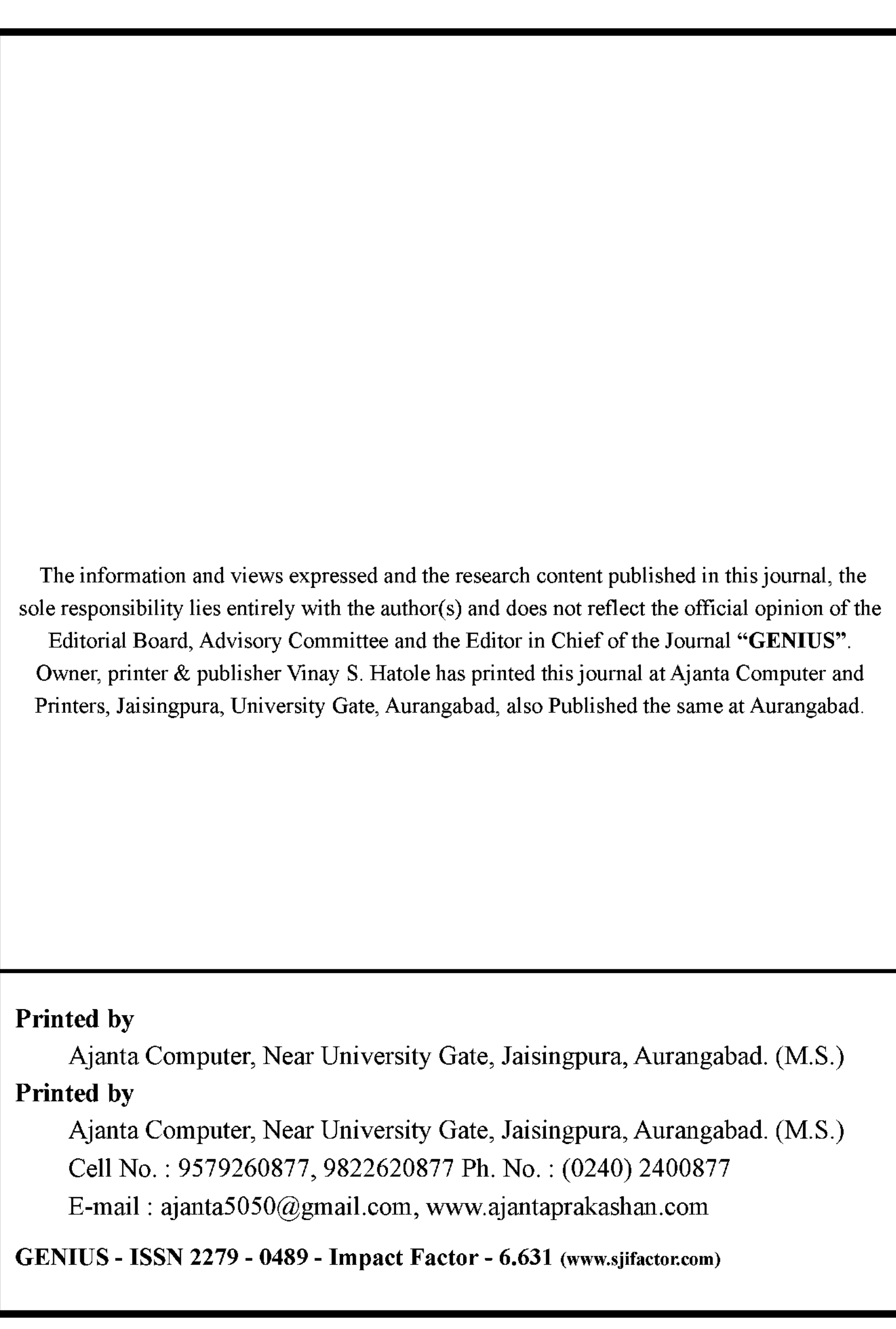

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1. Estimation of Antibacterial Properties in Hair Dyes

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Abstract

Hennotannic acid also known as lawsone is found in Herbal dyes which reserves hair with its antibacterial properties. The hydrogen peroxide, Paraphenylenediamine (PPD) and parabens present in Non-herbal hair dyes are hazardous for hair scalp. The method that was used to estimate its antibacterial properties was the cork borer well diffusion method. This experiment focuses on comparing both the hair dyes and estimate its growth against the two most common microbes found on the skin i.e. Klebsiella pneumoniae and Escherichia coli. These two bacteria mainly grow where there is moisture and can multiply rapidly. The samples were loaded by 1% v/v dilution in the well following the incubation for 24hrs. The growth was observed and which evidently showed that Herbal hair dyes had antibacterial properties with visible zone of inhibition whereas Non-herbal hair dye had minimal visibility concluding that antibacterial properties in non-herbal hair dyes are not safe enough for hair cuticles and can lead to hair shafting.

Keywords - Hair dyes, Antibacterial, Grey hair, Hair Damage, Scalp.

1. Introduction

Nowadays people are very conscious with their physical appearance and in which hairs play an important role. Hair styling and colouring is very common and flushes out a lot of penny from our pocket. But does that actually do any good after spending a fortune? Just to know your hair is damaged and completely destroyed. There are many hair products in the market that help in maintaining and nourishing our hair even after using harmful chemicals. Some are grooving

products while some are actually healthy products. Many factors influence the growth and density of hairs such as lifestyle, genes, overuse of chemical products etc. Nowadays, teenagers go through premature greying because of their unhealthy and stressful lifestyle. Hair greying is a real issue between all age group and the concern for the right product can be hectic and confusing. There were time when people followed ayurvedic practices in their daily life and had healthy diet with proper exercise. This can be a reason why people those days had comparatively healthy and voluminous hairs. Today's generation has a hectic schedule where they don't get time to look after their health and leads to an unhealthy lifestyle with overstress.

All this factors can lead to weak hairs. Weak hair follicles and decreasing melanin level can cause hair greying which can be controlled at an early age. Hair nutrition in the scalp is absorbed by the bacteria which in turn can result into dry scalp, dandruff, itching, inflammation etc. All this factors can be major issues resulting into greying of hairs. Choosing the right products to treat hair is very essential and the number of options in the market can put oneself in a loophole. There is no proven method that can reverse the greying of hair and thus the measures that can be taken is to avoid any more greying and maintaining the melanin level of hairs.

When choosing a hair dye, it is important to look up on the composition of each ingredients used in that specific dye and also to avoid any harsh chemicals. Hair dyes with ammonia can pull apart the layer of hairs resulting in hair shafting. Apart from chemicals, a hair dye should also help in cleaning the pre-existing bacteria present on the scalp. Antibacterial hair dyes are the go-to solutions to prevent any extra damages to hair and maintain its nutrition level. This experiment is mainly going to focus the efficiency level between herbal and non-herbal hair dyes reacting with two common bacteria found on the scalp i.e.; Klebsiella and Escherichia Coli. These are 2 are among the most commonly found microbes and which is also easy for laboratorial experiments. Klebsiella pneumoniae is a Gram-negative bacterium which is non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium.

Klebsiella bacteria are normally found in human intestines and also in human stools. It can also be found on the moist part of your skin which leads to skin infection. They are suitable to room temperature and multiply rapidly in numbers. They can survive against the defence of immune system. It can also survive in oxygenic or anoxic conditions. Escherichia coli are Gram-negative rod-shaped bacteria. It is commonly found in intestines of warm blooded animals and also found in the gut of some animals. They are mostly harmless and keep the digestive tract

healthy but it can be harmful in some cases depending on the strain. It can cause food poisoning, pneumonia and urinary tract infections.

E.coli survives in room temperature and multiplies conveniently. Both these bacteria can also be found on hair scalps as it builds up due to the frequent use of chemicals or poor treatment of hair. Dampness on hair for a longer period can result into bacterial growth and it is necessary to get rid of it. The difference between herbal and non-herbal products is quite evident but it doesn't prove that whether the herbal products kills the mildest bacteria on our skin or just preserve hair. The ingredients of beauty products and its composition should be considered precisely before buying a product. It is advisable to know your allergens and then shop for it. This is the common mistake that occurs between customers that they are not aware about their allergies and end up using the ingredients that cause infections or rashes on their body. This experiment thus focuses on identifying the aftercare in both herbal and non-herbal products.

2. Materials and Methods

1.1 Preparation of Dye Samples

Samples were differentiated into 2 categories i.e. Herbal and Non-Herbal. Herbal hair dyes consisted of Garnier and Godrej which is a well-known brand in the market. For Non-Herbal hair dyes, Black rose and Safe 2 shine were used which is made up of henna leaves. 1gm

of each samples were diluted in 10ml of saline solution (1% v/v) to form a semi-liquid consistency.

1.2 Inoculation of Bacteria

Klebsiella Pneumoniae and Escherichia Coli were isolated and inoculated in saline solution and kept in incubator for 1hr to observe a matte growth. After Incubation, the saline suspension was pipetted out from a sterile 10ml pipette and loaded in agar plates. The bacteria was then spreaded equally to alll sides using a sterile spreader.

1.3 Sample Loading

Using quadrant method, wells were made in the agar plates with a sterile cork borer. The quadrants were numbered and the samples were loaded into the wells precisely.

1.4 Observing Growth

The agar plates were kept in incubation for 24hrs. After Incubation, It was observed that Herbal hair dyes showed visible zone of inhibition whereas Non-herbal samples had minimal yet near to negligible zone of inhibition.

Observation

Fig A: Escherichia Coli

Fig A.1: Escherichia coli
(Zone of inhibition)

Fig B: Klebsiella pneumoniae

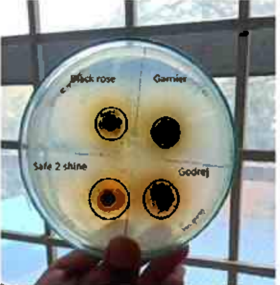
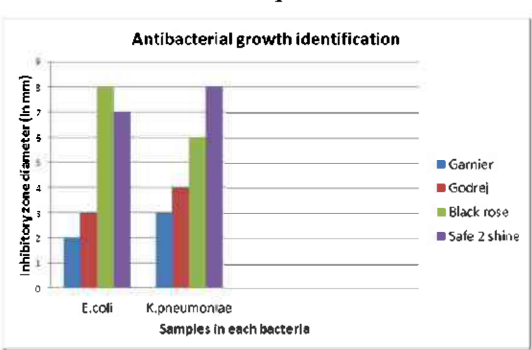


Fig B.1: Klebsiella pneumoniae
(Zone of inhibition)

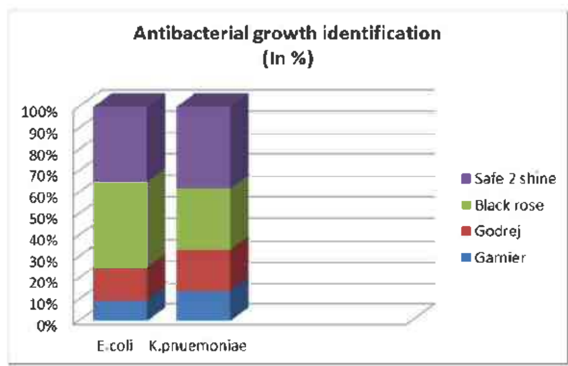
Observation Table

Bacteria	Hair dye samples	Inhibitory zone diameter(In mm)
Escherichia Coli	Safe 2 shine	7mm
	Black rose	8mm
	Godrej	3mm
	Garnier	2mm
Klebsiella pneumoniae	Safe 2 shine	8mm
	Black rose	6mm
	Godrej	4mm
	Garnier	3mm

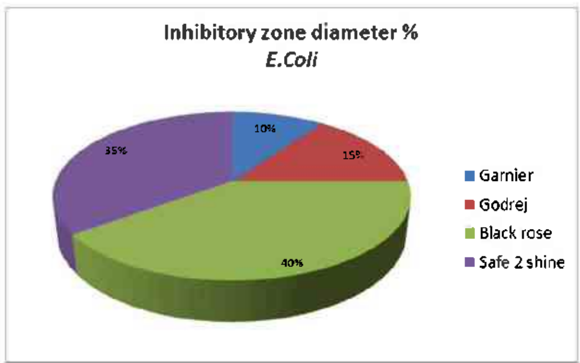
Graph



Bar Diagram



Pie Diagram



Result

After incubation, zone of inhibition were observed around the well of each samples. Herbal hair dyes showed visible zone of inhibition in both the agar plates under the used bacteria wiz Escherichia.coli and Klebsiella pneumoniae. Non-herbal hair dyes showed slight zone of inhibition which was near to negligible.

Comparative Study between E.Coli & K. Pneumoniae

Hair dye samples	E.coli	K.pneumoniae
Garnier	10%	14%
Godrej	15%	19%
Black rose	40%	28%
Safe 2 shine	35%	39%

Conclusion

This experiment concluded stating that herbal hair dyes have antibacterial properties in it and it is much effective than Non-herbal hair dyes. Thus herbal hair dyes are absolutely safe for the hairs and regular use of it can help in strengthening of hair follicles and root firmness.

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2. Comparative Study of Antibacterial Properties in *Azadirachta Indica* (Neem) and *Ocimum Sanctum* (Tulsi) against *Pseudomonas Aeruginosa* and *Klebsiella Pneumoniae*

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Abstract

This research was focused on to compare the antibacterial properties of concentrated sample of traditional herbs used in Ayurveda. With the increasing antibiotic resistance exhibited by microbes, in today's world it has become important to come up with new medicines and vaccines to cure diseases. In current epoch quick results are expected; hence haphazard use of synthetic antimicrobial drugs is copious these days which is now resulting in multiple drug resistance and evidences of serious adverse effects are noted in various studies. Therefore natural herbs are gaining importance in overcoming this problem as the traditional herbs are found to be more economical and having lesser side effects than synthetic drugs. We have chosen Neem (Azadirachta indica) and Tulsi (Ocimum sanctum) leaves to study their antibacterial properties towards antibiotic resistant bacteria's like Pseudomonas aeruginosa and Klebsiella pneumoniae. Two kinds of samples were prepared; (i) concentrated sample of Neem and tulsi leaves ash was prepared using distilled water (iii) concentrated sample of Neem and Tulsi leaves paste was prepared using saline solution. By spreading bacterial inoculum of the above-mentioned microorganisms on the surface of the media, the nutrient agar plates were inoculated. Antibacterial property has been done by Well Diffusion Method; wells were punched in the agar

using a sterile stainless steel cork borer. Both concentrated samples of Neem and Tulsi leaves were allowed to diffuse into the medium. After an incubation period of 24 hours, limited to no zone of inhibition against Pseudomonas aeruginosa and Klebsiella pneumoniae was observed on the plates loaded with Neem and Tulsi leaves ash samples whereas concentrated samples of neem and Tulsi leaves paste have shown antibacterial properties against Pseudomonas aeruginosa and Klebsiella pneumoniae. A. indica and Ocimum sanctum have shown higher antibacterial activity against Klebsiella pneumoniae (indicated by the zone of inhibition) than against Pseudomonas aeruginosa.

Keywords - Antibiotics, antimicrobial resistance, Ayurveda, antibacterial properties, traditional herbs, synthetic drugs.

1. Introduction

A vast range of infectious disease causing microbes (bacteria, viruses, protozoa) have grown resistant to common antimicrobial drugs (antibacterial drugs like antibiotics, antiviral and antiprotozoal drugs). Antimicrobial resistance is a microbes ability to resist the effects of medication that once successfully treated the disease caused by the microbe. Microbes that have grown resistant are difficult to treat as alternative medicines or antimicrobials in higher doses are required. This approach can be fatal to human body in the long run. Environment has played a great role in spreading the resistance mainly including factors related to soil, animal husbandry and waste management. Several researches have proven that human misuse of antimicrobial drugs is one of the most contributing factor towards antimicrobial resistance. Practices like use of over the counter medicine without prescription from a medical practitioner or high doses prescribed by the practitioner. Resistance level in some individuals increases due to irrational use and this is a greater threat at community level. Resistant bacteria's like Enterococcus spp., S. Aureus, K. pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp have been involved in causing infectious diseases in humans. Pseudomonas aeruginosa is an opportunistic pathogen and is a leading cause of morbidity and mortality in cystic fibrosis patients and immunocompromised individuals. Due to its remarkable ability to resist antibiotics eradication of P.aeruginosa has become difficult, they are known to use their high levels of intrinsic and acquired resistance mechanisms to resist most antibiotics. Recently a new mechanism called Adaptive antibiotic resistance in P.aeruginosa has been characterized which leads to recurrence of infection. Klebsiella pneumoniae is an important gram-negative

opportunistic bacterium that causes nosocomial infections, infections in hospitalized or otherwise immunocompromised individuals contributing to substantial morbidity and mortality. K. pneumoniae is showing a high resistance to a wide spectrum of drugs like beta-lactam antibiotics, fluoroquinolones, and aminoglycosides resulting in a growing worldwide problem.

With a 62% increase over the last few decades India has become world's largest Antibiotics consumer. Antibiotics have various modes of action depending on the nature of their composition and their affinity to target sites within the bacterial cells;

i. They inhibit cell wall synthesis in bacteria's, examples: penicillins, bacitracin, cephalosporin and vancomycin.

ii. They inhibit functions of cell membrane but this can be fatal to the human host as well due to the presence of cell membrane in both eukaryotic and prokaryotic organisms, therefore their clinical usage is limited to topical applications, examples: polymyxin B and colistin.

iii. They inhibit synthesis of protein which leads to disturbance in normal metabolism of bacterial cell causing death of organism or inhibition of its multiplication and growth, examples: tetracyclines, chloramphenicol, lincosamides, macrolides.

iv. They inhibit nucleic acid synthesis by binding to components involved in RNA or DNA synthesis, examples: metronidazole, rifampin and quinolones.

As the development of resistance of microbial pathogens against currently available modern antibiotics is becoming widespread, medical science is now taking major steps to develop antibiotics that are novel. Traditional therapeutic methods (those that are used in Ayurveda) are considered to be better alternatives. Approaches used in Ayurveda act on the root cause rather than the symptoms. Ayurveda focuses on decreasing the spread of disease, reducing the consumption of antibiotics by people on community level and proper care after the antibiotic use. One of the most important factor here is strengthening the host body. Natural herbs like Neem (Azadirachta indica) belonging to family Meliaceae and Tulsi (Ocimum sanctum L.) belonging to the family Lamiaceae have been used for medicinal purpose since since antiquity in India. Its twig is used for brushing teeth in rural areas since decades and it is proven to have plaque preventing and several related antibacterial properties. Tulsi leaves are known to possess insecticidal and antibacterial activities. The plant and its extracts are of great use in curing numerous infections and also as a remedy for common cold and cough based on the traditional

experience. Decoctions and oils extracted from Neem leaf are characterized to have exceptional antiseptic and wide spectrum antibacterial activity action against Gram-negative and Gram-positive microorganisms, including M.tuberculosis and Streptomycin resistant strains.

2. Materials and Methods

2.1 Plant leaf Collection

The leaves of Azadirachta indica and Ocimum sanctum were collected from the garden of a housing society. Leaves were manually separated, cleaned and divided into two categories for the preparation of two concentrated samples i.e. (i) concentrated ash sample (ii) concentrated paste sample. One set of both neem and tulsi leaves was air-dried for 3-4 days. Subsequently the leaves were burnt and pulverized separately to a fine powder (ash)through mortar and pestle. The other set was ground separately into thick paste using distilled water through mortar and pestle.

2.2 Preparation of Samples

About 10ml of prepared saline solution (9g NaCl dissolved in 1000ml distilled water) was added to sterile 15ml test tubes.

- For the preparation of concentrated sample of ashes of leaves around 1gm of neem and tulsi leaves ash were taken in two separate watch glasses; to this approximately 1ml of saline solution from the test tube was added using a sterile pipette.
- For the preparation of concentrated sample of leaves paste, in two separate watch glasses neem and tulsi leaves paste was taken to which 1ml of prepared saline solution was added using a sterile pipette.

2.3 Test Organisms and Inoculation of Bacterial Cultures

Two bacterial cultures were used to evaluate the antimicrobial activity: Pseudomonas aeruginosa and Klebsiella pneumoniae. They were isolated and inoculated in saline solution and after an incubation period of 1hr the saline solution was pipetted out through a sterile 10ml pipette. Nutrient agar plates were inoculated with the above-mentioned microorganisms by spreading the bacterial inoculum on the surface of the media in all directions equally using a sterile spreader. Using a sterile cork borer, wells(6mm in diameter) were punched in the agar as per the quadrant method.

2.4 Sample Loading

Prepared concentrated samples of neem and tulsi leaves ash and paste was loaded into the wells in separate petri plates and were incubated for 24hrs at 37°C.

3. Observations

3.1 With concentrated sample of Azadirachta indica and Ocimum sanctum paste, zones of inhibition were observed against Pseudomonas aeruginosa and Klebsiella pneumoniae. For accuracy the zone of inhibition was measured in millimeters using suitable instruments.

Fig.1(a): Azadirachta indica
Zone of inhibition against
Pseudomonas aeruginosa

Fig.1(b)

Fig.2 (a): Ocimum sanctum
Zone of inhibition against
Pseudomonas aeruginosa

Fig.2 (b)

Fig. 3 (a): Azadirachta indica
Zone of inhibition against

Fig. 3 (b)

Klebsiella pneumoniae

Fig. 4 (a): Ocimum sanctum **Fig. 4 (b)**

Zone of inhibition against

Klebsiella pneumonia

Observation Table

d1 = diameter 1 and d2 = diameter 2

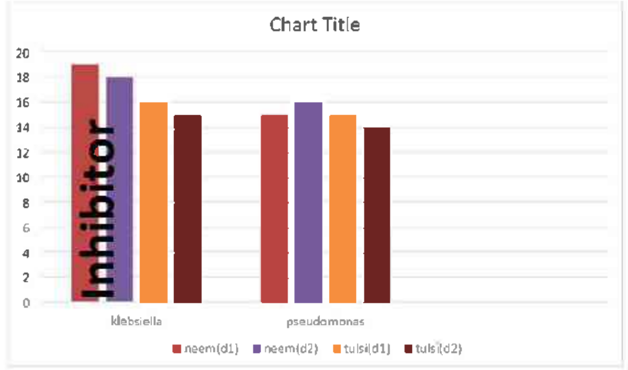
Bacteria	Leaf Sample	Inhibitory zone diameter (in mm)
Klebsiella pneumoniae	Neem (d1)	19mm
	Neem (d2)	18mm
	Tulsi (d1)	16mm
	Tulsi (d2)	15mm
Pseudomonas aeruginosa	Neem (d1)	15mm
	Neem (d2)	16mm
	Tulsi (d1)	15mm
	Tulsi (d2)	14mm

Graph

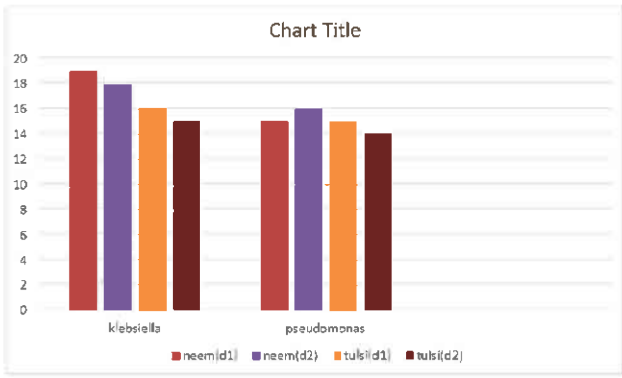
The graph was mounted based on the observations and values obtained from the antimicrobial test.

Antimicrobial Growth Identification

Inhibitory zone diameter Inhibitory zone diameter

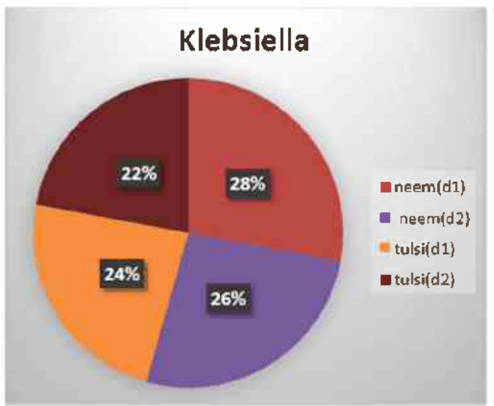


Bacterial growth in each Concentrated Samples Bar diagram



Antibacterial Growth Identification

Pie Chart



Inhibitory Zone Diameter

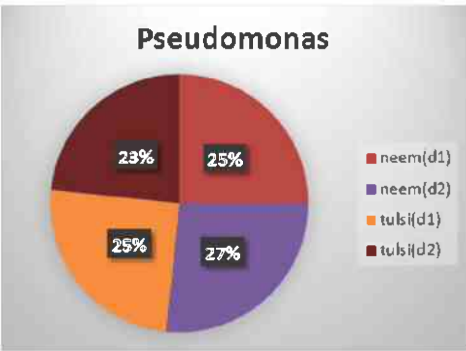
3.2 No zone of inhibition was observed on the plates loaded with concentrated sample of Azadirachta indica and Ocimum sanctum ash against Pseudomonas aeruginosa and Klebsiella pneumoniae.

No zone of inhibition observed against Klebsiella pneumoniae (Top 2 quadrants- neem leaves ash, Bottom 2 quadrants- tulsi leaves ash)

No zone of inhibition observed against Pseudomonas aeruginosa (Top 2 quadrants- neem leaves ash, bottom 2 quadrants- tulsi leaves ash)

Result and Discussion

A large number of people in low-income communities cannot afford soap hence use ash or soil instead for disinfecting their hands or utensils. Various studies have shown that Ash or soil is more effective than water alone. Ash was found as effective against bacteria as soap in short-term experiments with bacterial contaminated hands in Bangladesh and India. Ash is also not only used for cleansing but is also used as a disinfecting agent (alkaline) in certain cases it is recommended to use ash or sand as alternative to soap when needed, e.g. after emergencies. However, based on the results (no zone of inhibition against both the bacterias) obtained in this research it can be concluded that bacteria's like Pseudomonas aeruginosa and Klebsiella pneumoniae are resistant to the antibacterial properties of ashes of Azadirachta indica and Ocimum sanctum.



Comparative Analysis of Antibacterial Properties of Azadirachta indica and Ocimum Sanctum against Pseudomonas Aeruginosa and Klebsiella Pneumoniae

Bacteria	Leaf sample	Growth (in percentage)
Klebsiella pneumoniae	Neem (d1)	28%
	Neem (d2)	26%
	Tulsi (d1)	24%
	Tulsi (d2)	22%
Pseudomonas aeruginosa	Neem (d1)	25%
	Neem (d2)	27%
	Tulsi (d1)	25%
	Tulsi (d2)	23%

Impressive zones of inhibition were observed against both the bacteria's. Azadirachta indica showed a bigger zone of inhibition against Klebsiella pneumoniae (diameter 1= 19mm,

diameter 2= 18mm) than against Pseudomonas aaeruginosa (diameter 1= 15mm, diameter = 16mm) whereas Ocimum sanctum has proven to be more effective against Pseudomonas aeruginosa than Klebsiella pneumoniae (based on the pie chart drawn). Another conclusion that can be drawn from this research is that Azadirachta indica is more antibacterial against antibiotic resistant bacteria's like Pseudomonas aeruginosa and Klebsiella pneumoniae. Due to the overuse of antibiotics all over the world, bacteria's like Pseudomonas aeruginosa and Klebsiella pneumoniae have developed mechanisms resulting in resistance to antibacterial drugs and medicines. Due to factors such as evolution, adaptation and overuse of synthetic drugs against bacteria's over a long period of time, there has been an enormous increase in antibiotic resistance among various bacterias. Consequently, the need of developing potent yet safe antimicrobial drugs against the antibiotic resistant bacteria is arising and due to the rich plant diversity and phytochemicals containing secondary metabolites having antimicrobial activity, it can act as a great alternative for synthetic drugs prescribed in allopathy due to its inexpensive cost and comparatively fewer side effects. We can use these plants as a special formulation for preventing and curing various infections and diseases. Plant formulations created synthetically in labs can be used in food and pharmaceutical industry effectively.

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3. Estimations of Anti-Bacterial Properties in Paint and Pen

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Abstract

The wall is one place that will allow the insects and diseases to stay around because the environment is not clean on the outside. It can be quite difficult to get rid of them if you do not take care of the conditions. This is something that must be taken care of and there are several ways by which you can get this done. It is of utmost importance that you paint your home with anti-bacterial and anti-fungal paints. Pens are something that we come across every single day. Although they are quite useful, they don't get nearly as much attention as they deserve. Most pens studied had been shown to have multiple colonies of bacteria, and all were considered fomites, or inanimate objects capable of transmitting germs or viruses between people. Thus we must make a use of anti-bacterial pen This experiment focuses on weather paint and pen estimate its growth against the three most common microbes found on the skin i.e. *Escherichia coli, Staphylococcus aureus and aspergillus Niger*. These three bacteria mainly grow where there is moisture and can multiply rapidly. Thus, this experiment works on estimation of anti-bacterial properties in it. The two most recommended methods for this experiment were the disc diffusion method and cork borer method. The method that is used for this experiment is the cork borer agar well diffusion method. This method was used so that the sample that was loaded in the well reacts well with sample and show expected results. The samples were diffused in the well and incubated for 24 hours to observe the zone of inhibition that forms around the samples

Keywords - Paints, Pen, Anti-bacterial, Infection.

1. Introduction

Paint

For many thousands of years, paint has been used to decorate the spaces we live, work and play in. This, combined with the increased prevalence of antibiotic resistance and reports that cleaning agents are having a reduced effect on microbial colonisation, is why leading global brands are looking to source effective antimicrobial paint additives that can be integrated into their paint products. A growing concern for cleanliness in various environments has led to the continued rapid growth of this industry. According to a report by Grand View Research, the global antimicrobial coatings market was valued at $2.44 billion in 2015. Articles within hygiene-critical environments such as hospitals, schools, care homes and food production facilities are known to harbour pathogenic bacteria and other microbes for extended periods of time Antimicrobial paint additives are specific actives that can be introduced into a paint, coating, ink or lacquer during the manufacturing process to make it resistant to microbes.

Pen

Bacteria can thrive and survive on surfaces for many weeks, if not months. Your favourite pen is no exception. Used throughout the day, it gets placed on many different surfaces, we tuck them behind our ears, use them to pin up our hair and we may even chew them thoughtfully whilst we seek inspiration. Each time transferring bacteria to the pen and vice-verse. These can include worktops, door handles and yes you guessed it even pens. An antibacterial pen suppresses the growth and survival of bacteria on its surface. Thus preventing contamination been passed on from person to person. Antibacterial Pens are commonplace in the food manufacturing industry but they are also ideal for use in places where there is a higher risk of cross-infection. At the moment places such as care homes, doctors' offices, kitchens, hospitals,

and reception areas don't tend to have them as they use cheap stick pens or printed promotional pens. But there is a case that there may be some benefit if they were to switch to antibacterial pens,

The key feature of the Ant-Bac is an anti-bacterial additive embedded in the plastic of the pen. The additive remains inside the pen–it is not coated or lacquered–and is effective for 100% of the pen's lifetime. As soon as the pen comes into contact with bacteria, it begins a process that essentially destroys the bacteria through starvation, protecting and cleaning the pen's handlers from potentially threatening bacteria. Consequently, the pen re-cleans itself, and has thus been dubbed the "The Living Pen" by its creators. "Introduced during the manufacturing process, our antimicrobial additives exert, by either chemical or mechanical means, a negative effect on any contaminating microbes causing them to die." This is achieved by protein damage, cell membrane damage, Oxidative damage, and DNA interference.

Bacteria

Escherichia coli and Staphylococcus aureus are a serious cause of a variety of community- and hospital-acquired infections. Escherichia coli is one of the most common nosocomial pathogens that cause urinary tract infections (UTIs) (Miri et al., 2017)and Staphylococcus aureus is also an etiological infection agent responsible for significant levels of morbidity and mortality. According to Broad Institute. (2010), Escherichia coli accounts for 17.3% of clinical infections requiring hospitalization and is the second most common source of infection behind Staphylococcus aureus (18.8%) (Di Pinto et al., 2005). In recent years, the emergence of resistant Staphylococcus aureus and resistant Escherichia coli strain to many antibiotics has been observed worldwide. The filamentous fungus Aspergillus Niger is one of the main contaminants of the International Space Station (ISS). It forms highly pigmented, airborne spores that have thick cell walls and low metabolic activity, enabling them to withstand harsh conditions and colonize spacecraft surface (Chancellor et al., 2018)

2. Method and Materials

2.1 Inoculation of Bacteria

Escherichia coli, Staphylococcus aureus and Aspergillus Niger were isolated and inoculated in saline solution and kept in incubator for 1 hr to observe a matte growth. After

Incubation, the saline suspension was pipetted out from sterile 10 ml pipette and loaded in agar plates. The bacteria was then spread equally to all sides using a sterile spreader

2.2 Sample Loading

Using quadrant method, wells were made in the agar plates with a sterile cork borer. The quadrant were numbered and the sample were loaded into the precisely

2.3 Observing Growth

The agar plates were kept in incubation for 24 hrs. After Incubation, it was observed that Ancient Royal Paint showed visible zone of inhibition whereas in Luxor Anti-microbial Pen had minimal in two of pathogens and yet near to negligible zone of inhibition in one of the micro-organism

Sample

1. Royale Health Shield Anti-bacterial Paint
2. Luxor Antimicrobial Pen

Method

Day 1

1. Pour autoclaved nutrient agar in three petri plates in aseptic zone and let it aside to cool for 20-30 minute
2. Make a quadrant on the base of the petri plates as it solidifies
3. Pipette out 1ml saline suspension of Escherichia Coli, Staphylococcus Aureus, Aspergillus Niger
4. Add 1ml of Escherichia Coli on one agar plate and 1ml of Staphylococcus Aureus on second agar plate and 1 ml of Aspergillus Niger on another agar plate in aseptic zone
5. Sterile the spreader with alcohol and spread the bacteria on agar plate equally
6. Let it aside for 1hr for the bacteria to settle
7. After 1hr,sterile the cork borer and make wells in each quadrant
8. Take 0.5gm of each sample in spatula and load it inside the well
9. Incubate the agar plate for 24hrs

Day 2

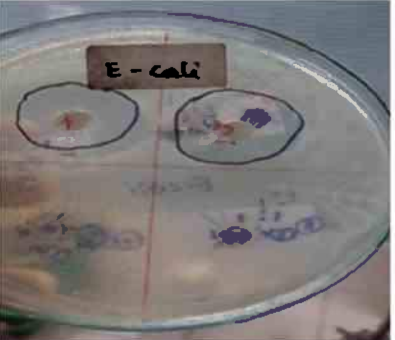
1. Measure the diameter of zone of the inhibition given by both the sample.
2. Plot the standard graph of the zone of inhibition (mm)

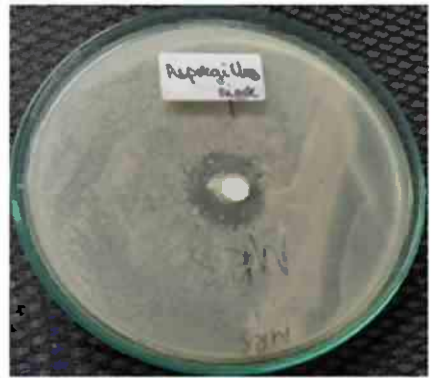
3. From this, we found the effect of the paint and pen on the micro-organism

3. Obsrvation Table

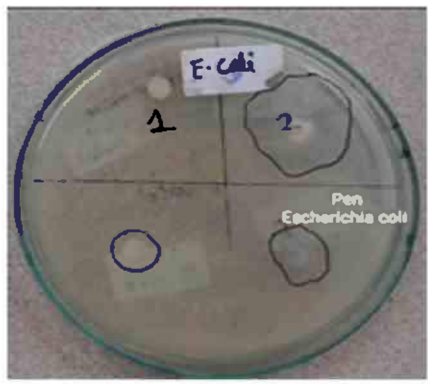
SAMPLE	BACTERIA	INHIBITORY ZONE DIAMETER (IN mm)
ANCIENT ROYALE PAINT	Escherichia coli	20mm
	Staphylococcus aureus	16mm
	Aspergillus Niger	5mm
LUXOR ANTI MICROBIAL PEN	Escherichia coli	3mm
	Staphylococcus aureus	2mm
	Aspergillus Niger	-

Observation **Paint**

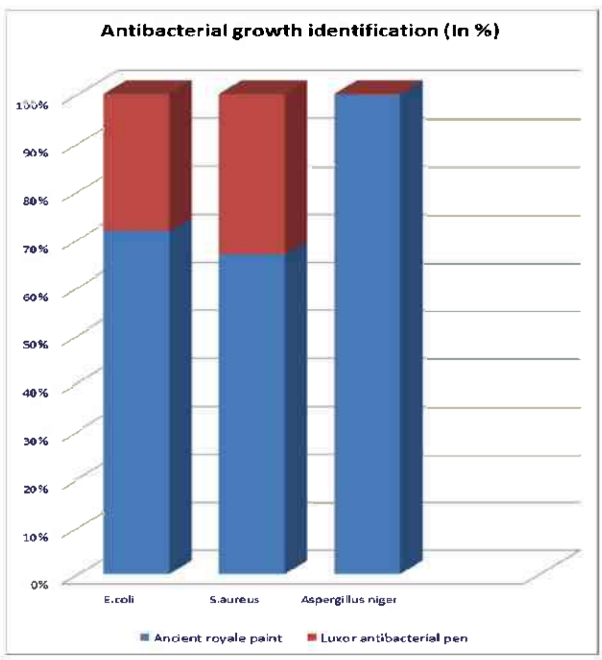


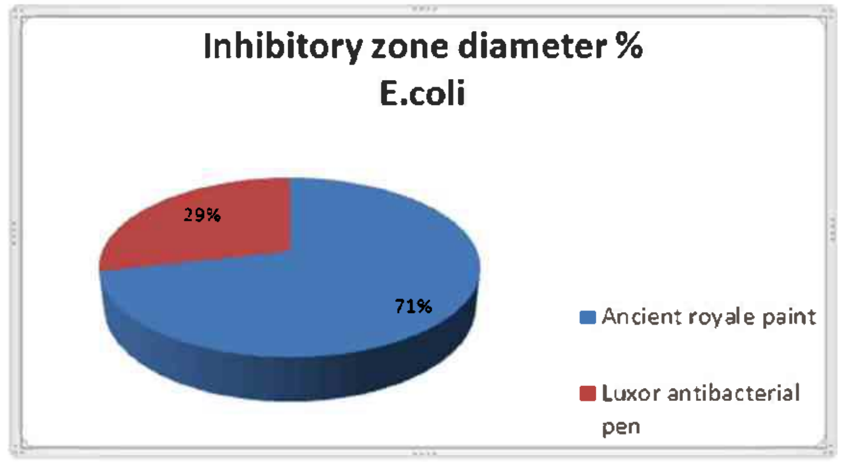


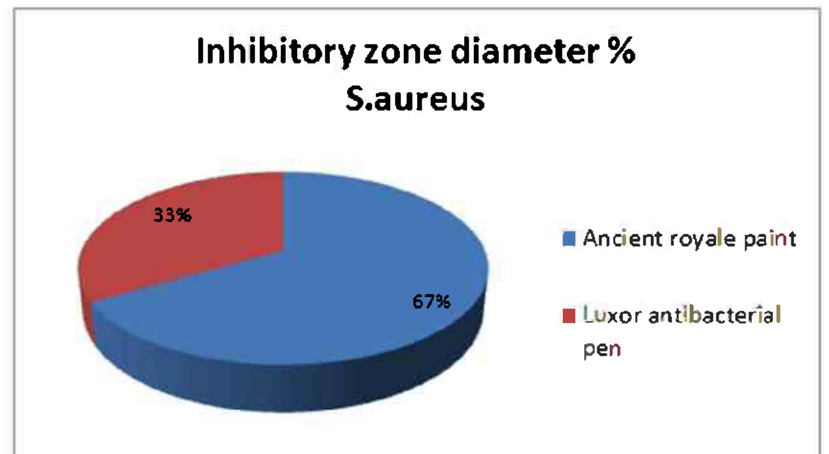
Pen

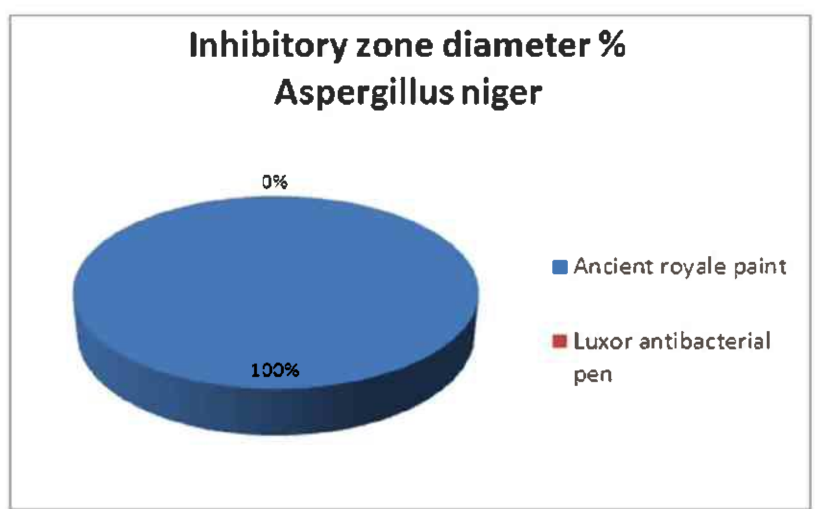


Graph




Inhibitory zone diameter %
E.coli
29%
71%
Ancient royale paint
Luxor antibacterial pen


Inhibitory zone diameter %
S.aureus
33%
67%
Ancient royale paint
Luxor antibacterial pen


Inhibitory zone diameter %
Aspergillus niger
0%
100%
Ancient royale paint
Luxor antibacterial pen

4. Result

After incubation, a zone of inhibition was observed around the well of each sample. Ancient Royal Health Shield Anti-Bacteria Paint showed amazing effect as compared to Luxor Anti-Microbial Pen showed a visible zone of inhibition in the agar plates under the used bacteria wiz Escherichia coli, Staphylococcus aureus and Aspergillus Niger. Luxor Pen showed limited zone of inhibition in bacteria Escherichia coli, Staphylococcus aureus whereas in Aspergillus Niger showed near to negligible zone of inhibition.

5. Conclusion

While concluding we can say that the famous brand ANCIENT ROYAL PAINT showed great zone of inhibition against all the three bacteria, hence it won't be false to say it is the best anti-bacterial paint among the rest.

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4. Identification of Bacteria in Human Earwax against *Escherichia Coli, Klebsiella Pneumoniae and Staphylococcus Aureus*

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Abstract

This topic was selected to identify the antibacterial properties of human earwax sample and to know the bacteria present in the earwax. Earwax is the common thing in all age groups. Different age groups have different types of earwax. Excess of earwax can lead to major Otitis problems. Having earwax has it own advantages and disadvantages. If anyone is having discharge from the ear, he/she should consult immediately to the doctor. Earwax can only be a problem when it can cause hearing impairment or through other symptoms. Cerumen forms naturally and separates itself from the skin of the ear canal as it can lead to ear damage. For this research paper, we are highlighting and identifying the effectiveness of *Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus* on the earwax sample which is the cork borer agar well diffusion method. The sample of the earwax was diffused in the well and incubated for 24 hours to observe the zone of inhibition that forms around the earwax sample. The material that was used was the human earwax to perform this experiment. This sample was tested on *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* which are the most commonly found microbes.

The ear canal is lined with hair follicles. Building up of earwax can block the ear canal. Blockage of earwax is one of the most general reason of hearing loss. In some people, the glands produce more wax which can be removed easily from the ears. Earwax is pretty important for

ears as it is produced by itself to protect the ears. Our diet can also affect wax build up. Gluten, dairy products, sweet foods, caffeine can also cause earwax buildup. Unhealthy diet can cause excessive buildup. Earwax safeguards our ears.

Key - Words - HUMAN EARWAX, CERUMEN, OTOSCOPE.

Inroduction

Earwax is also known as CERUMEN, it is a waxy substance secreted in the ear canal of human. It helps in cleaning and provides protection against bacteria and fungi. It consists of dead skin cells. It is a secretion of cerumen by ceruminous and sebaceous glands. The constituents of earwax are

1. Long chain fatty acids
2. Squalene
3. Alcohol
4. Cholesterol

Earwax causes a problem only if it starts showing ear related symptoms. The Japanese researchers, led by Koh-ichiro Yoshiura of Nagasaki university. The wet form of earwax is very common in Africa and in Europe.

The Wax is Classified Into Dry and Wet Types

- **DRY WAX-** It lacks of ceruminous secretion. This type of earwax comes out in flakes. Dry earwax is not only due to genetics. It can be a sign of health condition like Eczema.
- **WET WAX-** It consists of sticky earwax. This is because of a mutation of a gene. Wet ears typically means infection. Wet earwax has more lipids, which makes it sticky and thick.

Bacteria such as *aeruginosa Staphylococcus epidermis, Pseudomonas, Corynebacterium ,Staphylococcus aureus Escherichia coli and Klebsiella* pneumoniae are present in the earwax. Ear infections are usually caused by bacteria like Haemophilus influenzae and Streptococcus pneumoniae. They can also lead to middle ear infection.

Building up of earwax can happen to anyone as it occurs naturally and it acts as a filter. It is present in 5% in adults and 10% in children and children usually have softer and lighter colored earwax. A special instrument named “OTOSCOPE”. A special instrument is used to check whether the earwax is present or not. Earwax has an antibacterial property which is designed for protecting the ears.

Earwax acts as a protective shield between the external world and the eardrum. Earwax is acidic, it protects the tissues of the ear. Inner lining of the ear canal contains fine hairs called "CILIA".

Cerumen contains esters which have long aliphatic 4 chains of carbon molecules. If there is no earwax the possibility of ear infection would be less. It has been advised that cerumen is unable to prevent ear infection. On the other side, it has been said that cerumen may have antibacterial properties.

Earwax is very common out of all the substances that our body excretes and one should not be feel hasty to remove it. Though it is unpleasant it's important part of good health. It acts as a moisturizer and protects the ear canal. Earwax has it's own benefits. It works as a natural antibiotic. Stress can increase the production of earwax. Excessive earwax can be due to shape of individuals ears, water buildup, high amount of ear hair. The biggest culprit can also be constant use of headphones. It can also cause loss of hearing.

Escherichia coli- Escherichia coli is gram negative, rod-shaped, coliform bacterium of the genus. Escherichia that is found in the lower intestine of warm-blooded organisms. Some cause infection like urinary tract infection, pneumonia and other diseases. Viral infections that activate ear infection can also spread from one person to another.

Klebsiella pneumoniae- It is genus of gram negative, rod-shaped bacteria with polysaccharide based capsule. Klebsiella spp. are found almost everywhere in the nature. Recently, it was found that a 47 year old diabetic man with a swollen obliterated external canal with granulation tissue on the right ear. Later, it was proved that it was necrotizing external otitis caused by *Klebsiella pneumoniae.* Otherwise, it is mostly attributed to *Pseudomonas aeruginosa. Klebsiella pneumonia* was first discovered in 1882 by a German microbiologist and pathologist Carl Friedlander.

Staphylococcus aureus- This bacteria was discovered by Alexander Ogstan in 1880. *Staphylococcus aureus* is a gram positive, round shaped bacterium. It is a membrane of microbiota of body. It is found in upper respiratory tract on skin. This bacteria are very adaptable. It has quick micro-evolutionary rates. It can cause various soft tissue infection. *Staphylococcus aureus* is also responsible for food poisoning. *Staphylococcus aureus* infection in ears occurs when excess water in ear canal makes it's way at possible environment for *Staphylococcus aureus* bacteria to grow. Humid weather can also cause this bacteria to grow.

Staphylococcus aureus in ears can be treated by doctors consultation. Doctor's may suggest certain oral antibiotics or eardrops.

Fig- Human Earwax Sample

Color Classification of Earwax

Brown and Black

It specifies that the earwax is old and it contains dirt in which bacteria is confined in it. People need to clean their ears regularly to avoid overproduction of oxidized earwax. Elderly people usually have this kind of earwax.

Red Earwax

This indicates the sign of bleeding. There are multiple reasons for blood in earwax. It can further lead to serious infection head trauma and several eardrum problems. If this continues further one should immediately consult a doctor

Yellow Orange Earwax

It is a genetic trait, it is normally wet which is a sign of a healthy earwax. It sometimes includes earaches and itchiness. The earwax is not stale.

White Yellow Green Earwax

This white earwax designates that there is pus formation in your ear canal. Pus in ear indicates that one is suffering from serious ear infection and should immediately consult a doctor to avoid further harm.

White and Grey

This earwax comes out as flakes and it is the most common cause of white earwax. In some cases it could be a sign of atopic dermatitis. It causes pain and inflammation in ears. It piles up the dust particles which causes pain in the ear.

Causes

1. Unnecessary cleaning of ear.
2. Deafness.
3. Do not use pointed things such as bobby pin stick finger tips rolled napkin corner safety pin etc. for cleaning ear canal as it can cause cuts.
4. Do not insert cotton swabs directly into the ear.

Prevention

1. Earwax cannot be prevented it has been debarred to protect ear from dirt. If there is building up of wax often then it may lead to irritation or partial hearing.
2. instead use a dropper by applying baby oil /glycerin or gently was it with a soap or water.
3. If anyone has eardrum problem doctors don not recommend them to use eardrops.
4. Carbamide peroxide (Debrox earwax removal kit), Murine earwax, tea tree so sensitive ear cleaner, Soliwax eardrops, Ceflox dee optic are the types of earwax used for cleaning ears.
5. These drops are usually used for sensitive skin.
6. Fourderm cream is the cream that is used to treat ear infections. It reduces redness swelling of the ear. It also inhibits the growth of bacteria.

Otoscope

1. It is a device which is used to check the ears.
2. It is used to look over ear problems.

3. It gives an overview of the ear canal
4. It is used by doctor during regular checkup who is suffering from ear infected problems.
5. It is used to study the ear anatomy.

Symtomps

1. Earache
2. Difficulty in hearing
3. Tinnitus
4. Itchiness in the ear
5. Dizziness
6. Cold and cough.

Materials and Method

The sample that we used was the Human earwax. Then we dipped the earwax sample into the saline suspension///////// .The Glassware that we used for this research was the Sterile nutrient agar plate(24grams of nutrient agar was taken and 12grams of agar agar was added in it. Then 1ml f distilled water was poured. After mixing it was kept in the boiling water bath) autoclaved 1ml pipette, spreader. The equipment that were used are alcohol, nichrome loop, earbud and cork borer(Cork Borer usually comes In a nested sizes it is use to punch hole in agar plates. It performs certain experiments in microbiology. One should hold it cork precisely in the left hand and place the cork borer in the suggested area of the border. Dip the borer in the Ethanol before placing it on an agar plate. The cleaning process of the borer should be with the nitric acid or chromic acid to clean the dirt. It is use in various chemistry labs, biology labs, microbiology labs molecular biology labs. Agar well diffusion method that was used in this experiment was well diffusion method using Cork borer)Human earwax sample was collected from 20 different individuals, by providing them a sterile earbud. The earwax sample was dipped in the saline suspension in the test tube. *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* were the bacteria used . Pour autoclaved nutrient agar in two plates in aseptic zone and let it cool for 20-30mins. Make quadrant on the basis of the petri plate as it solidifies. Pipette out 1ml saline suspension of *Escherichia coli and Klebsiella pneumoniae and Staphylococcus aureus*. Add 1ml of all the three bacteria's in different sterile nutrient agar plates in aseptic zone. Sterile the spreader with alcohol and spread the bacteria on agar plate equally.

Let it aside for 1hour for the bacteria to settle. After 1hour, sterile the cork borer and make wells in the quadrant. Take 0.5gm of sample in the spatula and load it inside the well. Incubate the agar plates for 24 hours.

After incubation it was observed that zone of inhibition was seen on *Escherichia coli and Klebsiella pneumonia.*

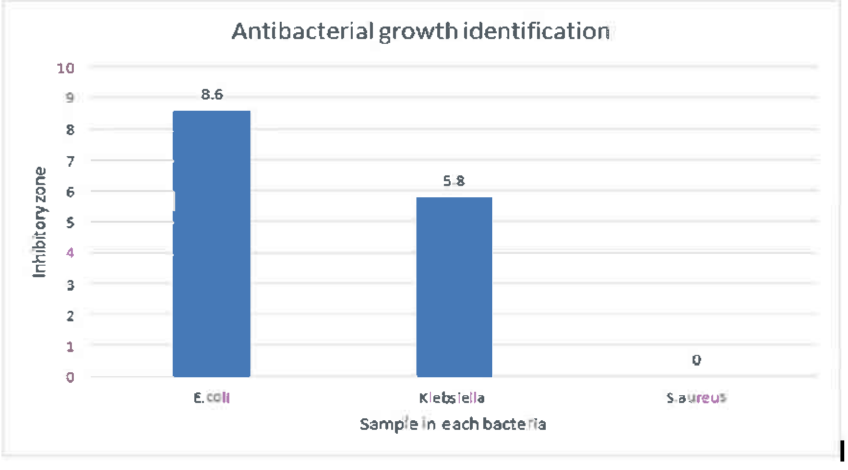
Fig A- Escherichia coli

Fig B- Klebsiella pneumonia

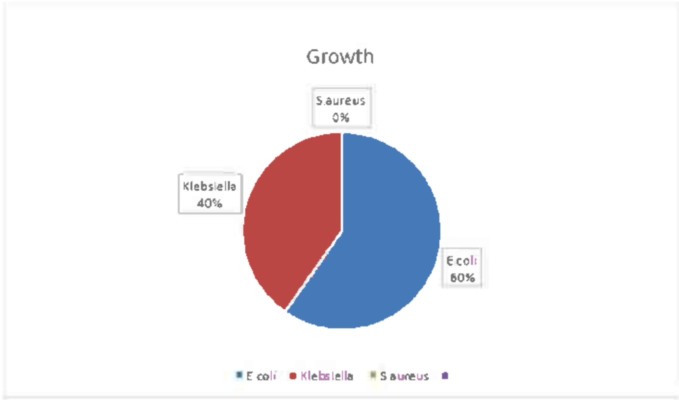
Observation Table

Bacteria	Sample	Inhibitory zone in diameter(in mm)
Escherichia coli	Human earwax	8.6mm
Klebsiella pneumoniae	Human earwax	5.8mm
Staphylococcus aureus	Human earwax	0

Graph



Pie Chart



Result and Discussion

Different earwax was studied and performed accordingly . After incubation, zone of inhibition was observed around the well of the samples. Zone of inhibition was observed in *Escherichia coli and Klebsiella pneumoniae.* The *Escherichia coli* and *Klebsiella pneumoniae* organism was effective against the human earwax sample. *Staphylococcus aureus* showed slight zone of inhibition was nearly negligible.

Sample	*Escherichia coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
Human earwax	60%	40%	0%

Conclusion

This experiment concluded stating that *Escherichia coli* and Klebsiella pneumoniae were effective. *Escherichia coli* showed zone of inhibition which is 8.6 mm in diameter and *Klebsiella pneumoniae* showed zone of inhibition of 5.8 mm.This conclude that ear wax have some antibacterial property against these bacteria.

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5. Estimation of Antimicrobial Properties in Indian Pickle

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ABSTRACT

The research aimed at the study of antimicrobial properties of Indian pickles on the most prevalent pathogens inhabitants in the household. We used the Agar well method and loaded the concentrated sample of pickle both A and B in this experiment. After incubating for 24 hours, we observed an impressive zone of inhibition in S. aureus and a limited area of inhibition in E. coli, Pseudomonas, Aspergillus niger. To obtain a uniform inhibition zone the Agar well method is occasionally beneficial for the assay of viscous materials. The Agar well diffusion method is extensively used to evaluate the antimicrobial activity of pickle extracts. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a certain amount of the desired concentration of antibacterial agent or extract solution is injected into the well. The agar plates are then incubated at the appropriate conditions for the test microorganism. The antimicrobial ingredient diffuses across the agar medium and stops the bacteria strain from growing.

KEYWORD

Pickle, antimicrobial, zone of inhibition, S. aureus, E. coli, Pseudomonas, Aspergillus niger

INTRODUCTION

Historically, pickling is one of the primitive preservation methods of distinct foodstuffs such as vegetables, fruits, fish, and meat. Exclusive and enticing metamorphose in flavor,

texture, and color occurs over time in fermented pickles by the process of pickling. A distinguishing characteristic is a pH of 4.5 or lower, an unexceptional way to kill most bacteria. This speculation focuses on the antimicrobial activities of various types of pickles.

The specimens utilized were sample A (sweet lime pickle) and sample B (mixed pickle)

Sweet lime pickle (A)

Ingredients utilized are sugar powder (50%), lime (40%), edible common salt, cumin, chili powder (1.2%), acidic regulator citric acid (INS 330), preservative sodium benzoate (INS 211) Edible common salt has antimicrobial properties that can annihilate or restrain the growth of organisms like *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens.*

Cumin illustrates vigorous antibacterial activity against bacteria like *Escherichia coli, Staphylococcus aureus, Klebsiella sp., and Pseudomonas aeruginosa and tenacious to moderate antifungal activity against fungi like (Aspergillus flavus, Candida albicans, and Cryptococcus sp.)*

Chilli powder has exhibited properties that diminished the load of *Enterobacteriaceae.* Acid regulator citric acid has inhibitory properties against *Salmonella. sp, E. coli, Klebsiella sp.*, etc.

Sodium benzoate acts counter to food spoiling yeast, *Escherichia coli. Salmonella sp.*

Mixed pickle(B)

Ingredients utilized are green mango (22.3%), lime (18.2%), Carrot 17.8%, Edible Common Salt, Edible Rice Bran Oil, Green Chilli (7%), Mustard, Chilli Powder, Fenugreek, Ginger 1.6%Turmeric Powder, Compounded Asafoetida, Preservative Sodium Benzoate (INS 211), Acidity Regulator Acetic Acid (INS 260). Green mango inhibits the growth of Proteus, *Salmonella, Pseudomonas, Klebsiella, Staphylococcus, E. coli, and Streptococcus.*

Bacillus spp., Staphylococcus spp., Salmonella spp., Proteus sp., Escherichia coli, Klebsiella sp., and Pseudomonas sp. exhibit antimicrobial effects in lime juice. Carrot inhibits the growth of *Shigella flexneri, E. coli, Staphylococcus aureus, and Klebsiella pneumonia.* Edible common salt has antimicrobial properties that can annihilate or restrain the growth of organisms like *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens.*

The efficient antimicrobial properties against *V. pneumonia* were found in rice bran extracts. Green chili inhibits the growth of *Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Pseudomonas aeruginosa, S. aureus.* P. *putida, E. coli, and S. Typhi* show inhibited growth because of mustard. Chilli powder has exhibited properties that diminished the load of *Enterobacteriaceae.*

Fenugreek exhibits antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, and Klebsiella pneumonia.* Ginger exhibits antimicrobial activity against *Salmonella typhi, Bacillus subtilis, Candida albicans, E. coli, M. avium, and M. tuberculosis.*

Turmeric shows inhibiting properties in contradiction to *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Listeria monocytogenes, Candida albicans, Vibrio cholera, Pseudomonas aeruginosa, and B. cereus.*

Acidity regulator acetic acid inhibits the growth of *Escherichia coli, P. Vulgaris, P. aeruginosa, A. baumannii, methicillin-resistant Staphylococcus aureus, Enterococcus faecalis, Staphylococcus epidermidis.*

Asafoetida exhibits symbolic outcomes contradictory to *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Aspergillus niger.* Sodium benzoate acts counter to, *Escherichia coli, Salmonella sp.*, food spoiling yeast.

Bacteria's

The most commonly found pathogens in the home are *Staphylococcus aureus, Pseudomonas sp., E. coli, Aspergillus niger, Salmonella sp., Streptococcus sp.*

1. *S. aureus* is a mediocre bacterium that causes disease in humans. It is the advancing source of skin and soft tissue infections such as cellulitis, abscesses (boils), and furuncles. Most infections caused by *S. aureus* are not severe. *S. aureus* can be the root of septicity like bloodstream infections, pneumonia, or bone and joint infections.
2. *Pseudomonas* infection is particularly universal among patients with burn wounds, organ transplants, and intravenous-drug addiction. The most severe septicity includes malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia.

3. *E. coli* can exhibit diarrhea, stomach pain, cramps, low-grade fever. Some E. coli infections can be dangerous (hemolytic uremic syndrome).
4. *Aspergillus niger* is an opportunistic fungus that causes the ruination of food.

Pickles Used

Materials and Methods

Devices used are stainless steel or ceramic cylinder. To obtain a uniform inhibition zone the Agar well method is applicable. In our laboratory, the agar-well method is occasionally beneficial for the assay of viscous materials. The Agar well diffusion method is extensively used to evaluate the antimicrobial activity of plants or microbial extracts. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a certain amount of the antibacterial agent or extract solution is injected into the well at the desired concentration. The agar plates are then incubated at the appropriate conditions for the test microorganism. The antimicrobial ingredient diffuses across the agar medium and stops the bacteria strain from proliferating. The spread plate technique is useful for separating microorganisms from a small sample volume dispersed over the surface of an agar plate. When the proper concentration of bacteria is used, distinct colonies are formed that are equally dispersed across the agar surface. In addition to using this technique for viable plate counts, in which the total number of colony-forming units on a single plate is enumerated and used to calculate the cell concentration in the

tube where the sample was plated. Spread-plating is beneficial in enrichment, selection, and screening experiments. In most cases, the desired outcome for these three tests is the same as for plate counts, in which the distribution of discrete colonies forms across the surface of the agar. The goal isn't to ensure that every viable cell forms a colony.

Instead, only those cells in a population with a specific genotype should be supposed to thrive. The spread plate procedure may be preferred over the pour plate technique for an enumeration experiment if the end goal is to isolate colonies for further analysis because colonies grow accessibly on the agar surface, it becomes embedded in the agar with the pour plate procedure.

1. Sample A (sweet lime)
2. Sample B (mixed pickle)
3. (Sample a and b are in pure concentration as well as in 1:1 concentration [1gm sample dissolve in 1ml distilled water])
4. 4 Petri dish
5. Nutrient agar
6. Cork borer
7. Pipette of 1 and 10 ml
8. Saline suspension of ***E. coli, Pseudomonas, Aspergillus niger, staphylococcus aureus***
9. Spreader
10. Distilled water
11. Nichrome loop

Day 1

Prepare two concentrations of the samples A and B of 1:1 and the other one is a pure concentration using sterile buffered distilled water.

Prepare four nutrient agar Petri dishes with the mentioned microorganisms by using the spread plate method and letting them rest for a few minutes.

Make 4 wells in each plate using a sterile cork borer.

Transfer 0.05 to 0.2 ml of samples A and B in each well of a plate aseptically using the pipette.

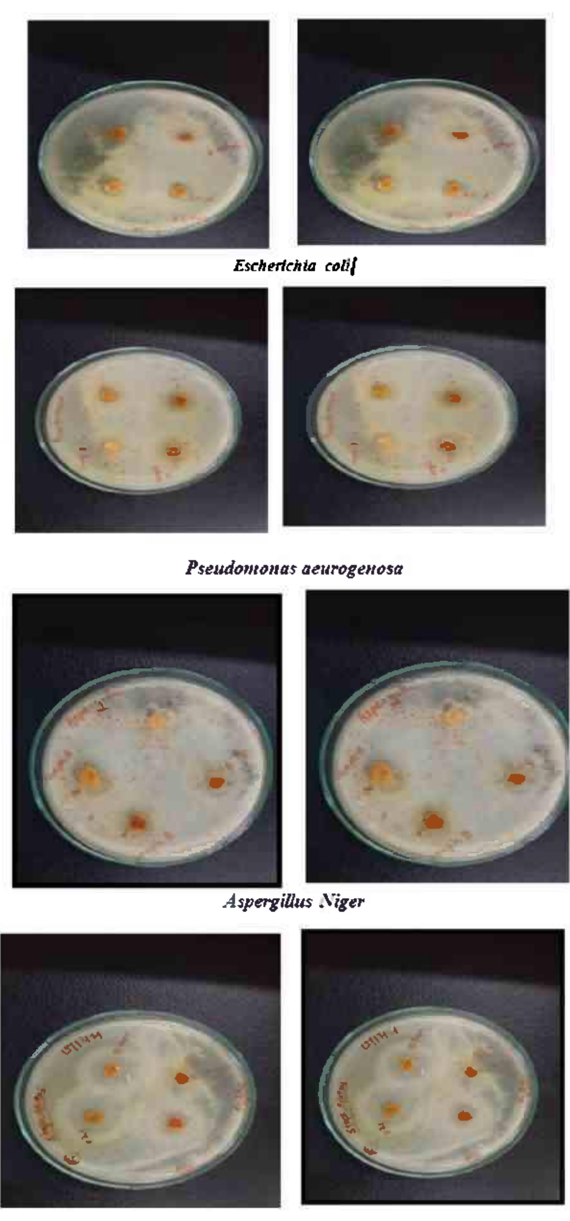
Incubate the plate in an incubator for 24 hours.

Day 2

Measure the diameter of the zone of inhibition given by both the sample of pickles. Plot a standard graph of the zone of inhibition (in mm) against the two concentrations of the sample of pickles.

From this, we found the effect of the pickle on the microorganism.

Observation



Escherichia coli

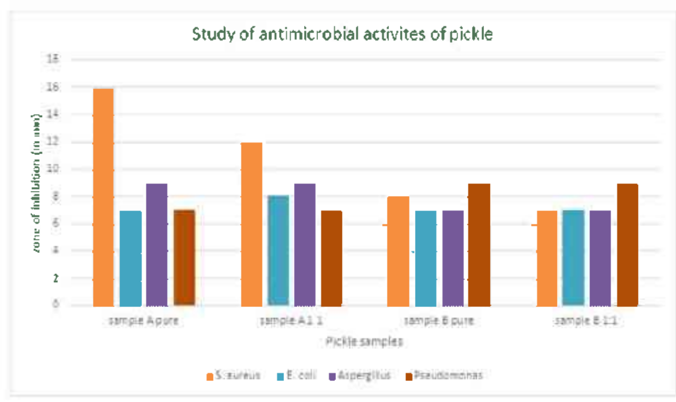
Pseudomonas aeurogenosa

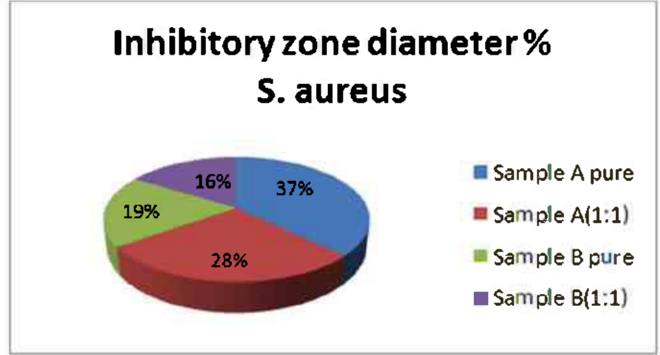
Aspergillus Niger

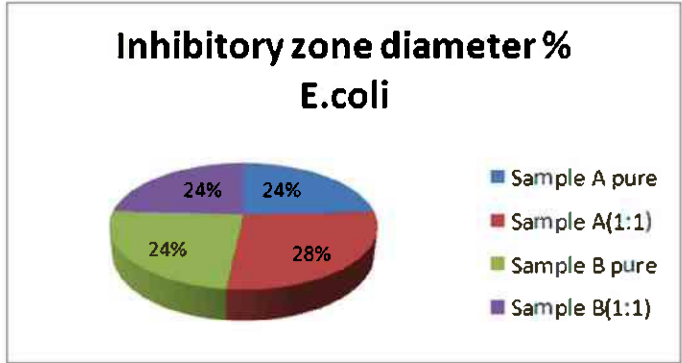
Observation Table

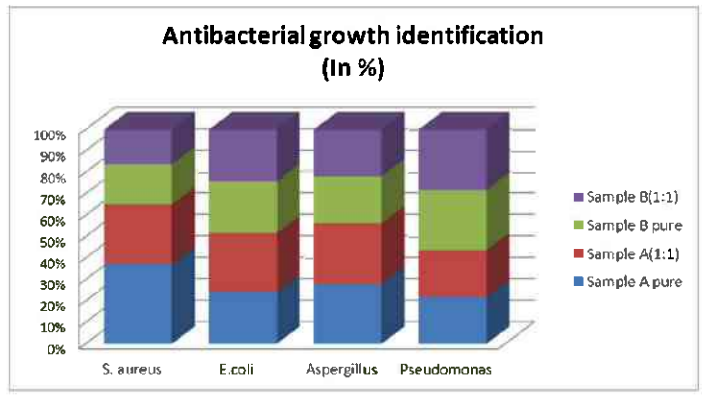
1	PICKLE SAMPLE	S. aureus	E. coli	Aspergillus	Pseudomonas
2	sample A pure	16 mm	7mm	9mm	7mm
3	sample A 1:1	12 mm	8mm	9mm	7mm
4	sample B pure	8mm	7mm	7mm	9mm
5	sample B 1:1	7mm	7mm	7mm	9mm

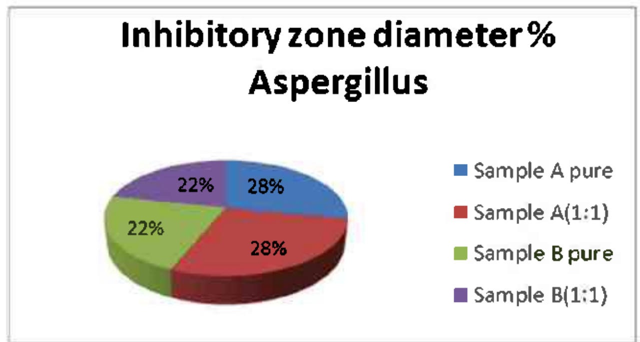
Graph

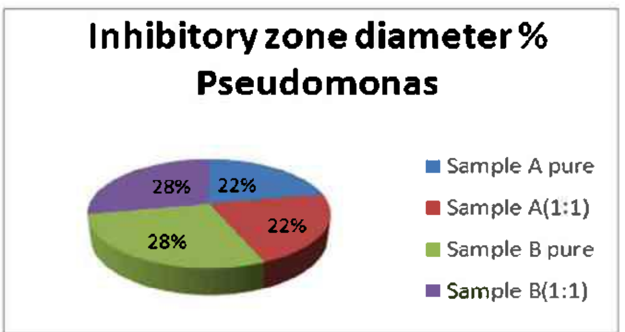












Result

After incubation, a zone of inhibition was observed around the well of each sample. The sweet lime pickle showed amazing affect s. aureus as compared to the mixed pickle and both pickles showed a limited zone of inhibition on E. coli, Pseudomonas, Aspergillus niger. Indian pickles are effective against the commonly found microorganism in our home. This research work states that Indian pickle shows antibacterial properties and are marvelously effective on the pathogen commonly found in our house. A comparative study was done between the sweet lime and mixed pickle

Conclusion

Pickles not only enhanced the flavor of any food but also contains many nutrients that contribute to overall health. They contain antimicrobial properties that kills commonly found microbes and also probiotics which keep our gut flourished with good microbial flora. We used the Ram bandhu Achar sample which is popular in India, we tested that it is beneficial to consume it in right amount as it help our immune system by killing and inhibiting the growth of most commonly found pathogens that enter our body everyday. However it is important to ensure that these pickles are consumed in moderate quantities to avoid any side effects.

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6. Comparative Study and Verification of Antibacterial Properties in Commercial Antibacterial Pads

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Abstract

Every month, Menstruation affects 8 billion people on the planet. Millions of these girls, women and non-binary persons are unable to manage their menstrual cycle in a dignified, healthy way.[11] National Family Health Survey 2015-2016 estimates that of the 336 million menstruating women approximately 121 million women in India (approximately 36% of the population) use sanitary napkins commercially produced. Women use menstruation pads to absorb period flow and preserve their clothing and belongings. Using sanitary pad is very easy and convenient. Considering the advantages women are still prone to a lot of bacterial infections and diseases in their genital areas due to an ample of reasons. Observations in the clinic imply that *E.coli* accumulating around the urethral orifice increases the risk of UTI. This bacteria normally inhabit the rectum and can cause infection if spread to the vagina.[7] *Staphylococcus aureus* forms the abnormal vaginal flora that contributes to the onset of aerobic vaginitis [9]. It is widely assumed that vaginal *lactobacilli* play a critical role in protecting the female genital tract by producing lactic acid, which lowers vaginal pH and prevents sexually transmitted infections.. Lactic acid is in equilibrium with lactate anion but an imbalance of vaginal bacteria, especially having too little *lactobacillus*, can cause fungus to grow out of control inside the vagina. The severity of a yeast infection can range from minor to severe. [8] With the intention of resolving such hazards recently brands have come up with sanitary pads withholding antibacterial

properties. In this research paper we aim to verify these antibacterial properties of the most easily available sanitary products . Surfing on various online shopping apps, sites as well as our traditional supermarkets we came across brands like SOFY, SELESTA and SIRONA who address their sanitary napkins as antibacterial products. We reacted the extracts accumulated from antibacterial layer of these pads with various harmful as well as useful bacteria like *Staphylococcus aureus*, *Lactobacillus acidophilus* and *E.coli*, respectively.. After thorough experiments and calculations by cork borer method our results suggested *E. coli* and *Lactobacilli* show satisfactory susceptibility to brands like Sofy and Selesta whereas Sirona acts as a shield for *S. aureus*.

Keywords - Sanitary napkins, menstrual cycle, antibacterial, bacterial infections, vaginal flora, *E.coli, S.aureus. Lactobacillus acidophilus*.

Introduction

The Menstrual Cycle describes the set of cyclic changes that occur in the reproductive system of a primate female's body every month, or 28 days in this case. [5]. The technical word for getting your period is menstruation. Females who have reached puberty will have menstrual blood around once a month. This occurs because the uterine lining has thickened and become richer in blood vessels in preparation for a prospective pregnancy. If pregnancy does not occur, this thickened lining is shed, accompanied by bleeding. Bleeding might last anywhere from 3 to 8 days. Menstruation follows a pretty predictable pattern for the majority of women. The length of time from the first day of period to the first day of the next period normally ranges from 21-35 days. The menstrual cycle is governed by a complicated symphony of hormones generated by two brain regions, the pituitary gland and hypothalamus, as well as the pituitary gland and hypothalamus. [10] Women's health has a significant impact not only on the women themselves but also on children's health, and the community. Menstruation is an important aspect of a woman's health. Individual experiences or perceptions of health, as well as societal and cultural influences, can influence attitudes toward it and its treatment. In India, despite advancements in women's social status, educational level, and their increased participation in economic activities, until relatively recently, menstruation-related issues were treated as taboo subjects not to be discussed publicly and therefore related research was insufficient . However, as people become more aware of the dangers of disposable menstruation pads, interest in menstrual hygiene and products has grown.

Disposable menstruation pads, reusable cloth menstrual pads, tampons, and the menstrual cup are all menstrual hygiene items for menstrual discharge. Disposable menstruation pads are essential for women of reproductive age all over the world, with an average woman using around 11,400 pads in her lifetime. Menstrual hygiene products must be of high quality and safety since they come into close touch with a woman's body on a monthly basis and for a long time (an average of 40 years of age) We acknowledge sanitary pads as a hygienic option for millions of girls and women to manage their periods. We do, however, raise some key points concerning the blind advertising of sanitary pads, which have an impact on the health of the young girls and women we aim to help. According to the latest National Family and Health Survey 4, 58 percent of young Indian women (15-24 years) utilise a sanitary method of protection (mainly sanitary pads), up from 12 percent in 2010. (as reported by the Plan and AC Nielsen study). This is undoubtedly a result of India's increased focus on menstrual hygiene management (MHM) in recent years. Corporate product lines have grown, as has their market reach. Several government and non-government programmes have promoted menstruation hygiene through health awareness campaigns and the provision of sanitary pads for free or at a reduced cost. [6]

Even after taking all precautions for our menstrual hygiene , women are still prone to bacterial infections. Bacterial vaginosis is a type of vaginal discomfort caused by an overabundance of bacteria in the vagina that disrupts the usual equilibrium. Kinds of bacteria that can be involved in bacterial vaginosis are *Lactobacillus, Bacteroides, Streptococcus, Fusobacterium, Eubacterium*, as well as a number of other types. When the many kinds of bacteria that naturally live in the vagina become imbalanced, a woman may have a foul-smelling vaginal discharge.[4] The vaginal flora is the bacteria that live inside the vagina .*Lactobacillus* species predominate in the typical vaginal flora. *Lactobacilli* species help to keep the vagina healthy by producing lactic acid, hydrogen peroxide, and other compounds that stop yeast and other undesirable organisms from growing. They maintain the vagina at a healthy pH of around 4. These bacteria play a crucial role in maintaining a healthy vaginal habitat. Bacterial vaginosis is caused by an excess of bacteria that ordinarily live in the vaginal area at low levels. These bacteria take control when the *lactobacillus* population is disturbed. [13] For the most part, *E. coli* lives harmlessly in your gut. But it can cause problems if it enters your urinary system, usually from stool that migrates into the urethra. UTIs are incredibly common. In fact, 6 to 8 million cases are diagnosed each year in the United States. While. The germs normally present in

the vagina can multiply and cause infection when the pH and hormone balance of the vagina and surrounding tissue are disturbed. *E.coli* bacteria live in the rectum and can travel to the vaginal area, causing illness.[12] Four studies assessed the frequency of vaginal *Staphylococcus aureus* colonization in healthy women and associated risk factors. An association was found in vaginal colonisation by *S.aureus*, as well as colonisation of the labia minora and anterior nares. A history of genital herpes simplex infection, tampon insertion without an applicator, and the use of Rely (Procter & Gamble) tampons were all found to be significant risk factors for vaginal *S.aureus* infection in at least one research. *S.aureus* recovery was reduced when systemic antibiotics were used within two weeks of the vaginal culture. The total prevalence of vaginal *S.aureus* was 9.2% in the 808 women in the four investigations. [3] With the intention of resolving and preventing such bacterial infections and maintain women's health some commercial brands have launched their antibacterial sanitary pads. In this research we have selected some easily available brands such as Sofy, Selesta and Sirona. The primary aim of this research is to test the efficiency of their antibacterial properties by testing them against bacteria like *E.coli, Staphylococcus aureus* and *Lactobacilli.*

Antibacterial Pads Used

2. Materials and Method

2.1 Preparation of Agar Plates

Agar plates are the standard solid support material for growing microorganisms. Microbial growth media contains nutrients and an energy source to fuel the microbes as they grow, and agar to keep the media in a semi-solid, gel-like state. Simultaneously, for preparation of agar plates make up the medium according to the recipe, then add the desired amount of agar powder (normally about 1% w/v) and stir. Only use 3/4 of the bottle's capacity while preparing the agar. While the agar is melting in the microwave, this creates space for bubbles to rise (and saves you cleaning up overflowing agar from the microwave). Set your medium to autoclave for 25 minutes. After autoclaving, you can store the medium-agar mix in a toughened glass bottle then melt it in a microwave or water bath when needed. Use toughened glass bottles whenever possible. When handling hot media fresh from the autoclave, remember to use heat-resistant gloves. Reduce the temperature of the medium-agar mixture to 55°C. Cool for a couple of hours in a 55°C water bath for consistently consistent outcomes. At around 50°C, agar begins to harden .When creating agar plates, using a water bath allows you to chill the slurry to just above the solidification temperature. Use about 20-30 mL of the agar-medium mix to create each plate when using 100-mm diameter dishes . The less agar-medium mix in each plate, the more easily they will dry out. We need 3 agar plates to be precise .[2]

2.2 Cork Borer Well Method

A cork borer can be used to punch holes on an agar plate and perform well diffusion assays to study bioactivity in microbiology. Hence to detect the antibacterial activities we use the cork borer method . In this method we sterilize a cork borer by autoclaving or disinfecting it by rising in alcohol followed by sterile water. We take the previously made nutrient agar plate and aseptically punch (4-mm) holes in the agar using a cork borer. Using a marker, mark the

underside of the Petri to label the wells. Each plate requires 3 holes as there are 3 samples to be tested . [1]

2.3 Requirements

- Sample 1 (Sofy antibacterial layer extracts + ethanol)
- Sample 2 (Sirona antibacterial layer extracts + ethanol)
- Sample 3 (Selesta antibacterial layer extracts + ethanol)
- Pure culture organisms – *Lactobacillus* , *S. aureus, E. coli*
- 3 sterile nutrient agar plates
- Ethanol
- Saline suspension : Loop full of bacteria in 6ml saline
- Nichrome loop
- Spatula
- Spreader

2.4 Procedure

Day 1: For preparing Sample 1 take extracts from the antibacterial layer of Sofy antibacterial pads and dissolve it in ethanol

For preparing Sample 2 take extracts from the antibacterial layer of Sirona antibacterial pads and dissolve it in ethanol

For preparing Sample 3 take extracts from the antibacterial layer of Selesta antibacterial pads and dissolve it in ethanol

Place the sample test tubes in warm water to aid in the dissolving of the substance.

Day 2 : Prepare 3 nutrient agar petri dishes by pouring the autoclaved agar in three petri plates in aseptic zone . Let it aside to cool for 20-30 mins. Divide each plate into three columns, address each column to a specific sample (eg. column 1 for sample 1 and so on)

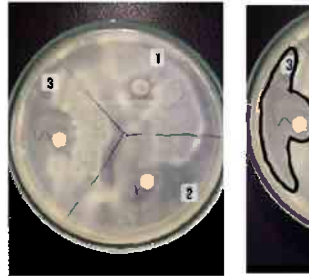
Prepare 3 saline suspensions with *E. coli, S. aureus* and *Lactobacillus acidophilus* in it respectively. Add 1ml of *E. coli* on one agar plate, *S. aureus* in the second plate and *Lactobacillus acidophilus* in the third. Do this under aseptic zone. Sterile the spreader with ethanol and spread the bacteria on agar plates equally. Let them rest for 15 minutes .Divide each plate into three columns, address each column to a specific sample (eg. column 1 for sample 1 and so on)

Make 3 wells in each plate within the range of respective columns using a sterile cork borer. Transfer a solid piece of sample 1,2 and 3 in columns addressed to it in an aseptic medium.

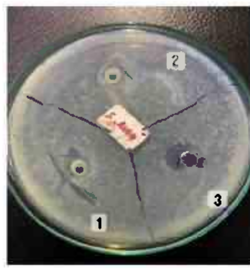
Incubate the plates for a total of 24 hours.

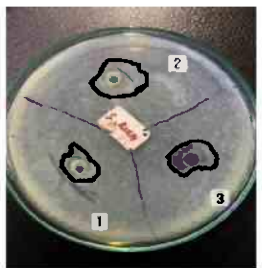
Day 3 : Measure the zone of inhibition caused by all the three samples in different bacteria cultured plates. Plot the standard graph of zone of inhibition (in mm) of these antibacterial pad extracts from the bacteria . From this we have found how these pads protects the female genitalia from various harmful bacterias and what possible change can it cause in vaginal flora.

3. Observation

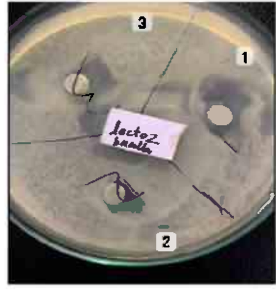


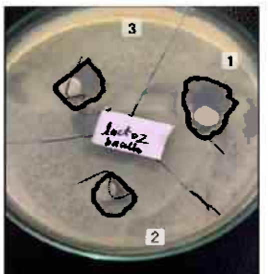
3.1 ***E. coli*** :1. Sofy's inhibition zone 2.Sirona's inihibition zone 3.Selesta's inhibition zone





3.2 ***S. aureus*** : 1. Sofy's inhibition zone 2.Sirona's inihibition zone 3.Selesta's inhibition



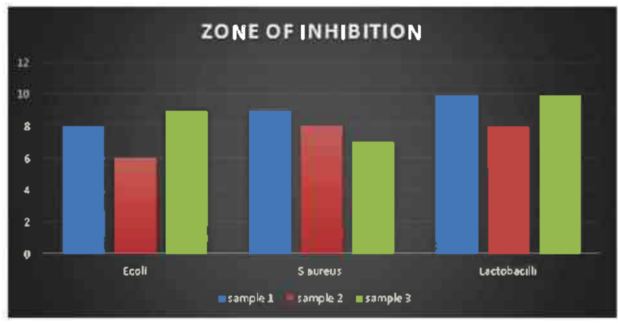


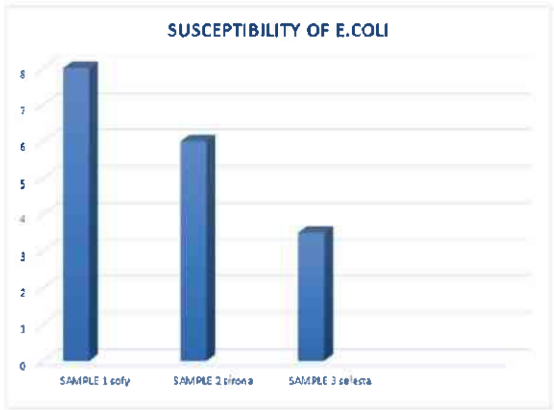
3.3 ***Lactobacillus acidophilus*** : Sofy's inhibition zone 2.Sirona's inihibition zone 3.Selesta's inhibition z

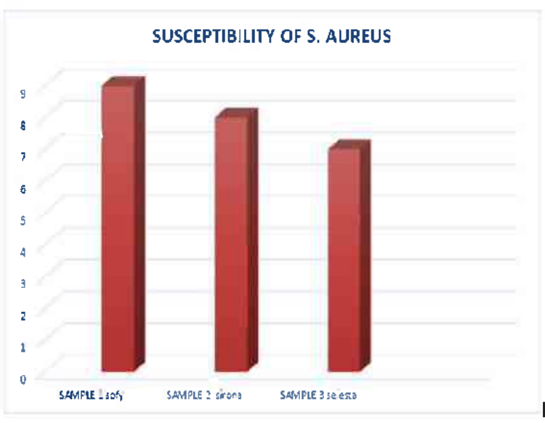
3. 4 Measurement of Zone of Inhibition

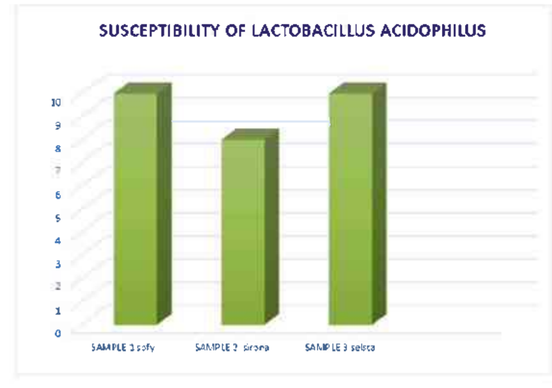
E. coli			
S. aureus			
Lactobacillus acidophilus			
	Sample 1 Sofy pad	Sample 2 Sirona pad	Sample 3 Selesta pad
E. coli	8mm	6mm	9mm
S. aureus	9mm	8mm	7mm
Lactobacillus acidophilus	10mm	8mm	10mm

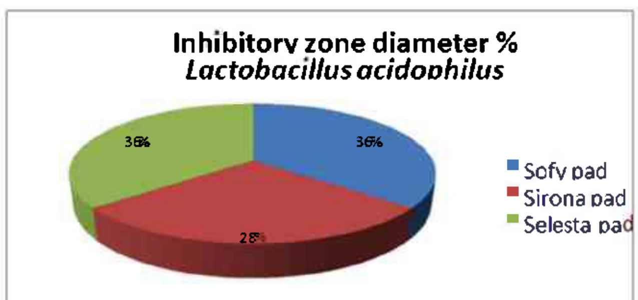
3.5 Graphical Representation

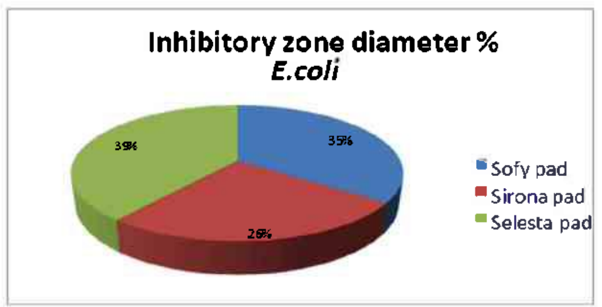


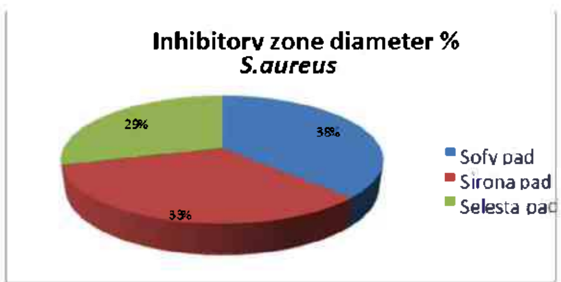












4. Result

After the incubation when we take a look at the results all the three brands that is SOFY , SIRONA and SELESTA show satisfactory antibacterial activities . Greater zone of inhibition is seen in brands SOFY and SELESTA against bacteria *E. coli* and *Lactobacillus* acidophilus . Whereas SIRONA and SOFY shows good inhibition for *S. aureus.*

5. Conclusion

While concluding we can say that the famous brand SOFY showed great zone of inhibition against all the three bacteria , hence it won't be false to say it is the best antibacterial pad among the rest. Whereas SELESTA following it . But it would safe to say all the three brands stand by their claim of having antibacterial properties .

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7. Detection of Microorganism's Present in Beauty Product's of Face Cream Skin and to Check Their Effect on Face

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Abstract

This topic has been chosen to identify the antimicrobial properties of face foundation (beauty product) and to know how face foundation can inhibit the growth of common microorganism found on the skin of face. There are many skin problems, like hyper pigmentations, pimples, dark spots and many other which is caused by different kind of microorganisms and to cover these problems many people use different kind of beauty products. these products can be of different kinds as they can be herbal and in some products they can be composed of chemicals also. So nowadays people mostly prefer to use herbal based things. There are many brands of herbal face foundations which claims that there herbal products are better then another brand. Herbal face foundations are mainly composed of wax, and some organic vegetables oil, fruit juices also which may be either natural or synthetic. Water-based foundation utilizes either a combination of oil and water or a blend of water and emollients or fatty alcohols to achieve a lightweight, easy-to-apply consistency. Non herbal foundations are mainly composed of chemical compounds such as Dimethicone, Isododecane, Cyclohexasiloxane, Glycerine etc.,

This research work is based on the comparison between products of different brand on the basis of there antimicrobial properties and to estimate its growth against the two most common microbes found on the skin i.e., Klebsiella pneumoniae and Escherichia coli. These bacteria are commonly found on skin flora and they causes pimples, acne on our face skin and

they can multiply very rapidly. And they can also cause itching on our face. So by the use of these antimicrobial skin foundation may help in to reduce these problems and beautify skin and to hide dark spots. These products are also water proof so that people using can put there foundation for long time.

Thus, this experiment focuses on comparing two different kinds of foundation herbal brands of Loreal and Lakme on the basis of there antimicrobial property to kill the microorganisms and to inhibit the growth of microorganism. The two methods for this experiment were the disc diffusion method and cork borer method. The method that is used for this experiment is the cork borer agar well diffusion method. This method was used so that the sample that was loaded in the well reacts well with the sample and show expected results. The sample of different foundation is diffused in the well and incubated for 24 hours to observe the zone of inhibition that forms around the microorganisms samples.

Keywords

Face foundations, Antimicrobial, Face problems, Herbal products, loreal foundation, nykaa foundation.

Introduction

in todays Era peoples are being very chosen towards what products they choose for there face and their skin and most of people think that the big brands provide them good quality of products such as antimicrobial and herbal. That's why most of the people think that buying high range of brand products can reduce their problems. In early days peoples uses natures product such as Multani soil, and some fruit peelings like oranges. In that they basically dry the orange peel and then they crush it and make paste of it and apply it on their face. and they also use turmeric and gram flour as face mask to glow their skin. And natural products were so cheaper than compared to nowadays branded products. And in today's Era big brands are selling their brands at very high cost and they are even assuring that their products are very good. But how do we know that their brand products can actually kill the micro-organism or inhibit the growth of organisms. There are many products available in market but we only want that products which suits our skin type and do not causes any kind of skin issue like pimple, acne, itching, and do not cause any side effect to our body.

But on these basis company do not provide any assurance with confidence but we the people thinks that's these are big brands they really care for us. But this is not true some

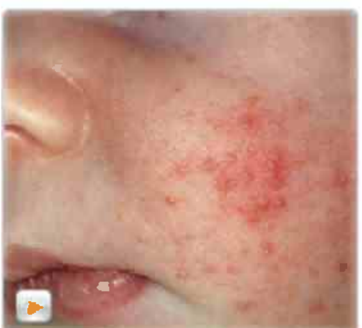
companies they mostly focus on their brand promotion and profit of there company. Acne and pimples are biggest problems nowadays for ladies as well as gents also they mostly prefer to use herbal products. Herbal products mainly consists of organic vegetable oils and some fruit extract. Pimples and acne is mostly common in between teenagers. They took a lot of care of their skin, as they have to look good because they have to go in every field of work as they are going to be next future of the country and they have to represent their country so they need to maintain their skin routine. Though, there is no proven method that can reverse the problem of pimples and acne. Today's generation has a hectic schedule where they don't get time to look after their health and leads to an unhealthy lifestyle with overstress. Some skin conditions have no known cause. Inflammatory bowel disease is a term for a group of intestinal disorders that cause prolonged inflammation of the digestive tract. Choosing the right products, to treat their skin is very essential, and the number of options in the market can put oneself in a loophole. There are many bacteria which is present on our skin and causes a lot of skin disorder and they also effect our hormones.

According to scientist jean M. Auel he states that "I had an idea for a story about a young woman who was living with people who were different, not just superficially different - such as hair colour, or eye colour, or skin colour but different in some significant way." He wants to explain that, to realize that we're all humans, despite differences in how we look or dress, or what we eat or celebrate. Games and activities offer a fun way for young children to learn about differences and similarities among people and to introduce the concept of diversity. All types of differences such as race, religion, language, traditions, and gender can be introduced this way. common bacteria found on the skin i.e.; Klebsiella and Escherichia Coli. These are 2 are among the most commonly found microbes and which is also easy for laboratorial experiments.

Klebsiella pneumoniae is a Gram-negative bacterium which is nonmotile, encapsulated, lactose-fermenting, facultative anaerobic, rod shaped bacterium. Klebsiella bacteria are normally found in human intestines and also in human stools. It can also be found on the moist part of your skin which leads to skin infection. They are suitable to room temperature and multiply rapidly in numbers. They can survive against the defense of immune system. It can also survive in oxygenic or anoxic conditions. The different species of *klebsiella* bacteria comprise of 7 bacteria, all of which are somewhat similar to one another. The different species of klebsiella bacteria are similar in DNA and you would find that they tend to be somewhat similar in action

too. In order to understand the action of klebsiella bacteria, you need to know about the 7 species that belong to this genus. The first is the klebsiella pneumoniae, the most common klebsiella bacteria in the medical world. The second is klebsiella oxytoca, another similar gram negative bacterium that can cause life threatening medical problems. Klebsiella ozaenae, klebsiella terrigena, klebsiella rhinoscleromatis, klebsiella planticola and klebsiella omithinolytica are the other 5 species that belong to the klebsiella bacteria group.

Escherichia coli are Gram-negative rod-shaped bacteria. Escherichia coli strains frequently are **isolated from skin and soft tissue infections** (SSTI); however, their virulence potential has not yet been extensively studied. In the present study, we characterized 102 E. coli SSTI strains isolated mostly from surgical and traumatic wounds, foot ulcers, and decubitus. *E.coli* survives in room temperature and multiplies conveniently. Antibiotic resistance is an old problem with new face as the rate of infections due to multidrug resistant bacteria is higher everyday and the number of new antibiotics to overwhelm the problem is becoming smaller. E. coli is the most frequent agent causing nosocomial or community-acquired bacteraemia being in our country 10% of them extended-spectrum beta-lactamases (ESBL) producing E. coli isolates. Nowadays the number of community- acquired or health-related infections caused by these ESBL producing E. coli is increasing. CTX-M has also become the most frequent ESBL compared to other enzymes. The role of these enzymes as a virulence factor increasing mortality in patients with bacteraemia due to E. coli is not well defined. The relevance of ESBL-E. coli seems to be related with the higher frequency of inadequate treatment and therefore the importance of identifying factors or features that might predict that the patient's infection is due to one of these isolates. In terms of prevention and control of infection measures, the role of patient's isolation is not clear but a proper prescription of antibiotics and antibiotic control policies are probably important to reduce the problem.



The sample were taken in experiment they both were herbal products namely, Lakme and Nykaa. These both brands are provide some herbal products for skin such as foundation. This concept is very ancient as mankind and civilized. Womens are obsessed with looking beautiful. The idea of to develop the herbal problems for skin came from at the time of ayurveda. At that time kings and queens use natures extract to make their skin glow and to keep their skin hydrated. They also used different kinds of herbs also. Nature has gifted many herbs and wood to the world in the world of cosmetics.

Material and Method

1. Sample A (Loreal face foundation)
2. Sample B (Nykaa face foundation) (Add 1gm of each face foundation sample in 10ml saline solution.)
3. 4 Petri dish
4. Nutrient agar
5. Cork borer
6. Pipette of 1 and 10 ml
7. Saline suspension of Klebsiella pneumoniae, Escherichia Coli
8. Spreader
9. Distilled water
10. Nichrome loop

Method

Day 1

Pour autoclaved nutrient agar in two Petri plates in the aseptic zoneand let it aside to cool for 20-30mins.Make a quadrant on the base of the Petri plate as it solidifies.Pipette out 1ml saline suspension of both E. coli and Klebsiellapneumoniae. Add 1ml E. coli on one agar plate and 1ml Klebsiella pneumoniaeon another in the aseptic zone.Sterile the spreader with alcohol and spread the bacteria on agarplate equally.

Let it aside for 1hr for the bacteria to settle.After 1hr, sterile the cork borer and make wells in each quadrant.spatulaTake 0.5gm of each sample in a spatula and load it inside the well. Incubate the agar plates for 24 hours.

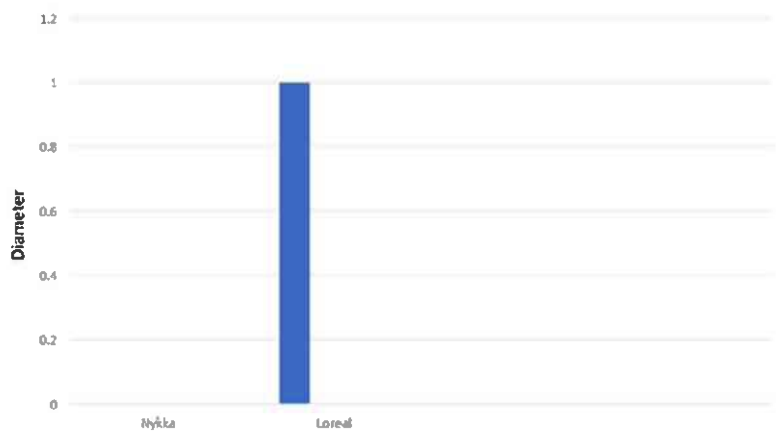
Day 2

Measure the diameter of the zone of inhibition given by both the sample of foundation Plot a standard graph of the zone of inhibition (in mm) against the concentrations of the sample of foundation. From this, we found the effect of the foundation on the microorganism

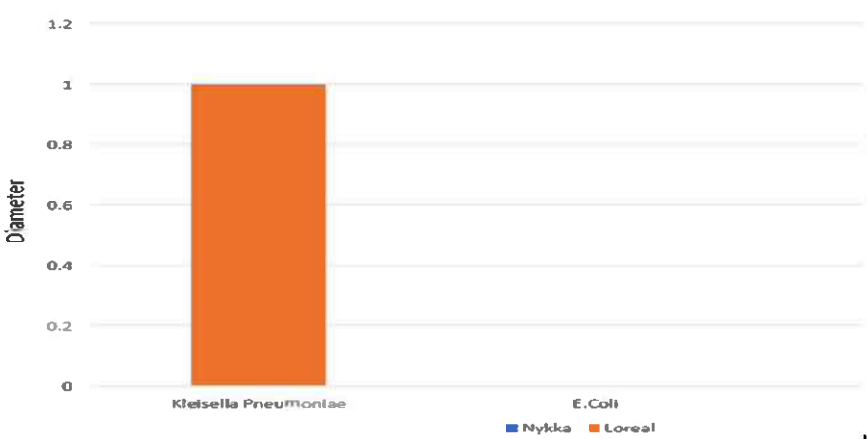
Observation Table

BACTERIA	SAMPLES	Inhibitory zone diameter (In mm)
Escherichia Coli	Nykaa	0 mm
Klebsiella pneumoniae	Loreal	1 mm

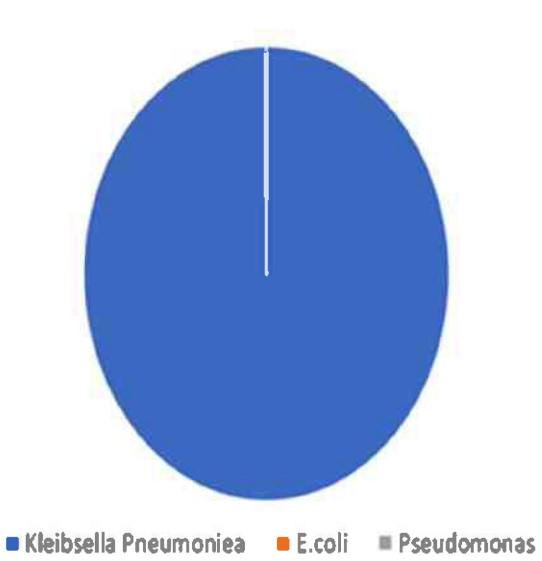
Graph



Bar Graph



Pie Diagram



Result

After incubation, zone of inhibition were observed around the well of each samples. Loreal showed visible zone of inhibition in the agar plates under the used bacteria wiz *Klebsiella pneumoniae*. And in *Escherichia Coli* Nykaa showed slight zone of inhibition which was near to negligible.

Conclusion

This experiment concluded stating that herbal products have antibacterial properties in it and it is much effective than Non-herbal but all herbal products are not effective. Thus loreal herbal foundation shows more effectiveness then nykaa herbal foundation. And regular use of it can help in reducing of pimple, acne and also reduce ageing of skin.

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8. Estimation of Antibacterial Properties in Ginger, Garlic and Ginger-Garlic Paste against Food-Poison Causing Bacteria

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Abstract

Many of the spices used in our daily diets have been noted to have great medicinal values and antibacterial properties as well. Ginger (*Zingiber officinale*) and garlic (*Allium sativum*) are one of these spices which are very useful and important and are used as therapeutic agent against many infections. The aim of this research paper was to estimate the antibacterial properties found in ginger, garlic and their paste against three strains of food poisoning bacteria such as *Staphylococcus, Salmonella, Escherichia coli.* The method that was used for this was the cork borer well diffusion method. The samples were loaded by 1% v/v dilution in the well following the incubation for 24hrs. The growth was observed which evidently showed that garlic has the most antibacterial properties against all three bacteria's whereas ginger and ginger-garlic paste have minimum antibacterial properties against these bacteria.

Keywords - Ginger, Garlic, Antibacterial properties, Food poison, Infection, Inhibition.

Introduction

Food poisoning, also called foodborne illness, is an infection that spreads through food and drinks. Food poisoning or foodborne disease (FBD) is one of the major problems in public health worldwide. According to WHO, each year 600 million people around the world become ill after consuming contaminated food. In most cases food poisoning is acute, meaning it happens suddenly and lasts a short period of time. Most of the cases last less than a week and some of the

people get better on their own without treatment. While in certain cases it can last longer lead to some serious complications. Chemicals, fungi, viruses, bacteria and parasites can cause food poisoning. Though most of the food poisoning reports are associated with bacterial contamination. More than 90% of the cases each year are caused by bacteria like *Staphylococcus. Salmonella, Clostridium, Perfringens, Campylobacter, Bacillus Cereus and Escherichia coli.* Commonly prevention of food spoilage is achieved by the use of chemical preservatives. But repeated application of these chemical preservatives may result into some negative effect such as accumulation of chemical residues in food, acquisition of microbial resistance to the applied chemicals and unpleasant side effects of these chemical preservatives on human health. Because of such reasons, consuming food which have antibacterial properties naturally can help us avoid such situations at certain level. This experiment focuses on the antibacterial properties found in simple spices like ginger and garlic which is used in our food commonly. Including them in our food increases the shell life of our food. Thus this help us built some percent of immune against food-poison causing bacteria by consuming them through our daily diet making our food healthier and safer.

Materials and Method

I. Preparation of Samples

Samples were prepared using two ingredients which are ginger and garlic. Ginger and garlic was first finely crushed making a thick paste of it. Then three samples were prepared from it which included ginger paste, garlic paste and one mixing both of them together forming ginger-garlic paste. After which 1gm of each sample were diluted in 10ml of saline solution (1% v/v) to form a semi-liquid consistency.

II. Noculation Of Bacteria

Salmonella, Staphylococcus and *Escherichia coli* were isolated and inoculated in saline solution and kept in incubator for 1hr to observe a matte growth. After incubation, the saline suspension was pipetted out from a sterile 10ml pipette and loaded in agar plates. The bacteria was then spreaded equally to all sides using a sterile spreader.

III. Sample loading

Using quadrant method, wells were made in the agar plates with a sterile cork borer. The quadrants were numbered and the samples were loaded into the wells precisely.

IV. Observing growth

The agar plates were kept in incubation for 24hrs. After incubation, it was observed that garlic showed visible zone of inhibition against all the bacteria's whereas ginger and ginger-garlic paste also showed very minimal zone of inhibition against the used bacteria.

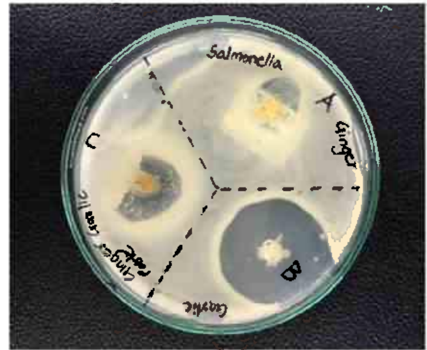


Fig.1: *Salmonella*

Fig.2: *Staphylococcus*

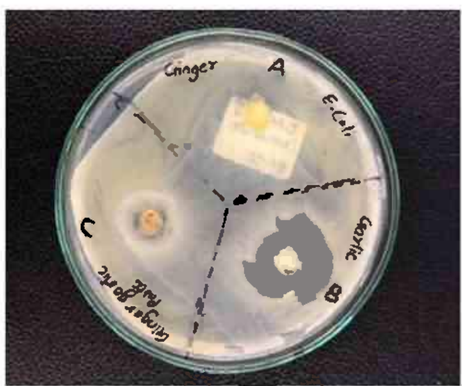
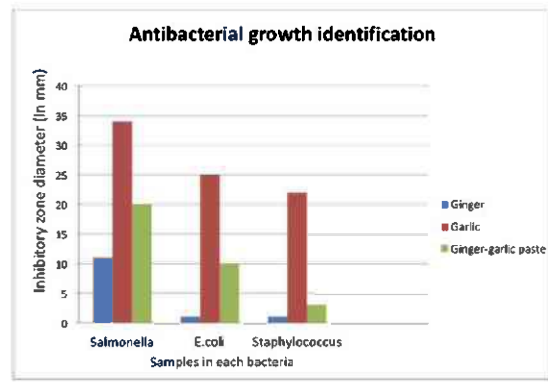


Fig.3: *Escherichia coli*

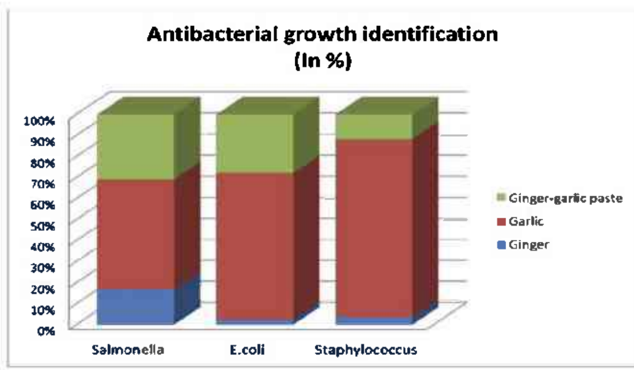
Observation Table

Bacteria	Sample	Inhibitory zone diameter (in mm)
	Ginger	11mm
Salmonella	Garlic	34mm
	Ginger-garlic paste	20mm
	Ginger	1mm
Escherichia coli	Garlic	25mm
	Ginger-garlic paste	10mm
	Ginger	1mm
Staphylococcus	Garlic	22mm
	Ginger-garlic paste	3mm

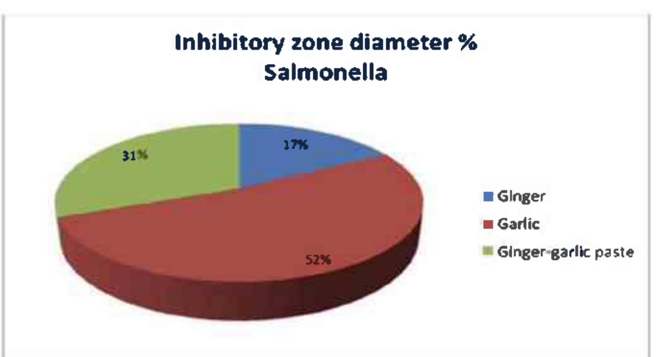
Graph

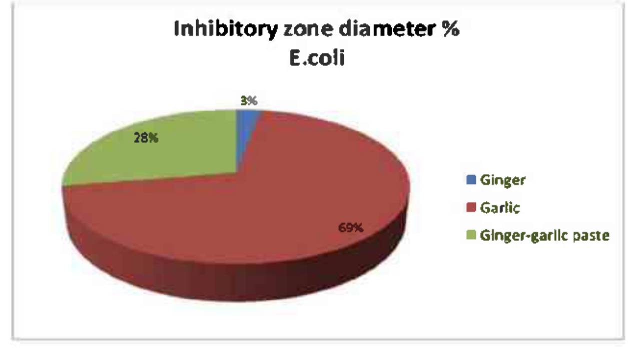


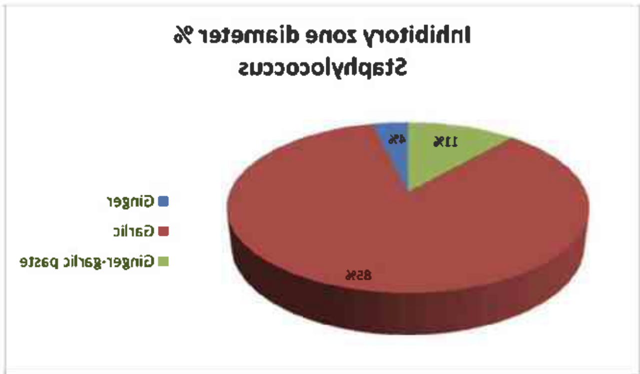
Bar Graph



Pie Diagram







Result

After incubation, zone of inhibition were observed around the well of each samples. Garlic showed visible zone of inhibition in all three agar plates under the used bacteria wiz *Salmonella, Escherichia coli* and *Staphylococcus.* Ginger and ginger-paste showed minimal zone of inhibition.

Conclusion

This experiment concluded stating that naturally available spices like ginger and garlic possess antibacterial properties which can be effective against food-poison causing bacteria like *Salmonella*, *Escherichia coli* and *Staphylococcus*. So We can use these to prepare natural sanitizer also. These substance will be helpful in increasing our immunity and fight against manu infectious bacteria. As, thses are having organic source, there will not be any side effect which can be seen in case of antibiotics. Finaly, according to my research thses substance are useful.

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9. Effects of Growth of Bacteria on Rancid Oil

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Abstract

We use oil on regular basis which composed of triglycerides.The triglycerides are unit molecules that contain carbon, chemical element and gas, and its structure includes glycerin and 3 fatty acids. once heated repeatedly or incorrectly stored changes in physical look of the oil can occur like redoubled consistency and darkening in color , which can alter the carboxylic acid composition of the oil so many times instead of using oil unknowingly we consume rancid oil through street food. The process in which oils and fats become partially or completely oxidized after exposure to air or even light sometimes it called rancidity of oil. If the oil gives fermented smell and taste it is probably because it has turned rancid. Each community of bacteria is specially adapted for the environment where it is living, and studies have found that bacteria consume oil most quickly when they are kept in conditions similar to their natural environment. The aim of this experiment is to find out bactericidal properties of rancid oil on *Salmonellatyphi* and *Psuedomonas aeruginosa* with the help of agar well diffusion method This method was used to estimate effects of growth of bacteria on rancid oil by cork. The samples were loaded by 1%v/v dilution in well following the incubation for 24hrs. The growth was observed and which evidently showed that rancid oil dose not effect on growth. It is harmful to human body but not to the bacteria.

Introduction

The major use of vegetable oil is in cooking it functions as a heat transfer medium and contributes flavor and texture to foods. oils area unit composed of triglycerides. The triglycerides

area unit molecules that contain carbon, chemical element and gas, and its structure includes glycerin and 3 fatty acids. The fatty acids in triglycerides is also constant or of various varieties. The physical characteristics of the triglycerides are unit directly connected with its fatty acids composition, thus vegetable oils can have carboxylic acid with chains of hydrocarbons of twelve to twenty two carbons, being the foremost common sixteen and eighteen carbons, with or while not unsaturated carbon atoms, that is, with presence of double links. so these fatty acids are often called saturated, monounsaturated and unsaturated. (KNOTHE, 2003). These unsaturation, over modifying the molecular mass of the triglycerides, act directly on the physical and chemical characteristics of vegetable oils. .but once fats in vegetable oil area unit exposed or are available in contact with extreme heat, light, or oxygen, they begin to decay and our oil begins to smell and tastre completely different , once heated repeatedly, changes in physical look of the oil can occur like redoubled consistency and darkening in color , which can alter the carboxylic acid composition of the oil. Heating causes the oil to bear a series of chemical reactions and chemical process In different words – it turns rancid and becomes unsuitable for any consumption. rancidity is reaction or autoxidation of fats into short-chain aldehydes , ketones and free fatty acids, this term usually use to denote un-pleasant odors and flavors in food. There are 3 completely different mechanisms of rancidity could occur, that is oxidative, hydrolytic, and microorganism activity . Oil quality is reduced by aerophilous and hydrolytic degradation accelerated during cooking ,longer storage, heat exposure, wet content. the kinds of rancidity area unit.

a. Hydrolytic Rancidity : Hydrolytic Rancidity is defined as the smell or odor that occurs when free fatty acids are released and triglycerides are hydrolyzed. It generally causes an unpleasant smell. Triglyceride is a combination of three fatty acids, present in oily food. They release free fatty acids and produce glycerol when they react with water. These fatty acids form toxic compounds and may further undergo oxidative rancidification. Hydrolytic Rancidity occurs more quickly in the presence of enzymes like lipase and with moisture and heat. It results in the hydrolysis of the fats with the liberation of either one or more volatile fatty acids.

b. Oxidative Rancidity : Oxidative Rancidity is defined as a reaction that causes oxygen damage to a food substance. It occurs with unsaturated fats. The natural oil structure is damaged and interrupted by oxygen molecules in such a way that it changes its color, taste, and smell. It leads to the formation of toxic compounds like peroxides that damage Vitamin A and E in foods.

In Oxidative Rancidity. The unsaturated fatty acids of glycerides are oxidized at their double bonds. Thus, this reaction causes the release of ketones, volatile aldehydes, and acids. It can be prevented by an oxygen-free atmosphere, light-proof packaging, and the addition of antioxidants.

C. Microbial Rancidity: Microbial Rancidity is that type of rancidity where microorganisms such as molds or bacteria use their enzymes like lipases to break down chemical structures of fat in oil-producing unwanted smells and taste. This process can be reduced by inhibiting microorganisms or by destroying them. It can also be reduced by pasteurization and the addition of antioxidant ingredients like vitamin E

Every community of microorganism is specially tailored for the surroundings wherever it's living, and studies have found that microorganism consume oil most quickly after they area unit unbroken in conditions just like their natural environments. There are unit species of marine microorganism in many families, together with Marinobacter, Oceanospiralles, genus *Pseudomonas*, and *Alkanivorax*, that may eat compounds from crude oil as a part of their diet. In fact, there area unit a minimum of seven species of microorganism that may survive exclusively on oil. Each community of bacteria is specially adapted for the environment where it is living, and studies have found that bacteria consume oil most quickly when they are kept in conditions similar to their natural environment

Rancid oils work on the cells of our body and weaken them. They deplete the body's vitamin B and E resources, have harmful health effects and are known to be linked to **Diabetes**. **Botulism and other digestive disorders** Consuming rancid fats and oils may reduce the nutritive value of the food, There is also evidence to show that decomposition products produced by oxidized oil may be detrimental to health and have been linked to the development of neurodegenerative conditions

Material and Method

A. Collection of Samples

Used cooking sunflower oil and mustard oil were taken which got rancid due to moisture or by any other factors is used as a sample. The color of these oils was pale yellow to dark yellow . The samples were divided and stored in two packaging plastic bottles then capped and labeled properly and placed in laboratory for analysis.

B. Isolation of Bacteria

The purpose of streaking for isolation is to produce isolated colonies of an bacteria on an agar plate and to find out effect of rancid oil on growth of bacteria.

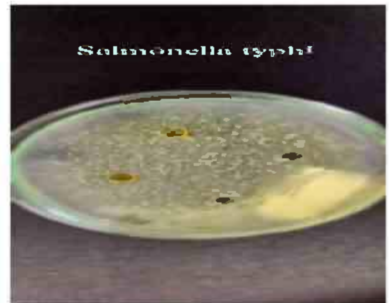
One loop-full of the working culture of *Pseudomonas aeruginos* and *Salmonella typhi* was suspended in 10ml of saline solution (1% V/V) after which 2 ml was used to inculcate on Petri dish by using spread plate method and then resting for 30 minutes. Made 4 wells in each plate using sterile crock borer. Then Oil samples 0.1 ml added in each well with the help of pipette and incubated at 37^0 C for 24 hours.

C. Screening by Agar well Diffusion Method

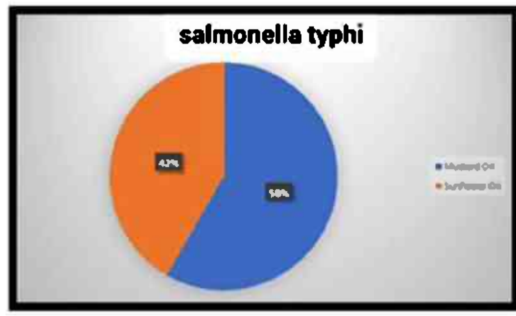
Antimicrobial activity of cell free extract was evaluated by the agar well diffusion method against the indicator organisms. A lawn of bacteria was made over the surface of agar plates by spreading 0.1 ml of 24 h old bacteria. The crude cell free extract of all isolates of 100 ûl quantity was incorporated in respective wells and plates were incubated at 37° C for 24 hours. The results were recorded by observing and measuring zone of inhibition. Amongst isolates those were selected showing broad spectrum antibacterial activity expressed in terms s od zone of inhibition.

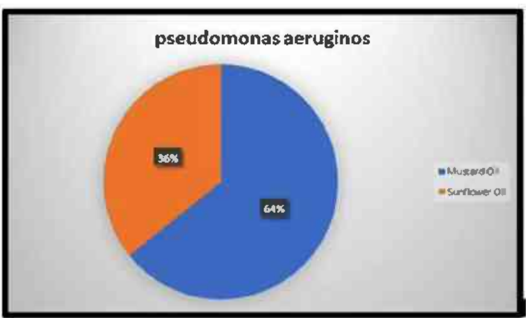
Observation

Growth of Bacteria in Presence of Rancidoil

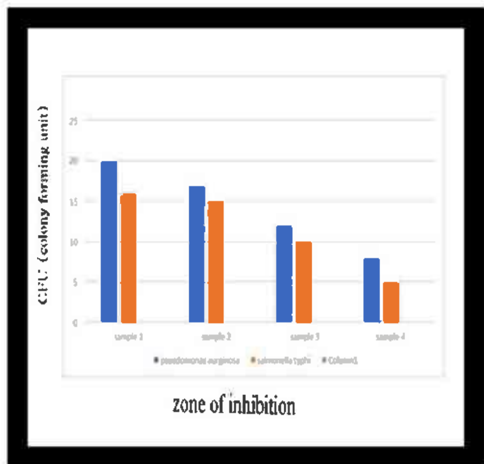


Graph and Pie Chart





Growth of Salmonella Typhi on Both the Samples



Growth of Pseudomonas Aeruginos on Both the Samples

Observation Table

Samples	*Psuedomonasaeruginos*	*Salmonella typhi*
Sunflowers oil	36%	42%
Mustard oil	64%	58%

Bacteria	Samples	Growth
Psuedomonasaeuroginas	Sunflower oil	No zone of inhibition observed
	Mustard oil	No zone of inhibition observed

Salmonellatyphi	Sunflower oil	No zone of inhibition observed
	Mustard oil	No zone of inhibition observed

Result and Discussion

Zone of Inhibition tests do not necessarily indicate that microorganisms have been killed by an antibacterial product just that they have been prevented from growing. But After incubation, there were no zone of inhibition observed around the well of each samples which showed rancid oil is not antibacterial it do not affect growth of bacteria the result of our experiment showed that the harmful rancid oil dose

Consuming rancid fats and oils may reduce the nutritive value of the food by destroying vitamins such as Vitamin A and E.14, 15 There is also evidence to show that decomposition products produced by oxidized oil may be detrimental to health and have been linked to the development of neurodegenerative conditions such as Alzheimer's and Parkinson's Disease.16-19.

In animal studies, exposure to oxidized oils and fats has been shown to cause harm including growth retardation, organ toxicity, accelerated atherosclerosis, reduced immunity parameters, high newborn mortality and increased maternal insulin resistance.20-23 Evidence into the impact of consuming rancid oils in humans is mixed. A 2016 Randomized Control Trial (RCT) in healthy subjects suggested that short-term consumption of oxidized fish oil may not have a negative impact at the molecular level .

Conclusion

As zone of inhibition was not found in any of the Petri plate, indicate that rancid oil is unable to kill any of the the microorganism selected for the experiment. This indicate that the rancid oil does not have any bacteriocidal or bacteriostatic property.The growth of of bacteria like pseudomonas sps in salmonella sps. increased. This indicate that rancid oil can be used as the source of lipid for the bacteria. This indicate that oil can be growth promoter for the bacteria.

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10. Isolation of Animal Fat Degrading Bacteria

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Abstract

Animal fats and oils are lipids derived from animals. Oils are liquid at room temperature, and fats are solid. Although many animal parts and secretions may yield oil, in commercial practice, oil is extracted primarily from rendered tissue fats from livestock animals like pigs, chickens and cows. Fat oil deposition is one of the major problems that harm the environment and cause dissatisfaction for human beings . So if it's not controlled properly it may leads to injurious to Human health. Uncontrolled and un-pre-treated Fat could lead development of various disorders. The Problems include the interference of fat with the aerobic microorganisms that are responsible in treating the wastewater by reducing oxygen transfer rates and for anaerobic microorganisms; their efficiency could also be reduced due to the reduction of the transport of soluble substrates to the bacterial biomass. Biodegradation could be one of the effective means to fat.

This topic was chosen to study about the fat degrading bacteria present in our environment. The genera of bacteria such as *Pseudomonas, Staphylococcus*, *Klebsiella*, *salmonella* are known to have the ability to degrade animal fat .In this experiment *Pseudomonas, Staphylococcus*, *Klebsiella*, *salmonella* are subjected to test the degration of animal fat. The main objective of this study is to isolate bacterial strains which can degrade the fat oils and identify the strains that are capable in the activities of degradation of fat oil.So we first melted the fat into the oil the autoclaved and then poured it into petriplate and them freezed the petriplate containing the fat oil and the we inclocated the bacterial sample to study the effect of different Bacteria on fat oil and test the efficiency of bacteria to degrade the fat oil.

Keywords - Sterilization, inoculation, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella Typhi, E. Coli .

Introduction

Fats, oils and greases (FOGs) are released into the environment with wastewater derived from the food processing industry, restaurants and kitchens or by accidental spills of oils. If not treated, they provoke an environmental effect similar to that of petroleum oil due to common physicalproperties. In natural environments, FOGs result in coating of animals and plants with oil, reduction of oxygen transfer rate and a high chemical oxygen demand (COD) in waste-water.

The main constituents of FOGs are animal fats and vegetable oils. They also comprise a combination of glycerol and free fatty acids whenever hydrolysis has taken place. When FOGs are spread in ecosystems, they may be used by indigenous lipid-degrading microorganism as an additional source of carbon and energy. Many microorganisms capable of degrading FOGs have been isolated from soil and water samples. Other genera of bacteria, such *as Pseudomonas, Burkholderia, Acinetobacter, Escherichia,* and fungi (*Candida, Rhodotorula*) are known to have the ability to degrade contaminants as well as Bacillus. However, the natural microbial degradation of FOGs is slow due to their low lipolytic activity. Furthermore, the biodegradation of lipids is often limited by the antimicrobial effect of hydrolysis products such as long-chain fatty acids .

Selection of microorganisms with a high degradation activity for FOGs and their application for the removal of pollutants is one of the ways to enhance biodegradation. Oils and fats, being naturally produced materials found in all living cells, are readily broken down both in situ as well as when in the environment. World production of oils and fats is currently over 70 millions tonnes/year (Gunst one 1989) and is predicted to rise at about 2.5 to 3.0 million tonnes per year throughout the 1990s (Mielke 1992) mainly due to increases in world population but also because of the continual industrialization of developing nations. About 75% of the total fats and oils are derived from plants, of which soybean, palm, sunflower and rape oils account for over 70%, and the remainder are from animals which encompass lard, tallow and marine oils.

Chemically, both fats and oils are composed of triglycerides. Dairy products yield animal fat and oil products such as butter. Certain fats, such as goose fat, have a higher smoke point than other animal fats, but are still lower than many vegetable oils such as olive or avocado. Animal

fats are commonly consumed as part of a western diet in their semi-solid form as either milk, butter, lard, schmaltz, and dripping or more commonly as filler in factory produced meat, pet food and fast-food products. Fatty acid degradation is the process in which fatty acids are broken down into their metabolites, in the end generating acetyl-CoA, the entry molecule for the citric acid cycle, the main energy supply of animals. Degradation of fats by bacteria and then using the enzyme of those bacteria to degrade the excess fats present in the body of individual person. This could be an interesting study.

Materials and Methods

- **Procurement of the Material**: Firstly the 500 gm fats was buyed from the Butcher Shop.
- **Extraction of oil from fat**: Firstly the fat was melted at High temperature for 3 hours to 4 hours at High temperature then we founded the thick oil of fat . After the extraction of the oil from the fat we proceeded with our experiment which was to isolate the bacteria which will degrade the fat.

Preparation of Media

1. After the preperation of the 100 ml oil we added agar agar powder to the oil and autoclaved the oil to sterlize the oil and to kill the other microorganisms present in the oil so we get accurate result.
2. Once the sterlization of oiled sample was done we poured the 5 ml oiled in the 15 petriplates each and then they were refrigerated in the freezer for 24 hours to arrest the growth of other microorganisms and for the settlement of the oil in the petriplates.
3. After the Refrigeration we prepare the saline suspension of microorganisms which has to to poured and cultured on the Oil plates. Firstly from the pure culture of microorganisms we prepared the sub culture of the microorganisms.
4. We took Total 5 different types of the microorganisms to identify their properties of degrading the bacteria and identify the bacteria which will degrade the microorganisms.
5. The bacteria which where taken are as follows
   a. Pseudomonas aeruginosa
   b. Staphylococcus aureus
   c. Klebsiella pneumoniae
   d. Salmonella Typhi

e. E. Coli

6. Once the preperation of sub culture was done we removed the refrigerated fat oil which was keep in freezer.
7. We started the pouring of the bacterial culture 0.3 ml in petriplates contains the fat oil in the aspetic condition between the two burner. After the pouring of bacter we spreaded it in the petriplates with the help of sterlized speader.
8. This process was repeated for 4 time for each petriplates.
9. After the inoculation of the fat oil with bacteria was done then the petriplates was keept inside the incubator at 37°C.
10. Once they are keept in the incubator they were stayed undistured for 5 intervals of the days.
11. On the 5th day, 10th day, 15th day we removed the petriplates from the incubator for short span of time to observe the result of the experiment.

Observation Table

We observed the the Petriplates of fat oil with Gap of 5 days we saw the following results on the each day on different plates containing the different microorganisms they are as follows.

DAYS	5th Day	10th Day	15th Day
Pseudomonas aeruginosa	Less Oil Was Degraded	Growth Of Colonies	Degrade Of Oil Seened
Staphylococcus aureus	Less Oil Was Degraded	Large Amount Oil Degraded	Large Amount Oil Degraded
Klebsiella pneumoniae	No Effect On Oil	Less Oil Was Degraded	Medium Oil Degraded
Salmonella typhi	Less Amount Oil Degraded	Less Amount Oil Degraded	Less Amount Oil Degraded
Escheretia coli	No Effect On Oil	No Effect On Oil	No Effect On Oil

The above table shows the results which were observed on the oil plates.

Amount of oil Obtained from Petri Plate

DAYS	5th Day	10th Day	15th Day
Pseudomonas aeruginosa	4.5 ml	4.0 ml	3.5 ml
Staphylococcus aureus	4.0 ml	3.0 ml	1.0 ml
Klebsiella pneumoniae	5.0 ml	4.1 ml	3.7 ml
Salmonella typhi	4.5 ml	4.3 ml	4.0 ml
Escheretia coli	5.0 ml	5.0 ml	5.0 ml

Observation of

Pseudomonas aeruginosa

Observation of

Staphylococcus aureus

Observation of *Kle*

bsiella pneumonia

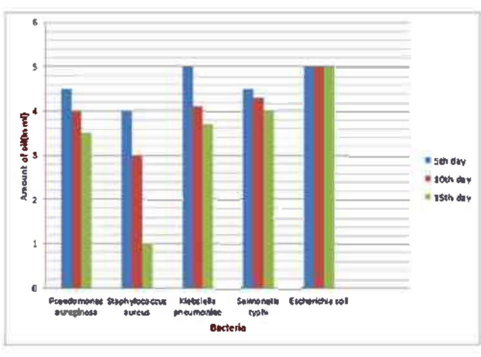
pneumoniae

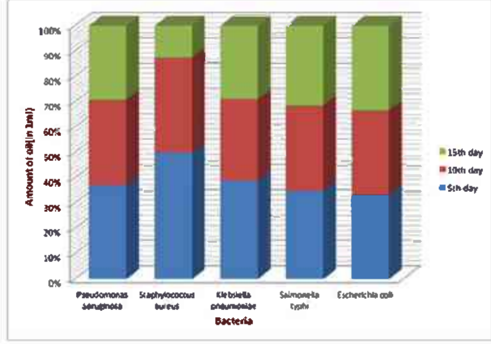
Observation of *Escherichia coli*

Observation of *Salmonella Typhi*

Graph

A. According To Oil Remain Inpetry Plate





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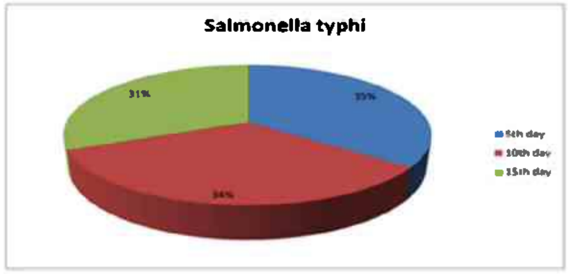
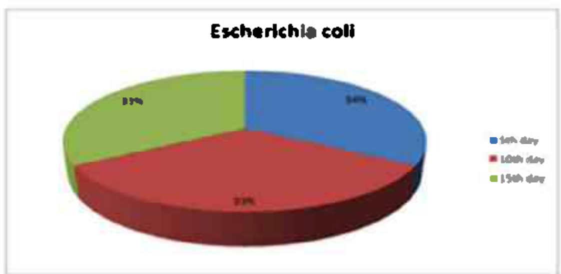
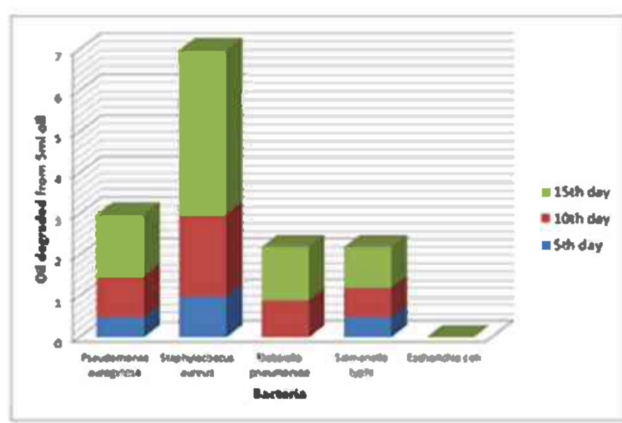
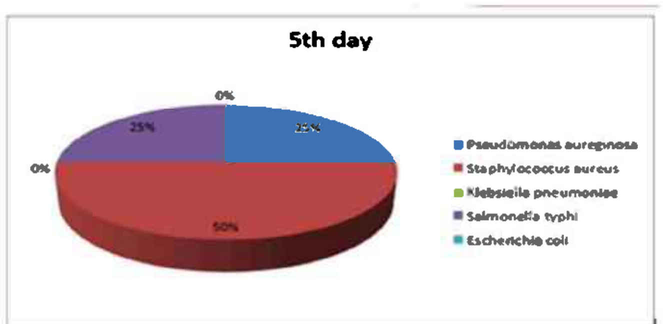
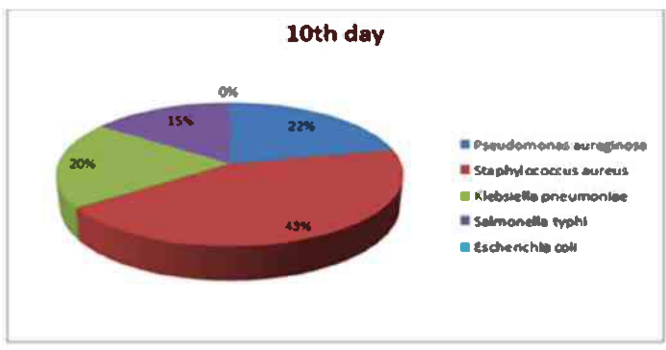
B.

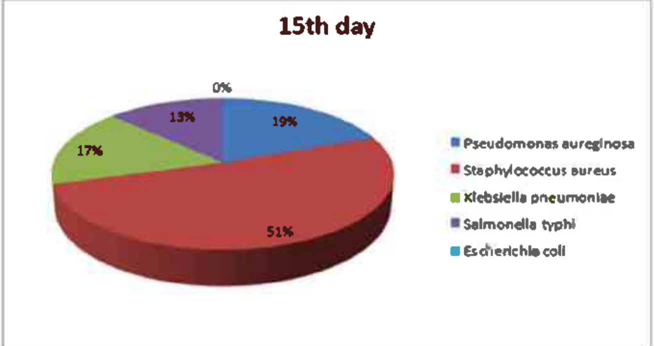

Salmonella typhi
31%
5th day
10th day
15th day


Escherichia coli


15th day
10th day
5th day


5th day
0%
25%
25%
0%
50%
Pseudomonas aureginosa
Staphylococcus aureus
Klebsiella pneumoniae
Salmonella typhi
Escherichia coli





Results

Staphylococcus aureus degraded the more amount of Oil compare to others Bacteria. After that *Staphylococcus aureus*, *Pseudomonas aeruginosa* degraded the medium amount of oil and other Bacteria also degraded the oil but it was less.

E.coli was the only Bacteria which doesn't degraded the fat oil.

The results of the experiments is tabulated in percentage as follows

DAYS	5th Day	10th Day	15th Day
Pseudomonas aeruginosa	10%	20%	30%
Staphylococcus aureus	20%	40%	80%
Klebsiella pneumoniae	0%	18%	26%
Salmonella typhi	10%	14%	20%
Escheretia coli	0%	0%	0%

Conclusion

The experiment performed by us conclude that the staphylococcus aureus show positive attitude by degrading maximum fat oil. On the other hand e coli and klebsiella pneumonia shows minimum oil degradation. Pseudomonas aeruginosa and Salma typhi show average fat oil degradation.

Since staphylococcus aureus so maximum oil degradation this indicate we can extract meaning all degrading enzyme from staphylococcus aureus. And we can use this bacteria for further experiments.

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11. To Study the Antimicrobial Properties of Mint (Pudina)

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Abstract

The present study, an antimicrobial activity of the aqueous extract of *Mentha species* was assessed using both well diffusion and microdilution method in multi-well micro-titre plates. Mint extract investigated for its antibacterial activity against seven selected pathogenic bacteria: *Bacillus fastidiosus, Staphylococcus aureus, Proteus mirabilis. Proteus vulgaris, Salmonella choleraesuis, Escherichia coli, Pseudomonas aeruginosa. Klebsiella pneumoniae and Serratia odorifera.* Menthe extract at different concentrations (1:1, 1:5, 1:10, and 1:20) was active against all tested bacteria except for *S.aureus*, and the highest inhibitory.

Introduction

Mentha is also known as mint. The genus has subcosmopolitan distribution throughout Europe, Africa, Asia, Australia, and North America. The most widely used species in India is the Hot Piperita . Most grow best in wet and moist soils. Minutes grow 10-120 cm in height and can spread to a permanent location. Due to their tendency to unchecked streaming, some mints are considered invasive. Introduction- Mint is a leafy plant that is probably best known for its association with fresh breath due to the cooling sensation it creates in the mouth. Toothpaste, mouthwash, respiratory mint, and chewing gum are all often flavored with mint.In addition to refreshing breath, mint is also used to add flavor to food and drink. Mint is known for adding fresh flavors to mint chocolate chip ice cream, mojito cocktails, and lamb dishes.There are many varieties of mint plants, and many of them fall under the genus Mitera. Because mint plants spread quickly, growers often grow them in containers. If planted directly in the ground, they can

invade and take over the garden. Some plants called "mint" also fall under the Monarda genus. Both Mentha and Monarda belong to the same family, called the Lamiaceae. Monarda mint includes horsemint, catmint, and stonemint. Mints are fragrant, almost endless. They have broad underground trunks and straight, square, with branches. The leaves are arranged in opposing pears, from oblong to lanceolate, usually low, and have a fixed border. The colors of the leaves range from dark green and gray to purple, blue, and sometimes pale yellow, The flowers are white to purple and are produced by a false whorl called verticilate. The corolla has two lips with subequal lobes, the upper lobe being usually larger. The fruit is a nut, which contains one to four seeds. Mint has square stems and fragrant leaves. Many can be spread in the natural order by stolons and can be aggressive in gardens. The small flowers are usually pale purple, pink, or white in color and arranged in clusters, whorls or dense in the final spike.

Antimicrobial Properties of Mint Essential Oils

Essential oils found in two types of mint, *Mentha pulegium* and *Mentha spicata*, have shown anti-bacterial properties against eight Gram-positive and Gram-negative bacteria. Essential oils of these types of mint at high concentrations (1/100 dilution) were extremely disinfected, while low concentrations (1/1000) caused a dose-dependent decrease in bacterial growth rates. The main components of p-menthane essential oils tested show a dynamic level of antibacterial activity not only between different types of bacteria but also between different types of the same bacteria. Antibacterial effects of mint species, especially peppermint oil from *Mentha piperita*, spearmint oil from *Mentha spicata* var. crisps and corn mint oil from *Mentha arvensis*, have great antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes and B.*

Keywords

1. Mentha longifolia
2. Good source of vitamin A
3. Methanol Pharmological effect

Materials

1. Fresh leaves of pudina
2. St. Test tubes
3. St .petri dishes
4. St. Pipettes

5. Alcohol
6. Sterile Muller Hinton Agar

Bacteria

1. *Escherichia coli*

Test Organism - Fresh strains of *Escherichia coli (e.coli)* were obtained from the laboratory of Microbiology Department

Well Diffusion Method

Antibacterial activity was tested by agar well diffusion method where different concentrations of selected plant extract were used. The organisms were poured in sterile Petri plates using medium by softly mixing of 1 ml of the 24 hrs. fresh culture along with 20 ml Sterile Muller Hinton Agar. 7 mm diameter wells were made using sterile borer after hardening the agar of Petri plates. All wells were filled with 0.1 ml of extract using the micropipette and then incubated at 37 °C for 24 hrs. The diameter of the inhibition zone around of each well was measured to detect the antibacterial activity. The experiment was done in triplicate and mean diameter of inhibition zones were recorded.

Results

The purpose of this study on mint was to determine the antibacterial activity of mint episodes. It showed a large block area at high altitudes and a small area of prevention as concentration decreases.

Escherichia coli Staphylococcus aureus

Sample (Pudina Extract)	**Zone of inhibitions**	**Zone of inhibitions**
Dilution	*Escherichia coli*	*S. aureus*
Undiluted	15 mm.	10 mm.
10^{-1}	13 mm.	8 mm
10^{-2}	6 mm.	6 mm.
10^{-3}	-	5 mm.

Conclusion

From the observation it is clear that Escherichia coli and S. aureus i.e. gram positive and gram negative both type of organisms are sensitive for Pudina extract. This study has shown the usefulness of natural products in reducing antibiotics germs resist, becoming a threat to human health. As a result, it is important that the drug plants with unknown structures become a priority for senior executives in development countries where citizens can always afford expensive orthodox medicines. In this study, several secondary metabolites are observed in mint leaves. Crop plants can also be used to treat many ailments, including skin infections, according to research. findings support the traditional use of mint leaves in the treatment of microbial infections and suggest that it can be used to produce novel antibacterial drugs.

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12. To Study the Antibacterial Properties of Onion

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Abstract

Onion is one of the oldest cultivated plants which are used for multiple purposes. It has many nutritional effects; the antioxidants and antimicrobial activity of onion has been proposed and can be more researched. The purpose of this study was to establish Onion's antibacterial efficacy and effectiveness. The disc diffusion method was employed to test the antibacterial activity of a concentrated onion extract. The presence of zone of inhibition was discovered against Bacteria's like *Escherichia coli & Klebsiella.* To assess the zone of inhibition, fresh samples of *E. coli* and *Klebsiella* were swabbed on the plates and subjected to concentrated onion. The zone of inhibition on the agar plates were measured as 12mm and 11mm in diameter. The results obtained in this experiment give credence to the effectiveness & antimicrobial properties of onion.

Keywords

- Onion
- Antibacterial properties
- *E. coli*
- *Klebsiella Spp.*
- Effectiveness

Introduction

One of the earliest cultivated vegetables all over the world was the *Allium* family which has over 500 species of various taste, color and shape. The most common type of *Allium's* members is: *Allium cepa* (Onion) and *Allium stivum* (Garlic), *Allium Fistulosum, Allium Ampeloprasum, and Allium schoenoprasm*. Onion was one of the oldest cultivated vegetables in

6000 years B.C and its common name was called *Allium cepa.* Onion contains good amount of minerals and a few vitamins. It also has various and many types of medicinal uses.

The onions not only have many uses throughout in culinary but also in therapeutic uses. Onions are healthy to consume due to its polyphenol molecules or phytonutrients which has many antioxidants and antibacterial properties. Onions have active components like Sulphur which is the most useful substance found in onions as it acts as inflammatory, other things like thiosulfates acts as anti-thrombotic and superoxide-dismutase which is an antioxidant. The most crucial antimicrobial agent in onion isquercetin and allicin (thio-2-propene-1-sulfinic acid-5-allyl-esters). This study investigates the antimicrobial activity of onion (*Allium cepa) against E. coli* and *Klebsiella* using; Minimum inhibitory concentrations (MIC), Minimum bactericidal concentrations (MBC), and disc diffusion.

Onions is a rich source of antimicrobial agent; the French researcher Louis Pasteur described the antimicrobial effects of onion and garlic. The flavonoids found in vitro are very helpful antimicrobial agents against microorganisms like bacteria and virus. Onions contains compounds that are antibacterial, antiviral, and antifungal.

Materials and Methods

a. Onions

For this research the red onion (*Rouge Amposta)* was used.

b. Preparation of Onion

About a medium sized onion was peeled off and chopped into fine pieces and then crushed into a mixer/blender. Once the extract was crushed it was filtered using whatmann filter paper, collected in a test tube and getting the onion extract ready for antimicrobial testing.

c. Selected Bacterial Strains

Fresh strains of *Escherichia coli* (*E. coli*) and *Klebsiella* sp. were obtained from the laboratory of the Microbiology department.

d. Media

Sterile Mueller-Hilton Agar

Mueller Hinton Agar is used for detecting antibiotic susceptibility tests (MIC, MBC).

This medium provides perfect growth for most pathogens due to its low concentration of sulfonamide.

Ingredients	In Gram/Litre
Beef Extract	2.00 gm
Acid Hydrolysate of Casein	17.50 gm
Starch	1.50 gm
Agar	17.00 gm
Distilled Water	1000 ml

e. Miscellaneous

- Test tubes
- Blender
- Sterile petri plates
- Sterile cotton swabs
- Sterile antimicrobial discs

Antibacterial Activity of Red Onion

First, we peel off a medium sized red onion and cut it into small pieces and then crushed into a mixer/blender. Once the mixture turns into a thick liquid the onion extract was pressurized and filtered using Whatman filter paper and collected in a sterile test tube. A series of dilution ranging from 10^{-1}, 10^{-2}, 10^{-3} was made, using distilled water as diluent. Sterile Mueller–Hinton agar media was prepared and poured into sterile petri plates. These plates were allowed to cool and solidify at room temperature.

After solidifying, the Mueller–Hinton agar plates were swabbed with strains of *Escherichia coli* and *Klebsiella* by using a sterile cotton swab on their respective plates. In the meantime, the sterile discs were aseptically immersed in their respective dilutions of red onion extract for 1 hour. After the immersion these discs were then transferred in their quadrants respectively. The plates were transferred in an incubator, for the incubation at 37^0C for 24-48 hours.

Result

The purpose of this study on Onion was to determine the antibacterial activity in red onion extracts. It showed a larger zone of inhibition at higher concentrations and a smaller zone of inhibition as concentrations are reduced.

The undiluted red onion extract showed the zone of inhibition in Escherichia coli (12mm), klebsiella (11mm). The red onion extract at dilution 10^{-1} showed the zone of inhibition

in Escherichia coli (9mm), klebsiella (8mm). Red onion extract at dilution 10^{-2} showed no zone of inhibition was observed in Klebsiella spp and E. coli. Inhibition zones less than or equal to 6mm On 10^{-3} no zone of inhibition observed, were considered to have no antimicrobial effect.

Escherichia coli ***Klebsiella***

Sample		**Zone of inhibition in diameter**	
Red	Dilutions	Escherichia coli	Klebsiella
onion	*Undiluted*	12mm	11mm
extract	10^{-1}	9mm	8mm
	10^{-2}	No zone of inhibition	No zone of inhibition
	10^{-3}	No zone of inhibition	No zone of inhibition

Table: Antimicrobial activity of Onion

Discussion

Onions have been used for many years due to its culinary, therapeutic and antimicrobial properties. The aim of this study was to investigate possible antimicrobial effects of onions, in which red onion (*Rouge Amposta)* was used. Based upon which we used common infectious causing bacteria, Escherichia coli (E. coli) and Klebsiella Spp. With onions being natural biological compounds and development in medical use, so we study this research to know the possible antibacterial effects of onion. The *allium* is known to be a natural preservative which can be used as a method to control pathogens. As useful as they are in nutritional properties, they're also antibacterial and antifungal against many gram-negative and gram-positive bacterias. Onion extract has been proven effective against pathogens and bacterias such as *E coli, Klebsiella* spp, *Salmonella* and *Bacillus subtilis*. It has also been found to be antibacterial against *S.aureus.*

Whereas in antifungal effects of onion has been observed in *Fistulosin* which has been extracted from welsh nion which has been proven to have antifungal properties.

Conclusion

The extract of red onion has shown antibacterial properties against tested organisms. This study demonstrated the usefulness of natural products in reducing antibiotic resistant bacteria, which has posed a threat to human health. As a result, it's vital that medicinal vegetables with unknown properties become a top priority for senior management in developing countries where residents can't always afford expensive orthodox medicine. In this study, several secondary metabolites were identified in the different types of Allium family. Onion extracts can also be

used to treat a number of preservatives, including antibacterial ad antifungal infections, according to research. The findings back up the traditional use of *Allium cepa* for microbial infection treatment and suggest that it could be exploited to produce novel antibacterial drugs. The onion extract has shown us the antibacterial activity, so the amount of antimicrobial activity of onion depends upon the type of onion, in which we used the red onion which showed us 50% and above antibacterial activity. Further study might help us in understanding more properties through researching.

Acknowledgement

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13. Antimicrobial Activity of Ginger Extract

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Abstract

This study investigated the antimicrobial activity of ginger (*Zingiber officinale*) extracts on three pathogenic microorganisms using the agar well diffusion method. These bacteria include; *Klebseilla* pneumoniae, *E.coli* and *Salmonella typhi.* Four different extracts were obtained from the rhizomes of ginger. There were zones of inhibitions around the wells which indicate that the organisms were sensitive to both water and ethanol extracts of ginger. The result showed that the isolates behaved differently in their sensitivity to the different extracts added to their growth medium. Water extract of *Zingiber officinale* produced the highest zone of inhibition.

Keywords - Ginger, Water, Antimicrobial, Antibiotics.

Introduction

Increasing multidrug resistance of pathogens forces to find alternative compounds for treatment of infectious diseases (Gull *et al.,* 2012). Antimicrobial agents with selective toxicity are especially useful as a chemotherapeutic agent in treating infectious diseases and may be a function of specific receptor requirement for drug attachment or it may depend on the inhibition of biochemical events essential to the pathogen but not to the host (Omoya and Akharaiyi, 2010). Many investigators have demonstrated the antimicrobial activity of the constituents of some higher plants and quite a number of chemical compounds of plant origin have been shown to possess antimicrobial activities (Rocio, 1982). Presently, the search for antifungal and antibacterial drugs has received attention mainly as a result of considerable drawbacks in the use of major antibiotics. These include those of limited antimicrobial spectrum that will cause serious side effects and high incidence of resistance in bacteria.

Ginger is the rhizome of the plant *Zingiber officinale*, consumed as a delicacy, medicine, or spice. It lends its name to its genus and family (Zingiberaceae). Other notable members of this plant family are turmeric, cardamom, and galangal (NPGS/GRIN, 2011). Preliminary research indicates that nine compounds found in ginger may bind to human serotonin receptors, which may explain ginger's extensive effects on the gastro-intestinal tract and suggesting a mechanism for its effects on anxiety (Nievergelt *et al.*, 2010). Many scientists have reported antimicrobial properties of several plants. The antimicrobial, antitumour (Khalil *et al.*, 2005; Akroum *et al.*, 2009; Omoya and Akharaiyi, 2012), anti-inflammatory and anti-necrotic (Lin and Huang, 2002; Omoya and Akharaiyi, 2012) activities have been reported from the use of plants extracts. The most well-known member of *Zingiber* (ginger) is *Zingiber officinale*. In many parts of the world, *Z. officinale* has medicinal and culinary values (Omoya and Akharaiyi, 2012). The volatile oil gingerol and other pungent principles not only give ginger its pungent aroma, but are the most medically powerful because they inhibit prostaglandin and leukotriene formation, which are products that influence blood flow and inflammation (Longe *et al*, 2005; Omoya and Akharaiyi, 2012). Ginger has been found to be more effective than placebo in multiple studies for treating nausea caused by seasickness, morning sickness and chemotherapy (Ernst and Pittler, 2000), though ginger was not found superior to placebo for presumptively treating postoperative nausea (Omoya and Akharaiyi, 2012). These studies also show superiority of odansetron over ginger in the treatment of chemotherapy related nausea. Ginger compounds are active against a form of diarrhea which is the leading cause of infant death in developing countries. Zingerone is likely to be the active constituent against enterotoxigenic *Escherichia coli* heat-labile enterotoxin-induced diarrhea (Ernst and Pittler, 2000; Chen *et al.*, 2007).

The aim of this study is to determine the antimicrobial activity of *Zingiber officinale* (ginger) extracts on different pathogenic bacteria and to carry out phytochemical screening of the extracts so as to evaluate the bioactive constituents responsible for their antimicrobial activity.

Materials and Methods

Preparation of Plant Extract

The fresh forms ginger were collected from the local market. It was washed thoroughly and all the dirt remnants were removed. The compound was then chopped and transferred in a blending jar then it was blended in a fine paste. The paste was filtered through a fine cloth and

then again filtered through the Whattman's fiter paper. The extract was transferred in an air tight container for after use.

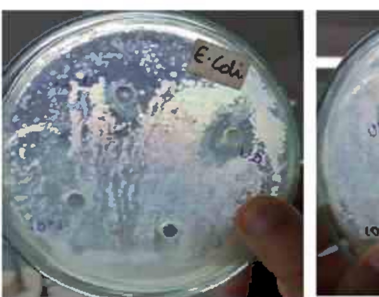
Screening For Antibactrial Activity

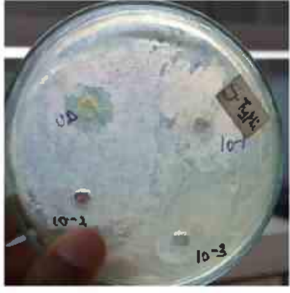
Antibacterial activity was tested by agar well diffusion method where different concentrations of selected plant extract were used. The organisms were poured in sterile Petri plates using medium by softly mixing of 0.1 ml of the 24 hrs. fresh cultures alone with 20 ml of sterile Muller Hinton agar. 7 mm diameter wells were made using sterile borer after hardening the agar of Petri plates. All wells were filled with 0.1 ml of extract using the micropipette and then incubated at 37 °C for 24 hrs. The diameter of the inhibition zone around of each well was measured to detect the antibacterial activity. The experiment was done in triplicate and mean diameter of inhibition zones were recorded.

Bacteria and Bacteria Culture

Prior to sensitivity testing, *Escherichia coli, Kebseilla pneumoniae* and *Salmonella typhi* strains were cultured onto Nutrient Agar and incubated for 24 h at 37 °C. A single colony was then cultured in 5 ml Nutrient Broth for 4 h at 37 °C. The density of bacteria culture required forthe test was adjusted to 0.5 McFarland standard, (1.0 x 108 CFU/ml) measured using the Turbidometer.

Observations





Result

1. The result shows that the ginger extract is the least reactive for *Salmonella typhi* and the zone of inhibition for only seen in the undiluted sample to *Salmonella typhi*.
2. It is a bit more reactive to *E.coli* than with *Salmonella typhi* and shows the zone of inhibition for only 10^{-1} concentration.
3. For *Klebseilla pneumoniae* it shows the highest zone of inhibition than the other two organisms and the zone of inhibition can be seen in all the concentrations i.e. Undiled, $10^{-1,}$ 10^{-2}, 10^{-3}

The result showed that the ginger extracts behave differently for different organisms.

Conclusion

The result of the study emphasizes the usefulness of *Zingiber officinale* (ginger) in the treatment of diseases and the need to enhance its exploitation on this regard. This is particularly of urgent interest when the growth rate of multi-resistant drug strains of bacteria worldwide is considered (Prescott *et al*., 2005). The results of present study have provided the justification for therapeutic potential of spices. The practice of using spices as supplementary or alternative medicine in developing countries like Nigeria will not reduce only the clinical burden of drug resistance development but also the side effects and cost of the treatment with allopathic medicine. This study also showed that ginger extracts possess differences in antibacterial activities. Ginger in its spicy nature with free radical inhibitions index performs other toxic factors which of course responded to the antibacterial effect observed in the study. While some phytochemical constituents known for inhibition of microorganisms were observed in *Zingiber officinale*, it did not possessed traces of sapinin and cardiac glycoside. However, the importance of ginger (*Zingiber offficinale*) cannot be over emphasized as regards their rule in health remedy. In fact, the findings revealed that the knowledge of the antimicrobial activity of the extracts obtained from ginger can be very useful and can be applied in different areas of research such as the pharmaceutical and food industries.

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14. To Study the Antimicrobial Properties of Aloe Vera

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Abstract

The purpose of this study was to establish Aloe Vera's antibacterial efficacy and effectiveness. The disc diffusion method was employed to test the antibacterial activity of a concentrated Aloe Vera extract. The presence of zone of inhibition was discovered against Bacteria's like *Escherichia coli* & *Klebsiella*. To assess the zone of inhibition, fresh samples of *E. coli* and *Klebsiella* were swabbed on the plates and subjected to concentrated Aloe Vera. The zone of inhibition on the agar plates were measured as 10mm and 11mm in diameter. The results obtained in this experiment give credence to the effectiveness & antimicrobial properties of Aloe Vera.

2. Keywords

- Aloe Vera
- Effectiveness
- Antimicrobial properties
- *E.coli*
- *Klebsiella*

3. Introduction

Plants are acknowledged as the primary medicinal source in some underdeveloped nations for the treatment of numerous infectious diseases. Plant extracts are the result of a never-ending search for new antimicrobial compounds. Approximately 20% of all plants discovered on the planet have been subjected to pharmacological or biological testing and a significant percentage of novel antibiotics are derived from natural or semisynthetic sources (Mothana and Linclequist, 2005). Aloe Vera (Aloe barbadensis miller) is a succulent plant that belongs to the

Liliaceae family. It has a whorl of elongated, pointy leaves (Strickland *et al.*, 2004; Beckford and Badrie, 2000). The term Aloe Vera comes from the Arabic word 'Alloeh,' which meaning 'bitter,' and refers to the bitter flavor of the liquid contained in the leaves. Aloe Vera thrives in arid environments and is found in India, and other arid regions. Herbal medicine is widely indicated as an application for the species.

Aloe Vera is a drought-resistant, perennial succulent plant. It features stiff green lance-shaped leaves with a core mucilaginous pulp containing clear gel. Its large leaves provide the plant with enough water to endure lengthy periods of drought (Foster,1999). When the green epidermis of a leaf is peeled, a clear mucilaginous substance appears that comprises fibers, water, and the chemical that keeps the water in the leaf from evaporating. The gel is 99.3% water, while the remaining 0.7 percent is made up of solids, with carbohydrates accounting for the majority of the components, proteins (Glycoprotein), amino acid (7 essential amino acids and 20 amino acid found naturally), enzymes (cellulase, carboxypeptidase, catalase, bradykinase, oxidase, amylase etc.), vitamins (B1, B2, B6, niacin, ascorbic acid, carotenoids, folic acid, etc.), minerals (copper, iron, zinc, magnesium, etc.), other constituents such as phenolic compounds, organic acids (mallic acid, succinic acid etc.) and phytosterol (campesterol, β-sitosterol, etc).

Aloe contains compounds that are antibacterial, antiviral, and antifungal. Above all, the presence of two organic acids, cinnamic and chrysophanic acid, also known as chrysophanol, allows Aloe Vera to inhibit the growth of bacteria and fungi. Aloin, oleic acid, and aloe-emodin are all anthraquinone compounds with anti-inflammatory and antibacterial properties.

4. Materials and Methods

4.1 Test organism

Fresh strains of *Escherichia coli (E.coli*) and *Klebsiella* were obtained from the laboratory of the Microbiology department.

4.2 Preparation of Extract

Leaves of Aloe Vera were washed and cut opened, the skin was removed and fresh gel was collected. The aloe juice was extracted by pounding the aloe vera gel in mortar by using pestle. The aloe juice was then filtered by filter paper and fresh aloe juice was collected in a test tube.

4.3 Media

Sterile Mueller–Hinton agar

Ingredients	grams/ liter
HM infusion B	300
Acicase	17.5
Starch	1.50
Agar	17.0
Final pH	7.3±0.1

4.4 Miscellaneous

Test tubes, Mortar and Pestle, sterile petri plates, sterile cotton swab, sterile disc.

5. Antimicrobial Activity of Aloe Vera

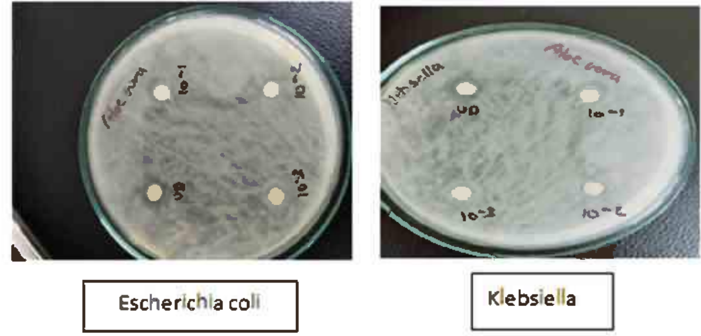
First the aloe vera leaves were washed carefully, the leaves were cut opened and the skin was removed gently by using a blade. Then the aloe vera gel was collected and was transferred in mortar. The aloe juice was extracted by pounding the aloe vera gel continuously in mortar by using pestle. The fresh aloe juice was collected and filtered by using a filter paper. This fresh aloe juice was collected in a sterile test tube. A series of dilution ranging from 10^{-1}, 10^{-2}, 10^{-3} was made, using water as diluent. Sterile Mueller–Hinton agar media was prepared and poured into sterile petri plates. These plates were allowed to cool and solidify at room temperature.

After solidifying, the Mueller–Hinton agar plates were swabbed with strains of *Escherichia coli* and *Klebsiella* by using a sterile cotton swab on their respective plates. In the meantime, the sterile discs were aseptically immersed in their respective dilutions of aloe vera juice for 1 hour. After the immersion these discs were then transferred in their quadrants respectively. The plates were transferred in an incubator, for the incubation at 37^0C for 24 hours.

6. Result

The purpose of this study on Aloe vera was to determine the antibacterial activity Aloe Vera extracts. It showed a larger zone of inhibition at higher concentrations and a smaller zone of inhibition as concentrations are reduced.

The undiluted aloe vera extract showed the zone of inhibition in *Escherichia coli* (12mm), *Klebsiella* (11mm). The aloe vera extract at dilution 10^{-1} showed the zone of inhibition in *Escherichia coli* (8mm), *Klebsiella* (7mm). Aloe Vera extract at dilution 10^{-2} and 10^{-3} showed no zone of inhibition was observed.



Sample		Zone of inhibition in diameter	
Aloe Vera extract	**Dilutions**	**Escherichia coli**	**Klebsiella**
	Undiluted	12mm	11mm
	10^{-1}	8mm	7mm
	10^{-2}	-	-
	10^{-3}	-	-

Table no 1. Antimicrobial activity of Aloe Vera

7. Discussion

Many medicinal plants, the most notable of which is Aloe Vera, are widely utilized in India to treat wounds as possible sources of new antibacterial chemicals. These findings on antibacterial activity are consistent with those of Antonisamy *et al.*, who discovered that DMSO gel extracts of Aloe Vera had varying antibacterial and antifungal activity against five bacterial cultures of *Bacillus subtilis, Salmonella typhi. Escherichia coli*, and *Staphylococcus aureus*, as well as three fungal cultures of *Aspergillus fumigatus, Candida albicans, and Penicillium.*

Aloe Vera demonstrated the highest level of action against the pathogens tested, and the level of inhibition varied depending on the extract concentration. The elements of gel and leaf have been confirmed to be separate by scientific data. Some researchers feel the gel is more active than the leaf, but there is no doubt that the gel and leaf may complement each other in terms of therapeutic properties. Although the recognized chemicals in Aloe Vera leaf gel are present in little amounts (approximately 1%), they can be connected to operate together and provide a synergistic effect, resulting in a complete effect that is bigger than the sum of its parts.

8. Conclusion

This study demonstrated the usefulness of natural products in reducing antibiotic-resistant bacteria, which has posed a threat to human health. As a result, it's vital that medicinal plants with unknown properties become a top priority for senior management in developing countries

where residents can't always afford expensive orthodox medicine. In this study, several secondary metabolites were identified in the leaves of Aloe Vera. Plant extracts can also be used to treat a number of ailments, including skin-transmitted infections, according to research. The findings back up the traditional use of Aloe Vera for microbial infection treatment and suggest that it could be exploited to produce novel antibacterial drugs.

9. Acknowledgement

I Suraj shukla, would like to thank my professor, Mrs. Sonali Joshi, for assisting and mentoring me throughout the process, as well as my college, ZSCT's Thakur Shyamnarayan Degree College, for providing me with a platform to present our research papers.

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15. Lipase Catalyzed Process for Biodiesel Production

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Abstract

Biodiesel is a climate agreeable and inexhaustible fuel delivered by transesterification of different feedstocks. Albeit the lipase- catalysed biodiesel creation has quite a large number benefits over the traditional salt catalysed process, its modern applications have been restricted by significant expense and low-security of lipase compounds. This audit gives a general outline of the new advances in lipase designing counting both protein change and creation. Later progresses in biotechnology, for example, in protein designing, recombinant strategies and metabolic designing have been utilized yet will be yet to affect lipase designing for cost effective creation of biodiesel. An outline of the current difficulties and points of view for potential arrangements are also provided.

Keywords - Biodiesel; lipase; enzyme; transesterification; protein engineering; lipase production

Introduction

As of late, inferable current utilization rate, the accessible inventory of petroleum products might keep going for <50 years (Shariff *et al.*, 2010). In expansion, expanding CO2 discharges because of copying petroleum products set tension on the biological cycle and may account for worldwide environmental change. In this specific circumstance, biodiesel, as an elective fuel from sustainable sources which contain free unsaturated fats (FFAs) and fatty substances (TGs), can give an incomplete by esterification of FFAs or transesterification of TGs (Andrade *et al.*, 2011; Meher *et al.*, 2006). The regular synthetic responses associated with biodiesel creation. Right now, the fundamental feedstock for biodiesel creation is virgin oil, for example, soybean and rapeseed oil (Bartet *et.al.* 2010; Moser, 2011). For biodiesel from palatable oil sources, the expense of the feedstock addresses 70-80% of aggregate biodiesel

creation costs (Demirbas, 2009). From this setting, non-eatable oils (e.g., castor bean, jatropha, pongamia, and so forth), low worth lipids (e.g., creature fat, squander cooking oils, and so forth) and microalgae have as of late drawn in extensive interest (Azocar *et al.*, 2010; Bart *et al.*, 2010; Hama and Kondo, 2013; Lai *et al.*, 2012; Moser, 2011; Olmstead *et al.*, 2013; Zhang *et al.*, 2003b). The determination of fitting feedstock relies upon the territorial accessibility and financial aspects. Biodiesel is a CO2-impartial fuel since its essential feedstock starts from carbon dioxide in the air. When contrasted with mineral diesel, biodiesel contains next to no sulphur and sweet-smelling compounds, hence contrarily affects air quality (Andrade *et al.*, 2011; Meher *et al.*, 2006). For these reasons, biodiesel is presently generally acknowledged as a practical option in contrast to diesel fuel for transportation applications. The current world inventory of biodiesel comes nearly only from compound catalysed change processes, utilizing salt impetuses like NaOH or KOH (Kaieda *et al.*, 1999; Meher *et al.*, 2006;) (Srivastava and Prasad, 2000; Zhang *et al.* 2003b). The salt catalysed response, in any case, a lot of cleanser which hinders division among FAAE and glycerol (see Fig. 1a). Also, the substance process commonly uses huge amounts of water to eliminate salt impetus from the item, consequently create the waste water which adds a huge weight to the biological framework (Suehara *et al.*, 2005). Besides, on the grounds that the esterification and transesterification responses require different working conditions, a two-organized response framework is by and large needed for the synthetic cycle.

When contrasted with the substance catalysed process, the chemical (lipase)- catalysed process doesn't have the above noted downsides. In particular, lipases can change over both FFAs and TGsto deliver FAAE without cleanser development in a solitary reactor (Fjerbaek *et al.*, 2009; Vasudevan and Briggs, 2008). It is, subsequently, additionally more straightforward to adapt to changes in the inventory network because of market changes, for instance, soy bean oil cost increment. Moreover, the proteins immobilized in insoluble materials can be somewhat effectively isolated from the last item, which works on the downstream partition steps also diminishes cost. In spite of its extraordinary guarantee, there are major challenges in adjusting the lipase-catalysed cycle to modern scale, including execution, steadiness, recyclability, and creation of lipases. In particular, the paces of enzymatic responses are by and large low. Despite the fact that lipase is conceivably recyclable, it will in general lose movement later continuous

operation and can be deactivated by short-chain alcohols and glycerol (Chen and Wu, 2003; Chesterfield *et al.*, 2012; Lu *et al.*, 2012;

Salis *et al.*, 2005; Shimada *et al.*, 1999). The high cost of enzymes is also a barrier towards the industrial application of the enzyme-catalysed biodiesel production processes. Improving the performance and durability of lipase and reducing its manufacturing cost thus hold the key to large-scale commercialization of lipase-catalysed biodiesel production.

Protein Engineering Strategies

Despite the fact that lipases got from normal sources can be utilized in biodiesel creation, they ordinarily miss the mark on advantageous elements that are reasonable for modern scale responses. In particular, normal lipases have greatest reactant exercises in the temperature range 30-50C (Fjerbaek *et al.*, 2009). At these temperatures, the transesterification response has low reaction rate which makes the interaction tedious and less monetarily cutthroat. Expanding the working temperature scope of lipases by further developing thermostability, thusly, a basic part of lipase designing. Second, attributable to corruption, regular lipases ordinarily have restricted lifetimes what's more must be supplanted oftentimes in modern reactors. The lipase lifetimes can be additionally abbreviated when short chain solvents are utilized in the change responses (Chen and Wu, 2003; Chesterfield *et al.*, 2012; Lu *et al.*, 2012; Salis *et al.*, 2005; Shimada *et al.*, 1999). It is hence alluring to engineer a lipase with delayed lifetimes by improving its protection from both normal and short-chain worked with corruption pathways. Third, the lipase-catalysed transformation responses require restricting and unbinding of substrates to the synergist focuses and the response rate is regularly controlled by the availability or dispersion boundaries to the synergist focuses. Low response rates are for the most part seen in lipase catalysed processes. Expanding the response rate Finally, most natural lipases have been evolved to target a specific type of substrate with defined chain lengths, while for industrial processes adaptability to various feedstocks with distinctive compositions is desirable. To be commercially competitive, a natural lipase has to be redesigned to possess enhanced features in all of the above-mentioned aspects. Two significant protein-designing methodologies, in particular reasonable plan and coordinated development, have been applied to further develop the applicable properties recorded previously. In spite of the fact that the two methodologies can work on utilitarian properties of lipases, the decision of strategy relies upon the accessibility of information, for example, the design work relationship of a explicit lipase and high throughput screening draws near .The judicious plan of

proteins requires deduced information of the construction work connection of a protein. Brady *et al.* (1990)

The first used X-beam crystallographic investigation to comprehend the construction of Rhizomucor miehei lipase. The constructions of other significant lipase types have been distinguished in ongoing years, including *Bacillus thermocatenulatus*, *Candida antarctica*, *Pseudomonas Cepacia*, and *Bacillus subtilis* (Carrasco Lopez *et al.*, 2008; Ericsson *et al.*, 2008; Kim *et al.*, 1992; Ransac *et al.*, 1994). This data gives a substantial information base to judiciously choose potential change destinations on lipases. The new advances in PC helped protein plan by sub-atomic unique reproduction instruments have further empowered forecasts of point mutation(s) on practical properties of lipases (Guieysse *et al.*, 2008). On the off chance that the underlying data of a particular kind of lipase is feeling the loss of, the construction of a homologous catalyst can be used to work with the adjustment process (Bordes *et al.*, 2009; Bornscheuer and Pohl, 2001; Kazlauskas, 2000). For instance, the construction of Burkholderia cepacia lipase (PDB:3LIP (Schrag *et al.*, 1997). For most lipases, admittance to the dynamic site containing a serine, histidine and aspartate set of three, is safeguarded by a cover area. This cover comprises of a-helices, which are associated by a circle, connected to the body of a lipase. In the open also dynamic type of the lipase, the cover moves away by pivoting pivot district and makes the dynamic site open to the substrate. This versatile cover district probably adds to the and action of the lipase and has been the "problem area" for lipase designing lately.

Thermostability

Since they can be deactivated due to thermal denaturation, thermostability is an important requirement for commercial lipases. In general, increased reaction temperature enhances the solubility of alcohols in oil, which promotes a faster transesterification reaction. Thus, enzymatic catalysts used at high temperatures could increase transesterification yield and require shorter reaction time. Several types of lipases, particularly those originating from thermophilic organisms, such as *Bacillus subtilis*, *Thermomyces lanuginose, Rhizopus orzae.* and *Psedomonas sp.*, have been reported to have heat resistance up to 90C (Bouzas *et al.*, 2006; Haki and Rakshit, 2003). The current performance of lipases, however still misses the mark regarding modern assumptions as far as long term thermostability.

Both normal plan and coordinated development techniques have been utilized to upgrade thermostability. To act as an illustration of the previous, Santarossa *et al.* (2005) distinguished

three polar deposits (T137, T138, and S141) in the top area of cold adapted *Pseduomonas fragi* lipase utilizing homologous design. They tracked down that those deposits contribute fundamentally to upgrade thermostability of the lipases (Santarossa *et al.*, 2005). In a coordinated development approach, Yu *et al.* (2012b) further developed thermostability of lipase from Rhizopus chinensis essentially by two rounds of ep-PCR and two rounds of DNA rearranging. They found that, owing fundamentally to expanding the hydrophilicity and extremity of the protein surface and making hydrophobic contacts inside the protein, the softening temperature of a variation was 22°C higher and half-lives at 60°C and 65°C were 46- and 23-time longer, as contrast with the parent. They additionally investigated the connection between top inflexibility and lipase action by presenting a disulfide bond in the pivot area of the top of lipase (Yu *et al.*, 2012a). They viewed that as, when contrasted with the wild-type, the cross-connected variation showed 11-overlay expansion in half-life at 60°C and 7°C increment of dissolving temperature. Reetz *et al.* (2006) presented the alleged B factor iterative test (B-FIT) to decide the thermostability enzymes. Their approach was based on the observation that thermostability can often be related to the rigidity of the protein. A higher B-factor means that an amino acid residue has a low number of contacts with other amino acids and is considered to be more flexible and more thermo-unstable (Radivojac *et al.*, 2004). By an iterative saturation mutagenesis of seven sets of residues from *Bacillus subtilis* lipase A with a high B-factor, they were able to shift the temperature stability (T50°C, 60°C: 50% activity after 1 h at the defined temperature) from 48o°C (wild type) to 93°C (Reetz and Carballeira,2007).

Stability in Organic Solvents

Low solvency of short-chain alcohols in oil prompts lipase inactivation. Expansion of natural dissolvable to the combination of liquor and oil works on the security by improving the solvency just as diminishing the thickness of the response combination. Nonetheless, lipases can be denatured in natural solvents and along these lines action of the lipases can be restricted in frameworks containing natural solvents (Brocca *et al.*, 2003). Both coordinated advancement and objective plan approaches have been utilized to improve strength of lipase within the sight of natural solvents .Through ep-PCR, freaks of *Psedomonas aeruginosa* LSt03 lipase have displayed higher half-life in solvents, for example, dimethyl sulfoxide (DMSO), cyclohexane, n-octane, and decane (Kawata and Ogino, 2009). The underlying examination of these variations uncovered that a huge part of changes were situated on the protein surface. In light of this data,

they utilized a site-coordinated mutagenesis strategy (Kawata also Ogino, 2010). Five changes (S155L, G157R, S164K, S194R, and D209N) were recognized to further develop solidness of lipase within the sight of natural solvents by actuating underlying changes which prompted a superior pressing of the deeply. As of late, it has been recommended that the circles situated on the outer layer of *Bacillus subtilis* lipase play a basic job in resistance to natural solvents like DMSO (Yedavalli and Rao, 2013). They screened 18,000 clones, in light of site immersion mutagenesis of every one of the 91 amino acids in the circle district and observed that a variation has multiple times higher reactant turnover in 60% DMSO.

Current Challenges and Perspectives in Protein Engineering

Since specific structural domains have been recognized and linked to the catalytic activity and substrate specificity of lipases (Boersma *et al.*, 2008; Brocca *et al.*, 2003; Fernandez *et al.*, 2008; Hidalgo et al., 2008; Martinelle *et al.*, 1995; Santarossa et al., 2005; Secundo et al., 2004; Skjot et al., 2009; Yen *et al.*, 2010), rational design is found to be more efficient in designing these types of lipase specificities. Nevertheless, properties such as thermostability and solvent-tolerance are commonly affiliated with the global folding of the protein (Chakravorty *et al.*, 2012; Kawata and Ogino, 2010; Santarossa *et al.*, 2005; Yedavalli and Rao, 2013; Yu *et al.*, 2012a,b). Introducing a single mutation or altering a single structural domain is thus unlikely to significantly affect the global folding and stability. Although modern molecular simulation tools can provide insightful suggestions regarding the modifications sites, the agreement between .Later improvements have zeroed in on making more modest libraries (<100). This approach alludes to the randomization of all amino acids at a characterized position or to the synchronous randomization of at least two positions in a compound. For this situation, the succession libraries become more modest and subsequently quicker to screen. In this unique circumstance, the way to achievement lies rather in the proficient blend of coordinated development and objective protein configuration draws near, as proposed beforehand (Morley and Kazlauskas, 2005). Regardless of extraordinary advances of designed lipases in later years, the current lipases actually need adequate soundness for long term ceaseless activity to be monetarily achievable to rival salt impetuses. Albeit present day protein designing procedures can be utilized to work on some specific part of lipase execution, generally specialized challenges are partnered with the security of the chemical that requires worldwide advancement of the general protein structures. An orderly screening approach consolidating all cycle contemplations, for example, dynamic

temperature range, natural dissolvable solidness, synergist movement, substrate selectivity and so on, will accordingly be needed to upgrade lipases for business scale biodiesel creation.

Lipase Production

Lipases are ubiquitous in nature and found in plants, animals and microorganisms. Among them, microbial lipases are the most commonly used in industrial applications due to their selectivity, stability and broad substrate specificity. In spite of improvements in lipase properties in recent years, the high manufacturing cost of lipases is still the major roadblock for commercialization of lipase-catalyzed biodiesel production processes. In this segment, we examine the new advances in lipase creation utilizing host strain and metabolic designing strategies.

Host Strain Selection

Creation of useful lipases utilizing heterologous approaches is the most encouraging procedure to bring down the expense of lipases (Valero, 2012). A wide range of animal categories have been formed into proficient host strains for heterologous articulation of lipases in the previous ten years,. Beneath, we give an overall outline of the most usually utilized articulation has for the improved production of recombinant lipases.

Bacteria

For an assortment of reasons *Escherichia coli* stays the most famous articulation have for recombinant protein articulation. *E. coli* is more versatile for hereditary control and furthermore has high change effectiveness and quick development rates. This prokaryotic host has been utilized for communicating an assortment of lipase started from microbes (Akbari *et al.*, 2010), yeast (Jung *et al.*, 2011), and filamentous growths (Di Lorenzo *et al.*, 2005). Because of the absence of appropriate collapsing instruments, nonetheless, *E. coli* framework ordinarily results in intracellular aggregation of dormant or insoluble incorporation bodies. A number of strategies have been utilized to dodge this constraint. For instance, dynamic type of Lipase B from *Candida antarctica* (CalB), perhaps the most broadly created protein in biocatalysis businesses, can be communicated in *E. coli* by changing the response medium or altering the lipase (Blank *et al.*, 2006; Narayanan and Chou, 2009). Combination of lipases with a polycationic amino corrosive tag can likewise expand the solvency of communicated proteins in *E. coli* (Blank *et al.*, 2006). Moreover, a few sorts of lipases require the arrangement of explicit disulfide bonds to work with the collapsing of utilitarian proteins. This issue can be tended to by utilizing a specific

E. coli Origami (DE3) strain or co-articulation of the Dsb-family protein (e.g., DsbA) where disulfide bond development.

Yeasts

Yeasts offer various benefits as articulation frameworks forcomplex proteins, including solid development limit, permitting disulfide bond arrangement, simple hereditary control, and post-translational handling of proteins (Darvishi, 2012; Shockey *et al.*, 2011). Saccharomyces cerevisiae is nonpathogenic and has been utilized as a host for heterologous lipase creation for some time (Yu *et al.*, 2007). Shockey *et al.* what's more Darvishi changed Yarrowia lipolytica lipase 2 (LIP2) qualities into S. cerevisiae with PEX11 advertiser. They effectively produced S. cerevisiae strains that emit dynamic Lip2 lipase (Lip2p) into the development media (Darvishi, 2012; Shockey *et al.*, 2011). Despite the fact that S. cerevisiae articulation framework permit control and significant degree of heterologous protein articulation, it additionally has a few downsides, for example, helpless plasmid steadiness, low discharge limit, trouble in increase, and hyper-glycosylation.

Fungi

Growths like genera *Mucor, Rhizopus, Geotrichum, Rhizomucor, Aspergillus and Penicillium*, are the significant lipase-creating sources. As contrasted and microorganisms and yeasts, the filamentous parasites has are considered as a beneficial methodology. Filamentous parasites enjoy a few benefits including higher plasmid duplicate number, plasmid solidness and higher capacity to discharge extracellular proteins when contrasted with other heterologous. Among various organisms species, *Aspergillus sp.* And also *Trichoderma sp.* is broadly utilized for lipase creation in modern applications (Adachi et al., 2011, 2013). Prathumpai *et al.* (2004) revealed two recombinant strains of *Aspergillus niger* creating a heterologous lipase from Thermomyces lanuginosus utilizing the TAKA amylase advertiser from *Aspergillus oryzae.* The most concentrated on filamentous growths have is *Aspergillus oryzae*, for instance, CalB with high esterification action has been heterologously created by *Aspergillus oryzae* and immobilized for entire cell biocatalyst for enzymatic biodiesel creation (Prathumpai *et al.,* 2004). What's more, Trichoderma reesei has drawn consideration for recombinant protein creation as of late utilizing cbh1 advertiser (Wang and Xia, 2011), henceforth is viewed as an selective host for recombinant lipase creation.

Current Challenges and Perspectives in Lipase Production

Among somewhere in the range of 4,000 proteins known to date, lipase is perceived as one of the omnipresent compounds of significant modern potential. Presently, business lipases are for the most part acquired from microorganisms that produce a wide assortment of extracellular lipases. The worldwide interest of business compounds, around 75% of which are hydrolytic proteins (counting lipases), is relied upon to ascend by around 5% in the following ten years. Lipase interest in China expanded enormously starting around 2003 as a defining moment, and the creation limit has expanded by 10% yearly. In 2010, the assembling limit of lipases has reached around 2,500 tons. Notwithstanding, the current stockpile of lipases misses the mark to meet the expanding request. This challenge can be tended to by evaluating for novel lipase-creating microorganisms and performing metabolic designing. Moreover, the advancement of new lipases creation processes, by using lowered maturation, blended activity modes, new high-proficiency bioreactors, and numerical and factual advancement models, is additionally viewed as a compelling methodology for upgrading lipase creation. Among these, high-throughput screening techniques and engineered science are bound to work on the efficiency of lipase creation. Moreover, later progresses in screening procedures have empowered quick identification of high return microorganisms. Engineered science, on the other hand, can broaden and alter the conduct of living beings for better lipase efficiency. Use of manufactured science for lipase creation is relied upon to outperform conventional engineering procedures by mixing the best highlights of regular and fake microbial frameworks with objective plans that are extensible, thorough, and effective.

Conclusion

Lipase-catalyzed biodiesel creation from sustainable sources enjoys a few upper hands over the regular chemical catalyzed process, including lower natural worries furthermore energy utilization. The low steadiness and significant expense of lipase, notwithstanding, have been the principle obstacles for the industrialization of lipase-catalyzed biodiesel creation. In this specific circumstance, protein designing and further developed lipase creation framework alongside the streamlined metabolic process are fundamental for address the difficulties noted previously. Both levelheaded plan and coordinated advancement strategies have been effectively used to design lipase catalysts for upgraded execution. Propels in displaying and computational apparatuses for successive and underlying examination just as screening frameworks will additionally work with

improvement of high performance lipases. Moreover, enhancement of lipase creation frameworks can build usefulness while decreasing item cost. For enormous scope commercialization of lipase catalyzed process, catalyst immobilization and streamlining of the interaction will be likewise required, which can further decline complete item cost. It is reasoned that a coordinated research program which consolidates lipase designing and metabolic designing for high lipase usefulness, and response designing for process heightening, is probably going to yield promising result for boundless use of the lipase-catalyzed biodiesel creation process

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16. To Study the Antimicrobial Effects of Citrus Sinensis Peel Extracts on Different Types of Bacteria

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Abstract

There is abundant medicinal flora which is Unrevealed through research. The study was conducted to assess the in vitro antimicrobial potential and also determine the minimum inhibitory concentration (MIC) of *Citrus sinensis* peel extracts with a view of searching a novel Extract as a remedy for pathogens Which can be used to reduce the effect of the pathogens with the formation of the sever product like handwash and sanitizer.

Material and Method

The peels were carefully washed under running tap water Followed by sterile distilled water. These were air dried at room temperature (30°C) for two days, pulverized to a fine powder using a sterilized mixer grinder and stored

In air-tight bottles various test were performed to know the chemical composition of the peel extract like 1)Test for tannins, 2)Test for saponins, 3)Test for flavonoids, 4)Test for Tarpenoids, 5)Test for Alkaloids, 6)Test for phenols, which shows the presence of these compounds in peel extract of *Citrus Sinensis*

Then Four different bacteria which are

1. *Staphylococcus aureus*
2. *Salmonella Typhi*
3. *Klebsiella pneumoniae*
4. *Pseudomonas aeruginosa*

Were procured from Microbial Type Culture Collection, These microorganisms were subcultured on the specific media, incubated aerobically at 37°C. Identification of all strains was confirmed by standard biochemical and staining methods.

Firstly the Nutrient agar was prepared then autoclaved and the they were poured on sterile pertriplate then the Extracts of the *Citrus sinensis* were used for the antimicrobial screening by Using the agar well diffusion method. The different types of microorganisms were inoculated on the different petriplate with the help of the sterlized spreader The media was Punched with 7mm diameter wells with the help of borer and were filled with various concentrations of the extracts 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and observed plates were then incubated at 37°C for 24 hours. After incubation, zone of growth inhibition for each extract was observed.

Conclusion

Citrus sinensis peels extract demonstrated in vitro antimicrobial activity against pathogens warranting for further in vivo clinical studies to determine the exact dosages and its effectiveness in practical situations. Toxicity studies should also be done to determine safety. Need of the hour is to execute more and more screening of natural products or plant parts to set a primary platform for further phytochemical, pharmacological and in vivo studies that may open the possibilities of finding new clinically effective antibacterial compounds against bacterial resistant pathogens. We can also prepare some product like handwash and sanitizer which be used to reduce the effect of the pathogens on body

Keywords – Agar well diffusion, antimicrobial activity, *Citrus sinensis*, zone of inhibition.

Introduction

There are certain resistant bacteria which represent a challenge in the treatments of various well-known infections and they necessitate the need to find new substances with antimicrobial properties to be used against these microorganism. Plants have always anchored to the mother earth long before man can set his feet on earth. Mankind has been gifted with Sources for existence much earlier than arrival of life on Earth.

The World Health Organization (WHO) estimates that about 80% of the population still depends upon herbal medicines for the treatment of various diseases due to easy availability, economic reasons and lesser side effect.

Medical pharmacology have been constructed by herbal remedies for ages and have formed a basis of traditional systems of medicines Popularity gained by herbal medicines is due to better patient acceptance. Availability of medicinal plants is not a problem especially in

developing countries like India, which is having rich agro climatic, cultural and ethnic biodiversity. India is the largest producer of medicinal Herbs and is a appropriately called the botanical garden of world. Orange, the tasty, juicy fruit, belonging to the family Rutaceae is botanically known as *Citrus sinensis. Citrus sinensis* is one of the most important and widely grown fruit crops, with total global production reported to be around 120 million tons. Orange trees are widely cultivated in tropical and subtropical climates for its tasty juice and medicinal value. Many medicinal properties of orange peel extract, such As against colic, upset stomach, cancer, diuretic, cormunative, immuno enhancing, stomachic, tonic to digestive system, immune system and skin has been listed.

Hence, the Present Study was Undertaken with the Following Objectives

1. To assess and compare the in vitro antibacterial properties of different extracts of *Citrus sinensis* against various Pathogens
2. To determine the minimum inhibitory concentration (MIC) of each extract of both the plants against each pathogen with a view of searching a novel extract as remedy for different types of pathoges.

Taxonomical classification

- **Kingdom: Plantae**
- **Clade: Tracheophytes**
- **Clade: Angiosperms**
- **Clade: Eudicots**
- **Clade: Rosids**
- **Order: Sapindales**
- **Family: Rutaceae**
- **Genus: *Citrus***
- **Species: *sinensis***

Plant part which are used: PEEL OF CITRUS SINENSIS

Materials and Method

Procurement of Plant Material

Oranges (*Citrus sinensis*) were purchased from local market and orange peel were obtained.

Extraction

The peels were carefully washed under running tap water followed by sterile distilled water. These were air dried at room temperature (30°C) for two days, pulverized to a fine powder using a sterilized mixer grinder and stored in air-tight bottles. 10 g amount of the pulverized peel was separately soaked in 100 ml of ethanol (96%) and cold Sterile distilled water for 24hrs.

The same amount (i.e. 10 g) of pulverized peel was immersed in 100ml of hot sterile distilled water (100°C) and allowed to stand For 30 min on a water bath with occasional shaking and Kept undisturbed for 24 hEach preparation was filtered through a sterilized Whatman filter paper No.1.

Different types of test were also performed to know the constituents present in the extract of peel of *Citrus sinensis*.

Qualitative Analysis on Phytochemical Constituents

Test for Tannins

0.5g of powdered sample of each plant was boiled in 20ml of distilled water in a test tube and then filtered.

The filtration method used here was the normal method, which includes a conical flask and filter paper. 0.1% $FeCl3$ Is added to the filtered samples and observed for brownish green or a blue black colouration, which shows the presence of tannins.

Test for Saponins

2g of powdered samples of each plant was boiled separately with 20ml of distilled water in a water bath and filtered. 10ml of the filtered sample was mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicated the presence of Saponins

Test for Flavonoids

A few drops of 1% $NH3$ solution was added to the aqueous extract of each plant sample in a test tube. A Yellow coloration confirms the presence of flavonoid Compounds.

Test for Terpenoids

5ml of aqueous extract of each plant sample was mixed With 2ml of $CHCl3$In a test tube. 3ml of concentrated $H2SO4$ was carefully added to the mixture to form a layer. An interface forms with a reddish brown coloration if terpenoids constituent is present.

Test for Alkaloids

200mg plant material in 10ml methanol, filtered; a 2ml Filtrate + 1% HCl + steam, 1ml filtrate + 6 drops of Dra-Gendroff reagent, orange precipitate indicated the presence of respective alkaloids.

Test for Phenol

To 2-3 ml of aqueous or alcoholic extract few drops of 5% FeCl3 solution was added. Formation of deep blue-Black colour indicated the presence of phenols.

Test for Microorganisms

Four different bacteria which are 1) *Staphylococcus aureus, 2)Salmonella Typhi, 3)Klebsiella pneumoniae, 4)Pseudomonas aeruginosa* were procured from Microbial Type Culture Collection, These microorganisms were subcultured on the specific media, incubated aerobically at 37°C. Identification of all strains was confirmed by standard biochemical and staining methods.

Firstly the sterile nutrient agar was prepared then autoclaved and then 20ml. of sterile molten NA poured on sterile pertriplate then the extracts of the *Citrus sinensis* were used for the antimicrobial screening by Using the agar well diffusion method. The different types of microorganisms were inoculated on the different petriplate with the help of the sterlized spreader. The media was Punched with 7mm diameter wells and were filled with various concentrations of the extracts 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and observed plates were then incubated at 37°C for 24 hours. After incubation, zone of growth inhibition for each extract was observed.

Result

Pathogens were found to be resistant against extracts of *Citrus sinensis*. Concentration of all Extracts was significantly ($p \leq 0.05$) associated with mean Zone of inhibition. An increase in zone of inhibition was observed with increase in concentration of the extract Minimum inhibitory concentration of *Citrus sinensis* peel ranged between 12-15 mg/ml against both the dental caries pathogens.

Fig 1 MIC of citrus sinensis on *Staphylococcus aureus*

Fig 2 MIC of citrus sinensis on *Salmonella Typhi*

Fig 3 MIC of citrus sinensis on *Klebsiella pneumonia*

Fig 4 MIC of Citrus Sinensis on Pseudomonas Aeruginosa

Discussion

There are Many herbal remedies Have been tried on in vitro and in vivo against different pathogens and have been found to be effective.

Similarly Orange peels can be an alternative use in food, Pharmaceutical and Cosmetic industries. This finding can form the basis for the studies to prepare an optimize preparation of the herbal extract. Recycling of fruit waste is one of the most important means of utilizing it in a number of innovative ways yielding new products and meeting the requirements of essential products required in human, animal and plant nutrition as well as in the pharmaceutical industry.

There are various compound and nutrients and other chemical in the Pell extract of the Citrus sinensis which is very beneficial for human.

CHEMICAL CONSTITUENTS	CITRUS SINENSIS
1) ALKANOIDS	PRESENT
2) TANNINS	PRESENT
3) SAPONINS	PRESENT
4) FLAVONOIDS	PRESENT
5) TERPENOIDS	PRESENT
6) PHENOLS	PRESENT

Table 1 Chemical Constituents of Citrus Sinensis Peel

TYPES OF BACTERIA	ZONES OF INHIBITION
1) Staphylococcus aureus	FORMED
2) Salmonella Typhi	FORMED
3) Klebsiella pneumoniae,	FORMED
4) Pseudomonas aeruginosa	FORMED

Table 2 Formation of Zone of Inhibition of Bacterias

Antimicrobial efficacy is usually determined by examining minimum inhibitory concentration, bactericidal Effects and other test that commonly utilize various microbial culture techniques. In the present study cultural Method employed was agar well diffusion method which Offered several advantages such as selective quantification of microorganisms but are laborious and only enumerate bacteria that can grow on agar .

The mean zone of inhibition by orange peel extracts against different microorganisms ranged From 8 mm to 15 mm at all concentrations.

The antimicrobial potency of plants is believed be due to tannins, saponins, phenolic compounds, essential oils and flavonoids. These compounds are known to be biologically active and therefore aid the antimicrobial activity of the plants

Conclusion

Citrus sinensis peels extract demonstrated in vitro antimicrobial activity against pathogens warranting for further in vivo clinical studies to determine the exact dosages and its effectiveness in practical situations. Toxicity studies should also be done to determine safety. Need of the hour is to execute more and more screening of natural products or plant parts to set a primary platform for further phytochemical, pharmacological and in vivo studies that may open the possibilities of finding new clinically effective antibacterial compounds against bacterial resistant pathogens. We can also prepare some product like hand wash and sanitizer which be used to reduce the effect of the pathogens on body

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17. To Determine the Various Phytochemical Compounds Present in Citrus Limetta and Study the Antimicrobial Effects of Citrus Limetta Peel Extracts on Different Types of Bacteria

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Abstract

The rapid emerging flow and spread of multidrug resistant (MDR) pathogens is continuously decreasing the effect of the of common antibiotics. This has necessitated the search for novel bioactive compounds from natural products. In this study, a MDR Gram –ve bacterial pathogens were subjected to their sensitivity against peel extracts of *Citrus limetta.*
There is abundant medicinal flora which is unrevealed till now through research. The study was conducted to assess the in antimicrobial potential and also determine the minimum inhibitory concentration (MIC) of *Citrus limetta* peel extracts with a view of searching a novel Extract as a remedy of these pathogens Which can be used to neutralized the effects of these pathogens and production of the different types of natural products.

Firstly the peels were carefully washed under running tap water Followed by sterile distilled water. These were air dried at room temperature (30°C) for two days, pulverized to a fine powder using a sterilized mixer grinder and stored

In air-tight bottles various test were performed to know the chemical composition of the peel extract like 1)Test for tannins, 2)Test for saponins, 3)Test for flavonoids, 4)Test for Tarpenoids, 5)Test for Alkaloids, 6)Test for phenols, which shows the presence of these compounds in peel extract of *Citrus limetta*

Then Four different bacteria which are

1. *Staphylococcus aureus*,

2. *Salmonella Typhi*,
3. *Klebsiella pneumoniae*,
4. *Pseudomonas aeruginosa*

Were procured from Microbial Type Culture Collection, These microorganisms were sub cultured on the specific media, incubated aerobically at 37°C. Identification of all strains was confirmed by standard biochemical and staining methods.

Firstly the Nutrient agar was prepared then autoclaved and the they were poured on sterile pertriplate then the Extracts of the *Citrus limetta* were used for the antimicrobial screening by using the agar well diffusion method. The different types of microorganisms were inoculated on the different petriplate with the help of the sterlized spreader. The media was Punched with 7mm diameter wells with cork borer and were filled with various concentrations of the extracts 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and observed plates were then incubated at 37°C for 24 hours. After incubation, zone of growth inhibition for each extract was observed.

We observed *that Citrus limetta* peels extract demonstrated in vitro antimicrobial activity against pathogens warranting for further in vivo clinical studies to determine the exact dosages and its effectiveness in practical situations. Toxicity studies should also be done to determine safety. Need of the hour is to execute more and more screening of natural products or plant parts to set a primary platform for further phytochemical, pharmacological and in vivo studies that may open the possibilities of finding new clinically effective antibacterial compounds against bacterial resistant pathogens. We can also prepare some product like hand wash and sanitizer which be used to reduce the effect of the pathogens on body

Keywords – Agar well diffusion, antimicrobial activity, *Citrus limetta* zone of inhibition.

Introduction

Infectious diseases are still one of the leading causes of mortality and morbidity globally. Excessive and indiscriminate use of antibiotics in medical, veterinary and agricultural practices has resulted into high level of emergence and spread of multidrug resistant bacterial pathogens necessitate the need to find new substances with antimicrobial properties to be used against these microorganism . Plants have always anchored to the mother earth long before man can set his feet on earth. Mankind has been gifted with Sources for existence much earlier than arrival of life on Earth.

The World Health Organization (WHO) estimates that about 80% of the population still depends upon herbal medicines for the treatment of various diseases due to easy availability, economic reasons and lesser side effect.

Medical pharmacology have been constructed by herbal remedies for ages and have formed a basis of traditional systems of medicines. Popularity gained by herbal medicines is due to better patient acceptance. Availability of medicinal plants is not a problem especially in developing countries like India, which is having rich agro climatic, cultural and ethnic Biodiversity. India is the largest producer of medicinal Herbs and is an appropriately called the botanical garden of world. Sweet lime, the tasty, juicy fruit, belonging to the Family Rutaceae is botanically known as *Citrus limetta*. *Citrus limetta* is one of the most important and widely grown fruit crops, with total global production reported to be around 120 million tons. Sweet lime trees are widely cultivated in tropical and subtropical climates for its tasty juice and medicinal value. Many medicinal properties of Sweet lime peel extract, such As against colic, upset stomach, cancer, diuretic, cormunative, immuno enhancing, stomachic, tonic to digestive system, immune system and skin has been listed.

Hence, the present study was undertaken with the following objectives

1. To assess and compare the in vitro antibacterial properties of different extracts of *Citrus limetta* against various pathogens.
2. To determine the minimum inhibitory concentration (MIC) of each extract of both the plants against each pathogen with a view of searching a novel extract as remedy for different types of pathogens.

Taxonomical classification

- **Kingdom** : Plantae
- **Subkingdom :** Viridiplantae
- **Infrakingdom :** Streptophyta
- **Super division :** Embryophyta
- **Division:** Tracheophyta
- **Subdivision :**Spermatophytina
- **Class :** Magnoliopsida
- **Super Order :** Rosanae
- **Order :** Spindale's

- **Family :** Rutaceae
- **Genus :** *Citrus*
- **Species :** *limetta*
- **Plant part which are used**: Peel of *Citrus limetta*

Materials and Method

Procurement of Plant Material

Sweet limes (*Citrus limetta*) were purchased from local Market and Sweet lime Peel were obtained

Extraction

The peels were carefully washed under running tap water followed by sterile distilled water. These were air dried at room temperature (30°C) for two days, pulverized to a fine powder using a sterilized mixer grinder and stored in air-tight bottles. 10 gm. amount of the pulverized peel was separately soaked in 100 ml of ethanol (96%) and cold Sterile distilled water for 24hrs.

The same amount (i.e. 10 gm.) of pulverized peel was immersed in 100ml of hot sterile distilled water (100°C) and allowed to stand for 30 min on a water bath with occasional shaking and kept undisturbed for 24 hrs. Each preparation was filtered through a sterilized whatmann filter paper No.1.

Different types of test were also performed to know the constituents present in the extract of peel of *Citrus limetta*.

Qualitative Analysis on Phytochemical Constituents

Test for Tannins

0.5g of powdered sample of each plant was boiled in 20ml of distilled water in a test tube and then filtered.

The filtration method used here was the normal method, which includes a conical flask and filter paper. 0.1% $FeCl_3$ Is added to the filtered samples and observed for brownish green or a blue black coloration, which shows the presence of tannins.

Test for Saponins

2g of powdered samples of each plant was boiled separately with 20ml of distilled water in a water bath and filtered. 10ml of the filtered sample was mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed

with 3 drops of olive oil and observed for the formation of emulsion, which indicated the presence of Saponins.

Test for Flavonoids

A few drops of 1% NH3 solution was added to the aqueous extract of each plant sample in a test tube. A Yellow coloration confirms the presence of flavonoid compounds.

Test for Terpenoids

5ml. of aqueous extract of each plant sample was mixed with 2ml of CHCl3 in a test tube. 3ml of concentrated H2SO4 was carefully added to the mixture to form a layer. An interface forms with a reddish brown coloration observe if terpenoids constituent is present.

Test for Alkaloids

200mg plant material in 10ml methanol, filtered; a 2ml filtrate + 1% HCl + steam, 1ml filtrate + 6 drops of Dra-Gendroff reagent, Sweet lime precipitate indicated the presence of respective alkaloids.

Test for Phenol

To 2-3 ml of aqueous or alcoholic extract few drops of 5% FeCl3 solution was added. Formation of deep blue-Black colour indicated the presence of phenols.

Test for Microorganisms

Four different bacteria which are

1. *Staphylococcus aureus*
2. *Salmonella Typhi*
3. *Klebsiella pneumonia*
4. *Pseudomonas aeruginosa*

Were procured from Microbial Type Culture Collection, These microorganisms were subcultured on the specific media, incubated aerobically at 37°C. Identification of all strains was confirmed by standard biochemical and staining methods.

Firstly the nutrient agar was prepared then autoclaved and the they were poured on sterile pertriplate then the extracts of the *Citrus limetta* were used for the antimicrobial screening by using the agar well diffusion method. The different types of microorganisms were inoculated on the different petriplate with the help of the sterlized spreader. The media was punched with 7mm diameter wells and were filled with various concentrations of the extracts 5mg/ml,

10mg/ml, 15mg/ml, 20mg/ml and observed plates were then incubated at 37°C for 24 hours. After incubation, zone of growth inhibition for each extract was observed.

Result

Pathogens which we used were found to be resistant against extracts of *Citrus limett Concentration of all Extracts was significantly (*$p \leq 0.07$*) associated with mean zone of inhibition. An increase in zone of inhibition was observed with increase in concentration of the extract minimum inhibitory concentration of Citrus limetta* peel ranged between 13 to 16 mg/ml against both the dental caries pathogens.

Fig 1 MIC of *Citrus limetta* on *Staphylococcus aureus*

Fig 2 MIC of *Citrus limetta* on *Salmonella Typhi*

Fig 3 MIC of *Citrus limetta* on *Klebsiella pneumoniae*

Fig 4 MIC of *Citrus limetta* on *Pseudomonas aeruginosa*

Discussion

There are Many herbal remedies have been tried on in vitro and in vivo against different pathogens and have been found to be effective.

Similarly sweet lime peels can be an alternative use in food, pharmaceutical and cosmetic industries. This finding can form the basis for the studies to prepare an optimize preparation of the herbal extract. Recycling of fruit waste is one of the most important means of utilizing it in a number of innovative ways yielding new products and meeting the requirements of essential products required in human, animal and plant nutrition as well as in the pharmaceutical industry.

There are various compound and nutrients and other chemical in the peel extract of the *citrus limetta* which is very beneficial for human.

CHEMICAL CONSTITUENTS	CITRUS LIMETTA
1) ALKANOIDS	PRESENT
2) TANNINS	PRESENT
3) SAPONINS	PRESENT
4) FLAVONOIDS	PRESENT
5) TERPENOIDS	PRESENT
6) PHENOLS	PRESENT

Table 1 Chemical Constituents Of Citrus Limetta Peel

TYPES OF BACTERIA	ZONES OF INHIBITION
1) Staphylococcus aureus	11mm.
2) Salmonella typhi	-
3) Klebsiella pneumoniae,	-
4) Pseudomonas aeruginosa	-

Table 2 Formation of Zone of Inhibition of Bacterias

Antimicrobial efficacy is usually determined by examining minimum inhibitory concentration, bactericidal effects and other test that commonly utilize various microbial culture techniques. In the present study cultural method employed was agar well diffusion method which offered several advantages such as selective quantification of microorganisms but are laborious and only enumerate bacteria that can grow on agar.

From observation it is chear that *Citrus limetta* is only sensitive to Staphylococcus aureus. Other organisms like *Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa* are resistsnt to *Citrus limetta*

The antimicrobial potency of plants is believed be due to tannins, saponins, phenolic compounds, essential oils and flavonoids. These compounds are known to be biologically active and therefore aid the antimicrobial activity of the plants

Conclusion

Citrus limetta peels extract demonstrated in vitro anti-microbial activity against pathogens warranting for further in vivo clinical studies to determine the exact dosages and its effectiveness in practical situations. Toxicity studies should also be done to determine safety. Need of the hour is to execute more and more screening of natural products or plant parts to set a primary platform for further phytochemical, pharmacological and in vivo studies that may open the possibilities of finding new clinically effective antibacterial compounds against bacterial resistant pathogens. We can also prepare some product like handwash and sanitizer which be used to reduce the effect of the pathogens on body

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18. Checking Quality of Water of Municipal Corporation Water from Mumbai Suburban

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Abstract

Water is possibly the maximum treasured natural aid after air, although the floor of the earth is frequently consists of water, best a small a part of its miles usable, which makes this useful resource very restricted. This precious and restrained resource, consequently, have to be used with prudence. As water is needed for unique purposes, the suitability of it needs to be checked before use. Also, resources of water have to be monitored frequently to determine whether or not they're in sound fitness or now not. Poor condition of water our bodies aren't best the indictor of environmental degradation, it is also a threat to the ecosystem. In industries, incorrect fine of water might also reason dangers and severe financial loss. As a result, the first-rate of water could be very crucial in each environmental and financial component. For that reason, water fine analysis is essential for the use of it in any motive. After years of studies, water high-quality analysis is now consists of some trendy protocols. There are guidelines for sampling maintenance and evaluation of the samples. Right here the usual chain of action is discussed in brief so that it may be beneficial to the analysts and researchers.

Introduction

Water test may be described as because the chemical, bodily and biological characteristics of water, usually in recognize to its suitability for a designated use. Water may be used for endeavor, drinking, fisheries, agriculture or industry. Each of those particular makes use of has different defined chemical, physical and organic requirements necessary to help that use. For instance, there are stringent standards for water to be used for drinking or swimming as

compared to that utilized in agriculture or industry. After many years of research, water excellent requirements are installed location to make sure the suitability of green use of water for a delegated motive. Water great evaluation is to measure the required parameters of water, following preferred strategies, to check whether they're according with the same old. Water test analysis is required particularly for tracking motive. A few importance of such assessment consists of:

1. To test whether or not the water great is in compliance with the requirements, and hence, appropriate or now not for the designated use.
2. To display the efficiency of a gadget, running for water exceptional maintenance.
3. To check whether upgradation / trade of a present system are required and to determine what modifications ought to take place?
4. To monitor whether water great is in compliance with rules and regulations.

Water Take a Look at Analysis is of Extremely Necessary within the Sectors of

1. Public health (especially for drinking water)
2. Industrial Use

Procedure of Water Quality Analysis

A. Selection of parameters
B. Selection of method
C. Precision and accuracy of method selected as per requirement
D. Proper labeling
E. Preservation
F. Analysis
G. Reporting

A. Selection of Parameters

The parameters of water quality are selected entirely according to the need for a specific use of that water. Water was selected from the places where the supply is from Municipal Corporation. It can use for drinking purpose and various household chores.

B. Selection of Methods

The strategies of water quality analysis are selected in line with the requirement. The elements gambling key function for the choice of strategies are:

i. Extent and wide variety of sample to be analysed

ii. Cost of analysis

iii. Precision required

iv. Promptness of the evaluation as required

C. Precision and Accuracy of Method Selected as Per Requirement

What precision and accuracy to be maintained against a specific method is selected according to the need. The factors influencing this decision consist of:

i. Fee

ii. Parameter

iii. Use

D. Proper Labelling

Proper labelling prevents sample misidentification and ensures the duty and responsibility of the collector. The sample should be labelled well, preferably via attaching an accurately inscribed tag or label. As a substitute, the plate can be labelled without delay with a water-evidence marker. Barcode labels also are to be had in recent times. Statistics on the pattern or the tag ought to include at least:

i. Sample code quantity (figuring out area)

ii. Date of sampling

iii. Source and kind of sample

iv. Pre-remedy or upkeep executed at the sample

v. Sampler's name

E. Preservation

There is usually a put off between the collection and analysis of a pattern. The nature of the pattern can be modified for the duration of this era. Consequently right preservation is required within the way to laboratory after collection and inside the laboratory up to when analysis starts off evolved. Complete and unequivocal preservation of samples, whether or not home wastewater, commercial wastes, or herbal waters, is a practical impossibility because whole stability for every constituent by no means may be executed. At satisfactory, upkeep strategies simplest retard chemical and biological adjustments that unavoidably continue after sample series. No single method of protection is totally first-rate; the preservative is chosen with due regard to the determinations to be made. Preservation strategies are constrained to pH

control, chemical addition, the use of amber and opaque bottles, refrigeration, incubator and freezing.

F. Analysis

The samples are analyzed, according to the requisite parameters, following standard methods and protocols.

G. Reporting

The ultimate procedure of this water test analysis to make proper report out of it. The repost must be authenticated. Microbes found in water samples must be observed properly. As the present study was aimed at analyzing the quality of the water and presence of different microbes such as bacteria collected from different areas.

Methodology

A. Collection of Samples

Water samples were collected from four different locations which are Borivali, Kandivali, Dahisar, Virar. Water samples from all the sites were collected in sterile glass bottles, brought to the laboratory, processed within 2-3 hrs.

B. Preparation of Spread Plate for indentification of Microbes

Four plates of each SCDA (Soyabean Casein Digest Agar) and SDA (Sabouraud Dextrose Agar) was prepared in a spread plate. With the help of a spreader 0.1 ml of water sample was spread in each plate of both agars. Then we kept SCDA for 3 days and SDA for 5 days in the incubator.

C. **Preparation of Medium**

SCDM (Soyabean Casein Digest Medium) was prepared in four flasks with 100 ml of SCDB (Soyabean Casein Digest Broth) and added 10 ml water sample in it from different sites. After that each flask was kept for incubation at 30-35°C for 24hrs. Then Agar plates were prepared to determine the microbes present in it.

D. Identification of Microorganisms through Streaking

- ***For Salmonella Checking:-*** Sterile RVSEB (Rappaport Vassiliadis Salmonella Enrichment Broth) 100ml was taken in a flask with 0.1 ml of water samples and was kept in an incubator for 24hrs. After completion of incubation four sterile XLD (Xylose Lysine Deoxycholate Agar) plates were prepared and a loopful of

water sample from the incubated SCDM was streaked on four plates of sterile XLD. All plates were kept for incubation for 24hrs.

- ***For E.coli Checking:-*** MB (Macconkey's Broth) 100ml was taken in a flask with 0.1 ml of water samples and was kept in incubator for 24hrs.After completion of incubation four Macconkey's Agar plates were prepared and loopful of sample from incubated SCDM were streaked on four plates of Macconkey's Agar. All plates were kept for incubation for three days.
- ***For Pseudomonas Checking:-*** In this Four sterile Cetrimide Agar plates was prepared and then loopful of water samples were streaked on all four plates of Cetrimide agar. After that it was kept for incubation for 3 days.
- ***For Staphylococcus Checking:-*** In this four Mannitol Salt Agar plates were prepared and then a loopful of water samples were streaked on all four plates of Mannitol Salt Agar. After that it was kept in an incubator for 3 days.

Result

Collection of Samples

Location from where water samples were collected are shown in this table.

SR. No	Locations
1.	Virar
2.	Dahisar
3.	Borivali
4.	Kandivali

Preparation of Spread Plate for Identification of Microbes

It was observed that in SDA plate Fungi was grown after 5 days of incubation and in SCDA plate Bacteria was grown after 3-5 days of incubation.

Indentification of Microorganisms through streaking:-

Microorganisms which were identified through streaking in the agar plates are shown in this table.

AGAR	MICROORGANISMS FOUND
XLD	Salmonella typhi
Macconkey's Agar	E.coli
Cetrimide Agar	Pseudomonas
Mannitol Salt Agar	Staphylococcus

Observation

Virar

a. SDA

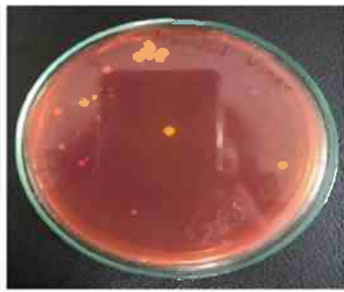
b. SCDA

c. XLD Plate

d. Macconkey's Agar plate

e. Cetrimide Agar plate

f. Mannitol Salt Agar plate



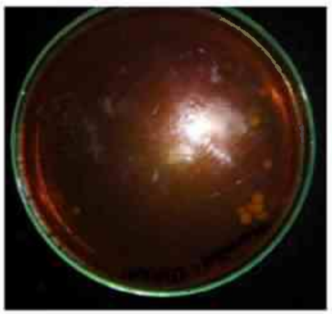
Dahisar

a. SDA

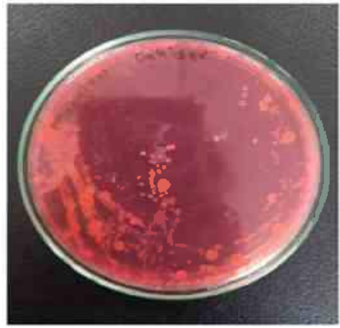
b. SCDA

c. XLD Plate

d. Macconkey's Agar plate



e. Mannitol Salt Agar plate

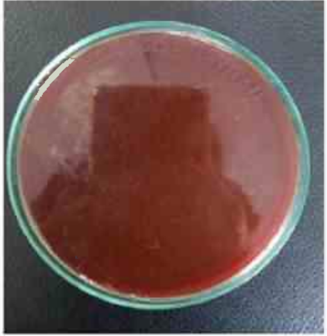


Borivali

a. SDA

b. SCDA

c. XLD plate



d. Macconkey's Agar Plate

e. Cetrimide Agar plate

f. Mannitol Salt Agar plate

Kandivali

a. SDA

b. SCDA

c. XLD

d. Macconkey's Agar plate

e. Cetrimide Agar plate

f. Mannitol Salt Agar plate

Conclusion

Assessment of water quality is essential to check the suitability of a water source for the designated use.

Several water quality parameters are assessed and compared with their standard values to determine the acceptability of the source of water and microbes found in it. After prolonged research, the procedures for the assessment of the water have also been standardized for different purposes. In this analysis such guidelines are discussed concisely in one place for the convenience of the researchers and analysts. Thus, it may be helpful for to get an overview of the water quality assessment standards and its upprocedures.

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19. Identification of Microorganisms from Different Surfaces

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1. Abstract

Exposure to the microorganism in day to life on different surfaces is now a very common thing which can also be dangerous in many ways, like damp, moisture-filled places may host microbes which are dangerous for our health and maybe pathogenic as well as can cause harm to us, so places like Kitchen surfaces, School labs, Doorknobs, table and chairs can be some of the many things which are common to human touch and may get contaminated by the touch or by any route of transmission which can be host harmful gram-negative microbes like *Acinetobacter spp., Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa, Serratia marcescens, or Shigella spp.* which can be a matter of concern.

So the following research focuses on Microorganism found on various surfaces which are susceptible to exposure and day to day touch of humans, so 3 samples were collected sample 1st was collected from a laptop, sample 2nd from Lab floor and Sample 3rd was collected after proper planting and streaking on nutrient plate Sample 2's Nutrient plate showed the growth of *E.coli* in EMB agar plate which was identified with the help of Gram Staining and various biochemical tests. This contamination can be cleaned by simple wiping with a cloth or with the help of application of 70% Alcohol. So it is very important to check whether the surface is sterilized or not because it can be a matter of concern.

Keywords: Gram staining, Contamination, Nutrient plate and biochemical test.

2. Introduction

Due to the ubiquitous nature of bacteria and fungi, the microbial-mediated contamination of food, air, water, and surfaces of common households may cause specific site-borne illnesses. (Manu V. Gajanan *et.al.*, 2013). Surfaces are something that are exposed to environment for 24 hours and can be easily contaminated by unwanted microbes and entities. There can be microbes like *Salmonella, Shigella and Escherichia coli* which are common among surface contamination, which can cause serious disease and fatalities.(Chiara Falciani *et.al.* 2012) The most common inner, airborne molds spores (e.g. Penicillium, Aspergillus, Cladosporium, and Alternaria) deposit on humans, food particulars, faves, and ménage Particulars, causing antipathetic conditions while at the same time causing non-allergic symptoms.(Bush RK. *et.al.* 1989). It is also very lucid to find locations that are not maintained and cleaned and which can be good place for the growth of microbes and can even cause life threatening diseases, even they can stay undetectable as they do not produce any texture, color or odor with their growth. (Lothar Beutin. *et.al.*, 2012)(Jitesh A Soares. et.al., 2011)

Above stated points motivated us to research and study more about surface contaminants and there harmful effects and even to examine the cleaning of surfaces of household and labs.

3. Source of Transmission

The word source of infection refers to the normal growth niche of the microbe a point in the body of the mortal or beast host. Objects and materials contaminated with live but temporarily inactive microorganism may act as transport or reservoirs of infection agents and not sources of infection. Human related diseases may be acquired or taken in by the mechanisms of exogenous infection that is from non-indoor sources such as school laboratory (windows, sinks, doors, and benches), from human patients admitted in hospitals with clinical infections (Nosocomial), healthy human carriers of the pathogenic microorganisms in public spaces and also from fomites present in the laboratory. On the other contrary, Endogenous infection refers to number of sources of a clinical infection within the patient's own body (Smith and Pearce, 1986). For example *Pseudomonas* and *Bacillus* species are of particular importance in a hospital setting, where they are the most common cause of infection.

4. Routes of Transmission

This is referred to the action of mechanism by which an infection and non-infection agent is transferred from one individual to the other or from the reservoir to a new host. This may

occur by contact through individual to individual, or indirectly through contaminated object or material (fomites).

Transmission of infections are more likely to take place in dense areas, where there are many people, movement of people in that direction, low sunshine and also tend to be more marked in urban areas such as school, colleges, railway station and public spaces, than in a rural area where we have less population. As reported and noticed by (F.J Baker *et al*., 2002), Some highly contagious infection can spread through casual contact in public spaces, the type of contact which occurs in day to day activities at home, work, school or college. Laboratory workers occasionally become infected due to exposure from artificial cultures or from infected diagnostic materials collected from experimental animals during research or experiment.

5. Advantages Disadvantages of Bacterial attachment to Surfaces

Adhering to surfaces provides bacteria with many advantages. Attachment to horizontal surfaces stimulates bacterial growth (particularly in nutrient-poor environments) as organic material suspended in liquid settles, is deposited on surfaces, and increases the local concentration of nutrients. (Claude E. Zobell *et.al*., 1943) Also, adding the substrate face area (e.g., by adding glass globules to a culture vessel) provides further area on which nutrients can adsorb, enabling cells to grow at nutrient attention that would typically be too low to support growth. (Heukelekian H *et.al*, Heller A. J. Bacteriol *et.al*, 1940) *Caulobacter crescentus* is an excellent example of a bacterium that takes advantage of surface attachment to optimize nutrient uptake.

Adhering or attaching to surfaces to surfaces also has many disadvantages, including the inhibition of motility, often due to a "switch" in the activation of genes involved in adhesion and motility: for instance, genes coding for flagella may be turned off by the same transcriptional regulator that turns on genes for extracellular matrix production. (Krasteva PV *et.al*, Fong JCN *et.al*, 2010)(Caiazza NC *et.al*, Merritt JH *et.al*,. 2007)

6. Antimicrobial Coating

An antimicrobial coating is nothing but an application of a chemical agent on a surface that has the ability to control the growth of disease-causing micro-organisms. Apart from increasing the surface's durability, appearance, corrosion resistance, and several other properties, these coatings also protect us from harmful disease-causing microbes. (Painting and coating industry PCI, 2020) Several different types of materials are used in making of antimicrobial

coating like copper, as well as its alloys like bronze, brass, copper-nickel-zinc and cupronickel, are well known for their natural antimicrobial property, dendrimers are also used because of their special ability to traverse the cellular membrane, polymer brushes, Silver nanoparticles, Polycationic hydrogel and Graphene-like two-dimensional materials. (Science Direct, 2019) Antimicrobial coatings are substantially applied to counters, walls and door handles even used in movie theatres as well as other high- touch areas mechanicals and HVAC reflections and numerous further shells. In other occasions, they're scattered onto gloves, masks, carpeting, and fabrics. Antimicrobial coatings are also used by makeup manufacturers who apply them to their manual products and wall makeup to constrain pathogens growth in installations. In addition to this, medical installations and hospitals are using the antimicrobial coating on their medical bias (Mixer Direct, 2019). (Markets and Markets, 2018)

7. Uses of Antimicrobial Coating

- Long-lasting protection against several types of pathogenic and dangerous microbes present around us including SARS types of virus, fungi, mold, and bacteria. The formula prevents the growth of microbes effectively on surfaces.
- The fine and thin invisible coating when applied to a metal surface including wooden and plastic-coated or any other surfaces, the lifespan of the articles increases manifold. There would be no lumps, stains, or stenches on the as long as the coating remain effective on that particular surface.
- Common public places like hospitals, theatres and banks where the gathering is always high these coatings keep the places clean and hygienic over a long period of time.
- This is one of the most cost-effective ways of sanitization. The public places that require frequent sanitization or sanitization almost every day can be kept sanitized with this form of treatment for a long time. (droom, 2020)

8. Survival on Inanimate Coating

Many different kinds of microbes are found on surfaces which can persist and survive on surfaces from a wide range of time for example like *Corynebacterium diphtheriae* can survive for 7 days to 6 months in environment (Walther *et.al* and Ewald.*et.al*,2007), *Escherichia coli* can survive for 1.5 hrs to 16 months (Guan *et.al* and Holley *et.al*, 2003)(Erickson *et al.* 2010), *Klebsiella sp.* with range of 2 h to >30 months, ≤144 h in detergent solution (Beadle *et.al* and Verran *et.al*, 1999), *Mycobacterium tuberculosis* with surviving from 1 day up to 4 months

(Walther *et.al* and Ewald.*et.al*,2007), *Pseudomonas aeruginosa* with 6 hrs. up to 16 months in environment ,on dry floor: 5 weeks and in aerosol for few hours (Clifton *et al.* 2008), *Staphylococcus aureus* can survive for 7 days up to 1 year (in-vitro), 9–12 days on plastic surfaces, 72 h on stainless steel, 6 h (copper) (Oie *et.al* and Kamiya *et.al* 1996)(Wagenvoort *et.al* and Penders *et.al* 1997) , (Huang *et al.* 2006)

9. Methodology

9. a Collection of Sample

3 samples were collected from 3 different locations,

- Sample 1 was collected from Laptop,
- Sample 2 was collected from Lab floor
- Sample 3 from Table surface

9. b Isolation and Enrichment of Samples

Cotton swabs were taken in saline water of around 10 ml and streaked on different Nutrient plates, Nutrient broth, EMB agar, Mac-Conkey's Agar, and Centrimide agar.

After 24 hours several colonies were spotted from the sample taken from lab floor and streaked against the nutrient plates

9. c Identification of Microorganism by Gram Nature and Biochemical Technique

Morphological and Cultural Identification

Strain of interest was studied and gram stained for its colonies developed on EMB agar were characterize by observing various parameters viz, shape, size, color, elevation, margin, surface etc.

Biochemical Characterization

Several tests were carried out like Oxidase Test, Catalase Test, H_2S production test, Nitrate Reduction Test, Gelatine Hydrolysis Test and Urease Test.

- **Oxidase Test -** Dissolve all the ingredients except the indicator and sugar solution in water. Adjust the pH. Add the indicator and steam sterilize the medium is a flask at 121°C for 30 min. The sugar solution is sterilized separately by filtration and then added as given in the composition. The medium is then distributed in the sterile tubes to depth of about 4 cm in aseptic condition. (Microbiologyinfo, 2018

- **Catalase Test -** Transfer growth from centre of colony on the surface of glass slide. Add one drop 3% H2O2, and observe bubble formation. (Student Health Centre Manuals, 2016
- **H_2S production Test -** Firstly inoculate the organism into labelled tube by means of stab inoculation in SIM medium then incubate the inoculated tubes at 37°C for around 24-48 hours then properly observe for the formation of black precipitate on the medium carefully. (Microbionotes, 2018)
- **Nitrate Reduction Test -** AtStartInoculatetestorganisminnitratebroththenincubate at 37°C for 24 hours then add 1ml of ά -naphthylamine and 1 ml of sulfanilic acid. (Microbiology info.com, 2021)
- **Gelatin Hydrolysis Test -** First inoculate known Gelatinase positive organisms eg. Staphyloccus aureus, Pseudomonas sp. then incubate at 37°C for 24-48 hrs then chill the tubes at 4°C for 20 minutes and if the medium remain in liquid state, it is a positive test. (Microbesnotes.com, 2020)
- **In IMViC Test -** Indole test, Methyl- red test, Voges-Proskauer Test and Citrate Utilization test were carried out.
- **Indole Test** - First inoculate tryptophan broth with test culture and incubate it at37°C for around 24 hours then add around 15 drops of Kovac's reagent along the side of the test tube very carefully after that there should be formation of red ring on the top of medium. (Microbiology info.com. 2021)
- **Methyl Red Test -** Before the inoculation, allow medium to equilibrate to room temperature, then using organisms taken from an 18-24 hour culture, slightly inoculate the medium very carefully then incubate it at aerobically at 37 degrees for around 24 hours.After 24 hours of incubation, aliquot 1ml of the broth to a clean test tube carefully. Then reincubate the remaining broth for an more additional 24 hours. Then carefully add 1 to 3 drops of methyl red indicator to aliquot then carefully observe for red color immediately. (Microbeonline.com,. 2021)
- **Voges-Proskauer Test-** At first inoculate test organisms in St. Glucose phosphate broth then inoculate at 37°C for 24 hours then, add 0.5 ml of 5% a-naphthol and 0.2 ml 40% KOH, shake gently and carefully (Onlinebiology.notes, 2018).

- **Citrate Utilization Test -** First prepare Simmon's Citrate agar and pour it in test tube and keep it at a slant position then inoculate test culture on surface of summons's citrate slant by streaking and let it incubate at 37°C. (Microbenotes.com, 2020) Test for Glucose, Maltose, Mannitol, Lactose, Xylose and Sucrose were carried out.

At first saline water was taken in a 500 ml flask and 10 ml of peptone was added and mixed properly and poured into 6 flasks with each flask holding a quantity of 50ml.
Then in each flask certain required grams of Glucose, Sucrose, Maltose, Mannitol Lactose and Xylose was added. Then 10 ml was taken from each flask and poured into 6 different test tubes, this was done until we had 3 test tube filled of each sugar. Then Durham's tube was inserted properly and in a way that avoided the formation of bubble in the Durham's tube. After this pH was checked with the help of pH paper and if it is found to be acidic then NaOH is added and if it is found to be basic then HCl is added. All the test tubes were sterilized for 121°C for 20 mins. After sterilization cooled in and inoculate loop full of suspension of the microorganism in test tubes, this is to be done in interest of the microorganism we founded so that it can be identified.

10. Result and Discussion

10. a Collection of Samples

Sample and its Location from where it is Collected is Shown in Table No. 1

Table No. 1

Sr. No	Location
1	Laptop
2	Lab floor
3	Table Surface

10. b Isolation and Enrichment of Water Sample

Out of 3 samples, Sample with lab floor showed significant growth on EMB Nutrient Plate and was taken in for the further process of Gram staining and Identification.

10.c Identification of Microbe

Gram of the Microbe has been shown in table no. 2

Table no. 2

Colony Characteristics	Colony 1
Size	1 to 2 µm

Colour	Beige color
Shape	Rod Shaped
Margin	Entire
Opacity	Opaque
Consistency	Butyrous
Elevation	Low convex
Gram nature	Gram negative
Morphology	Short rods

Biochemical Testing of Bacteria Shown in Table No. 3

Table No. 3:

Characteristics	**Result**	**Characteristics**	**Result**
	Morphological Characteristics		
Shape	Rod Shaped	Gram Staining	Gram Negative
Size	1.0-2.0 micrometers	Motility Test	Motile
Colour	beige	Spore	Non Sporulating
	Decomposition/Enzymatic studies		
Oxidase Test	Negative	Beta-galactosidase Test	
Catalase Test	Positive	H_2S production test	Negative
Nitrate Reduction Test	Positive	Gelatine Hydrolysis Test	
		Urease Test	Negative
	IMViC Set		
Indole test	Positive	Methyl- red test	Positive
Voges-Proskauer Test	Negative	Citrate Utilization test	Negative
	Sugar Fermentation Test		
Glucose	Positive	Lactose	Positive
Maltose	Positive	Mannitol	Positive
Sucrose	Variable	Xylose	Positive

From Above results and tables we can conclude that microorganism present is none other than *Escherichia Coli.*

Similar experiment was carried out by Jan Dirk van Elsas *et.al* and Alexander V Semenov *et.al* and found out that *E.coli* being found in surfaces but it can survive on surface only for a varying period of time on the surface depending on the strain (Jan Dirk van Elsas *et.al* and Alexander V Semenov *et.al,* 2010). Even in the research conducted by A P Williams *et.al*, L

M Avery *et.al* , K Killham *et.al*, and D L Jones *et.al* they detected the presence of *E.coli* and concluded that it can survive there for a long period of time, if conditions are favourable and not the opposite(A P Williams *et.al*, L M Avery *et.al* , K Killham *et.al*, and D L Jones *et.al,* 2005). Experiments conducted by S A Wilks *et.al*, H Michels *et.al*, C W Keevil *et.al* showed that the strain of E.coli that is E.coli O157 can survive for over 28 days at both refrigeration and room temperatures on stainless steel which can easily contaminate the surface (S A Wilks *et.al*, H Michels *et.al*, C W Keevil *et.al,* 2005).

Conclusion

According to Present Result, The *E.Coli* is Found in the Sample Taken from Lab Floor

Ways to Clean Surface and Prevent it from Contamination

- Clean high- touch surfaces regularly or as demanded and after you have guests in your home.
- Focus on high- touch surfaces similar as doorknobs, tables, handles, light switches, and countertops where human incoming and outcoming is frequent.
- Clean other surfaces in your home when they're visibly dirty or as demanded. You might also choose to disinfect.
- One can also take use of 70% alcohol to wipe any kind of surface.
- Clean surfaces using a product suitable for each face, following instructions and guidelines on the product label and be careful as it may damage the product. (Centre for Disease Control and Prevention CDC, 2021)(MedLinePlus, 2022)

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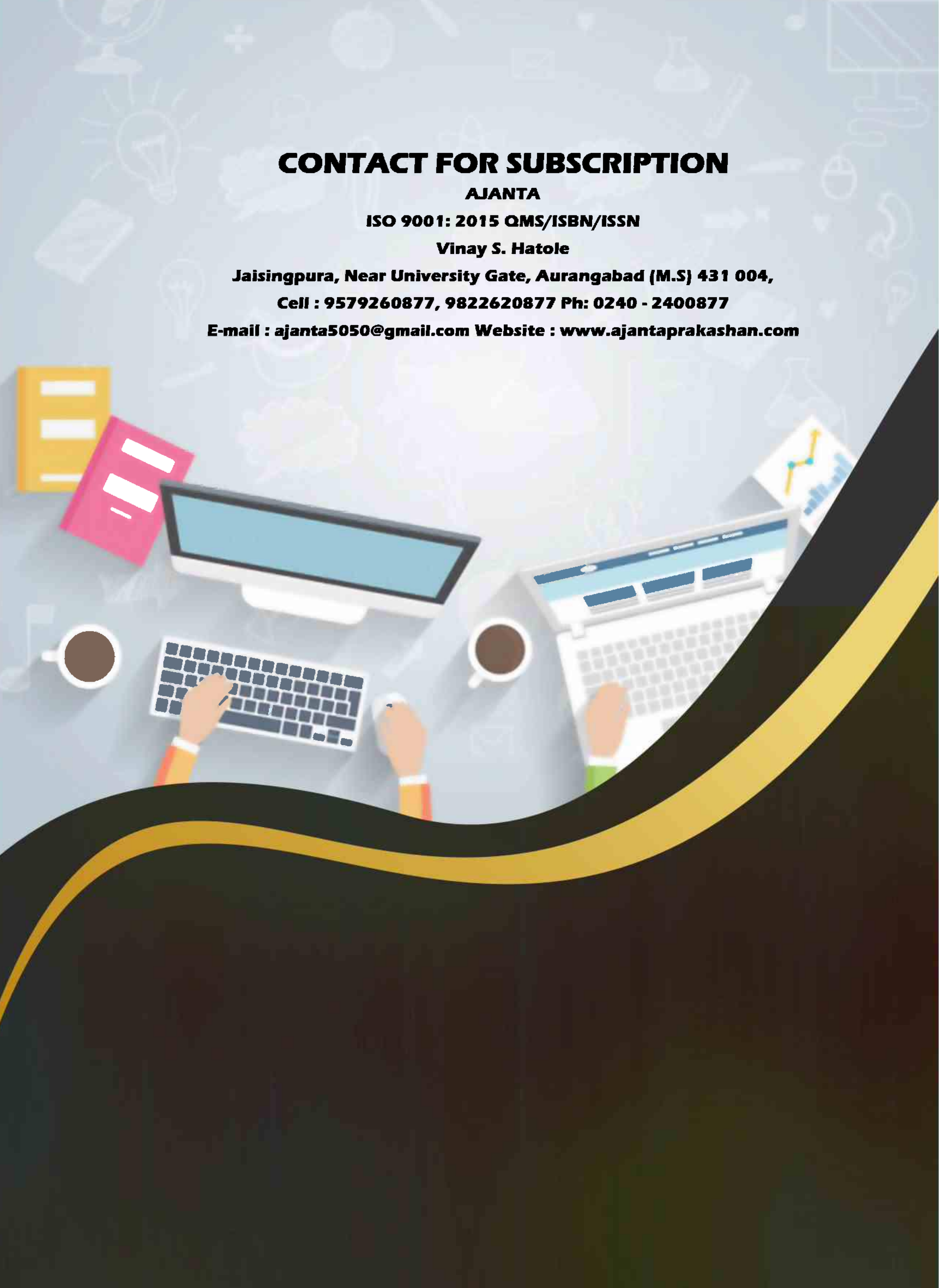

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