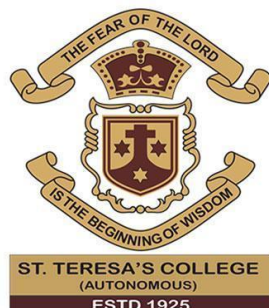


ANTIBACTERIAL EFFECT OF FRUIT EXTRACTS ON DIFFERENT BACTERIA



Project work by
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Affiliated to Mahathma Gandhi university, Kottayam
in partial fulfilment of requirement for the
Degree of Bachelor of Science in Zoology
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CERTIFICATE

This is to certify that the project report entitled “**ANTIBACTERIAL EFFECT OF FRUIT EXTRACT ON DIFFERENT BACTERIA**” submitted by Ms.VARSHA ROSHAN ,Reg No:- **AB20ZOO039** in partial fulfilment of the requirement of Bachelor of Science degree of Mahatma Gandhi University, Kottayam, is a bonafide work under my guidance and supervision and to my best knowledge, this is her original effort.

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EXAMINERS

1)

2)

DECLARATION

I, hereby declare that this project work entitled “**ANTIBACTERIAL EFFECT OF FRUIT EXTRACTS ON DIFFERENT BACTERIA**” is submitted to St. Teresa’s College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam in partial fulfilment of the requirements of Bachelor of Science degree in Zoology. This work has not been undertaken or submitted elsewhere in connection with any other academic course and the opinions furnished in this report are entirely my own.

NAME: VARSHA ROSHAN

REGISTRATION NUMBER: AB20ZOO0039

SIGNATURE

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The success and final outcome of this project required a lot of guidance and assistance from many people and I am extremely privileged to have got this all along the completion of my project. All that I have done is only due to such supervision and assistance and I would not forget to thank them.

I thank God Almighty for showering his blessings upon me abundantly and for giving me the strength, ability, and opportunity to understand and complete this project work.

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My project work would not have been a success without the constant encouragement from my parents and friends

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SYNOPSIS

Microbiology is the study of microorganisms, a large and diverse group of microscopic organisms that exist as single cells or cell clusters. Antibacterial activity can be defined as the ability to inhibit the growth of bacteria. Fruits are good sources of fibre, vitamins, minerals, and carbohydrates. Fruit extract shows antibacterial activity and can be used in the treatment of many diseases, including cancer, and it also helps in the overall improvement of the health.

The project titled "Antibacterial Effect Of Fruit Extract" is done by using fruits such as grapes, lemon, orange, mossambi, tamarind, avertrhoa bilimbi, banana, pineapple, pomegranate, and papaya on the bacterial strains *Enterococcus faecalis*, *Staphylococcus aureus*, *E. coli*, and *Klebsiella pneumoniae*.

Current results indicate that lemon is either more sensitive or acts as a powerful antibacterial agent. After lemon, tamarind and mossambi also showed a promising result as antibacterial agents. All these fruits are rich in many organic acids and compounds. Banana, papaya, pomegranate, and pineapple had no antibacterial effect on any of the bacterial strains. Among the 10 fruits we used, lemon showed the most potent antibacterial activity. The present study suggested that citrus fruits like lemon, followed by tamarind and mossambi, have greater potential as antibacterial agents against different pathogenic bacteria.

INTRODUCTION

Microbiology is the study of microorganisms, a large, diverse group of microscopic organisms that exist as single cells or cell clusters. It also includes viruses that are microscopic but not cellular. Out of the many microorganisms that exist, bacteria is one of them. These are the only organisms with prokaryotic cellular organisation. They are also capable of nitrogen fixation (Al-Mohanna, Moshtaq, 2016). Antibacterial activity can be defined as the ability to inhibit the growth of bacteria. Antibacterial activity is the most important characteristic of medical textiles to provide adequate protection against microorganisms, biological fluids, and aerosols, as well as disease transmission (F. Alihosseini 2016).

In this project entitled "Antibacterial Activity of Fruit Extract on Different Bacteria," a study was conducted using the fruit extracts of pineapple, lemon, orange, pomegranate, banana, tamarind, grapes, avertrhoa bilimbi, papaya, and mossambi on the bacterial strains *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterococcus faecalis*

Escherichia coli (*E. coli*) bacteria normally live in the intestines of healthy people and animals. Most types of *E. coli* are harmless or cause relatively brief diarrhoea. Enterotoxigenic *Escherichia coli* (ETEC) is frequently found in diarrheal stools worldwide and can be the main source of disease-causing agents in water. So it is recommended as a water quality indicator (Betina Hebbelstrup et al. 2017).

Streptococcus pneumoniae bacteria cause pneumococcal diseases. But they are also commonly found in the respiratory tract of healthy people, especially children. They are gramme-positive, facultative anaerobic bacteria with more than one hundred known serotypes. A rare case of multiple vertebral osteomyelitis due to *Streptococcus pneumoniae* was reported. A 73-year-old man admitted for back pain and a low-grade fever was found in laboratory studies to have severe leukocytosis and increased C-reactive protein, but neither computed tomography (CT) nor vertebral magnetic resonance imaging (MRI) clarified the cause of infection in the painful hip lesion, and paralysis developed (Kenichi Izumi et al., 2008).

Enterococcus faecalis is a gramme-positive commensal bacterium that inhibits the gastrointestinal tract of humans and other animals. *E. faecalis* can cause a life-threatening

infection in humans (Haydan et al., 2006). There are at least 18 different species of these bacteria. These bacteria also live in the mouth and vagina. They are very resilient, so they can survive in hot, salty, or acidic environments.

Klebsiellapnuemoniae, belonging to the family *Enterobacteriaceae*, is a natural inhabitant of the gastrointestinal tract microbiome of healthy humans and animals. It has a variety of mechanisms for antibiotic resistance and is a common pathogen causing hospital-acquired surgical wound infections, digestive tract infections, and community-onset infections, which can cause outbreaks of nosocomial infection. It is a gramme-negative, non-motile, encapsulated, lactose-fermenting, facultatively anaerobic, rob-shaped bacterium (Dunga Kingsley Excel et al., 2023)

Fruits are essentially part of a healthy diet. They are good sources of fibre, vitamins, carbohydrates, and vitamins. Fruits also reduce the risk of many diseases. Fruit extracts contain antioxidants, vitamins, and minerals that are essential for human beings and play an important role in the prevention of heart diseases and cancer. Numerous studies have proven that fruit extracts and their active components have anticancer and cytotoxic activities. The extracted juices from fruits contain most substances that are found in the original ripe state. The high potassium and low sodium characteristics of most fruit extracts help maintain a healthy blood pressure. Vitamin C is naturally present in the extracts, which are essential for the body to form collagen, cartilage, muscles, and blood vessels. It also helps in the absorption of iron. Fruit extracts have been proven to be associated with the treatment of various diseases. Natural compounds or extracts from fruits are important leads in the development of anticancer drugs that will not have the side effects of chemotherapy.

Lemon belongs to the family of Rutaceae and it contains 5% to 6% of citric acid with a pH around 2.2, giving it a sour taste. It was reported that the juice of a citrus *lemon* contained flavonoids, alkaloids, steroids, saponins, terpenoids, reducing sugars, and cardiac glycosides. This investigation suggested that this juice had beneficial antimicrobial roles that could be controlled by the unwanted microbial growth (Oikeh et al., 2016). Among fruits, citric acid is most concentrated in lemons, which comprise as much as 8% of the dry fruit's weight. Citric acid is a weak tricarboxylic acid. It is naturally concentrated in citrus fruits. When compared to other acids. It is effective in killing the bacteria at the core of biofilm microorganisms and inhibiting microbial growth.

Tamarind is used as a flavouring in many dishes. It may also have medicinal properties and can be used as a tarnish remover. Tamarind pulp can also be used as a metal polish. It contains tartaric acid. In studies conducted in Nigeria, flavonoids and polyphenols, in association with alkaloids, were linked to the antimicrobial activity of tamarind leaves (Doughari JH. 2006). In tamarind, the acid present is tartaric acid, which is mainly found in grapes and bananas. Dihydroxybutane-dioic acid is another word for tartaric acid. The main organic acid present in Bilimbi is oxalic acid. The high level of this acid accounts for its extremely low pH.

The active substance content of Averrhoa bilimbi fruit ethyl acetate extract gel, which contains flavonoids, alkaloids, saponins, terpenoids, and tannins, can function as an antibacterial (Dewi Pertiwi et al., 2020).

In the case of Mossambi, the phytochemical analysis showed the presence of alkaloids, cyanogenetic, cardiac, and steroidal glycosides, tannins, saponins, flavonoids, and water-soluble vitamins (Malachy Ifeanyi Okeke et al., 2015). The presence of these compounds accounts for its antibacterial activity.

Grapes are a rich source of bioactive molecules, including phenolic acids, flavonoids, anthocyanins, stilbenes, and lipids. These are the compounds that contribute to the health benefits of grapes and grape-derived products. They possess antioxidant, antimicrobial, anti-inflammatory, and anti-carcinogenic activities and have wide applications in the food and nutraceutical industries (Ali Sabra 2021). In the study, grapes were only effective against *Staphylococcus aureus*.

Citrus fruits such as oranges are a good source of bioactive compounds with antioxidant properties, such as vitamin A (mainly in the form of ascorbic acid), provitamin A (carotenoids, such as β -carotene), vitamin E (mainly in the α -tocopherol isoform), limonoids, phenolic compounds (flavonoids and phenolic acids), and anthocyanins, an important bioactive compound in blood oranges. In addition, many citrus fruits could be considered sources of dietary fibre, mainly soluble fibre fraction, and low energy value (mainly due to the low lipid content). Therefore, citrus fruit consumption should be promoted as part of the Mediterranean diet (Laura Cebadera et al., 2020). In this study, orange was only effective against *Klebsiella pneumoniae*. Other fruits used in the study, such as banana, papaya, pomegranate, and pineapple, did not show any inhibition zone against any of the pathogenic bacteria.

Fruit extracts that show antibacterial activity can be used in the treatment of many diseases, including cancer, and they also improve overall health. Some of the extracts had good potential for therapeutic use against the bacterial pathogens or for application in treating diarrhoea. The antibacterial effects of fruit extracts can have various aftereffects, depending on the specific fruit and the type of bacteria being targeted. Reduction of bacterial growth: Fruit extracts with antibacterial properties can inhibit the growth of harmful bacteria, reducing the risk of infection or illness. Regular consumption of fruits with antibacterial properties may improve the body's immune system, making it more resistant to bacterial infection. Overuse of antibiotics can lead to the development of antibiotic-resistant bacteria. Using natural fruit extracts with antibacterial properties can reduce the need for antibiotics and help prevent the development of resistance. Some fruits with antibacterial properties, such as cranberries and blueberries, can help promote healthy digestion by reducing the growth of harmful bacteria in the gut. Certain fruits with antibacterial properties, such as pomegranates and cherries, have been found to have anti-inflammatory effects, which may help reduce the risk of chronic diseases.

Objective: To find out the antibacterial activities of various fruit juices on selected bacterial species.

REVIEW OF LITERATURE

A study was conducted by Sulthana et al. in 2022 on the antimicrobial activity of some citrus fruits and non-citrus fruits against six pathogenic bacteria. The MIC values of these bacterial species were determined by measuring the OD via a spectrophotometer. In this study, lime (*Citrus aurantiifolia*) showed the highest effect against the test organisms such as enteropathogenic *E. coli*, enterotoxigenic *E. coli*, *V. parahaemolyticus*, *S. aureus*, and *Salmonella spp.* The present study suggested that the citrus fruits and pineapple juices have great potential as antimicrobial agents against different pathogenic bacteria.

Sheikh et al. studied crude ethanolic and methanolic extracts from *Carica papaya L. seeds* in 2022 and investigated their antibacterial and cytotoxic potentialities. The average zone of inhibition for an ethanolic extract of *C. papaya* seed ranged from 6.25 to 9.75mm at 8.14mm. The methanolic extracts zone of inhibition ranged from 7.75 to 8.50 for a particle size of 11.25 to 14.50 mm; these findings could be correlated with the traditional medicinal uses of papaya seeds and showed the rationale for further investigation for screening out possible bioactive constituents.

Arianto et al. studied the antibacterial activity and determination of total phenol and flavonoids in *Carica papaya* in 2023. Antibiotics from plants are possible. Papaya leaf carpaine alkaloid may be antibacterial. The study determined papaya leaf compound content, flavonoids total phenol content, and optimal concentration of papaya leaf ethanol extract that inhibits *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Mansour et al. studied to evaluate the antimicrobial activity of essential oils and extracts of lemon citrus. Mandarin (*Citrus reticulata*) and pummelo (*Citrus grandis*) against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhi*. The result showed that there was no antimicrobial cavity effect in any of the extracts tested on the tested

bacteria. In this study, the antibacterial effect of the essential oil of citrus leaves on four strains of pathogenic microorganisms was confirmed.

Konera et al. in this study aimed to assess the in vitro antibacterial effects of extracts from four wild food fruits, such as *Balanites aegyptiaca*, *Saba senegalensis*, *Ziziphus mauritiana*, and *Raphia sudanica*, in 2023. These findings support the local therapeutic properties of these fruits. They also demonstrate that, in addition to their nutrition values, these edible fruits could be used for developing antibiotics to treat infectious diseases and food poisoning.

Kodasi et al. studied the adept green synthesis of CU₂O nanoparticles using kiwi fruit juice and studied their cytotoxic activity and antimicrobial evaluation in 2023. Nanoparticles have gained a lot of interest in recent years for their antibacterial and cytotoxic potential, particularly the copper oxide nanoparticle. The CU₂O nanoparticles manifested excellent cytotoxicity against HEK-293 and MCF-7 cell lines, and their antimicrobial activity was superlative, with exceptional zones of inhibition against *S. aureus*, *E. coli*, *Candida albicans*, and *Aniger*, revealing outstanding effectiveness at all concentrations.

Lu Wang et al. in 2022 focused on the relationship between the content level of phytochemicals and the biological activities of noni (*Morinda citrifolia*) fruit extract prepared with traditional solvents and a deep eutectic solvent. This study provides valuable information on the efficient extraction of phytochemicals from noni fruits, and DESs are promising green solvents for the extraction of bioactive compounds from noni fruits. (Solvent effect on phenolics, iridoids, antioxidants, and pancreatic lipase inhibition activity of *Morinda citrifolia* fruit extracts.

Aboudia et al. studied the three-step approach to assess the efficacy of alternative chemical treatments to preserve fruit juice in 2021. Fresh pineapple juice exhibits a shelf life of a few

days, limited by the development required to assess the efficacy of treatments of juice based on three successive steps. The extracts were mainly composed of the monoterpene carvacrol and inhibited fungal growth in the culture medium. The efficiency of treatment was assessed in fresh pineapple juice harbouring its natural microbial population. A three-step approach to assessing the efficiency of alternative chemical treatments to preserve fresh fruit.

Khanal et al. (2022) studied the in vitro antibacterial activity of lemon juice against clinical isolates of *Klebsiella* having specific genes. A total of 667 clinical specimens were processed. The tested varieties of lemon juice showed potential inhibitory activity against *Klebsiella* species, indicating that lemon juice has the potential to be used as an antibacterial agent.

Oliveria et al. studied the in silico antibacterial activity of a peptide from a trypsin inhibitor isolated from tamarind seed. Bacterial infections have become a global concern, stimulating the growing demand for natural and biologically safe therapeutic agents with antibacterial action. This study evaluated the genotoxicity of the trypsin inhibitor isolated from tamarind seed and the antibacterial effect of TTI theoric model 56 and conformation 287 and derived peptides in silico.

Aguirre et al. studied the analysis of the antimicrobial and antioxidant activity of the essential oils of royal lemon in 2022. The essential oil was extracted from the fruit and leaves of the royal lemon (*Citrus limonum*) by means of steam entrainment and fractional distillation. The antimicrobial activity was evaluated against the strains of *Esterichia coli*, *Salmonella*, *Listeria*, and *Arcobacter*. It was determined that oils obtained from the royal lemon have antimicrobial activity against *Listeria* and *Arcbacter*; for the antioxidant activity, at a concentration of 50 mg/ml, it presents a value of 48.82%, and at a concentration of 100 mg/ml, it presents 47.29% of sequestered peroxide. In *E. coli* and *Salmonella bacteria*, there is low antimicrobial activity.

Hasan et al. in 2019 extracted and studied the crude ethanol and methanol extracts of *Tarminidus indica* seeds to evaluate their antibacterial and cytotoxic potentialities. The average zone of inhibition of ethanol extracts of tamarind seeds ranged from 7.5-13.5mm. And for methanol extracts, the zone of inhibition ranged from 6.5-13.25mm. From the minimum inhibitory concentration, 1.25 mg/mL was found for ethanol and 0.625 mg/mL for methanol extract. The study confirms that the antibacterial activity of *T. indica* seed extracts is associated with moderate cytotoxic activities. Because they showed a lower concentration level than 100

Kamala et al. studied the antibacterial activity of lemon peel extract by the agar-well diffusion method against *Bacillus spp.* and *Escherichia coli* in 2021. The antibacterial activity was evaluated by measuring the zone of inhibition. It was observed that, with an increase in concentration of lemon peel extract, the antibacterial property of the extract also increased. The increase in antibacterial property with an increase in concentration may be because of the presence of more phytochemicals and because more extract gets dissolved in a higher concentration as compared to a lower concentration. The zone of inhibition against the microorganisms also increased. The results also confirmed that the study reported an increase in the zone of inhibition for increased concentrations (8 and 16%) of an ethanolic extract of *Tridax procumbens*.

Saguie et al. in 2020 studied the identification of compounds present in grape pomace, seed, and skin extracts and the evaluation of their antibacterial activity against *Xanthomonas citri* in vitro and in vivo using preventive and curative methods. The screening of the antibacterial activity demonstrated that all extracts have antibacterial activities against *X. citri*. The MIC and MBC values of the tested extracts were between 350 and 700 g/mL. The in vivo test showed that the preventive method was more effective than the curative method. The grape pomace extracts could be reused by applying them as a natural antibacterial agent against *Xanthomonas citri*, the etiologic agent of citrus canker in lemons.

In 2023, Umar et al. studied evaluating the potential antibacterial and antioxidant activity of ecoenzyme solutions derived from papaya, pineapple, and Kasturi orange. In this study, pieces of papaya, pineapple, and Kasturi orange were fermented with brown sugar for 10 days and 3 months to evaluate their inhibitory abilities against the growth of *Escherichia coli* and *Staphylococcus aureus* using the well diffusion method. Molecular docking studies revealed that *hesperidin* and *ciprofloxacin* interacted more strongly with the DNA gyrase of *S. aureus* than with *E. coli*. *Hesperidin* can therefore function as a potent antimicrobial. The present study concluded that EE solution fermented from papaya, pineapple, and Kasturi orange exhibits the potential to serve as a source of both antibacterial and antioxidant compounds.

Huynh et al. (2022) studied how to utilise discarded orange peels to extract essential oils and evaluated their physiochemical properties, antibacterial, antifungal, and antioxidant activities. Essential oils were extracted via a distillation system using a cleverger apparatus and gas chromatography; mass spectrometry analysis was employed to characterise their chemical components; antibacterial and antifungal tests were evaluated using a well diffusion method; and antioxidant activity was determined based on the DPPH radical's scavenging effect and ferric reducing antioxidant power (FRAP). The obtained essential oils had a yield of 3.29–0.24%, in which limonene was found to be the most abundant compound in the EOs (90.42%), followed by α -myrcene (4.7%), and α -pinene (1.22%). The result showed that gramme-positive bacteria (*Bacillus cereus*) were more susceptible to 50% EOs than gramme-negative bacteria (*Escherichia coli*) with respect to inhibitory zone diameters of 15.00–0.58 mm and 11.33–0.58 mm, respectively. The orange peel EOs could be a promising alternative to synthetic preservatives in the food industry due to their antimicrobial and antifungal activity as well as their antioxidant activity.

Thuray et al. 2020 studied the invitro antibacterial and phytochemical activities of ripe and unripe orange peel extract on selected microorganisms. These microorganisms include *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus spp.*, *Proteus spp.*, and *Enterobacter spp.* A stock concentration of each successive orange peel extract was obtained using alcohol (95% ethanol + 5% methanol) and

100% methanol (absolute). These extracts, along with positive and negative controls, were tested for the presence of active phytochemical zones of inhibition, which were significantly higher with methanol extract than alcohol extract against the test organisms. The minimum inhibitory concentrations of the methanol and alcohol peel extracts ranged between 6.25 and 12.5 mg/ml, while the minimum bacteriocidal concentrations ranged between 12.5 and 25 mg/ml. Therefore, they confirmed that *Citrus sinensis* and *Citrus aurantium* peels have antimicrobial potential against pathogenic bacteria.

Yusuf et al. studied the physicochemical properties, antioxidants, and antimicrobial activities of unripe green and yellow-ripened banana fruit peels and pulp oil extracts in 2022. The oil extraction was done in a Soxhlet apparatus using petroleum ether as a solvent. Then, the oil extracts were assessed based on the determination of oil yield, acid value, percent free fatty acid, peroxide value, and free radical scavenging activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide, and ascorbic acid. The antioxidant and antimicrobial activities were determined based on ascorbic acid content, DPPH, and hydrogen peroxide scavenging activities. The highest oil yield (2.600.21%), acid value (2.660.20%), and free fatty acids (1.340.10%) were recorded for green peel oil extract. Significantly, the highest DPPH radical scavenging activity was recorded for green peel (5.85%), followed by green pulp (4.80), and the least for yellow peel (4.50). Ascorbic acid and hydrogen peroxide scavenging activities were significantly higher for yellow peel and green pulp oil extracts than green peel oil extract. The strongest antibacterial activity with the maximum zone of inhibition (15.5mm), minimum inhibitory concentration (0.125 g/ml), and corresponding minimum bactericidal concentration (0.25 g/ml) was recorded for ripened peel oil extract against *Staphylococcus aureus*. The result indicated that the banana fruit peel oil extracts demonstrated differential antioxidant and antimicrobial potentials. Thus, the banana peel waste oil extracts proved to have potential sustainable applications in nutritional and drug development technologies.

Mulia et al. studied the antibacterial activity of an ethanol extract of banana, cassava, and pineapple peels against the fish pathogen *Aeromonas hydrophila* in 2011. Parameters observed included phytochemical compounds and MIC and MBC values of banana, cassava,

and pineapple peels. Phytochemical screening was performed using thin-layer chromatography (TLC) and the foam test. The microdilution method carried out the antibacterial activity of banana peel, cassava peel, and pineapple peel against *A. hydrophila*. It determines the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values. Phytochemical screening results showed that banana, cassava, and pineapple peel extracts contained flavonoids, alkaloids, terpenoids, tannins, and saponins. The MIC values of banana peel, cassava peel, and pineapple peel extract were 600, 400, and 200 g/mL, respectively. An extract of pineapple peel exhibited better antibacterial activity than banana and cassava peels. However, the three waste extracts indicated the antibacterial potential to be developed as natural bactericides to control *A. hydrophila* bacterial disease.

Bachar et al. (2011) studied the antibacterial and antioxidant activities of different parts of local seeded banana fruit in vitro. The dried peels, pulps, and seeds of the fruit were extracted with hexane, ethyl acetate, and ethanol. The antibacterial properties of the extracts were evaluated against four Gramme-positive and four Gramme-negative bacteria using the disc diffusion technique. Ethyl acetate and ethanol extracts of both pulp and peel exhibited antibacterial activity, with zones of inhibition ranging from 9 to 24 mm. On the other hand, only the ethyl acetate extract of seeds showed antibacterial activity, and the zone of inhibition ranged from 8.5 to 10 mm; interestingly, none of the hexane extracts of the three banana parts exhibited a zone of inhibition. High free radical scavenging activity was observed with ethyl acetate extracts of banana seed, peel, and pulp, with an ascorbic acid equivalent antioxidant capacity (AEAC) value of 1238.33, 1011.43, and 588.03 mg AA/100 g extract.

METHODOLOGY

MATERIALS REQUIRED

Conical flasks, Petriplates, test tubes, watch glass, breaker, glass rod, forceps, inoculating loop, cotton, filter paper disc, nutrient agar, nutrient brother, distilled water, measuring jar, sterilized swab, autoclave, newspaper, pen, rubber bands etc.

ANTIBACTERIAL FRUIT EXTRACT

Banana, Pomegranate, Lemon, pineapple, Tamarind, Orange, Grapes, Averrhoa bilimbi, Mossambi, Papaya.

BACTERIAL STRAINS

Staphylococcus aureus, *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae*, *Enterococcus faecalis*.

NUTRIENT BROTH CULTURE

1.3g of nutrient broth was weighed, it was added to 100ml distilled water and mixed well, and the broth was poured into test tubes, each having 5ml and it was plugged with cotton. It was then sterilized by autoclaving at 15 psi for 15 minutes and cooled to room temperature.

PREPARATION OF BACTERIAL INOCULA

The laminar air flow chamber was wiped with 70% alcohol and UV light was switched on for 15 minutes. The cotton plug of the bacterial culture tube was removed and the mouth was flamed.

The cooled sterilized loop was inserted into the tube and bacterial culture was taken. The mouth of the tube was plugged back again after flaming. By using similar procedure for removing the cap, the loop was inserted into the test tube with nutrient broth and shaken to remove bacteria into the broth. The loop was then withdrawn; mouth of the test tube was flamed and recapped. The inoculating loop was then re-sterilized and the broth culture was gently rotated to mix the contents properly. This was done for all bacterial strains and the tubes were labelled with the name of respective microbes. After inoculating each broth, they were incubated for 12-16 hours for sufficient bacterial growth.

PREPARATION OF NUTRIENT AGAR

For preparing nutrient agar medium, nutrient agar containing 2% agar was weighed out and added to 180 ml distilled water in a 250 ml conical flask and was plugged with cotton. Then the flask was sterilized for 15 minutes in an autoclave at 15psi. The medium was allowed to cool to pinna bearable temperature, poured into sterilized petri plates and allowed to set.

PREPARATION OF FILTER PAPER DISCS

down the whole agar plate. The plate is rotated 90 degrees and the swab was moved again from Filter paper discs which were about 6mm in diameter were prepared using a punching machine and sterilized by autoclaving. 0.5ml of each of floor cleaners (Dettol, Blossom, Ultrapower and Lysol) were taken in a test tube. They were diluted with equal volume of sterile distilled water to make 1:1 dilution.

The method used for antibacterial sensitivity is Kirby bauer disc diffusion method (Bauer et al., 1966) The nutritive media after sterilization was cooled down to ear bearing heat and was poured into petriplates. A lawn culture of each bacterium is prepared from the broth by swabbing using separate cotton swabs. For this, a sterile swab was dipped into the bacterial suspension, and then it was moved from side to side side to side. This is done once more with the swabs rotated 45 degrees. This method i done for each bacterium and the petriplates were labele appropriately.

Filter paper disc were soaked in each fruit extracts and kept on the petriplates using sterile forceps. The name of the fruit extract was marked. The plates were incubated at 37° C for 18-24 hours and each plate was examined for sensitivity (zone of inhibition). The radius of each zone was measured using a standard ruler in millimetre. Particular strains of bacteria were found to be sensitive to each fruit extract, if the bacterium shows no growth or feeble growth around the disc. Accordingly observations made.

PREPARATION OF FRUIT EXTRACT

The fruits were peeled off and were cutting to small pieces and juices were extracted by using grinder and the extract obtained were filtered using a sieve.

OBSERVATION AND RESULT

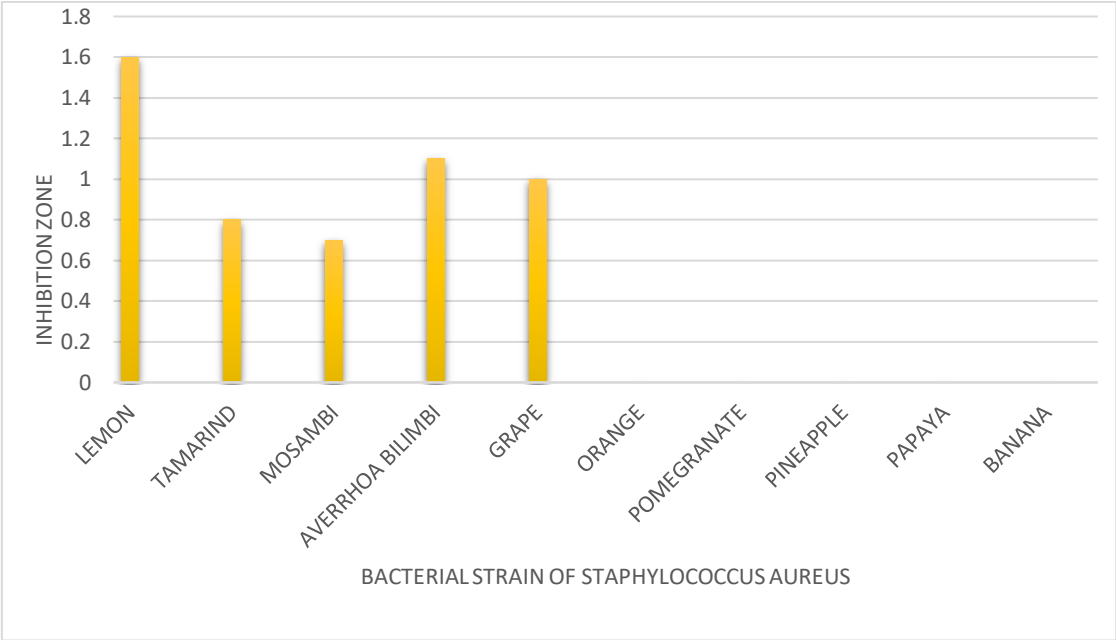


Fig 1

EFFECT OF FRUIT EXTRACTS ON *Staphylococcus aureus*

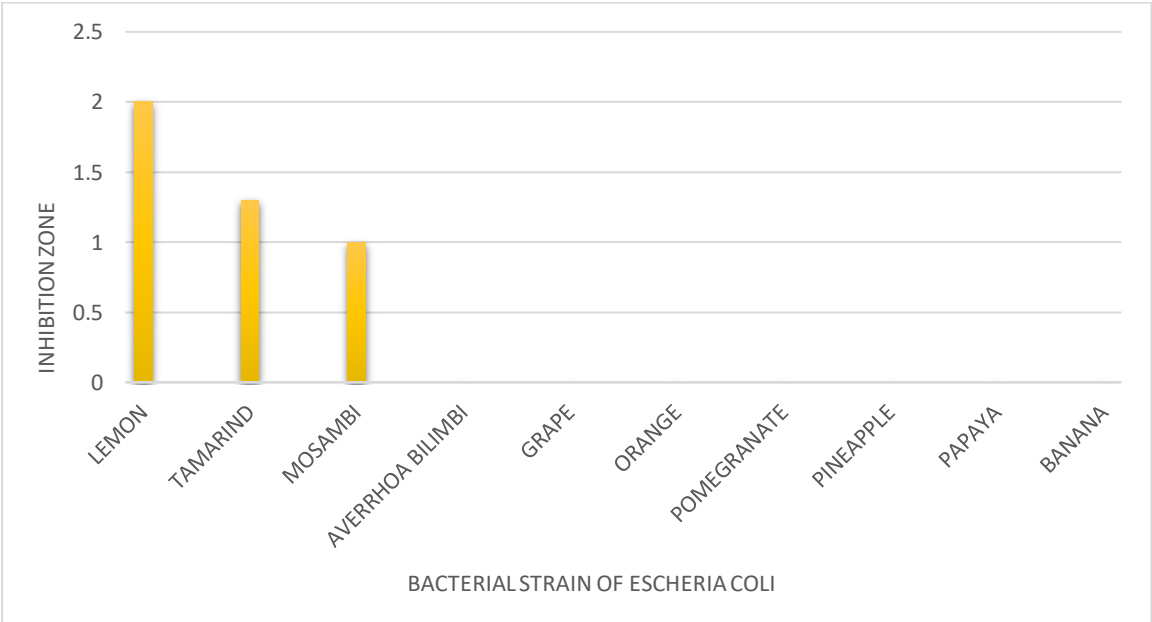


Fig 2

EFFECT OF FRUIT EXTRACTS ON *E. coli*.

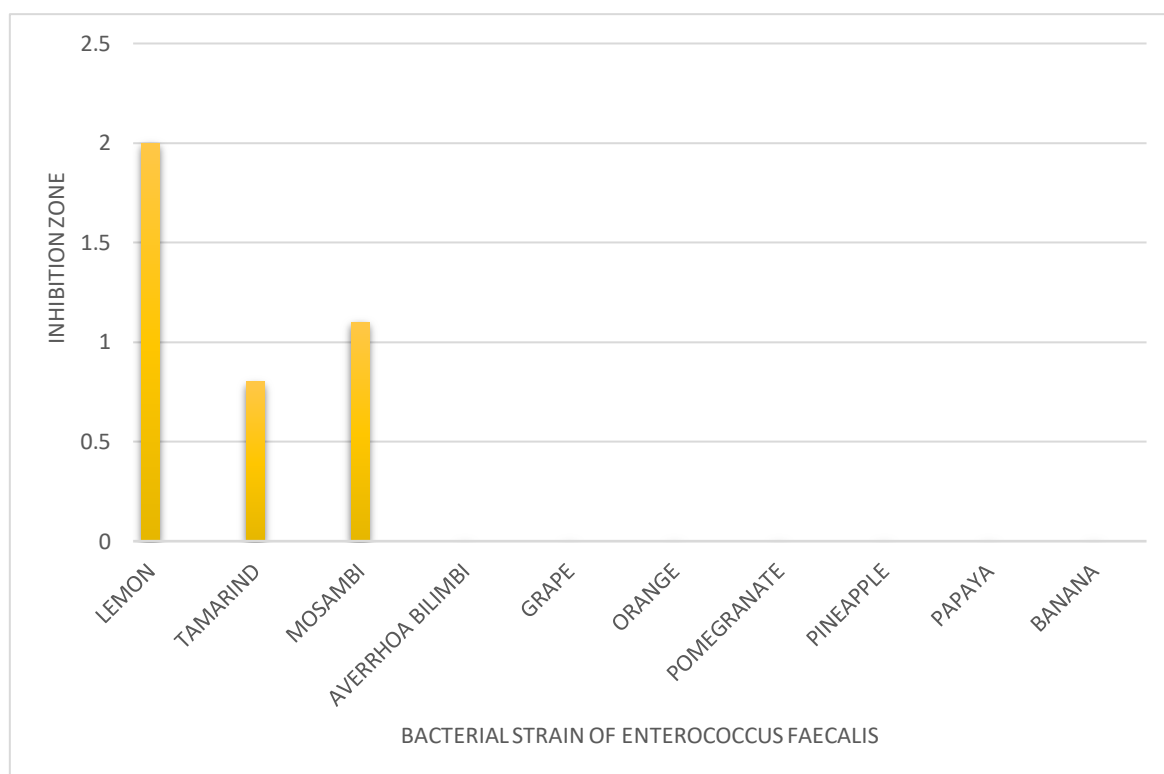


Fig3

EFFECT OF FRUIT EXTRACTS ON *Enterococcus faecalis*.

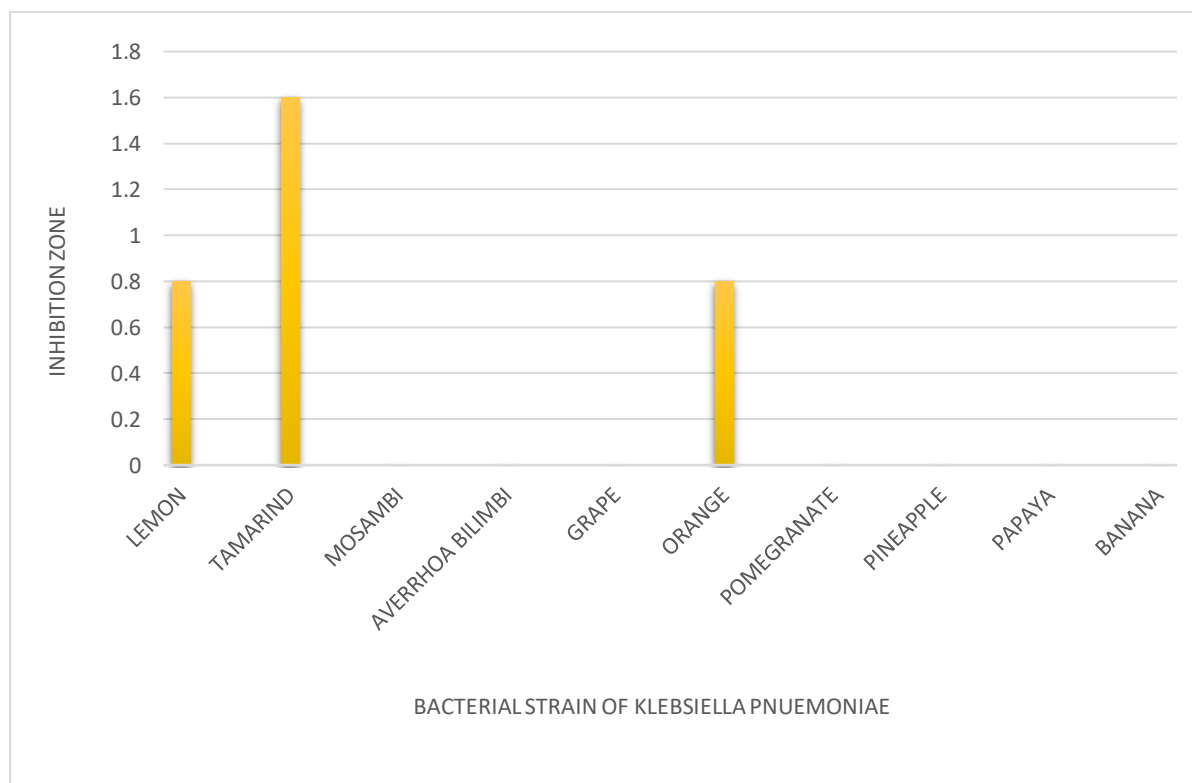


Fig 4

EFFECT OF FRUIT EXTRACTS ON *Klebsiella pneumoniae*.

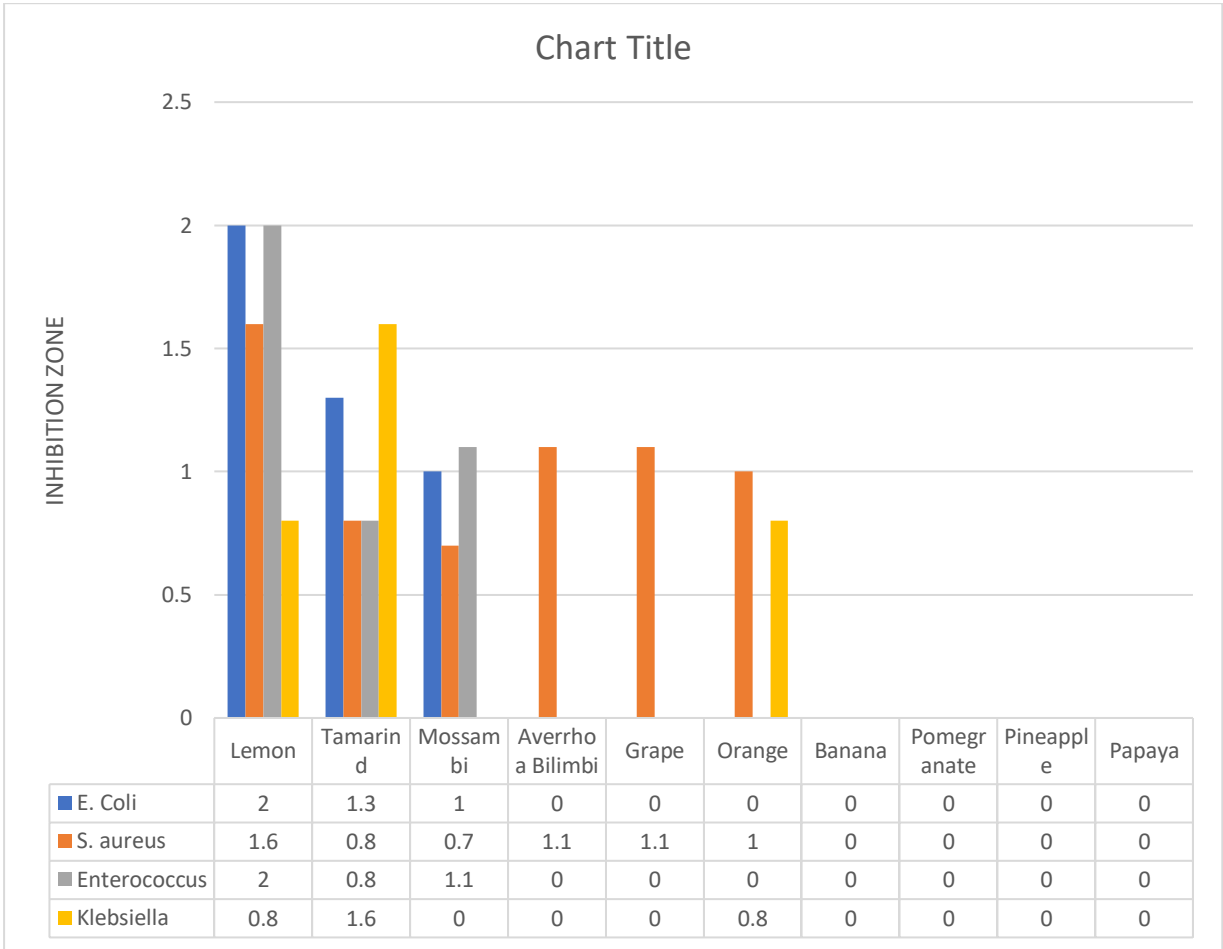


Fig 5

ANTIBACTERIAL ACTIVITY ON DIFFERENT FRUIT EXTRACT

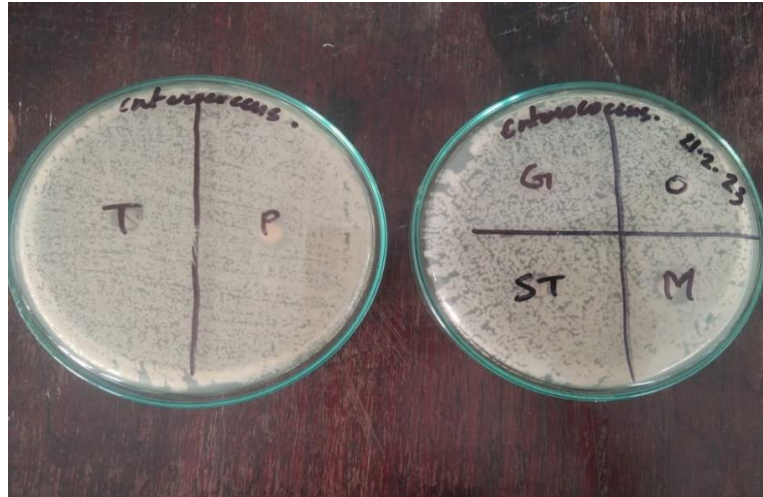


Fig 6

EFFECT OF FRUIT EXTRACTS ON ENTEROCOCCUS



Fig 7

EFFECT OF FRUIT EXTRACTS ON *S. AUREUS*



Fig 8

EFFECT OF FRUIT EXTRACTS ON *KLEBSIELLA*

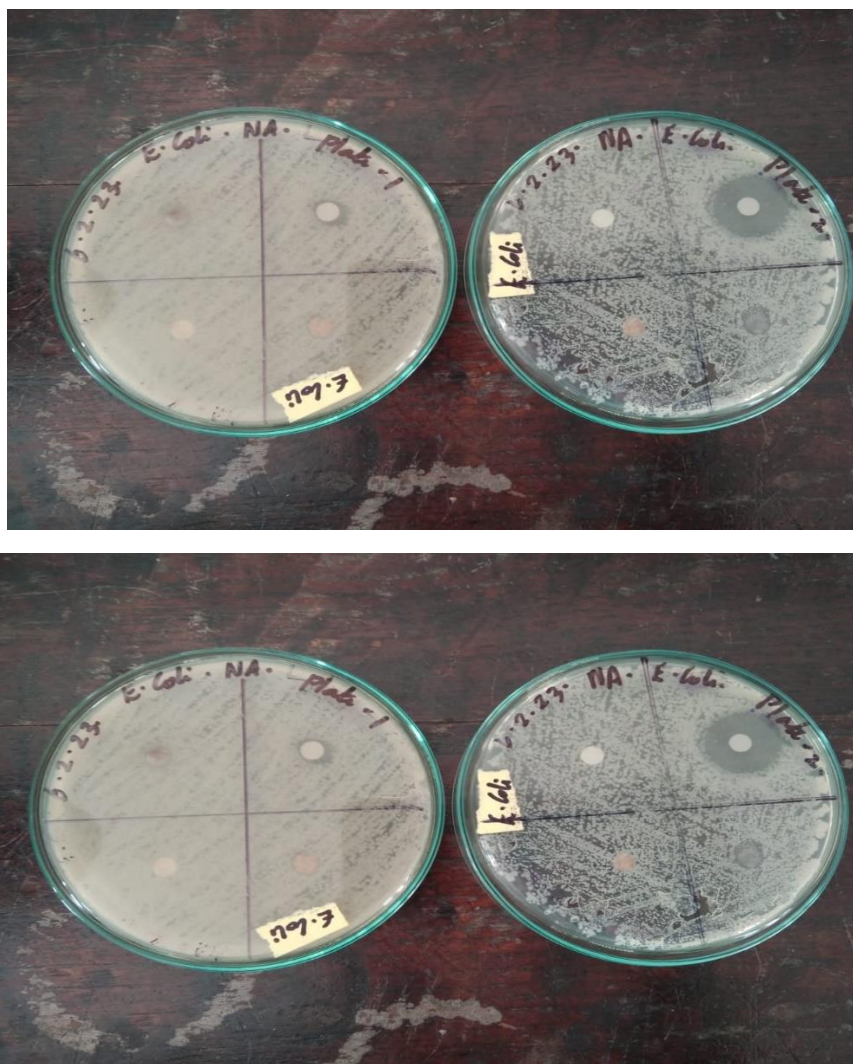


Fig 9

EFFECT OF FRUIT EXTRACTS ON *E. COLI*

The project titled "Antibacterial effect of Fruit Extract on Different Bacteria" is done by using fruits such as grapes, lemon, orange, mossambi, tamarind, averrhoa bilimbi, banana, pineapple, pomegranate, mossambi on the bacterial strains *Enterococcus faecalis*, *Staphylococcus aureus*, *E. coli*, and *Klebsiella pneumoniae*.

Results indicate that lemon is the most efficient antibacterial agent against all the pathogenic bacteria that were taken for the study. Lemon is followed by tamarind and mossambi. Lemon was equally effective against *E. coli* and *Enterococcus faecalis*, with an inhibition zone of

2mm. Lemon was moderately effective against *S. aureus* with an inhibition zone of 1.6mm and least effective against *Klebsiella* with an inhibition zone of 0.8mm.

Tamarind showed the highest effect against the test organism, *Klebsiella pneumoniae*, with a zone of 1.6 mm, and towards *E. coli*, it showed 1.3mm. *Staphylococcus aureus* and *Enterococcus faecalis* also had an equal effect, showing an inhibition zone of 0.8mm.

Mossambi, which is rich in citric acid, showed an antibacterial effect on *E. coli* and *Enterococcus faecalis* with inhibition zones of 1mm and 1.1 mm, respectively.

In the case of Averrhoa bilimbi, which is rich in oxalic acid, and grapes, which are rich in tartaric acid, the antibacterial effect was shown only on *Staphylococcus aureus*. Averrhoa bilimbi was only effective against *Staphylococcus aureus* with an inhibition zone of 1.1mm.

Grape was only effective against *Staphylococcus aureus* with an inhibition zone of 1mm.

Orange showed an inhibition zone of 0.8mm towards *Klebsiella pneumoniae*.

Banana, papaya, pomegranate, and pineapple had no antibacterial effect on any of the bacterial strains. Among the 10 fruits we used, lemon showed the most potential antibacterial activity. The present study suggests that citrus fruits and tamarind have greater potential as antibacterial agents against different pathogenic bacteria.

In the present experiment, it was observed that citrus fruits such as lemon, followed by tamarind and mossambi, showed the highest antibacterial activity. Therefore, citrus fruits and tamarind have greater potential as antibacterial agents against different pathogenic bacteria.

DISCUSSION

The present titled "ANTIBACTERIAL EFFECT OF FRUIT EXTRACT ON DIFFERENT BACTERIA" was done using different fruits such as lemon, tamarind, mossambi, papaya, orange, grapes, averrhoa bilimbi, pomegranate, pineapple, and banana on the bacterial strains of *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Enterococcus faecoli*. It was evident that the citrus fruits, like lemon, followed by tamarind and mossambi, were effective against all the strains of pathogenic bacteria when compared to other fruits that were taken for the study.

The results indicate that lemon was effective against all the strains of pathogenic bacteria. Lemons contain 5%–6% citric acid with a pH of around 2.2. Lemon is also determined by its high content of phenolic compounds, mainly flavonoids (e.g., diosmin, hesperidin, limocitrin) and phenolic acids (e.g., ferulic, synapic, p-hydroxybenzoic acids). The essential oil is rich in bioactive monoterpenoids such as D-limonene, -pinene, and -terpinene. There is scientific evidence to prove that the antibacterial properties of lemons can destroy the bacteria that cause fever and colds. The body requires lots of immune-boosting vitamin C to eliminate the viruses that cause the cold and flu. Lemon extract, when combined with water, is a helpful remedy for internally supplying vitamin C to the defence mechanism. Due to its antiviral properties It can also be used externally on the mucous membranes in the nose and throat. due to its anticancer properties. The lemon extract has the potency to kill cancer cells. Lemon was equally effective against *E. coli* and enterococci and also showed a moderate effect on *Staphylococcus* and the least antibacterial activity towards *Klebsiella*.

After lemon, it was tamarind that showed the most antibacterial activity. The aerial parts of this plant have demonstrated the presence of tartaric acid, succinic acid, and acetic acid; gum; tannins; sugars; pectin; flavonoids; sesquiterpenes; alkanoids; and glycosides. Tamarix is a rich source of magnesium. It also contains more calcium than many plant foods. The combination of these two minerals, plus weight-bearing exercise, could help prevent osteoporosis and bone fractures. Its pulp has been used in many traditional medicines as a laxative, digestive aid, and remedy for bile disorders.

Mossambi is rich in vitamin C and also contains minerals like phosphorus, folate, riboflavin, niacin, and magnesium. Because of its high content of vitamin C, it can be very effective in relieving inflammation and swelling. It plays an important role in the treatment of symptoms of osteoarthritis and rheumatoid arthritis. It also boosts calcium absorption. Mossambi extract also stimulates bone formation in cells and promotes overall bone health. Mossambi was more sensitive to *E. coli* and enterococci and moderately effective against staphylococci.

Phytochemicals contained in *Averrhoa bilimbi* include alkaloids, saponins, tannins, flavonoids, phenols, and triterpenoids. This plant's pharmacology is used as an antibacterial to reduce the activity of *Staphylococcus aureus*, which infected human mouths. This plant is principally cultivated for medicinal purposes in many tropical and subtropical countries of the world. A literature survey about this plant shows that *A. bilimbi* is mainly used as folk medicine in the treatment of diabetes mellitus, hypertension, and as an antimicrobial agent. It is also used to treat chickenpox, intestinal parasites, headaches, and other illnesses. Bilimbi showed maximum sensitivity towards *Staphylococcus aureus*.

Orange juice contains an array of potent antioxidants, including flavonoids (hesperetin and naringenin, predominantly as glycosides), carotenoids (xanthophylls, cryptoxanthins, carotenes), and vitamin C, in addition to other beneficial phytochemicals, such as folate. In addition to vitamin C, oranges have other nutrients that keep your body healthy. The fibre in oranges can keep blood sugar levels in check and reduce high cholesterol to prevent cardiovascular disease. The bacterial strain *Klebsiella* was moderately effective against orange

Grapes are a rich source of bioactive molecules, including phenolic acids, flavonoids, anthocyanins, stilbenes, and lipids. These are the compounds that contribute to the health benefits of grapes and grape-derived products. They help to lower the blood pressure and also protect against heart disease. It protects against diabetes and reduces high cholesterol. It was sensitive only to *Staphylococcus aureus*.

The mineral composition of banana extract consists of phosphorus, iron, calcium, magnesium, and sodium. Zinc, copper, potassium, and manganese were found in very low concentrations. It may improve digestion and also improve heart health. It also supports bone health and helps muscles recover after exercise. In this experiment, it hasn't shown any antibacterial activity against any of the pathogenic bacterial strains used for the study.

Pomegranate is a potent antioxidant. This fruit is rich in flavonoids, anthocyanins, punicalic acid, ellagitannins, alkaloids, fructose, sucrose, glucose, simple organic acids, and other components and is antiatherogenic. Antioxidants protect cells from damage and reduce inflammation and the effects of ageing. Studies have suggested that pomegranates can protect your heart in many ways, including by lowering blood pressure and reducing blood sugar levels. In this study, it was not effective against any of the pathogenic bacteria.

Carica papaya leaf contains active components such as alkaloids, glycosides, tannins, saponins, and flavonoids, which are responsible for its medicinal activity. Additionally, the leaf juice of papaya increases platelet counts in people suffering from dengue fever. In this study, it was not effective against any of the pathogenic bacteria.

Pineapple is high in vitamin C, which helps your immune system, the body's defence against germs, keep us healthy. It also has vitamins A, B6, E, and K. Calcium In this study, it was not effective against any of the pathogenic bacteria.

Fruit extracts contain antioxidants, vitamins, and minerals that are essential for human beings and play an important role in the prevention of heart diseases and cancer. Some of the extracts had good potential for therapeutic use against the bacterial pathogens or for application in treating diarrhoea. The antibacterial effects of fruit extracts can have various aftereffects, depending on the specific fruit and the type of bacteria being targeted. Reduction of bacterial growth: Fruit extracts with antibacterial properties can inhibit the growth of harmful bacteria, reducing the risk of infection or illness. The variations in the antibacterial property in different fruits extract is due to the different chemical components present in them.

CONCLUSION

The present project, titled "Antibacterial Activity on Fruit Extract," was studied using the Kirby Bauer Disc Diffusion Method. The effectiveness of different fruit extracts was used for the study. Citrus fruits such as lemon, followed by tamarind, and then Mossambi showed the highest potential for the antibacterial activity.

The antibacterial effects of fruit extracts can have various after effects, depending on the specific fruit and the type of bacteria being targeted. Fruit extracts with antibacterial properties can inhibit the growth of harmful bacteria, reducing the risk of infection or illness. The variations in the antibacterial property of different fruit extracts are due to the different chemical components present in them.

The bacterial strains of *E. coli* and *Enterococcus faecalis* were more sensitive to lemon. However, it was moderately sensitive to *Klebsiella* and *Enterococcus*. The bacterial strains *Klebsiella* and *E. coli* were more sensitive to tamarind and were moderately sensitive to *Staphylococcus aureus* and *Enterococcus faecalis*. In the case of mossambi, the bacterial strains *E. coli* and *Enterococcus* showed the highest sensitivity when compared to *Staphylococcus*. It doesn't show any antibacterial activity against *Klebsiella*. Averrhoa bilimbi and grapes were effective against only *Staphylococcus aureus* and didn't show any antibacterial activity against any other pathogenic bacteria. Orange was only effective against *Klebsiella*. No antibacterial activity was found in fruits such as banana, papaya, pomegranate, and pineapple against any other strains of pathogenic bacteria that were used for the study.

From the study, it can be concluded that lemon is the most effective antibacterial agent. Tamarind and Mossambi also showed a promising result; therefore, these are also good antibacterial agents. Least antibacterial activity was found in Averrhoa bilimbi, grapes, and oranges. Less or no antibacterial activity was found in banana, papaya, pomegranate, and pineapple.

The variations in the antibacterial property of different fruit extracts are due to the different chemical components present in them.

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