ANTIBACTERIAL EFFECTS OF SELECTED PLANT–PART EXTRACTS ON DIFFERENT BACTERIAL STRAINS

Project work by

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CERTIFICATE

This is to certify that the project report entitled "ANTIBACTERIAL EFFECTS OF SELECTED PLANT – PART EXTRACTS ON DIFFERENT BACTERIAL STRAINS" submitted by Ms. Shreishta Manoj, Reg No: AB21ZOO026 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.

Dr. Helvin Vincent	Dr. Soja Louis
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EXAMINERS:

1.

2.

DECLARATION

I, hereby declare that this project work entitled "ANTIBACTERIAL EFFECTS OF SELECTED PLANT – PART EXTRACTS ON DIFFERENT BACTERIAL STRAINS" is submitted to St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam in partial fulfilment of the requirement 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: Shreishta Manoj Signature:

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Shreishta Manoj

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ABSTRACT

The present project, titled " Antibacterial Effects Of Selected Plant – Part Extracts On Different Bacterial Strains", was focused on finding the antibacterial activity of 9 extracts of plant parts namely, leaves (Neem, Tulsi, Aloe vera), spices (Pepper, Cumin, Cinnamon) and modified stem (Ginger, Garlic, Onion). These were tested against 2 strains of bacteria of which one was gram-positive (*Staphylococcus aureus*) and the other one was gram-negative (*Escherichia coli*). These plant part extracts have been widely used as anti-inflammatory, antibacterial, antifungal, antimicrobial and antioxidants. They exhibit a broad spectrum of antibacterial activity. Antibacterial is an agent that selectively destroys bacteria by interfering with bacterial growth or survival. They are widely used in antibiotic medications for the treatment and prevention of infections. The present study showed the effectiveness of the plant part extracts against 2 different strains of bacteria. The method used was Magaldi's Well diffusion method (Magaldi *et al*., 2004). From the present study, it was concluded that all the extracts exhibited antibacterial activity against both strains of bacteria. An exception was neem which did not show a zone of inhibition against *S. aureus*.

INTRODUCTION

Bacterial effects on humans can vary widely, while certain bacteria are necessary for basic body processes, others can lead to infections and illnesses. Beneficial bacteria boost immunity, facilitate digestion, and improve general health. On the other hand, some pathogenic bacteria can seriously endanger your health, such as those that cause gastrointestinal, skin, or respiratory diseases. A wide range of symptoms, from minor discomfort to serious sickness, can result from infections caused by pathogenic bacteria. An increasing problem in medicine is antibiotic-resistant microorganisms, which can make treatment more difficult.

Natural compounds derived from plants have long been recognized for their therapeutic potential, including antibacterial properties. Numerous antibiotics have adverse effects that might occasionally harm vital organs including the kidneys and liver. As a result, a lot of people search for natural substitutes for these medications nowadays—fortunately, several plant parts function as organic antibiotics. Bioactive substances with antibacterial qualities found in plant leaves include alkaloids, flavonoids, and phenolics. Strong antibacterial qualities are exhibited by substances such as cumin aldehyde in cumin, piperine in pepper, and cinnamaldehyde in cinnamon. Modified stems are reservoirs of diverse bioactive compounds that exhibit various biological activities, including antimicrobial effects.

This project aims to investigate the antibacterial potential of various plant extracts against *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)*. Specifically, we focus on three categories of plant parts extracts: leaves (Neem, Tulsi, Aloe), spices (Cumin, Pepper, Cinnamon), and modified stem extracts (Onion, Ginge Garlic). Each of these plant parts possesses a rich reservoir of bioactive compounds known for their antimicrobial properties, making them compelling subjects for exploration in this study.

E. coli is a gram-negative bacterium commonly found in the intestines of humans and animals, while *S. aureus* is a gram-positive bacterium that can colonize various body sites and cause a range of infections. The choice of these bacterial strains is motivated by their clinical significance and prevalence in both community and healthcare settings. *E. coli* and *S. aureus* infections are associated with a wide spectrum of diseases, and the development of resistance against

conventional antibiotics underscores the urgent need for novel therapeutic interventions.

The methodology involves the extraction of bioactive compounds from selected plant parts, followed by screening for antibacterial activity against *E. coli* and *S. aureus* using standard microbiological assays using well diffusion method. The effectiveness of the antibacterial effects of the selected samples is based on the size of the zone of inhibition which depends upon the diffusability of the antibacterial agent, the size of the inoculum, type of medium and other factors.

Understanding the antibacterial sensitivity of root tuber extracts against *E. coli* and *S. aureus* is crucial for identifying potential candidates for further development as alternative antimicrobial agents.

Bacterial strains used in the experiment:

1. ***Escherichia coli (E. coli)*** – It is a rod-shaped, anaerobic, Gram-negative coliform bacterium found in the gut, aiding in physiological functions like resisting pathogenic bacteria, producing vitamin K2 for bone health and clotting, and fermenting dietary fibres. However, some strains can cause severe illnesses, from minor gastrointestinal issues to life-threatening conditions like septicemia and haemolytic uremic syndrome (HUS). Preventive measures are crucial to avoid outbreaks of foodborne and hospital-acquired infections, posing serious public health concerns.

2. ***Staphylococcus aureus (S. aureus)*** – It is a Gram-positive coccus bacterium typically found as a harmless commensal resident on skin and mucous membranes. While it helps maintain microbial balance, it can become pathogenic, causing various infections from mild skin issues to life-threatening conditions like pneumonia. *S. aureus* possesses virulence factors enabling it to evade the immune system, posing challenges in clinical management and public health.

Plant leaf extracts used in the experiment:

1. Neem - Neem extract, from the neem tree (*Azadirachta indica*), contains Nimbin, nimbidin, and azadirachtin, showing broad-spectrum antibacterial effects against pathogens like *Staphylococcus aureus, Escherichia coli,* and *Salmonella spp.*
2. Tulsi - Tulsi extract, from the sacred basil plant, contains eugenol, exhibiting antimicrobial activity against bacteria such as *E. coli* and *Staphylococcus aureus.* Its antioxidant properties also boost the immune system, aiding in fighting bacterial infections.
3. Aloe - Aloe vera extract is rich in anthraquinones and polysaccharides, which disrupt bacterial cell membranes, inhibit growth, and hinder biofilm formation, showcasing potent antibacterial effects.

Spice extract used in the experiment:

1. Cumin - Derived from Cuminum cyminum seeds, cumin extract contains cuminaldehyde and thymoquinone, showing potent antibacterial effects against foodborne and respiratory bacteria.
2. Pepper - Extracted from black, white, or green peppercorns, rich in bioactive compounds like piperine, pepper extract exhibits broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria.
3. Cinnamon - Compounds like cinnamaldehyde and eugenol found in cinnamon have been shown to inhibit the growth of various harmful bacteria, including *E. coli* and *Salmonella*. These antibacterial effects make cinnamon extract a promising natural alternative for combating bacterial infections and preserving food.

Modified stem extracts used in the experiment:

1. Garlic - Rich in organosulfur compounds like allicin, it exhibits broad-spectrum antimicrobial activity against various bacteria, including antibiotic-resistant strains. This natural remedy disrupts bacterial cell membranes, inhibiting their growth and proliferation.
2. Ginger - It contains bioactive compounds such as gingerol and shogaol, which exhibit antimicrobial effects against various bacteria. Ginger extract can inhibit the growth of bacteria by disrupting their cell membranes, interfering with their communication systems, and inhibiting their enzymes.
3. Onion – It is rich in compounds like allicin and quercetin, and demonstrates efficacy against various bacterial strains. Studies suggest onion extract can inhibit the growth of bacteria such as *Staphylococcus aureus* and *Escherichia coli* (Noha, 2014).

By exploring the antibacterial activity of plant part extracts, the current study seeks to identify promising natural alternatives to conventional antibiotics.

REVIEW OF LITERATURE

Bacteria are single-celled microorganisms with a simple cellular structure, typically comprising a cell wall, cell membrane, cytoplasm containing genetic material, and sometimes appendages like flagella and pili. Under a microscope, *Escherichia coli (E. coli)* appears as rod-shaped cells. It is a gram-negative bacterium. It is motile due to the presence of flagella and a sophisticated cytoplasmic structure that houses genetic material and plasmids. Conversely, under a microscope, gram-positive *Staphylococcus aureus (S. aureus)* appears as spherical cells or clusters that resemble grapes. Its resistance mechanisms and pathogenicity are attributed to its thick peptidoglycan cell wall and surface protein A.

Maragathavalli *et al*. (2012) in their study, investigated the antimicrobial activity of neem leaf extracts using varying concentrations against human pathogenic bacteria including *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Bacillus pumilus*. They employed the disc diffusion method and compared the results with gentamicin as a standard antibiotic. They found a maximum zone of inhibition in *S. aureus*.

In a study conducted by Raja *et al*. (2013) they compared the antimicrobial efficacy of aqueous extracts of leaf, bark and seeds of *A. indica* against human pathogenic bacteria (*Staphylococcus aureus, Enterococcus feacalis, Proteus mirabilis* and *Pseudomonas aeruginosa*) and fungi (*Aspergillus fumigatus* and *Candida albicans*). The agar well diffusion method and micro-broth dilution methods were used to determine the minimum inhibitory concentration (MIC). Results showed that leaf extract exhibited strong antimicrobial activity against bacteria and fungi at all the concentrations tested.

Rathnayaka (2013) evaluated the antibacterial activity of aqueous extract, oil extract, chloroform extract and alcohol extract obtained from leaves of *Ocimum sanctum* against four food-borne microbial pathogens, *Salmonella enteritica, Vibrio parahaemolyticus, Escherichia coli* and *Listeria monocytogenes*. In conclusion, Ocimum extracts were found to contain chemical compounds useful in food preservation and development of drugs against food-borne microbial pathogens.

Hanaa *et al*. (2016) examined the antimicrobial properties of essential oils distilled from Australian-grown *Ocimum tenuiflorum* (Tulsi), to quantify the volatile components present in flower spikes, leaves and the essential oil, and to investigate the compounds responsible for any activity. Broth micro-dilution was used to determine the minimum inhibitory concentration (MIC) of Tulsi essential oil against selected microbial pathogens. The oils completely inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* in certain concentrations, while the same concentrations only partly inhibited the growth of *Pseudomonas aeruginosa*. Out of 54 compounds identified in Tulsi leaves, flower spikes, or essential oil, three were proposed to be responsible for this activity; camphor, eucalyptol and eugenol. Since *S. aureus, P. aeruginosa* and *E. coli* are major pathogens causing skin and soft tissue infections, Tulsi essential oil could

be a valuable topical antimicrobial agent for the management of skin infections caused by these organisms.

Tulsi or *Ocimum sanctum* is a popular healing herb in Ayurvedic medicine. Its antimicrobial properties are widely used to treat several systemic diseases. A study by Arti *et al*. (2022) highlights the hidden antimicrobial potential of the phytochemicals and bioactive compounds derived from traditional medicinal plants like Tulsi as a good alternative.

Alessandra *et al*., (2005) worked on the biological effects of *Aloe vera* pulp extract, associated or not with UVA radiation, on *Escherichia coli*-deficient repair mutants and plasmid DNA, to test its genotoxic potential based on the idea that since people use *Aloe vera* topically, they could be exposed to solar ultraviolet light in addition and it might cause a cross damage effect between these agents. Data obtained from analysis of survival fractions, bacterial transformation and agarose gel electrophoresis suggest that *Aloe vera* has genotoxic properties, but it seems not to be able to damage the cell membrane.

Rafeef (2013) conducted experiments on the antibacterial effects on Cumin and proved that it exhibits strong antibacterial activity against four clinical bacterial isolates (*Escherichia coli, Staphylococcus aureus, Klebsiella sp.* and *Pseudomonas aeruginosa*) and strong to moderate antifungal activity against three fungal isolates (*Aspergillus flavus, Candida albicans* and *Cryptococcus sp.*). The minimum inhibitory concentration (MIC) of the cumin-extracted oil was applied against the clinical isolates of bacteria and E. coli was the most sensitive isolate, with the lowest MIC value.

In an experiment done by Shetty *et al*. (1994) Fungal (*Aspergillus and Penicillium spp.*) and yeast (*Saccharomyces and Candida spp.*) cultures were more sensitive to cumin volatile oil and cumin aldehyde than bacteria. Among Gram-negative bacteria, *Escherichia coli* was the most sensitive to the volatile oil while *Pseudomonas aeruginosa* was the most resistant. *Staphylococcus aureus* had an MIC almost double that of all other Gram-positive species tested, while the fungi had MIC values 10 to 20 times lower than those of the bacteria.

A study by Lan *et al*, (2015) explored the antimicrobial activity of black pepper chloroform extract (BPCE) against *Escherichia coli* and *Staphylococcus aureus*. The antibacterial mechanism of BPCE was elucidated by analysing the cell morphology, respiratory metabolism, pyruvic acid content, and ATP levels of the target bacteria. Scanning electron micrographs showed that the bacterial cells were destroyed and that plasmolysis was induced. BPCE inhibited the tricarboxylic acid pathway of the bacteria. The extract significantly increased pyruvic acid concentration in bacterial solutions and reduced ATP levels in bacterial cells. BPCE destroyed the permeability of the cell membrane, which consequently caused metabolic dysfunction, inhibited energy synthesis, and triggered cell death.

Black pepper (*Piper nigrum L*.) is known as the king of spices and its sharp taste is due to the presence of piperine which is the main bioactive alkaloid in the fruit. In a study by Dalia (2018), both piperine and black pepper oil in different concentrations were evaluated for their antimicrobial activity against *Staphylococcus aureus* (G+ coccoid-shaped bacteria*), Bacillus subtilis* (G+ long spore-forming bacteria), *Salmonella sp.* and *E. coli* (G- short rod bacteria). The inhibition activity was measured by using the agar well diffusion method. Piperine and black pepper oil showed antibacterial activity with all tested Gram-positive bacteria with zones ranging from 8.23-18.1mm and 3.14-10.43, respectively. The results showed that piperine is an excellent antibacterial agent with all tested bacteria.

Cinnamon essential oil (EO) exhibited effective antibacterial activity against foodborne spoilage and pathogenic bacteria in model systems using *Escherichia coli* and *Staphylococcus*. Overall, *S. aureus* was more susceptible to cinnamon EO than *E. coli* as proved in an experiment by Yunbin *et al*. (2016).

Antibacterial mechanisms of cinnamon and its constituents such as cinnamaldehyde and cinnamic acid, against pathogenic Gram-positive and Gram-negative bacteria, were described by Vasconcelos *et al*., (2018). The current knowledge of the primary modes of action of these compounds as well as the synergistic interactions between cinnamon or its constituents with known antibacterial agents was reviewed.

Antibacterial activity of extracts of *Allium sativum* (garlic) and *Zingiber officinale* (ginger) was evaluated by Bandna (2013) against four different bacteria namely *Escherichia coli*, *Salmonella typhi, Staphylococcus aureus* and *Bacillus cereus*. Two methods were used to determine the antimicrobial activity of garlic and ginger extracts namely the disc diffusion method and the agar well diffusion method. Garlic extract exhibited excellent antibacterial activity against all four test organisms while ginger extract showed antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus* only.

Serge *et al*. (1999) proved that Allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit i) antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxigenic strains of *Escherichia coli*; ii) antifungal activity, particularly against *Candida albicans*; iii) antiparasitic activity, including some major human intestinal protozoan parasites such as *Entamoeba histolytica* and *Giardia lamblia*; and iv) antiviral activity. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes.

The antimicrobial effects of garlic, ginger, carrot and turmeric paste against *Escherichia coli* O157:H7 in laboratory buffer and model food system were investigated by Shivani *et al*. (2005). Fresh ginger paste showed antimicrobial activity only at 8°C. Only commercial ginger

paste had antimicrobial activity in BPW at 4°C for 2 weeks. However, commercial ginger paste showed antimicrobial activity in ground beef at 3 days and after (about 1–2 log CFU/g) compared to control samples at 8°C for 2 weeks. Fresh garlic paste showed antimicrobial activity only in BPW (1.3 log CFU/g) at 8°C. These results indicate that the antimicrobial activity of these pastes is decreased in ground beef and laboratory buffer.

A study conducted by Induja *et al*. (2018) aimed to evaluate the antimicrobial activity of *Allium cepa* (Onion) against bacteria causing enteric infection. The screening of the antibacterial activity of extracts was carried out using the agar well diffusion method. Standard antibiotic discs ciprofloxacin was used as a positive control. The extracts at different concentrations exhibited antibacterial activity against all bacterial strains tested. The extract was more effective against *E. coli* and was least effective against *Pseudomonas aeruginosa. Staphylococcus aureus* showed a good zone of inhibition. They concluded that the extracts of *A. cepa* can be used as an antimicrobial agent against bacteria causing enteric infections.

A study of the antimicrobial potentials of *Allium cepa* (Onion) by Bakht *et al*. (2013) revealed that all the extracts from both fresh and old samples showed different ranges of antimicrobial activities. Ethyl acetate, chloroform, butanol, petroleum ether, ethanol and water sub-fractions were used. Among gram-positive microbes, *Bacillus subtilus* was the most susceptible bacteria inhibited by all extracts while the most resistant gram-positive bacteria was *Staphylococcus aureus*. *Erwinia caratovora* and *Klebsiella pneumonia* were the most susceptible gram-negative bacteria while *Pseudomonas aeurginosa* and *Salmonella typhi* were the most resistant bacteria.

Garlic (*Allium sativum*) has had an important dietary and medicinal role for centuries. It is a large annual plant of the Liliaceae family, which grows in most of Africa and in Ethiopia. Ethiopian garlic is used in traditional medicine for infectious diseases and some other cases. A study by Deresse (2010) tested the aqueous extract of garlic in vitro for its antibacterial activity. The extract showed concentration-dependent antibacterial activity against *Staphylococcus aureus.* The traditional use of Ethiopian garlic for infectious diseases and for controlling fever appears to be justified.

AIM

To investigate the antibacterial potential of various plant extracts against *E. coli* and *S. aureus*.

METHODOLOGY

Materials Required:

Nutrient broth, Agar Agar, Petri plate, spatula, glass rod, conical flasks, test tubes, beaker, mortar and pestle, watch glass, distilled water, forceps, filter paper, sterile swabs, weighing machine, cotton plugs, measuring cylinder, inoculating loop, newspaper, ethanol, etc.

Samples:

Leaves – Neem, Aloe vera, Tulsi

Spices – Cumin, Pepper, Cinnamon

Modified stems – Garlic, Onion, Ginger

Nutrient Broth culture:

0.65g of nutrient broth was weighed and added to 50ml distilled water taken in a conical flask and mixed well. The broth was then poured into 4 test tubes, closed using a cotton plug and placed in a beaker. It was sterilized by autoclaving for 15 minutes and cooled to room temperature.

Inoculating the broth:

The microbial culture tube was taken and the cotton plug was removed and the mouth was flamed. The sterilized inoculating loop was inserted into the culture tube carefully without touching the side to prevent contamination. A visible amount of inoculum was extracted using the loop and the cotton plug was placed back after flaming. The inoculum was then introduced into the culture tubes containing the sterile nutrient broth. The mouth of the culture tube was also plugged back carefully after flaming. The inoculating loop was re-sterilized and the broth culture was gently rotated for mixing of contents. The contents in each test tube were labelled with the names of respective microbes and the date was noted. Two cultures each of *E. coli* and *S. aureus* were made. For sufficient bacterial growth, inoculums were kept for 24 hours at 37°C.

Preparation of nutrient agar media:

The medium was prepared using 2.6g of nutrient broth and 4.0g of agar/ 200ml. Both the nutrient broth and agar were weighed properly and were added to 200ml distilled water taken in a conical flask. It was plugged with cotton and covered with newspaper to be sterilized for 15 min in an autoclave at 15psi. The medium was allowed to cool to pinna-bearing heat. The

nutrient agar was then poured into sterile petri dishes. It was allowed to set at room temperature. It was then kept upside down. These petri dishes were used for further study.

Preparation of extracts from the sample:

Samples were collected and washed with distilled water. Direct concentrated samples were taken from the leaves and the modified stems after crushing them using a mortar and pestle. Spices were crushed and 5g of each sample was weighed, and 10 ml water was added to it and mixed well. The extract was then filtered and collected in labelled test tubes.

Magaldi's Well diffusion method (Magaldi *et al*., 2004):

A lawn culture of each bacterium was prepared. A sterilized swab was dipped into each bacterial suspension and moved side to side from top to bottom leaving no space uncovered. The plate is rotated to 90° and the same process was repeated so that the plate was coated with bacteria. Once the lawn was made a sterilized syringe gel puncher was used to create three equidistant wells in each plate. The puncher was sterilized with ethanol before creating each well. The extracted agar was also disposed of into a beaker containing ethanol to prevent contamination. The plate was closed and neatly labelled with information like the name of the bacterial strain, the date of preparation of the plate and abbreviations of samples used. Using a micropipette, samples were loaded into the well one by one. New tips were taken to load each well and old ones were discarded. The plates were then incubated at 37°C for 48 hrs. The plate was then examined for sensitivity (zone of inhibition). The diameter of each zone was measured using a standard ruler in centimetres. A particular strain of bacteria is found to be sensitive to the sample extract if the bacteria showed no growth all around the disc. On the other hand, if the bacteria shows more number of colonies, then the bacterial strain is resistant to the sample extract. The diameter of the zone of inhibition was noted accordingly.

Killing and disposing of bacteria:

After the experiment, the bacteria were destroyed by decontaminating the plates for 15 min in an Autoclave. All the glass wares used for the experiment was also decontaminated to remove any bacteria if present. It was later washed with disinfectants and dried.

OBSERVATIONS AND RESULT

This study was conducted to observe the antibacterial effects of selected plant-part extracts. The following observations were noticed.

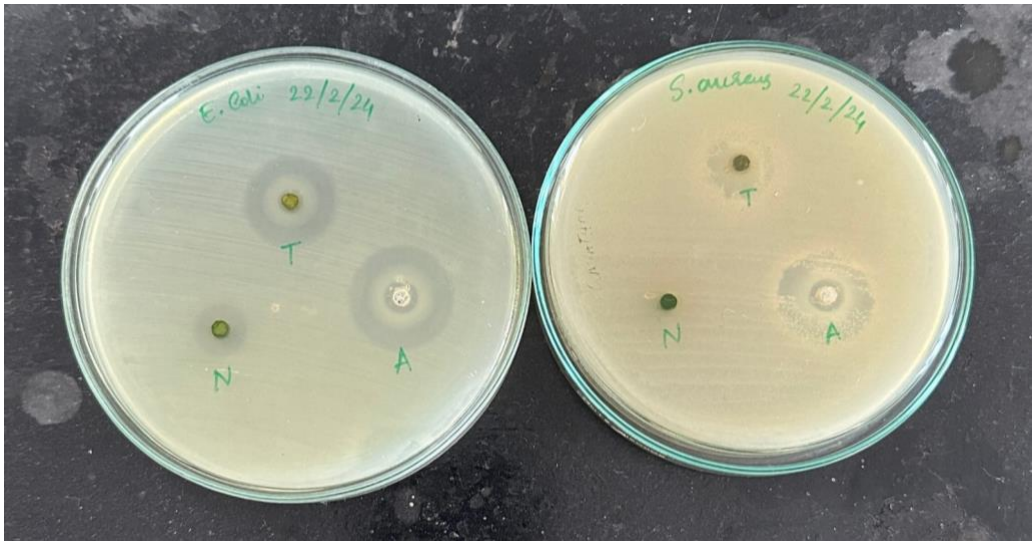


Figure 1: Zones of inhibition of leaf extracts in *E. coli* and *S. aureus*

It is clear from the figure that aloe vera has the largest zone of inhibition in both *E. coli* and *S. aureus* with a diameter of 2.4cm and 2cm respectively. Tulsi shows a good zone of inhibition in both the culture plates whereas neem shows a zone of inhibition only in *E. coli* with a diameter of 1cm.

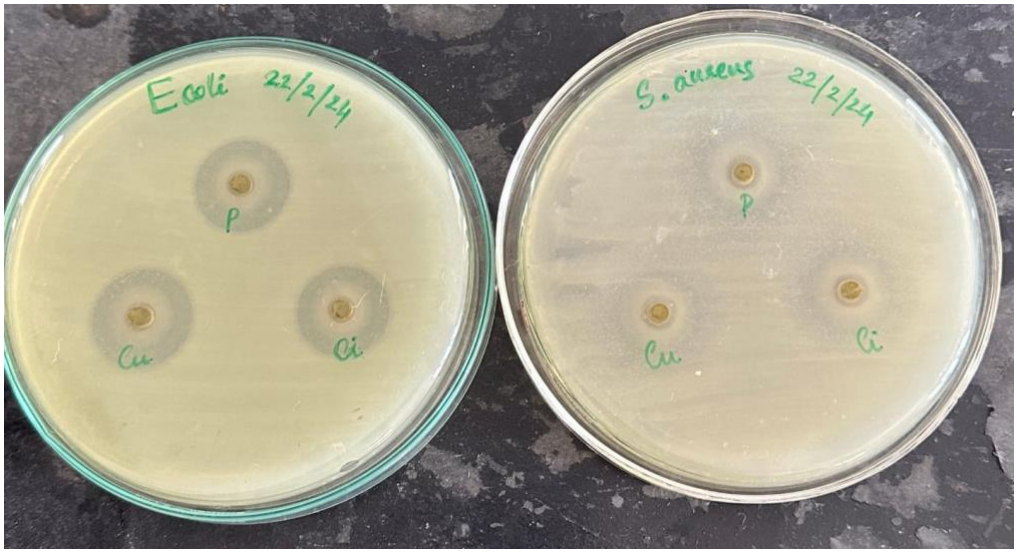


Figure 2: Zones of inhibition of spice extracts in *E. coli* and *S. aureus*

All of the selected spices showed a similar zone of inhibition in both the culture plates but they were distinct and more visible in *E. coli*. The highest inhibition zone was shown by Pepper in the *S. aureus* culture plate with a diameter of 2.5cm.

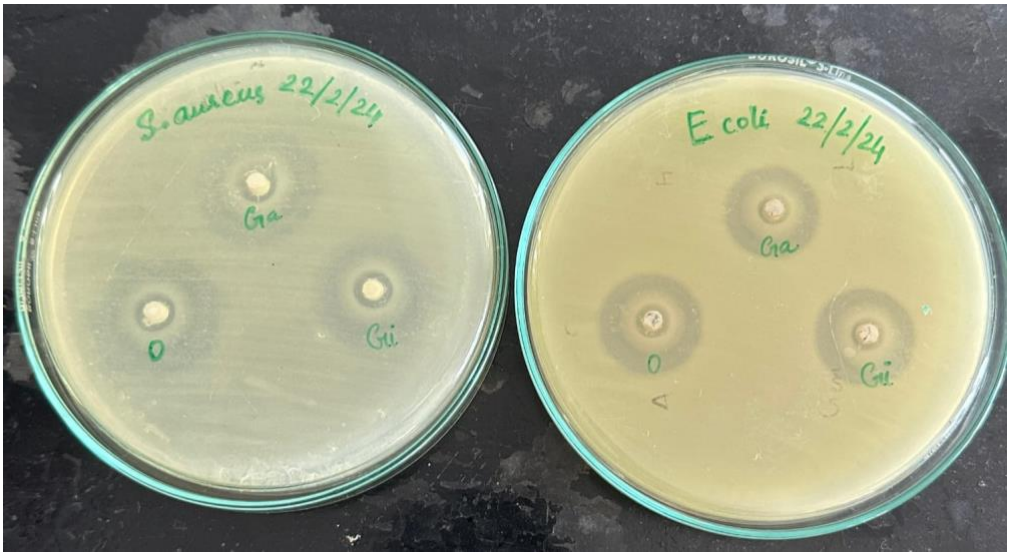


Figure 3: Zones of inhibition of modified stem extracts in *E. coli* and *S. aureus*

It can be observed that all of the stem extracts showed a good zone of inhibition in both *E. coli* as well as *S. aureus*. However, all of them showed the largest zone of inhibition in *S. aureus* when compared to *E. coli*. The largest zone of inhibition was shown by Garlic with a diameter of 2.2cm in the *S.aureus* culture plate.

The diameter of the zone of inhibition was measured and represented in the following table.

TABLE 1: DIAMETER OF ZONES OF INHIBITIONS OF SELECTED PLANT LEAF EXTRACTS

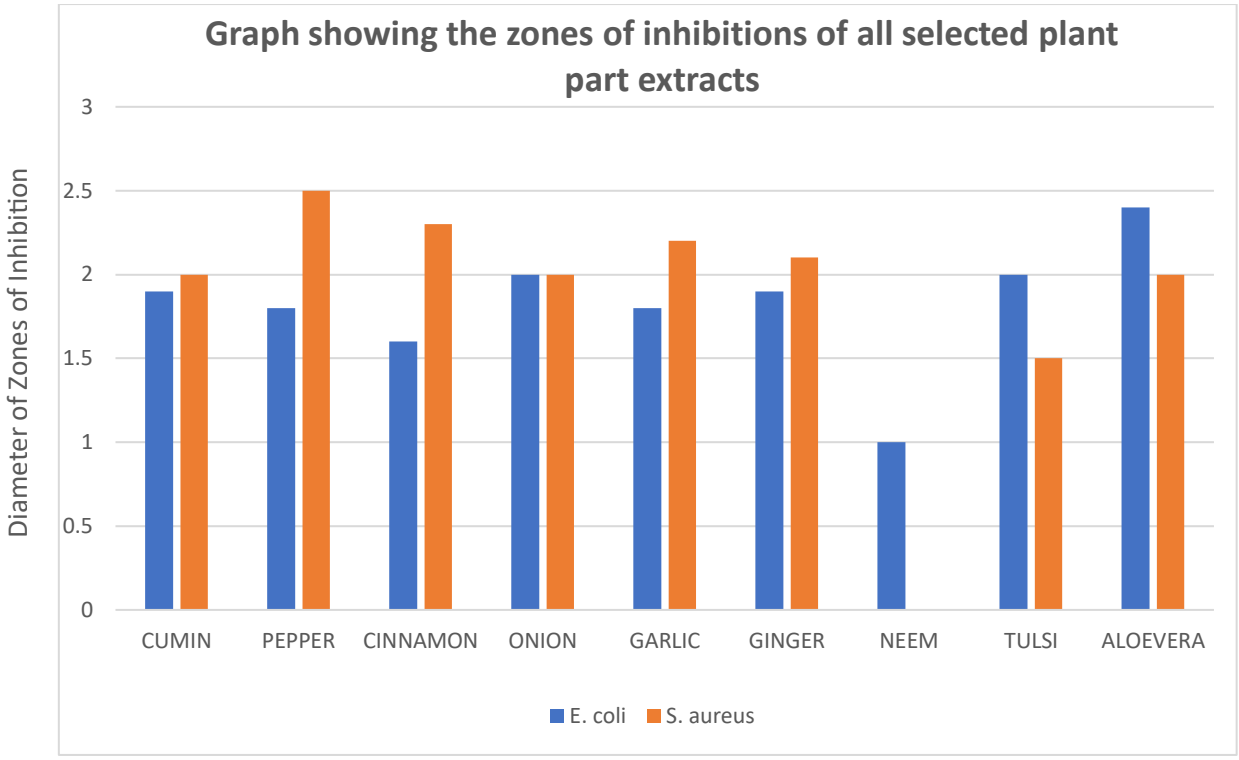
Sl. No.	Leaf Extract	Zone of inhibition (in cms)	
		Escherichia coli (E. coli)	***Staphylococcus aureus (S. aureus)***
1	Neem	1	-
2	Tulsi	2	1.5
3	Aloe	2.4	2

TABLE 2: DIAMETER OF ZONES OF INHIBITIONS OF SELECTED SPICE EXTRACTS

Sl. No.	Spice Extract	Zone of inhibition (in cms)	
		Escherichia coli (E. coli)	***Staphylococcus aureus (S. aureus)***
1	Cumin	1.9	2
2	Pepper	1.8	2.5
3	Cinnamon	1.6	2.3

TABLE 3: DIAMETER OF ZONES OF INHIBITIONS OF SELECTED MODIFIED STEM EXTRACTS

Sl. No.	Modified Stem Extract	Zone of inhibition (in cms)	
		Escherichia coli (E. coli)	***Staphylococcus aureus (S. aureus)***
1	Garlic	1.8	2.2
2	Ginger	1.9	2.1
3	Onion	2	2.1



GRAPH (y axis) SHOWING THE DIAMETER OF ZONES OF INHIBITIONS OF ALL SELECTED PLANT PART EXTRACTS

From the above graph, it can observed that, in the case of leaf extracts Aloe vera (*Aloe vera*) had the maximum clearing zone with 2.4cm and 2cm in *E. coli* and *S. aureus* respectively. Tulsi (*Ocimum tenuiflorum)* showed similar antibacterial activity in both strains of bacteria with 2cm and 1.5cm in *E. coli* and *S. aureus* respectively. In the case of Neem (*Azadirachta indica*), different results were obtained as compared to the other leaf extracts as it showed a zone of inhibition only in the *E. coli* culture plate whereas it didn't show any zone in *S. aureus* thereby showing antibacterial effects only against *E. coli.*

Upon examining the modified stem extracts, we can observe that each extract exhibited nearly identical zones of inhibition in both bacterial strains. There were 2 cm, 1.8 cm, and 1.9 cm zones of inhibition for *E. coli* shown by onion, garlic, and ginger, respectively. For *S. aureus*, the zones of inhibition for onion measured 2 cm, garlic measured 2.1 cm, and ginger measured 2.2 cm.

The outcomes for the spice extracts were nearly identical to those for the stem extracts. Cumin revealed a clearing zone for *S. aureus* and *E. coli* that measured 2 cm and 1.9 cm, respectively. For *S. aureus* and *E. coli*, pepper showed 1.8 cm and 2.5 cm, respectively. In *S. aureus* and *E. coli*, cinnamon demonstrated 1.6 and 2.3 cm. Compared to *E. coli*, *S. aureus* displayed a superior zone in these extracts

DISCUSSION

In recent years, there has been a growing interest in harnessing the therapeutic potential of natural compounds, particularly plant extracts, for combating bacterial infections. With the rise of antibiotic resistance posing a significant global health challenge, researchers are increasingly turning to alternative sources for novel antibacterial agents. Among these sources, plant extracts have emerged as promising candidates due to their diverse chemical composition and historical use in traditional medicine.

Plant extracts exert their antibacterial effects through multiple mechanisms, making them less prone to bacterial resistance compared to conventional antibiotics. Some compounds disrupt bacterial cell membranes, causing leakage of cellular contents and eventual cell death. Others interfere with essential microbial processes, such as cell wall synthesis, protein synthesis, or DNA replication. Additionally, certain plant compounds can modulate bacterial virulence factors, rendering pathogens less harmful to the host.

In the present study, we tested the antibacterial effect of 3 different leaf extracts- Neem, Tulsi (Holy Basil), and Aloe vera; spice extracts – Pepper, Cumin, Cinnamon; and modified stem extracts- Onion, Garlic, and Ginger. The two strains of bacteria taken for the study were *E.coli (Escherichia coli)* and *S.aureus (Staphylococcus aureus).* Both are common bacteria that can cause a range of infections in humans. The diameters of the zone of inhibitions were then measured.

A study done by Maragathavalli *et al*. (2012), investigated the antimicrobial properties of neem (*Azadirachta indica*) leaf extracts against various human pathogenic bacteria, including *E. coli, S.aureus, Pseudomonas aeruginosa, Salmonella typhimurium, and Bacillus pumilus.* Alcoholic extracts (methanol and ethanol) of neem leaves were utilized which were prepared using the disc diffusion method. Both extracts showed a zone of inhibition of 12 mm against *S. aureus* and none against *E.coli*. Out of the 2 strains of bacteria taken for our study, neem leaf extract showed a zone of inhibition of 1 cm against *E.coli* and none against *S.aureus*. According to Raja Ratna Reddy *et al*. (2013), aqueous extracts of neem leaf exhibited the highest antimicrobial activity compared with the bark and seed. The difference in the antimicrobial efficacy could be due to the variable distribution of phytochemical compounds in different parts.

Methanolic extract of Tulsi (Ocimum sanctum) was found to be most active against both *Staphylococcus aureus* and *Escherichia coli* bacterial strains. The zone of inhibition of methanolic extract against *S.aureus* and *E.coli* at 50 mg concentration was 16.0 and 18.0 mm respectively by the study conducted by Tahira Khaliq *et al*. (2018). In our study, a similar pattern was observed since tulsi extract showed a zone of inhibition of 1.5 cm against *S.aureus* and 2 cm against *E.coli.*

Aloe vera gel showed a zone of inhibition of 2.4 cm against *E.coli* and 2 cm against *S.aureus,* in our study. According to Alessandra A. Paes-Leme *et al*. (2005), Aloe vera has genotoxic properties against *E.coli*, but it seems not to be able to damage the cell membrane. Oscar

Chacon *et al*. (2019) found that few antibiotics when combined with Aloe vera, would allow for a dose reduction in antibiotics when treating clinical mastitis symptoms of dairy cattle caused by *S.aureus.*

In our study, cumin extract (*Cuminum cyminum*) showed a zone of inhibition of 1.9 cm against *E.coli* and 2 cm against *S. aureus*. Cumin extracted oil was applied against the clinical isolates of bacteria and *E.coli* was the most sensitive isolate, according to Rafeef (2013). The antibacterial activity of cumin is primarily attributed to its rich composition of bioactive compounds such as cumin aldehyde, cuminic acid, and thymoquinone.

Pepper extract (*Piper nigrum*) showed a zone of inhibition of 1.9 cm against *E.coli* and 2.5 cm against *S.aureus* in our study. The results of the study by P.V. Karsha *et al.* (2010) indicate excellent inhibition of the growth of Gram-positive bacteria like *S.aureus*, followed by *Bacillus cereus* and *Streptococcus faecalis*. Among the Gram-negative bacteria, *Pseudomonas aeruginosa* was more susceptible followed by *Salmonella typhi* and E.*coli*. Black pepper altered the membrane permeability resulting in the leakage of nucleic acids and proteins into the extracellular medium.

According to Yunbin Zhang (2016), after adding cinnamon at MIC (minimum inhibitory concentration) level, there were obvious changes in the morphology of bacteria cells indicating cell damage. In our study cinnamon extract showed a zone of inhibition of 1.6 cm against *E.coli* and 2.3 cm against *S.aureus.* Antibacterial activity of cinnamon is due to bioactive phytochemicals such as cinnamaldehyde and eugenol.

The study by Bandna Chand (2013) found that garlic (*Allium sativum*) extract displayed strong antibacterial effects against all four bacteria tested (*E.coli*, *Salmonella Typhi, S.aureus* and *Bacillus cereus*), while ginger (*Zingiber officinale*) extract showed activity against *Bacillus cereus* and *Staphylococcus aureus*. Additionally, the agar well diffusion method was more effective in detecting inhibition zones compared to the disk diffusion method for both extracts. These results suggest the potential of garlic and ginger as natural antimicrobial agents with implications for various applications. In our study ginger did show a zone of inhibition against both bacteria, i.e., 1.9 cm against *E.coli* and 2.1 cm against *S.aureus.* The zone of inhibition of garlic was 1.8 cm against *E.coli* and 2.2 cm against *S. aureus.*

Both garlic and onions contain sulfur compounds such as allicin in garlic and allyl sulfides in onions. These compounds are responsible for their characteristic odour and taste, as well as their antimicrobial properties. The present study showed that onion shows the zone of inhibition against *E.coli* and *S. aureus* of diameters 2 cm.

CONCLUSION

The present project, titled " Antibacterial Effects Of Selected Plant – Part Extracts On Different Bacterial Strains", was studied using the Magaldi's Well diffusion method (2004).

Plant extracts can exhibit antibacterial effects due to the presence of a diverse array of bioactive compounds. These compounds, such as polyphenols, alkaloids, saponins, and terpenoids, contribute to the plant's natural defence mechanisms against microbial pathogens.

In the present study, the antibacterial effect of 9 plant part extracts namely, leaves: Neem, Tulsi, Aloe vera; spices: Pepper, Cumin, Cinnamon; and modified stem: Ginger, Garlic, Onion; was tested against 2 strains of bacteria namely, *Escherichia coli* and *Staphylococcus aureus.*

All the extracts exhibited antibacterial activity against both strains of bacteria. An exception was neem which did not show a zone of inhibition against *S. aureus*.

It has been observed that there is no marked difference in the extent of antibacterial activity exhibited by these extracts or in other words, there is no significant variance in antibacterial activity among the observed extracts.

The antibacterial activity of plant extracts can vary depending on several factors, including the plant species, the part of the plant used for extraction, and the specific bacteria targeted. Different plants contain unique combinations and concentrations of bioactive compounds, leading to variations in their antibacterial properties.

Antibacterial effects observed in this study, underscore the importance of exploring alternative sources of antimicrobial agents, especially in the face of rising antibiotic resistance. Harnessing the therapeutic potential of plant extracts could offer novel strategies for the development of effective antibacterial treatment.

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