# ANTIBACTERIAL EFFECT OF SPICE EXTRACTS ON DIFFERENT STRAINS OF BACTERIA- A COMPARATIVE STUDY


Project Work By

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# CERTIFICATE

This is to certify that the project report entitled "**ANTIBACTERIAL EFFECT OF SPICE EXTRACTS ON DIFFERENT STRAINS OF BACTERIA- A COMPARATIVE STUDY** " submitted by **Ms. Ameesha Grace M P, Reg. No. AB20ZOO028** in partial fulfilment of the requirement of Bachelor of Science degree in Zoology of Mahatma Gandhi University, Kottayam is a bonafide work done under my guidance and supervision and to the best of my knowledge, this is her original effort.

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**EXAMINERS:**

1)

2)

# DECLARATION

I, Ms. AMEESHA GRACE M P, hereby declare that this project report entitled "**ANTIBACTERIAL EFFECT OF SPICE EXTRACTS ON DIFFERENT STRAINS OF BACTERIA- A COMPARATIVE STUDY**" is a bonafide record of work done by me during the academic year 2022-2023 in partial fulfilment of the requirements of Bachelor of Science degree in Zoology of Mahatma Gandhi University, Kottayam.

This work has not been undertaken or submitted elsewhere in connection with any other academic course and the opinions furnished in this report is entirely my own.

AMEESHA GRACE M P

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# SYNOPSIS

The present project "ANTIBACTERIAL EFFECT OF SPICE EXTRACTS ON DIFFERENT STRAINS OF BACTERIA- A COMPARATIVE STUDY" was focused to find the anti bacterial activity of five common spices such as Clove (*Synzygium aromaticum),* Cardomom *(Elettaria cardamomum),* Cinnamon *(Cinnamomum verum),* Pepper *(Piper nigrum)* and Cumin *(Cuminum cyminum)*. These spice extracts have been widely used as anti-inflammatory, anti bacterial, anti- fungal, anti-microbial and anti-oxidants. They exhibit a broad spectrum of anti bacterial activity. Anti-bacterial is an agent that selectively destroy 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 spices against six different strains of bacteria, of which three were gram positive (*Staphylococcus aureus, Enterococcus faecalis* and *Mycobacterium*) and three were gram negative (*Escherichia coli, Klebsiella pneumonia* and *Vibrio parahaemolyticus*). The method used was Kirby Bauer's disc diffusion method. From the present study, it was concluded that *Elettaria cardamomum* exhibited highest anti-bacterial activity against *Mycobacterium* with a zone of inhibition of 1.8 cm. *Cinnamomum verum* and *Cuminum cyminum* did not show any antibacterial effect against any of the six different bacteria tested.

# INTRODUCTION

Microbial pathogens in food may cause spoilage and contribute to foodborne disease incidence, and the emergence of multidrug resistant and disinfectant resistant bacteria—such as *Staphylococcus aureus* (*S. aureus), Escherichia coli (E. coli*), and *Pseudomonas aeruginosa (P. aeruginosa*) has increased rapidly, causing the increase of morbidity and mortality. Weak acids such as benzoic and sorbic acids, which are commonly applied in food industry as chemical preservatives to increase the safety and stability of manufactured foods on its whole shelf-life by controlling pathogenic and spoilage food-related microorganisms, can result in the development of microbiological resistance. Natural products, as substitutes of synthetic chemical preservatives, are increasingly accepted because they are innately better tolerated in human body and have inherent superiorities for food industry.

Spices have been used as food and flavoring since ancient times, and as medicine and food preservatives in recent decades. Many spices such as clove, oregano, thyme, cinnamon, and cumin have been applied to treat infectious diseases or protect food because they were experimentally proved to possess antimicrobial activities against pathogenic and spoilage fungi and bacteria. Moreover, the secondary metabolites of these spices are known as antimicrobial agents, the majority of which are generally recognized as safe materials for food with insignificant adverse effects. Therefore, spices could be candidates to discover and develop new antimicrobial agents against foodborne and human pathogens.

Bacterial Strains used:

| | |
|---|---|
| *Klebsiella pneumoniae* | Gram negative |
| *Vibrio parahaemolyticus* | Gram negative |
| *Escherichia coli* | Gram negative |
| *Staphylococcus aureus* | Gram positive |
| *Enterococcus faecalis* | Gram positive |
| *Mycobacterium* | Gram positive |

Spice samples used and their economic importance:

**Clove**: Clove is very aromatic and fine flavoured and imparts warming qualities. In all Indian homes, it is used as a culinary spice as the flavour blends well with both sweet and savoury dishes. Clove is used for flavouring pickle, curries, ketchup and sauces. It is highly valued in medicine as a carminative, aromatic and stimulant. Clove has stimulating properties and is one of the ingredients of betel chewing. In Jawa, clove is used in preparation of a special brand of cigarette for smoking.

**Cardamom**: Cardamom is used as a spice and masticatory, and in medicine. The seeds possess a pleasant aroma and a characteristic, warm, slightly pungent taste. It is used for flavouring curries, cakes, bread and for other culinary purposes. It is also used for flavouring liqueurs. In the Arab countries, cardamom is used for flavouring coffee and tea. In medicine, it is used as an aromatic, stimulant, carminative and flavouring agent.

**Cinnamon**: Cinnamon is generally used as a spice or ingredient of curry powder. It is used as a spice or condiment in curries and similar preparations. It is also used in medicine as a cordial stimulant. It is also used in bowel complaints such as dyspepsia, diarrhoea and vomiting. Powder cinnamon is a reputed remedy for diarrhoea and dysentery. The bark also yields oil of which the chief constituent is Cinnamic aldehyde.

**Cumin:** The economically important part of the plant is the dried fruit. It is used as a condiment in various cuisines of different cultures either as whole or in powdered form. It have some medicinal properties. Cumin oil is reported to have antibacterial activity. It is used in veterinary medicines and various other industries.

**Pepper**: Pepper fruits are used as spice or condiment. In Kerala, fresh green pepper is sometimes used for preparing pickles. Black and white pepper make the major condiments employed for seasoning freshly cooked and prepared foods. They are used for preserving meat. The whole fruits are added to pickles, certain types of sausages etc., but the bulk of the product is generally ground before use. Black pepper is mostly used for its characteristic aroma and pungent taste.

BACTERIAL STRAINS

***Klebsiella pneumonia*e**

*Klebsiella pneumoniae* is a gram negative, non-motile lactose fermenting bacteria that can grow with or without free oxygen, making it a facultative anaerobe. It is also surrounded by a capsule which increases its virulence by acting as a physical barrier to evade host immune response. It can be found in the mouth, skin and gastrointestinal tract where it is of the normal flora. Infection of *Klebsiella pneumonia* occurs in the lungs where they cause necrosis, inflammation and hemorrhage within the lung tissue.

***Vibrio parahaemolyticus***

*Vibrio parahaemolyticus* a curved, rod-shaped, Gram-negative bacterium found in the sea and in estuaries which, when ingested, may cause gastrointestinal illness in humans. *V. parahaemolyticus* is oxidase positive, facultatively aerobic, and does not form spores. Infection can occur by the fecal-oral route, ingestion of bacteria in raw or undercooked seafood, usually oysters, is the predominant cause of the acute gastroenteritis caused by *V. parahaemolyticus*. Wound infections also occur, but are less common than seafood-borne disease.

***Escherichia coli***

*E coli* is a gram negative, facultatively anaerobic, rod-shaped, coliform bacterium, commonly found in the lower intestine of warm blooded organisms. Most of the *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of normal flora of gut, and can benefit their hosts by producing vitaminK2 and preventing colonization of the intestine with pathogenic bacteria.

***Staphylococcus aureus***

*Staphylococcus aureus* is a gram positive round shaped bacterium that is a member of the Firmicutes, and it is a usual member of microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalyse and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. It is an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections, such as sinusitis and food poisoning.

***Enterococccus faecalis***

*Enterococci* are gram positive cocci bacteria that can survive harsh conditions in nature. They can be found in soil, water and plants. Some strains are used in manufacture of foodstuffs whereas others are cause of serious animal and human infections. They are associated with both community and human acquired infections. These infections may be local or systemic and include urinary tract and abdominal infections, wounds infections, bacteraemia and endocarditis.

***Mycobacterium sps***

Mycobacteria are Gram-positive, catalase positive, non-motile, non-spore forming rod-shaped bacteria (0.2–0.6 µm wide and 1.0–10 µm long). The colony morphology of *Mycobacteria* varies with some species growing as rough or smooth colonies. Colony colour ranges from white to orange or pink. Mycobacteria are slender, non spore forming, rod-shaped, aerobic, slow-growing, and free-living in soil and water.

Significance of the study

This project aims to investigate the antimicrobial activities of five spice extracts, namely clove, cardamom, cinnamon, cumin, and pepper, against six bacterial strains, including *Klebsiella pneumoniae, Vibrio parahaemolyticus, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis*, and *Mycobacterium*.

The significance of this project lies in demonstrating the effectiveness of these spice extracts in inhibiting the growth of pathogenic and spoilage bacteria, which could lead to the development of safer and more natural alternatives to conventional antimicrobial agents.

# REVIEW OF LITERATURE

Bacteria are single celled microbes. The cell structure is simpler than that of other organisms as there is no nucleus or membrane bound organelles. *E.coli* is gram negative, facultatively anaerobic, rod shaped bacterium that is commonly found in the lower intestine of warm blooded organisms. *Staphylococcus aureus* is a gram positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract. *Klebsiella pneumoniae*, a rod shaped bacterium and a common gut bacteria, causes problems when it moves outside the gut and causes infection. *Vibrio parahaemolyticus* is a curved, rod-shaped, Gram-negative bacterium found in the sea and in estuaries which, when ingested, may cause gastrointestinal illness in humans. Mycobacterium is classified into tuberculus and non tuberculus. *Mycobacterium tuberculosis*, also known as Koch's bacillus, is a species of pathogenic bacteria in the family Mycobacteriaceae and the causative agent of tuberculosis. *Enterococcus faecalis* is a gram positive commensal bacterium inhabiting the anointestinal tracts of humans and other mammals (Sloncezewski *et al*., 2009)

Rafeef (2013) reported that cumin oil exhibits a 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.).

Black pepper extracts reportedly inhibit food spoilage and food pathogenic bacteria. The antimicrobial activity of black pepper chloroform extract (BPCE) against *Escherichia coli* was studied. The antibacterial mechanism of BPCE was elucidated by analyzing the cell morphology, respiratory metabolism, pyruvic acid content, and ATP levels of the target bacteria (Lan *et al.*.,2015)

Antibacterial mechanisms of cinnamon and its constituents such as cinnamaldehyde and cinnamic acid, against pathogenic Gram-positive and Gram-negative bacteria were described. 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 (Vasconcelos *et al*., 2018).

Antibacterial and anti-inflammatory activities of cardamom (*Elettaria cardamomum*) extractswas studied and it provides evidence that cardamom fruit and seed extracts through their antibacterial and anti-inflammatory properties may be therapeutic agents of interest against periodontal infections (Mariam *et al*., 2020).

Antibacterial Effects of Clove Essential Oil by Nanoemulsion was studied. The aim of the study was to develop and evaluate nanoemulsion formulations of clove essential oil (CEO) for its antibacterial effects in comparison with pure CEO and standard amikacin antibiotic (positive control). Different nanoemulsions of CEO were developed by aqueous phase titration method via construction of pseudo
ternary phase diagrams and investigated for thermodynamic stability and self-nanoemulsification tests. The results indicated the potential of nanoemulsions for enhancing the therapeutic efficacy of natural active ingredients such as CEO (Khalid, 2014).

Study on Antimicrobial and Virulence-Modulating Effects of Clove Essential Oil on the Foodborne Pathogen *Campylobacter jejuni* demonstrates that the components of clove essential oil influence not only the expression of general stress genes but also the expression of virulence-associated genes. Based on this finding, alternative strategies can be worked on to control this important foodborne pathogen (Kovacs *et al*., 2016).

Antibacterial Effect of Clove Oil against Clinical Strains of *Escherichia coli* showed that clove oil did not show any activity against *E. coli*, having a negative effect on the same. Although it is said that clove oil produces antimicrobial properties, it is not very effective in inhibiting the growth of clinical strains of *Escherichia coli* (Jerusha, 2017).

Antibacterial Effect of Black Pepper Petroleum Ether Extract (BPPE) against *Listeria monocytogenes* and *Salmonella typhimurium* was evaluated and the results showed that the BPPE had a strong antimicrobial activity against *L. monocytogenes* and *S. typhimurium*, and 2-methylene-4,8,8-trimethyl 4-vinyl-bicyclo[5.2.0]nonane (9.36%) and caryophyllene oxide (4.85%) were identified as the two primary components of BPPE (Wenxue *et al*., 2019)

The inhibitory effect of alcoholic and aqueous extracts on some Gram positive (*Staphylococcus aureus)* and Gram negative (*Escherichia coli)* pathogenic bacteria was studied. Considering the antibacterial effects of alcoholic and aqueous extracts of cumin seeds on *S. aureus* observed in the investigation, continuing the research for studying the in-vivo effect of ant- microbial effects of *Cunminum cyminum* extracts on *Staphylococci* seems valuable (Motamedifar *et al*., 2012)

In the study on the antimicrobial effect of black pepper, Piperine showed antibacterial activity against all test bacteria with zone of inhibition ranged from 8mm-18mm. The maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (18mm) and *Bacillus subtilis* (14mm) than Gram negative bacteria *Pseudomonas aeruginosa* (9mm) and *Escherichia coli* (8mm). Maximum zone of inhibition was at 100ul for all the bacterial cultures. It indicates that zone of inhibition increases as the concentration of piperine increased. Piperine showed maximum antifungal activity towards *Fusarium oxysporum* (14mm), *Alternaria alternata* (17mm), minimum effect against *Aspergillus flavus* (30mm) and very least effect against *Aspergillus niger* (38mm) ( Rani *et al*., 2013)

In the study on Antibacterial Activity of Cinnamon Extract (*Cinnamomum burmannii*) against *Staphylococcus aureus* and *Escherichia coli*, it was observed that the efficacy of the cinnamon extract (*Cinnamomum burmannii*) as antibacterial against *Staphylococcus aureus* and *Escherichia coli*. It concluded that statistically cinnamon extract (*Cinnamomum burmannii*) is effective as an antibacterial against *Staphylococcus aureus and Escherichia coli* in vitro. MBC cinnamon extract (*Cinnamomum burmannii*) against *Staphylococcus aureus* is 5% with moderate zone inhibition zones. MBC cinnamon extract (*Cinnamomum burmannii*) to *Escherichia coli* is 10% with moderate zone inhibition zones.( Parisa *et al.,*2019)

On evaluating the antimicrobial potential of green cardamom essential oil focusing on quorum sensing inhibition of *Chromobacterium violaceum*, the study proved that cardamom essential oil is effective against *Staphylococcus aureus, Salmonella typhi, Candida albicans* and *Streptococci mutans*. Hence, it may play imperative role in the discovery of novel antibiotics due to its significant antibacterial properties (Abdullah *et al.*, 2017).

The antibacterial activity of black pepper (*Piper nigrum* Linn.) and its mode of action on bacteria were studied. The extracts of black pepper were evaluated for antibacterial activity by disc diffusion method. The results indicate excellent inhibition on the growth of Gram positive bacteria like *Staphylococcus aureus*, followed by *Bacillus cereus* and *Streptococcus faecalis*. Among the Gram negative bacteria

*Pseudomonas aeruginosa* was more susceptible followed by *Salmonella typhi* and *Escherichia coli* (Karsha and Lakshmi, 2010).

# METHODOLOGY

**Materials Required**: Nutrient broth, agar-agar, distilled water, filter paper, petri plates, conical flask, test tubes, forceps, cotton plugs, sterile swab, weighing machine, measuring cylinder, nichrome loop, ethanol, etc.

**Spice samples**:

Clove

Cardamom

Cinnamon

Pepper

Jeera

**Nutrient broth culture**: 0.7g of nutrient broth was weighed and added to 50ml distilled water and mixed well. The broth was then poured into test tubes and closed using a cotton plug sterilized by autoclaving for 15 min and cooled to room temperature.

**Inoculating the broth**: The inoculating loop was flamed to red hot and cooled by waving for few seconds. The cotton plug from the stock culture tube was removed and the mouth was flamed. The cooled sterilized loop was inserted into the culture tube carefully without touching the side to prevent contamination. A visible amount of inoculum was removed using the loop and the inoculum was introduced into the culture tubes with nutrient broth. The mouth of the tube was plugged back carefully after flaming. The inoculating loop was re-sterilized and the broth culture was gently rotated for proper mixing of contents. The contents in each test tube was labelled with the names of respective microbes and the date was noted. For sufficient bacterial growth, inoculums were kept for 18-24 hours of incubation.

**Preparation of nutrient agar** (culture media): The medium was prepared using 1.3g of nutrient broth and 2.0g of agar. Both the nutrient broth and agar was weighed out and was made into 100ml using distilled water. It was poured into a conical flask and sterilized for 15 min in an autoclave at 15psi. Cooled agar was poured into petri dishes and waited till it got set. 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, 8g of each sample was weighed, 10 ml water was added to it and grinded using mortar and pestle to obtain the extract. This extract was filtered and were collected in separate sterile bottles.

**Preparation of the discs**: 0.6 cm diameter filter paper discs were punched out and sterilized by autoclaving. It was then dipped with sample extract and used to test for antibacterial sensitivity.

**Method**: The method used for antibacterial sensitivity was Kirby Bauer disc diffusion method. A lawn culture of each bacterium was prepared using sterilized cotton swabs. A sterilized swab was dipped into 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 has been prepared the sterilized filter paper was dipped with sample extracts and placed on the plates. This plate was incubated at 37°C for 48 hrs. The name of the bacteria was labelled on each plate and was examined for sensitivity (zone of inhibition). The diameter of each zone was measured using a standard ruler in centimeters. A particular strain of bacteria is found to be sensitive to the sample extract, if the bacteria show only feeble 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. Observations were made accordingly.

**Killing and disposing**: After the experiment, the bacteria are destroyed by autoclaving the plates for 20 min. All the glassware used for the experiment were also autoclaved to remove any bacteria if present.

# OBSERVATIONS AND RESULT

The present work was conducted to study the effect of different spice extracts on different strains of bacteria.

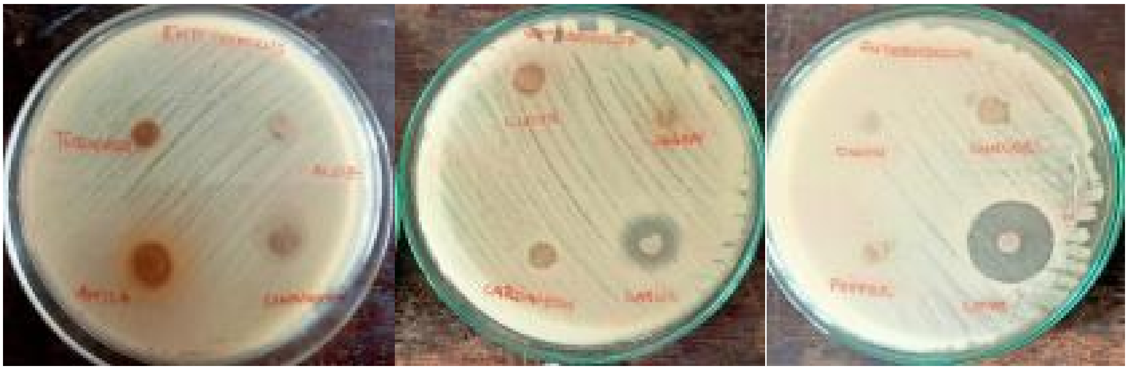

**Figure 1 showing clearing zone of *Enterococcus faecalis.***

From the figure it is clear that Cardamom, and Clove has clearing zone. Pepper, Cinnamon and Cumin has no clearing zone at all.

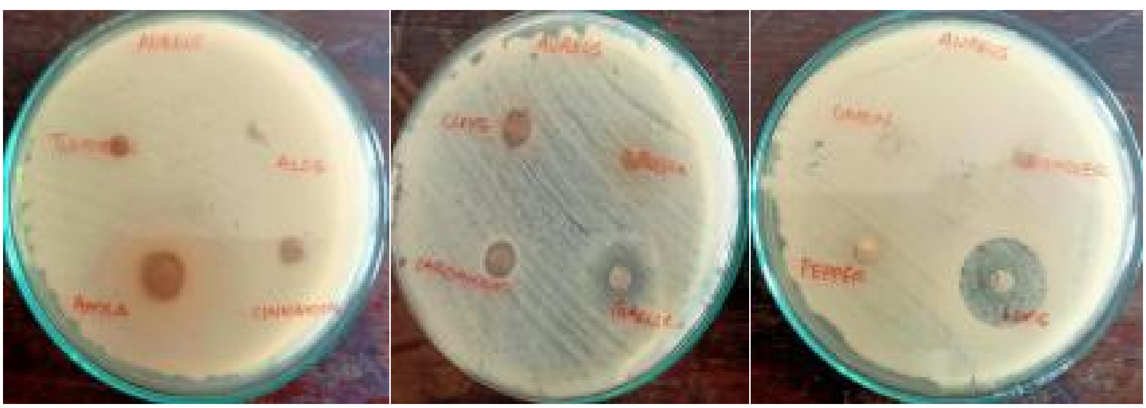

**Figure 2 showing clearing zone of *Staphylococcus aureus.***

From the figure it is clear that Cardamom and Clove had clearing zone. Pepper, Cumin and Cinnamon has no clearing zone at all.

**Figure 3 showing clearing zone of *Klebsiella pneumoniae***

From the figure it is clear that Cardamom, shows least clearing zone whereas Cumin, Cinnamon, Clove and Pepper shows no clearing zone at all.

**Figure 4 showing clearing zone of *Mycobacterium* sps.**

From the figure it is clear that Cardamom has least clearing zone whereas Pepper, Cumin, Cinnamon and Clove has no clearing zone at all.

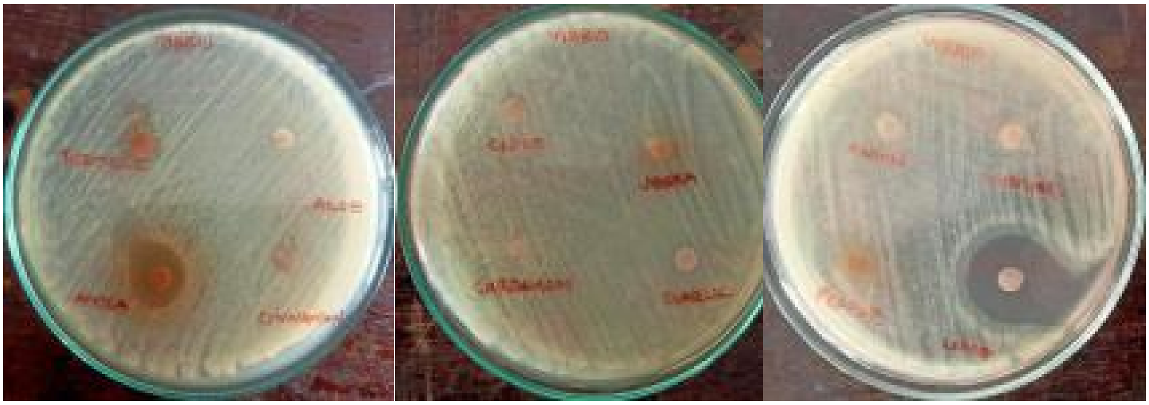

**Figure 5 showing clearing zone of *Vibrio parahaemolyticus*.**

From the figure it is clear that none of the spices extract shows the clearing zone against this particular strain of bacteria.

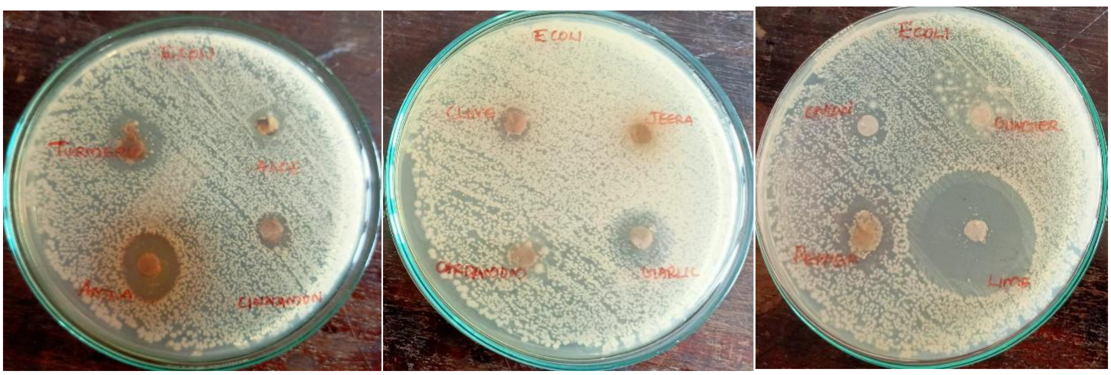


**Figure 6 showing clearing zone of *E. coli***

From the figure it is clear that Cinnamon and pepper shows least clearing zone than the others. Cardamom and Cumin has no clearing zone at all.

**Table 1 showing the diameter of zone of inhibition of various spices on different strains of bacteria**

| | **Zone of inhibition (cm)** | | | | | |
|---|---|---|---|---|---|---|
| **Spice sample** | ***Klebsiella*** | ***Vibrio*** | ***E. coli*** | ***Mycobacterium*** | ***S. aureus*** | ***Enterococcus*** |
| **Clove** | 0 | 0 | 0 | 0 | 0.8 | 0.7 |
| **Cardamom** | 0.9 | 0 | 0 | 1.8 | 0.8 | 0.7 |
| **Cinnamon** | 0 | 0 | 0.8 | 0 | 0 | 0 |
| **Pepper** | 0 | 0 | 0.8 | 0 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Jeera** | 0 | 0 | 0 | 0 | 0 | 0 |

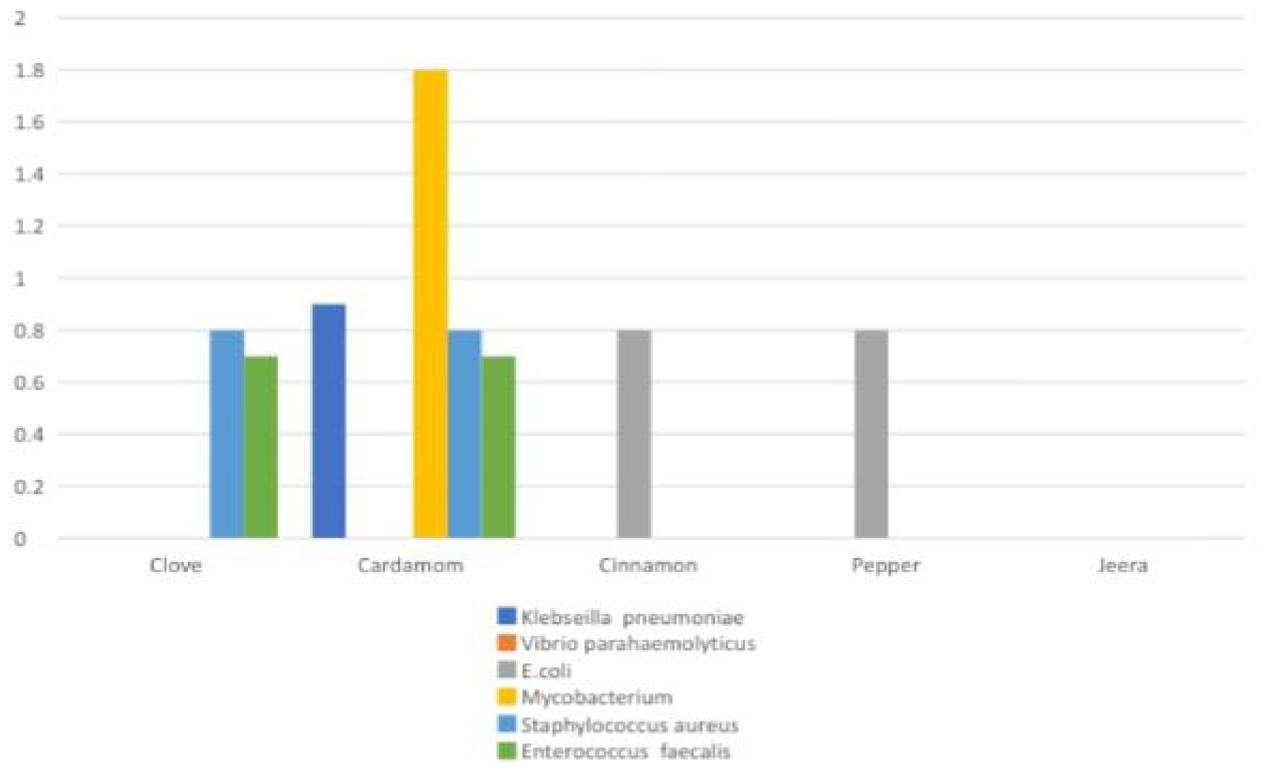


**Figure 7 showing the effect of various spices on different strains of bacteria**

From the graph it is clear that cardamom has the highest activity against *Mycobacterium* which produced an inhibitory zone of 1.8cm followed by a zone of 0.9cm against*,* 0.8cm against *S. aureus* and 0.7cm against *Enterococcus*. *Escherichia coli* and *Vibrio* was seen to be resistant to the samples tested. The second highest activity was shown by clove extract. *S.aureus* showed the greatest sensitivity with an inhibitory zone of 0.8cm followed by *Enterococcus faecalis* produced a zone of 0.7cm. *Mycobacterium*, *E.coli*, *Vibrio*, *Klebsiella* showed resistance.

In the case of pepper, the zone exhibited by *E.coli* was 0.8cm. All the other did not show sensitivity.

In case of cinnamon, the zone of inhibition was against the *E.coli* with a diameter of 0.8 cm. All the other did not show any sensitivity.

In the case of cumin there were no inhibitory zones at all.

# DISCUSSION

Antibiotics have long been trusted by physicians to treat bacterial infections. However, due to their overuse, antibiotic- resistant strains of bacteria now threaten the efficacy of many antibiotic types. As an alternative to the use of antibiotics, we can choose spices over it as many of them shows significant resistance against many bacterial strains.

In the present study, we tested the antibacterial effect of 5 different spices namely clove, cardamom, cinnamon, cumin, and pepper. Out of the 6 strains of bacteria taken for the study, pepper shows a zone of inhibition of 0.8 cm against *Escherichia coli.* A study done by Ganesh *et.al*. (2014) on Antibacterial Effect of pepper against *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Proteus sp. and Pseudomonas aeruginosa* shows that the presence of phytotochemicals in pepper is responsible for the antibacterial effect in them. In our case, pepper has shown zone of inhibition against *E. coli.*

In another study conducted on Antimicrobial Activity of Ethanolic Extracts of *Syzygium aromaticum and Allium sativum* Against Food Associated Bacteria and Fungi by Pundir *et.al*.,(2010) clove extract showed better antimicrobial activity than the garlic extract. The zone of inhibition in clove ethanolic extract against all the food associated bacteria was in the range of 25mm to 32mm while that for garlic was in the range of 20mm to 31mm. In the present study it was observed that clove showed a zone of inhibition of 0.8 cm against *S. aureus* and of 0.7cm against *E. faecalis.*

The study conducted by Nabavi *et.al*. (2015), on Antibacterial Effects of Cinnamon has shown that the antibacterial activity of cinnamon is due to bioactive phytochemicals such as cinnamaldehyde and eugenol. In the present study, it was observed that cinnamon had produced inhibition(0.8cm) towards *E.coli* only among the different strains that was taken for the experiment.

ElDin *et al*. (2020) examined effect of two plant extracts, black pepper and cumin extracts to inhibit the growth of foodborne *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* strains that were artificially inoculated into minced chicken meat samples. Results revealed that the used pepper and cumin extracts significantly reduced the count of tested strains by 102 CFU/g with reduction percent exceeded 99.0%, which proved that the used extracts were potentially effective with variable efficiency against the tested bacterial strains; so, it can be used as natural alternative preservatives to control food poisoning diseases and preserve food stuff avoiding health hazards of chemically antimicrobial agent applications. In the present study, cumin does not show any sensitivity to the above mentioned bacterial strains but pepper has a zone of inhibition of 0.8 cm against *E.coli.*

Rafeef (2013) reported that the cumin oil exhibits a strong antibacterial activity against four clinical bacterial isolates (*Escherichia coli, Staphylococcus aureus, Klebsiella sp. and Pseudomonas aeruginosa*). In the present study, cumin shows no antibacterial effect against the 6 bacterial strains taken.

In our study on antibacterial effect of various spices, cardamom inhibits the maximum number of bacterial strains taken. Abdullah *et al*., (2017), proved that cardamom has high resistance towards *Staphylococcus aureus, Salmonella typhi, Candida albicans and Streptococci mutans.* A similar observation was obtained in the present study, where the zone of inhibitions were 1.8cm, 0.9cm, 0.8cm and 0.7cm for *Mycobacterium*, *Klebsiella pneumoniae, Staphylococcus aureus and Enterococcus faecalis* respectively.

# CONCLUSION

In the present study, the antibacterial effect of 5 different spices namely clove, cardamom, cinnamon, cumin, and pepper was tested against six strains of bacteria tested namely, *Klebsiella pneumonia, Vibrio parahaemolyticus, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis*, and *Mycobacterium* sps.

Among the five samples of spices taken, *Elettaria cardamomum* exhibited the highest antibacterial activity against all the four strains of bacteria tested. Among this cardamom exhibited maximum inhibitory zone of 1.8 cm against *Mycobacterium*.

*Syzygium aromaticum* (clove) showed the second highest inhibition against *Staphylococcus aureus* and *Enterococcus faecalis* bacteria excluding other four.

Out of the six strains of bacteria, *Enterococcus faecalis, E.coli* and *S. aureus* were the most sensitive bacteria as they exhibited highest sensitivity to the most of the samples tested. *Piper nigrum* (pepper) and *Cinnamomum verum* (cinnamon) exhibited sensitivity only against *Escherichia coli.* Cumin did not show sensitivity in any of these six strains of bacteria.

The present study suggests that spices can inhibit the growth of some microorganisms to some extend. So inclusion of spices in food can prevent food borne microbes and can control infections to a greater extend. Moreover spices could be candidates to discover and develop new antimicrobial agents against foodborne and human pathogens.

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