



Efficacy of Spice Extracts Against Four Pathogenic Bacteria

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The antimicrobial effect of ethanol (60%), sterile distilled water and diethyl ether extracts on Syzygium aromaticum (Clove), Elleteria cardamom (Small cardamom), Nigella sativa (Black cumin), Cinnamomum zeylanicum (Cinnamon) against four pathogenic strains of bacteria: Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli and Shigella flexneri, were determined using Agar well diffusion method. The results obtained showed that all spices had antimicrobial properties. The ethanolic extract of black cumin was found to be the very effective and gave the widest zone of inhibition (29 mm) against Staphylococcus aureus and diethyl ether extract gave 32 mm of inhibition against Shigella flexneri. The sterile water extract of clove was found to be equally effective giving zones of inhibition of 24 mm and 23 mm against Escherichia coli and Shigella flexneri, respectively. Streptococcus pyogenes showed a greater resistance to the sterile water and ethanolic extracts of all the four spices. However, it was inhibited to an extent by the diethyl ether extract of cinnamon. The results further showed that sterile distilled water and diethyl ether were better solvent for extracting antimicrobial chemicals from the spices as compared to 60 % ethanol. Also, the antimicrobial activity was influenced to some extent by the extractive solvent.

Key words: Spice extract, Pathogenic bacteria, Extractive solvents

Introduction : India is the 'home of spices' and contributes 25- 30% of the total world trade. It is the largest producer and consumer of spices. Diverse agro-climatic zones, ranging from tropical to temperate are congenial for growing a number of crops, thus creating variety in availability of spices. But the research conducted in this direction is also negligible (Arya & Singh, 2000).

The spices cannot be grouped into the category of food, because they contain less nutritive value. However, they give a good flavour and aroma to food, stimulate the appetite and increase the secretion of gastric juices. The aromatic value of the spices is due to the presence of the essential oils. The spices are aromatic, pungent, vegetable produce used for flavouring and seasoning of food and drinks. Most of them are carminative, digestive, stimulant, stomachic and resolvent. Some of them are used as preservatives for maintaining the quality and extending the shelf life of food (Singhal, 1996). Since the use of natural products as a source of medicine is traditional, the need of the hour is to protect this fast disappearing indigenous

knowledge which is health security to millions in the rural and the urban communities. Although, the pathogenic microorganisms can be combated with the antimicrobial drugs available commercially, the need of new drugs has increased because the drug resistance in microorganisms has become common. Moreover, there is a growing apathy towards allopathic drugs due to their adverse side effects and high cost. In this perspective, the other alternative is herbal drugs which are easily available, cheap and free from side effects and can be easily used. Taking the above aspects into consideration, the present study was undertaken to determine the antimicrobial activity of some spices against four pathogenic bacteria.

Four spices selected for the present study were: *Syzygium aromaticum* (Clove)_Merill belongs to the family Myrtaceae. Cloves are very aromatic and are used as a culinary spice. They are stimulative, antiseptic and antispasmodic. The clove oil is also used as a local antiseptic in tooth ache. *Elleteria cardamom* Maton belongs to the family Zingiberaceae, and is native to India. Pharyngitis, sore throat and flu are cured by

gargling with an infusion of cardamom and cinnamon. Powdered seeds of cardamom boiled with tea water are used for scanty urination, dysentery, palpitation of the heart, exhaustion due to work, depression.

Nigella sativa also called mangraila belongs to the family Ranunculaceae and is an annual flowering plant, native to southwest Asia. Its oil contains nigellone which protects from histamine induced bronchial spasms (Mancini-Filho et. al, 1998). The presence of sterol, beta-sitosterol lends credence to its traditional use to treat abscesses and tumors of the eyes, abdomen and liver (Sansonettti, 2001). *Cinnamomum zeylanicum* is a small evergreen tree, 10-15 m tall belongs to the family Lauraceae and is a native to Sri Lanka (Shan et. al., 2005). Cinnamon is generally used as a spice or condiment in cookery. Cinnamon powder is a reputed remedy for bowel complaints such as, dyspepsia, diarrhoea and vomiting (Singhal, 1996). Cinnamon is high in antioxidant activity. It is also used in the preservation of certain food items.

The test organisms selected were: *Staphylococcus aureus* is the most common cause of staphylococcal infections. It is a spherical bacterium, frequently a part of the skin flora, is a facultatively anaerobic, gram-positive coccus which appears as grape-like clusters when viewed through a microscope. Symptoms include nausea, vomiting, diarrhoea and abdominal pain. *Streptococcus pyogenes* is a spherical, gram-positive bacterium that grows in long chains and is the cause of Group A Streptococcal infections They cause pharyngitis, strep throat or localized skin infections. *Escherichia coli* is a gram-negative bacterium that is commonly found in the lower intestine of warm blooded organisms. They are rod shaped, facultatively anaerobic and non-sporulating organisms.

Shigella flexneri is a gram-negative, rod-shaped bacterium closely related to *Escherichia coli*. 10–100 organisms are sufficient to cause the disease and transmission occurs from person to person. Shigellosis is very common in children aged between 1 and 5 years old (Beletsachew et.al.,2004).

Materials and Method:

Collection and processing of spice samples:

The spices, *Syzygium aromaticum* (Clove), *Elleteria cardamom* (Cardamom), *Nigella sativa* (Black cumin)

and *Cinnamomum zeylanicum* (Cinnamon) were powdered and 8 grams of each were dissolved in 80 ml of each solvent, separately. Three replicates for each spice were prepared. The suspended solutions were tightly covered and kept undisturbed for five days. After 5 days, the solutions were filtered out and were stored in amber colour bottles at 4°C.

Test Organisms:

The test organisms, *Staphylococcus aureus*(MTCC 96), *Streptococcus pyogenes*(MTCC 442), *Escherichia coli*(MTCC 119) and *Shigella flexneri*(MTCC 1457) were obtained from IMTECH, Chandigarh.

Antimicrobial test:

The antimicrobial activity was determined by Agar well diffusion method as described by Omenka and Osuoha (2000). The culture plates seeded with test organisms were allowed to solidify and punched with a sterile cork borer (7.0 mm diameter) to make open wells. The open wells were filled with 0.05 ml of the extract. The plates were incubated at 37°C for 48 hours. The zones of inhibition were measured and recorded.

Results and Discussion:

Tests were separately conducted on two categories of test organisms: Gram negative bacteria and Gram positive bacteria.

Effect on Gram negative bacteria:

The results obtained showed that the extracts of the spices have inhibitory effect, on the test strains of bacteria. Against *Escherichia coli*, the sterile water and alcoholic extracts of clove were found to form an inhibition zone of 24 mm and 21 mm respectively. The other spices gave negative results. In contrast the diethyl ether extract did not give an encouraging result (Table-I).

The diethyl ether extracts of black cumin, clove and cinnamon gave positive results against *Shigella flexneri*. The widest zone of inhibition 32 mm was shown by black cumin followed by clove with 17 mm. The aqueous extracts were equally effective against the organisms. The ethanolic extract of cinnamon only showed a zone of inhibition of 14 mm whereas, for the other spices no zone of inhibition was observed (Table I).

Effect on Gram positive bacteria:

Against *Staphylococcus aureus* the ethanolic extract of black cumin was shown to perform a maximum inhibition zone of 29 mm; the ethanolic extract of clove and cinnamon scored well behind with 19 mm and 17 mm, respectively. The least effective was the ethanolic extract of cardamom where the inhibition zone was 6mm. The sterile water extract of clove was found to create an inhibition zone of 15 mm followed by cinnamon with 14 mm. On the other hand, the sterile water extract of black cumin and cardamom gave negative results. A similar result was obtained for diethyl ether extracts (Table II).

Against *Streptococcus pyogenes*, the diethyl ether extract of cinnamon, cardamom and black cumin showed an inhibition zone of 14 mm, 11 mm and 10 mm respectively. The growth of test bacteria remained unchecked by ethanol and aqueous extracts of spices. (Table II)

Table I: Zone of inhibition (in mm) for Gram negative bacteria.

Organism	Spice	Sterile Water Extract	Ethanolic Extract	Diethyl Ether Extract
<i>E. coli</i>	Clove	24 mm	21 mm	Nil
	Cardamom	Nil	Nil	Nil
	Black cumin	Nil	Nil	Nil
	Cinnamon	Nil	Nil	15 mm
<i>S. flexneri</i>	Clove	23 mm	Nil	17 mm
	Cardamom	13 mm	Nil	Nil
	Black cumin	Nil	Nil	32 mm
	Cinnamon	16 mm	14 mm	10 mm

Table II: Zone of inhibition (in mm) for Gram positive bacteria.

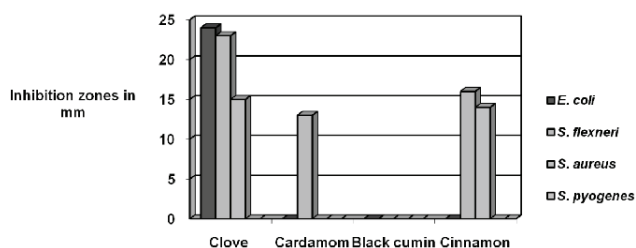
Organism	Spice	Sterile Water Extract	Ethanolic Extract	Diethyl Ether Extract
<i>S. aureus</i>	Clove	15 mm	19 mm	12 mm
	Cardamom	Nil	6 mm	Nil
	Black cumin	Nil	29 mm	Nil
	Cinnamon	14 mm	17 mm	11 mm
<i>S. pyogenes</i>	Clove	Nil	Nil	Nil
	Cardamom	Nil	Nil	11 mm
	Black cumin	Nil	Nil	10 mm
	Cinnamon	Nil	Nil	14 mm

Spices are source of secondary metabolites which exert physiological effect on the system of living organisms. Nickel (1959) reported the presence of antibiotic substances in 147 plant families out of 174 families which were surveyed. Crude spice extracts were tested for antimicrobial activity against *Staphylococcus*

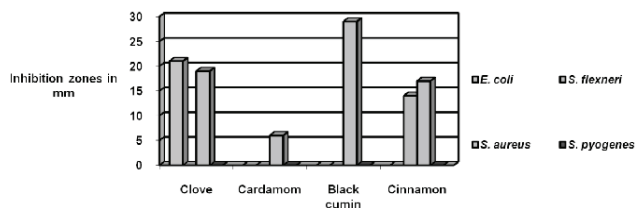
aureus, *E. coli*, *Streptococcus pyogenes* and *Shigella flexneri*. Review of literature (Mancini et. al., 1998, Samsonetti, 2001, Shan et. al., 2005) suggests *Syzygium aromaticum* for cure stomach and throat problems, *E. cardamom* for sore throat and flu, pharyngitis, vomiting and dysentery, *Nigella sativa* for skin conditions, respiratory and stomach ailments, *Cinnamomum zeylanicum* against diarrhoea and vomiting, are directly related to the diseases caused by the four bacterial species. Hence, these organisms were used for testing the antibacterial activity of the spice extracts.

The results obtained showed that the spice extracts in different solvents have bacteriocidal effects more or less to some extent on the four bacterial species. The results also agree with Takazawa et. al (1982), that there is a need to employ broad range of extractive solvents in the extractions of the possible phytochemicals from medicinal plants. Accordingly, the ethanolic extract of black cumin was found to be the most effective against *Staphylococcus aureus* and diethyl ether against *Shigella flexneri*. For *Escherichia coli* the sterile water extract of clove was found to be the most effective. *Streptococcus pyogenes* showed a great resistance to the sterile water and ethanolic extracts of all the four spices. However, it was inhibited to an extent by the diethyl ether extract of cinnamon.

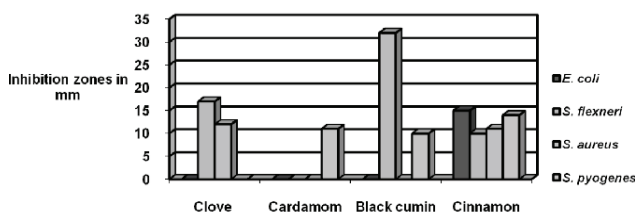
It can be clearly seen from Bar Diagrams 1, 2 & 3 that sterile distilled water and diethyl ether have been proved to be better solvents for extracting antibacterial chemicals from the spices as compared to alcohol. The widest zone of inhibition 32 mm and 29 mm was demonstrated by diethyl ether extracts of black cumin against *Shigella flexneri* and *Staphylococcus aureus*, respectively. For *E. coli* and *S. pyogenes* the sterile water extract and the diethyl ether extract showed a comparatively smaller zone of inhibition with 24 mm for clove and 14 mm for cinnamon. *S. pyogenes* was found to be a resistant strain to all the four spices and to all the three kinds of extracts.



Bar Diagram 1 : Comparative account of the effect of sterile water extracts of the four spices against four pathogenic bacteria.



Bar Diagram 2: Comparative account of the effect of ethanolic extracts of the four spices against four pathogenic bacteria



Bar Diagram 3: Comparative account of the effect of diethyl ether extracts of the four spices against four pathogenic bacteria.

The study is a preliminary evaluation of the antimicrobial activity of the spices. It indicates that the spices have the potential to be used for medical purposes. It may serve as the basis for the discovery of new classes of chemicals. Further studies are required to establish the types of compound responsible for the antimicrobial activity.

Acknowledgement:

We extend our gratitude to our Principal Dr. Sister Doris D'Souza A.C. for her constant support.

We are thankful to Mr. Vijay, our laboratory assistant, for his help throughout this project.

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