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Research Article

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Comparative studies on the antibacterial efficacy of clove and cinnamon extracts against clinical isolates

R. Ishwarya, D. Sangeetha*, J. Tharani, P. Ramya and M. Geetha

Department of Microbiology, Faculty of Science, Annamalai University,
Annamalai Nagar- 608002, Tamil Nadu, India.
*Corresponding author E-mail: *sangeethadau@gmail.com*

Abstract

Herbs and spices have been used since ancient times, not only as antioxidants and flavouring agents, but also for their antimicrobial activity against degradation induced by food borne pathogens and food spoilage bacteria. Many plants used in traditional medicine represent rich sources of natural bioactive substances with health promoting effects and no side effects. There has been a constant increase in the search of alternative and efficient compounds for food preservation, aimed at partial or total replacement of antimicrobial chemical additives. The chief significance of the below study was therefore to test the antimicrobial activity of clove and cinnamon and extract as an alternative to chemical preservative so as to minimize their side effects and simultaneously improving the shelf-life of the food products and also comparing the efficacy of these two spices in controlling bacterial growth in-vitro. The results showed that clove was much efficient controller of bacterial growth.

Keywords: spices, clove, cinnamon, antibacterial activity, agar well method, comparative study

Introduction

Nowadays, over 65% of the world population relies on traditional medicine for health care. Recently a large demand has risen for preservative free cosmetics and antimicrobial herbal extracts, aimed at reducing the risk of allergies connected to synthetic preservatives such as methylparabens. Among these secondary metabolites, polyphenols, terpenoids alkaloids, lectins, polypeptides and polyacetylenes are known to be antimicrobial agents; most of these metabolites are also approved as a GRAS (Generally Recognised as Safe) material for food products showing negligible side effect. *Syzygium aromaticum*, a medicinal plant commonly known as clove, is used to treat toothache, respiratory disorders, inflammation, and gastrointestinal disorders. From the flower buds of *S. aromaticum*, it is possible to obtain an essential oil comprised of a mixture of aliphatic and cyclic volatile terpenes and phenylpropanoids, being eugenol as the main component. Clove is gaining much importance among other spices, due to its

antimicrobial and antioxidant activities (Shan *et al*. 2005; Shrivastav *et al*. 2019) to cure toothache, gastrointestinal disorders and inflammation (El-Shouny *et al*. 2020; Santin *et al*. 2011; Oluwasina *et al*. 2019). Some of the studies mentioned the antibacterial potential of clove. The studies showed that phenolic extract of clove (*Syzygium aromaticum*) has antibacterial activities against the growth of *S. aureus* and *E. coli* in a concentration range of 50–100 µg/mL (El-Matti *et al*. 2016). *Syzygium aromaticum* seeds contain eugenol (component with antimicrobial activity) with a minimum inhibitory concentration (MIC) of 0.06 mg/mL, which affect the membrane permeability, and increase oxidative stress enzymes such as catalase and superoxide dismutase (Ajiboye *et al*. 2016).The genus Cinnamomum contains more than 300 evergreen aromatic trees and shrubs. Four species have great economic importance for their multiple culinary uses as common spices worldwide*: Cinnamon zeylanicum* Blume, *Cinnamon loureiroi* Nees, *Cinnamon burmanni* Blume, *Cinnamon aromaticum* Nees.

The term cinnamon commonly refers to dried bark of *C. zeylanicum* and *C. aromaticum* used for the preparation of different types of chocolate, beverages, spicy candies and liquors. Moreover, cinnamon is used in various savory dishes, pickles, soups and Persian sweets. Cinnamon bark, leaves, flowers and fruits are used to prepare essential oils, which are destined for use in cosmetics or food products. Moreover, according to traditional Chinese medicine cinnamon has been used as a neuroprotective agent and for the treatment of diabetes. Cinnamon has also been used as a health promoting agent for the treatment of disease such as inflammation, gastrointestinal disorders and urinary infections. Another potential medical use of cinnamon would be with regards to its antimicrobial properties, especially antibacterial activity (Ahuja et al 2015).It is well known that infection is one of the leading causes of morbidity and mortality worldwide. According to world Health Organization reports in 2011, there were more than 55 million deaths worldwide with infection being responsible for one-third of all deaths. The high prevalence of infection and long-term exposure to antibiotics has lead to the antibiotic resistance of micro organisms. Therefore, much attention has been paid to the discovery and development of new antimicrobial agents that might act against these resistant microorganisms and cinnamon could be an interesting candidate (Paliwal *et al* 2018). Thus the aim of the present study is to analyze antibacterial effects of cinnamon and cloves.

Materials and Methods

Bacterial culture:

The pathogenic bacteria *E. coli, Pseudomonas sp, Proteus sp, Salmonella sp, Staphylococcus aureus* were procured from the culture collection centre, Pondicherry.

Acquisition of spices and preparation of extract:

Cinnamon and cloves were procured from the local market. The spices were air dried at room temperature and grounded into fine powder. Three extracts namely ethanol, methanol and acetone were prepared. 10 grams of spice powder (cinnamon and cloves) was soaked separately in 100ml of ethanol, methanol and acetone in a conical flask, plugged with cotton and kept at room temperature for 3 days and filtered through Whatman No.1 filter paper. The filtrate was evaporated in petridishes at room temperature for 2-3 days till the volume was reduced to one fourth of the original volume of the solvent used and stored at 4°C in air tight bottles (Harborne, 1973).

Determination of antibacterial activity of spices powder

Antibacterial activity of methanol, ethanol, and acetone extract of the cinnamon and cloves powder was evaluated separately by agar well diffusion method (Aida et al., 2001). The test bacterial strains were inoculated into Mueller-Hinton broth and incubated at 37°C for 24 hours. After incubation a sterile cotton swab was immersed in the bacterial suspension and swabbed aseptically on the surface of Muller-

Hinton agar medium and allowed to dry for about 3 minutes. Well of 6mm diameter was punched into the agar medium and filled with 100 micro litre of crude spices (cinnamon & cloves) extracts (75 mg/ml in 10% DMSO), 100 micro litre and 150 micro litre of different concentration were used. Gentamicin (50 mg/ml) was used as positive control and 10% dimethyl sulfoxide (DMSO) was used as a negative control. The plates were incubated in an upright position at 37°C for overnight in an incubator. Antibacterial activity was detected by measuring the zone of inhibition around each well, excluding the diameter of the well in mm as low activity (<=6 mm) moderate activity (7-10mm) high activity (11-15mm) very active at (>= 16mm) and no activity (-) (Praveen *et al*, 2010).

Results and Discussion

The resistance to multiple drugs has become a common feature in which most of the organisms associated with diarrhoea and other enteric diseases. Urinary tract infection and wound infection (Uraih et al. 2004; Kumar et al. 2012).The present work was conducted to evaluate the antimicrobial potential of Indians spices namely *Syzgium aromaticum* (clove), *Cinnamon zeylanicum* (cinnamon). The spices were purchased in dried form and grinded before subjecting to antimicrobial activity. In present study, three solvents namely ethanol, methanol and acetone were selected for extraction. The methanolic extract of the two spices exhibited the maximum antimicrobial activity against pathogen. The ethanol and acetone extract showed less activity against the isolates. All extracts of spices showed antibacterial property. The maximum zone of inhibition (33±0.3mm) was attained from the methanolic extract of clove against *E. coli* in the concentration of 150 µl followed by *Pseudomonas sp* with the inhibition zone (24±0.2mm). The ethanolic extract showed maximum zone (31±0.4mm) against *Pseudomonas sp* followed by *E. coli* with the inhibition zone 26±0.4mm. Acetone extract showed less activity when compared with methanol and ethanol extracts.

Table 1: Antimicrobial activity of clove against bacterial pathogens

Bacteria/ Spice extract	Zone of Inhibition (millimetre in diameter)									Positive control (Gentamicin (50 mg/ml)
	Methanol (µl)			Ethanol (µl)			Acetone (µl)			
	75	100	150	75	100	150	75	100	150	
E.coli	26±0.3	29±0.4	33±0.3	22±0.2	25±0.2	26±0.4	16±0.2	18±0.3	20±0.2	34 ± 0.5
Proteus sp	16±0.2	17±0.2	18±0.2	18±0.4	19±0.3	20±0.3	16±0.2	17±0.2	18±0.4	30 ± 0.4
Salmonella sp	15±0.4	16±0.4	17±0.3	15±0.2	17±0.2	19±0.3	12±0.2	12±0.3	14±0.4	30 ± 0.2
Pseudomonas sp	20±0.2	22±0.2	24±0.2	25±0.4	28±0.2	31±0.4	15±0.3	17±0.4	18±0.2	30 ± 0.3
Staphylococcus sp	19±0.2	20±0.3	21±0.3	18±0.4	20±0.3	20±0.2	15±0.3	18±0.2	20±0.4	32 ± 0.4

Negative control – DMSO

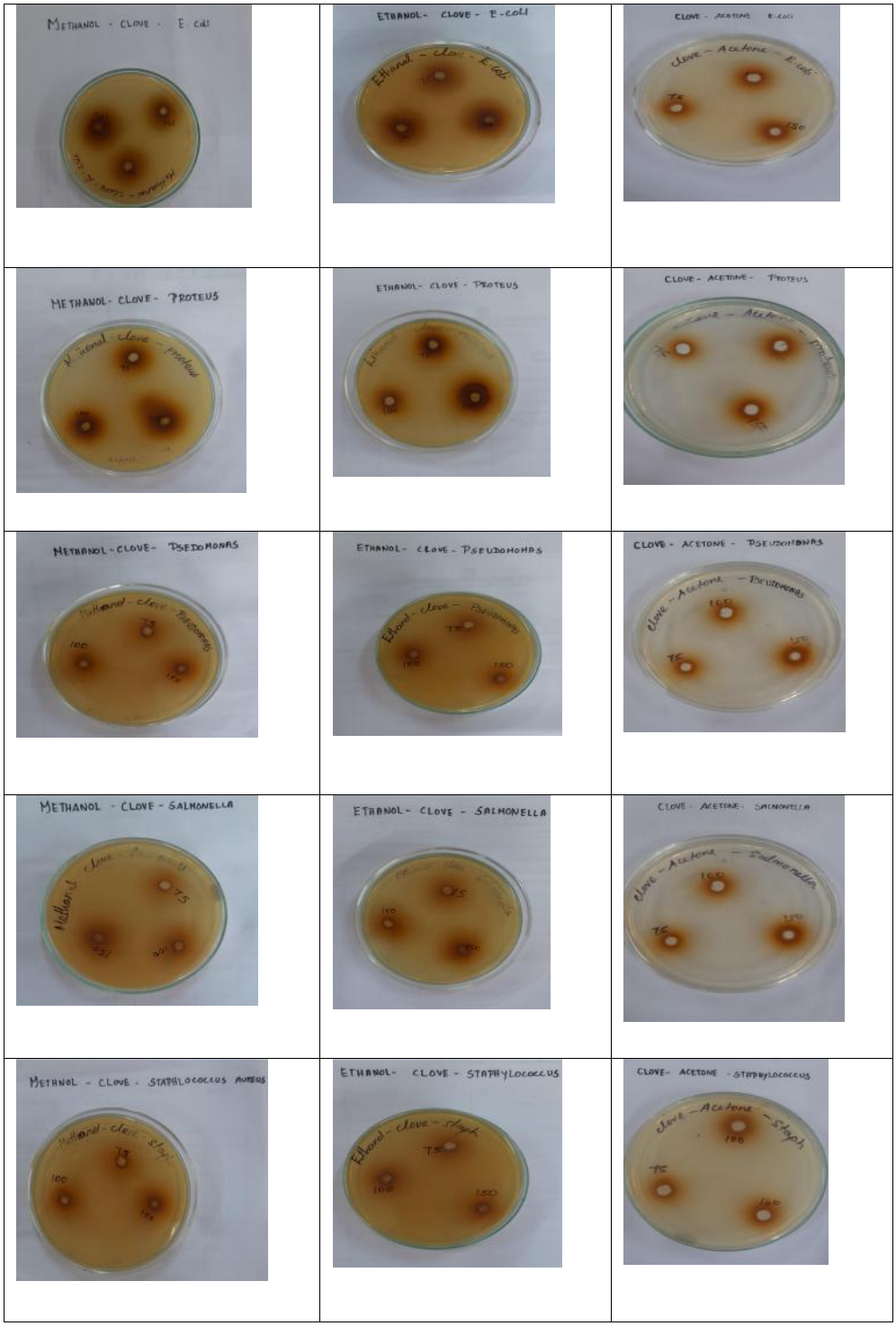

Fig 1. Antimicrobial activity of Clove against bacterial pathogens

Table 2: Antimicrobial activity of cinnamon against bacterial pathogens

Bacteria/extracts	Zone of Inhibition (millimetre in diameter)									Positive control (Gentamicin (50 mg/ml))
	Methanol (µl)			Ethanol (µl)			Acetone (µl)			
	75	100	150	75	100	150	75	100	150	
E. coli	27±0.2	28±0.2	29±0.3	17±0.3	19±0.2	22±0.2	14±0.3	17±0.2	18±0.4	30 ± 0.3
Proteus sp	15±0.4	16±0.4	17±0.4	15±0.4	16±0.4	17±0.3	14±0.3	15±0.3	15±0.3	27 ± 0.4
Salmonella sp	19±0.3	22±0.2	24±0.2	17±0.2	19±0.3	22±0.4	15±0.2	16±0.4	16±0.2	25 ± 0.5
Pseudomonas sp	20±0.2	21±0.3	22±0.3	16±0.3	18±0.2	20±0.3	16±0.2	18±0.2	20±0.4	26 ± 0.2
Staphylococcus sp	27±0.3	28±0.2	29±0.3	20±0.4	23±0.4	25±0.2	20±0.4	21±0.2	22±0.2	31 ± 0.4

Negative control - DMSO

The methanolic extract of cinnamon showed highest potential against *Escherichia coli* and *Staphylococcus sp* (29±0.3mm) followed by *Salmonella sp* (24±0.2mm) in the concentration of 150 µl. Ethanolic extract contributed the maximum zone of inhibition (25±0.2mm) against *Staphylococcus sp* followed by *Salmonella sp* (22±0.4mm) and acetone against Staphylococcus sp (22±0.2mm) followed by *Pseudomonas sp* (20±0.4mm). The ethanolic extract of cinnamon showed highest potential against *Escherichia coli*, methanolic extract against *Pseudomonas sp* and acetone against *Staphylococcus aureus.* The most potent antimicrobial constituents in many spices are aromatic phenolic compounds. It can be concluded that the antimicrobial efficacy of clove is due to eugenol while cinnamon is due to eugenol and cinnamon aldehyde (Rahman et al 1997).

The active constituent of spices may exhibit their antimicrobial effect either by degradation of cell wall, disruption of cytoplasmic membrane, leakage of cellular components, damage protein, interfere with enzymatic activities inside the cell, affect synthesis of DNA and RNA affect electron transport and nutrient uptake, leakage of cellular components, impair the energy production inside the cell, change the fatty acids and phospholipid constituents (Kuang *et al* 2011).

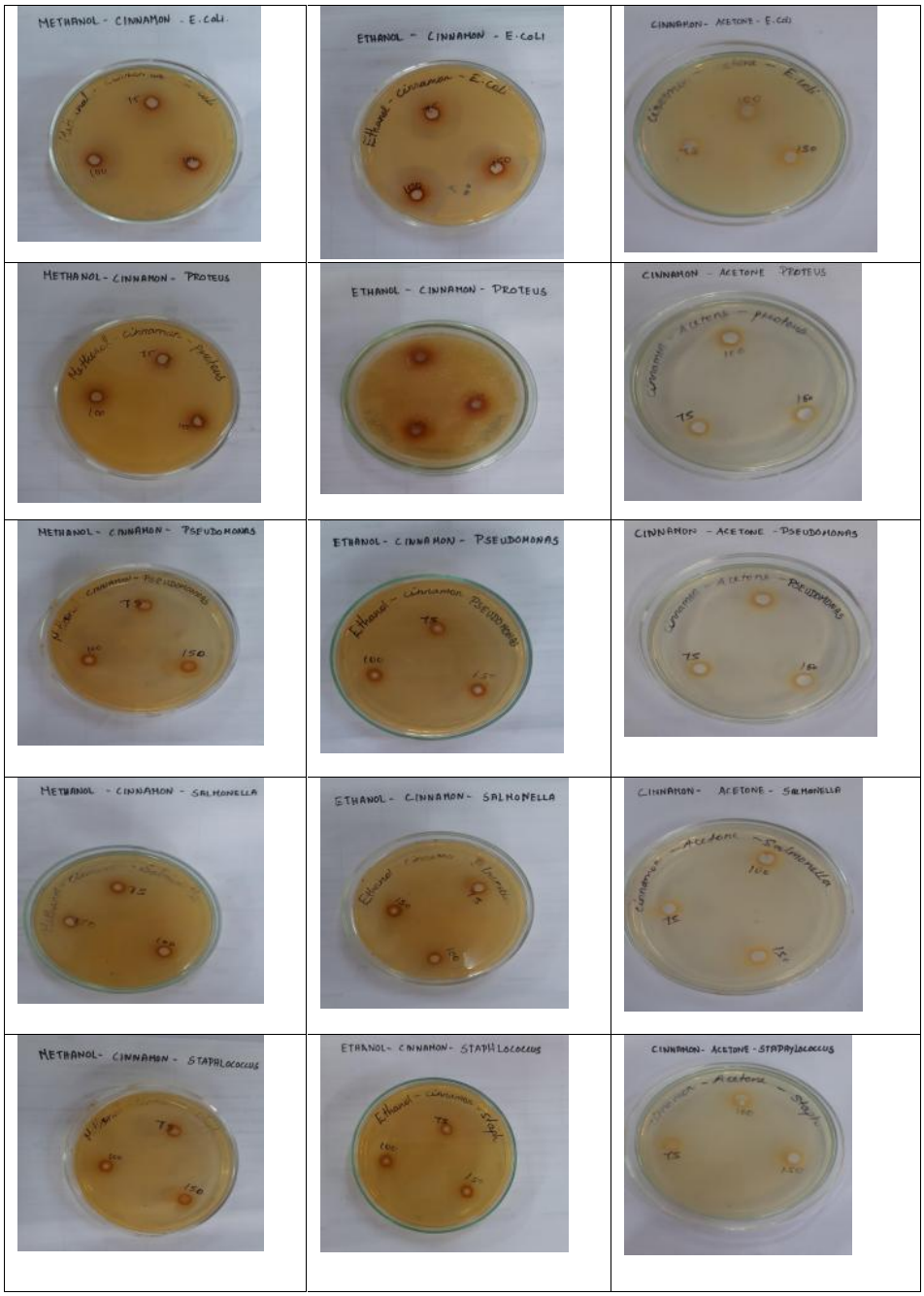

Fig 2. Antimicrobial activity of cinnamon against bacterial pathogens

Conclusion

Spices are an important part of human diet in India. They have been used for thousands of years to enhance the flavor, color and aroma of the food. Spices are well known for their preservative and medicinal values in households. It is however in recent year that the spices have drawn the attention of researchers due to increasing resistance against antibiotics amongst pathogens. From this study, it may be concluded that the methanolic extracts of spices can be used as a potential source of natural antimicrobial compound against pathogenic bacteria. When comparing the two spices cloves showed maximum antibacterial activity than cinnamon. This preliminary study can be further extended in determining the active components of the spices so that effective medicinal preparations can be made.

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