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Antibacterial Activity of *Syzigium aromaticum L.* (Clove)

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A B S T R A C T

Syzigium aromaticum L. (clove) is an aromatic historic spice, belongs to the family Myrtaceae. Essential oils are the main chemical constituents of the clove. Cloves are been used in the traditional and modern medicine preparations. It possess anti-helminthic, anti-inflammatory, anti-spasmodic, anti- pyretic, anti-allergic, anti-fungal, anti-carcinogenic, anti-allergic, anti-viral, antioxidant, anti-mutagenic, anti-arthritis, anti-parasitic properties. The extracts of clove were studied on 4 bacterial cultures i.e. gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. Gram negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. Growth inhibition was evaluated by the Agar well diffusion method. The antibacterial activity of clove was found statistically in gram positive bacteria *Staphylococcus aureus* (16mm) and *Bacillus subtilis* (12mm) than gram negative bacteria *Pseudomonas aeruginosa* (7mm) and *Escherichia coli* (6mm).Therefore the extracts of clove can be used as antibacterial activity.

Introduction

The plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Traditional healing systems around the world that utilise herbal remedies are an important source for the discovery of new antibiotics (Okpekon *et al*., 2004).

Spices have been defined as the plant substances from the indigenous (or) exotic origin, aromatic, with strong taste, used to enhance the taste of foods (Arora *et al*., 1999; Shelef, 1983). Spices posess antimicrobial activity due to the presence of Essential oil, alkaloids, glycosides etc. that are present in abundance herbs and spices commonly used in Indian food preparation. The presence of these bioactive substances is responsible for antimicrobial properties.

Clove (*Syzigium aromaticum L.*) is an aromatic spice, belongs tofamily *Myrtaceae*. Cloves are used in Ayurveda, Chinese medicine and Western herbalism. It has been shown that some components of clove are useful in bacterial and fungal infections (Zeng *et al*., 1992; Zhang *et al*., 1997).

The chemical constituents comprises of Eugenol (Chaieb *et al*., 2007; Daniel *et al*., 2009), acetyl eugenol (5.62%), beta – caryophyllene (1.38%) (oztürk *et al*., 2005; Jirovetz *et al*., 2006) and vanillin, crategolic acid, tannins such as bicornin (Kamatou *et al*., 2012) gallotannic acid, methyl salicylate (pain killer), the flavonoids eugenin, kaempferol, rhamnetin, and eugentin, triterpenoids such as oleanolic acid, stigmasterol, and campesterol and several sesquiterpenes, rhamnetin and vitamins (Aishwarya). The main constituent of clove is Eugenol comprises 72-90% of the essential oil the compound is most responsible for clove aroma.

Cloves possess anti-helmenthic, anti-inflammatory (Boulos, 1983), (Kim *et al*., 2003) anti-pyretic, anti-allergic (Feng *et al*., 1987), anti-fungal (Gayoso *et al*., 2005 Manohar *et al*., 2001**),** anti-carcinogenic, (Aisha *et al*., 2012) anti-allergic (Kim *et al*., 1998), anti-viral (Reichling *et al*., 2009) anti-mutagenic activity (Miyazawa *et al*., 2001), antioxidant insecticidal (Park *et al*., 2000) anti-spamodic, anti-arthritis (Kaur *et al*., 2012) anti-parasitic (32) properties, carminative (Odugbemi, 2006), to increase hydrochloric acid in the stomach and to improve peristalsis and an anaesthetic.

In the present study was to evaluate the in-vitro antimicrobial activity of essential oil of clove by using agar well diffusion method against selected 4 bacterial cultures i.e. gram negative bacterial organisms *Pseudomonas aeruginosa*, *Escherichia coli* and gram positive bacterial organisms i.e. *Staphylococcus aureus* and *Bacillus subtilis.*

Materials and Methods

Plant materials

Clove flower buds collected from the local market of Hyderabad, India.

Bacterial cultures

Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, Gram negative bacteria *pseudomonas aeruginosa* and *Escherichia coli* cultures are collected from Biotechnology department. University College for Women, Koti, Hyderabad, Telangana.

Hydro-distillation method for extraction of essential oil

The sample was submitted to hydro-distillation for 4 hours, using a Clevenger-type apparatus, according to the European Pharmacopoeia (European Pharmacopoeia). A 40g of powdered clove sample along with 500ml of distilled water was taken in a round bottom flask. The sample was heated to 600c for 4 hours on a heating mantle. The volatile distillate was collected over anhydrous sodium sulphate and refrigerated until time of analysis.

Agar well diffusion method

The antibacterial activity of essential oil of clove was evaluated by using agar well diffusion method. Bacterial cultures are mixed in nutrient agar medium and poured in Petri plates. Wells (or) cups of 5mm in size were made with sterile bower into water agar plates containing the bacterial inoculums.2mg of crude extract was completely dissolved in 2ml of Dimethyl sulphoxide (DMSO).Antibacterial activity was measured at different concentrations of extract ranging from 25, 50, 100, 150µl was poured into the wells of inoculated plates. DMSO served as control and antibiotic Amphicillin served as standard.

Results and Discussion

The antimicrobial activity of clove showed good inhibitory action against test bacterial

strains. Essential oil of clove showed antibacterial activity against all the test bacteria with zone of inhibition ranged from 6mm-16mm. The maximum zone of inhibition was against gram negative bacteria *Staphylococcus aureus* (16mm) and *Bacillus subtilis* (12mm), than gram positive bacteria *Pseudomonas aeruginosa* (7mm) and *Escherichia coli* (6mm). Maximum zone of inhibition was at 100µl for all the bacterial cultures. It indicates that zone of inhibition increase as the concentration of essential oil increased.

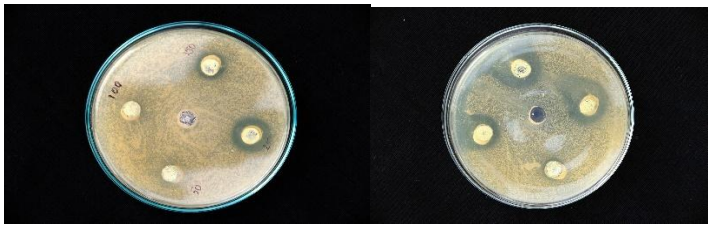
The data of significant inhibitory activity was observed in 4 selected test bacterial strains to the essential oil of clove given in the below table-1.

Table.1 Effect of essential oil of clove on growth of bacteria in vitro.

S. No	Name of Bacteria	Concentration(µl)	Zone of inhibition (mm)		
1.	*Staphylococcus aureus*	25µl	3	1	1
		50µl	5	2	1
		75µl	6	3	3
		100µl	16	14	13
2.	*Bacillus subtilis*	25µl	4	2	2
		50µl	6	3	2
		75µl	7	6	4
		100µl	12	7	7
3.	*Pseudomonas aeruginosa*	25µl	1	1	2
		50µl	2	2	2
		75µl	3	1	2
		100µl	7	4	4
4.	*Escherichia coli*	25µl	-	-	-
		50µl	3	2	2
		75µl	6	4	4
		100µl	6	5	4

Fig.1 I,II,III,IV showing the antibacterial activity of clove essential oil against pathogenic bacteria.

(I) Staphylococcus aureus (II) Pseudomonas aeruginosa



(III) Escherichia coli (IV) Bacillus subtilis

In the present study clove essential oil showed antimicrobial activity from the concentration 25µl-100µI for both Gram positive and Gram negative bacteria. Methanolic extracts of clove showed maximum zone of inhibition against *staphylococcus aureus* (16mm) and *Bacillus subtilis* (12mm) while minimum in against *Pseudomonas aeruginosa* (7mm) and *Escherichia coli* (6mm) was at 100µl for all bacterial cultures. On the contrary (Abd El Azim *et al*., 2014), He observed the methanolic extract of clove showed high inhibitory effect against *Escherichia coli* (7mm) at the 0.3ml concentration, while minimum resistance in *Staphylococcus aureus (*2mm) with 0.3 ml of extract concentration. *Clove* reported as antibacterial activity increases by the suspension of metal ions like Zn in the crude methanolic extract. In this study the organic extracts were more effective than aqueous extract. Most of the antimicrobial active compounds that have been identified were soluble in polar solvents such as methanol, ethanol etc., (Cowman, 1999) than in water. The result of antibacterial susceptibility assay shows promising evidence for the anti-bacterial effect of methanolic extracts of clove against three food borne associated *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, and Escherichia coli.*

In conclusion, the present study has confirmed antimicrobial properties of essential oil of clove that showed significant growth inhibition for above test bacterial strains. The results indicate the essential oil of clove can also use as natural antibiotic for the treatment of several infectious diseases caused by 4 bacterial strains.

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