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IN VITRO ANTIBACTERIAL ACTIVITY OF CLOVE AGAINST GRAM NEGATIVE BACTERIA

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Abstract

A study was carried out to investigate the potential of using aqueous infusion, decoction and essential oil of clove (*Syzygium aromaticum*) as natural antibacterial agents against 100 isolates belonging to 10 different species of Gram –ve bacilli viz., *Escherichia coli* (36), *Proteus mirabilis* (6), *Pseudomonas aeruginosa* (10), *Enterobacter aerogenes* (5), *Klebsiella ozaenae* (2), *Klebsiella pneumoniae* (24), *Serratia marcescens* (4), *Salmonella typhi* (3), *Shigella dysentriae* (5) and *Vibrio cholerae* (5). The screening was performed by standard disc diffusion method. The aqueous infusion and decoction of clove exhibited maximum activity against *P. aeruginosa* with 10.43 mm mean diameter of zone of inhibition ± 1.76 standard deviation and 10.86 mm mean diameter of zone of inhibition ± 1.46 standard deviation respectively. Essential oil of clove exhibited maximum activity against *V. cholerae* with 23.75 mm mean diameter of zone of inhibition ± 3.03 standard deviation. *K. ozaenae, K. pneumoniae, S. marcescens, S. typhi, S. dysentriae* and *V. cholerae* were found resistant to aqueous infusion and decoction while essential oil showed strong antibacterial activity against all bacterial isolates tested.

Introduction

Cloves (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*) are the aromatic dried flower buds of a tree in the family *Myrtaceae* (Srivastava & Malhotra, 1991; Chaieb *et al.,* 2007a). Cloves are used in Ayurveda, Chinese medicine and Western herbalism. Cloves are used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalis (Phyllis & James, 2000). It is also used in dentistry where the essential oil of clove is used as anadyne for dental emergencies (Cai & Wu, 1996; Prashar *et al.,* 2006). In addition,the cloves are antimutagenic (Miyazawa & Hisama, 2003), anti-inflammatory (Kim *et al.,* 1998), antioxidant (Chaieb *et al.,* 2007b), antiulcerogenic (Bae *et al.,* 1998; Li *et al.,* 2005), antithrombotic (Srivastava & Malhotra, 1991) and antiparasitic (Yang *et al.,* 2003).

The essential oil extracted from the dried flower buds of cloves is used for acne, warts, scars and parasites. Research has shown that clove oil is an effective mosquito repellent (Trongtokit *et al.,* 2005). The clove oil is also used as a topical application to relieve pain and to promote healing and also finds use in the fragrance and flavouring industries (Chaieb *et al.,* 2007a). However, clove oil is toxic to human cells (Prashar *et al.,* 2006). If ingested or injected in sufficient quantity, it has been shown to cause life-threatening complications, including Acute Respiratory Distress Syndrome, Fulminant Hepatic Failure and Central Nervous System disorder. The lethal oral dose is 3.752 g/Kg body weight (Kirsch, 1990; Lane *et al.,* 1991; Hartnoll *et al.*, 1993).

Several constituents of clove has been identified, mainly eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone (Chaieb *et al.,* 2007b), acetyleugenol, alpha-humulene, methyl salicylate, isoeugenol, methyleugenol (Yang *et al.,* 2003), phenyl propanoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnocitrin, myricetin,

gallic acid, ellagic acid and oleanolic acid (Cai & Wu, 1996). The main constituents of essential oil are phenylpropanoides such as carvacrol, thymol, eugenol and cinnamaldehyde (Chaieb *et al.,* 2007a). Several studies have demonstrated potent antifungal (Arina & Iqbal, 2002; Giordani *et al.,* 2004; Pawar & Thaker, 2006; Park *et al.,* 2007), antiviral (Chaieb *et al.,* 2007a) and antibacterial effects of clove (Cai & Wu, 1996; Bae *et al.,* 1998; Lopez *et al.,* 2005; Li *et al.,* 2005; Betoni *et al.,* 2006; Fu *et al.,* 2007).

The present study was therefore conducted to evaluate the antibacterial potential of aqueous infusion, decoction and essential oil of clove against 100 different isolates belonging to 10 different species of Gram-negative bacilli viz., *Escherichia coli* (36), *Proteus mirabilis* (6), *Pseudomonas aeruginosa* (10), *Enterobacter aerogenes* (5), *Klebsiella ozaenae* (2), *Klebsiella pneumoniae* (24), *Serratia marcescens* (4), *Salmonella typhi* (3), *Shigella dysentriae* (5) and *Vibrio cholerae* (5).

Materials and Methods

Maintenance of isolates: A total of 100 isolates belonging to 10 different species of Gram –ve bacilli (Table 1) isolated from different clinical specimens of stool, urine, blood and pus from wound were maintained on tryptone soy agar (TSA) (Oxoid).

Preparation of infusion: The aqueous infusion was prepared by taking 10 g clove in 100 ml distilled water and left for 24 hours at room temperature with occasional shaking and filtered to obtain clear infusion.

Preparation of decoction: The aqueous decoction was prepared by boiling 10 g clove in 100 ml distilled water in a flask for 20 minutes. The flask was removed from heat and allowed to cool. The content of flask was filtered to obtain clear decoction.

Table 1. Antibacterial activities of infusion, decoction and oil of clove.

S. No.	Name of organisms	No. of isolates	Mean zone of inhibition in mm ± Standard deviation		
			Infusion	Decoction	Oil
1.	*E. coli*	36	8.73 ± 1.18	9.07 ± 1.46	11.87 ± 3.22
2.	*P. mirabilis*	06	8.50 ± 0.87	8.00 ± 0.00	16.50 ± 0.50
3.	*P. aeruginosa*	10	10.43 ± 1.76	10.86 ± 1.46	18.86 ± 1.46
4.	*E. aerogenes*	05	9.40 ± 0.49	8.20 ± 0.40	14.20 ± 0.75
5.	*K. ozaenae*	02	-	-	14.50 ± 2.50
6.	*K. pneumoniae*	24	-	-	12.00 ± 3.15
7.	*S. marcescens*	04	-	-	14.25 ± 0.43
8.	*S. typhi*	03	-	-	18.00 ± 3.08
9.	*S. dysentriae*	05	-	-	16.50 ± 0.50
10.	*V. cholerae*	05	-	-	23.75 ± 3.03

-No activity

Essential oil: Essential oil of clove (Hamdard) was purchased from a local market of Karachi, Pakistan.

Screening of antibacterial activity: Screening of antibacterial activity was performed by standard disc diffusion method (Saeed *et al.,* 2007). Hundred sterilized discs of filter paper (6 mm diameter) were soaked in 1 ml of infusion, decoction and oil, seperately for 1-2 minutes and then used for screening. The potency of each disc was 10 µl. Mueller-Hinton agar (MHA) (Merck) was used as base medium and Mueller-Hinton broth (MHB) was used for the preparation of inoculum. Four to five isolated colonies of tested

organisms were picked by sterile inoculating loop and inoculated in tubes of MHB (5 ml each). The inoculated tubes were incubated at 35-37^{o} C for 24 hours and matched with 0.5 McFarland nephelometer turbidity standard (Saeed & Tariq, 2007). A sterile cotton swab was dipped into the standardized bacterial test suspension to inoculate entire surface of a MHA plate. Discs of infusion, decoction and oil were placed on the surface of inoculated plates with the help of sterile forcep. The inoculated plates were incubated at 35-37^{o} C for 24 hours. After incubation inhibition zone diameters were measured to the nearest millimeter (mm).

Statistical analysis: Mean diameter of zone of inhibition and standard deviations were calculated.

Results and Discussion

One hundred Gram-negative bacilli belonging to 10 different species viz., *E. coli* (36), *P. mirabilis* (6), *P. aeruginosa* (10), *E. aerogenes* (5), *K. ozaenae* (2), *K. pneumoniae* (24), *S. marcescens* (4), *S. typhi* (3), *S. dysentriae* (5) and *V. cholerae* (5), were used in the present study. The results of *In vitro* antibacterial activity of aqueous infusion, decoction and essential oil are presented in Table 1.

The aqueous infusion and decoction of clove exhibited maximum activity against *P. aeruginosa* with 10.43 mm mean diameter of zone of inhibition ± 1.76 standard deviation and 10.86 mm mean diameter of zone of inhibition ± 1.46 standard deviation respectively. Essential oil of clove exhibited maximum activity against *V. cholerae* with 23.75 mm mean diameter of zone of inhibition ± 3.03 standard deviation. *K. ozaenae, K. pneumoniae, S. marcescens, S. typhi, S. dysentriae* and *V. cholerae* were found resistant to aqueous infusion and decoction while essential oil showed strong antibacterial activity against all bacterial isolates tested. The results of the present study are in harmony to those reported by Burst & Reinders (2003) that clove oil was found effective against non-toxigenic strains of *E. coli* O157:H7. Similarly, in another study clove oil was found active against foodborne Gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis* and *Listeria monocytogenes*) and Gram-negative bacteria (*E. coli, Yersinia enterocolitica, Salmonella choleraesuis* and *P. aeruginosa*) (Lopez *et al.,* 2005). Furthermore, active constituents of clove (biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanoic acid) possess antibacterial activities against Gram-negative anaerobic periodontal oral pathogens, including *Streptococcus mutans, Actinomyces viscosus, Porphyromonas* and *Prevotella intermedia* (Cai & Wu, 1996). It has also been reported that the extract of clove potently inhibited the growth of *Helicobacter pylori* (Bae *et al.,* 1998; Li *et al.,* 2005). In a study carried out by Betoni *et al*., (2006) clove extract showed inhibitory effect against *S. aureus*.

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