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ANTIMICROBIAL ACTIVITY OF CLOVE (*SYZGIUM AROMATICUM)* OIL AGAINST FOOD BORNE MICROORGANISMS

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ABSTRACT
The present study was performed to isolate and identify various pathogenic bacteria and fungi from different spoiled fruits (orange and grapes), vegetables (capsicum, potato and tomato) samples. An attempt was also done to evaluate antimicrobial activity of clove oil (*Syzgium aromaticum*) against ten bacterial isolates present in fruits and vegetables belonging to different species of Gram positive and Gram negative bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* sp., *Pseudomonas* sp., *Salmonella* and *Streptococcus* sp. The fungal isolates identified in this study are *Aspergillus niger*, *Penicillium*, *Fusarium* and *Rhizopus*. Morphological identification and biochemical characterization were also performed on bacterial isolates. Antimicrobial activity of clove (*Syzgium aromaticum*) oil was tested against these bacterial isolates. Screening of antibacterial activity was performed by standard disc diffusion method and agar well diffusion method. The present study concluded that clove oil can be used as an effective antibacterial agent and natural food preservative.

KEYWORDS: Clove oil (*Syzgium aromaticum*), Antimicrobial activity.

INTRODUCTION

According to World Health Organization (WHO, 2007), consumption of contaminated food due to changed lifestyle, is the most widespread health problem and a major cause of the reduction in economic productivity affecting hundreds of millions of people. Various microorganisms such as *E. coli*, *Listeria*, *and Staphylococcus aureus*, *Salmonella*, *Campylobacter*, *Klebsiella* and *Pseudomonas* are responsible for food spoilage and food borne illness (Hemlata and Virupakshaiah, 2016). Due to increased consumptions of contaminated food, there has been a continuous increase in several food borne diseases caused by bacterial pathogens. This has initiated considerable research towards the discovery of potent antimicrobial agents. Due to consumer awareness regarding food safety, there is growing interest in using natural antimicrobial compounds, such as extracts and essential oils (EOs) of spices and herbs, which are perfect food preservative agents. Several constituents of clove has been identified (Saeed and Tariq, 2008), 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 clove as potent antifungal (Park *et al.*, 2007), antiviral (Chaieb *etal.,* 2007a) and antibacterial agent (Cai & Wu, 1996; Fu *et al.*, 2007). Antimicrobial properties of herbs and spices have been recognized and used since ancient times for food preservation as well as medicinal use (Dorman & Deans, 2000). Cloves (*Syzygium aromaticum*) are the aromatic dried flower buds of a tree in the family Myrtaceae. (Chaeib *et al*., 2007a). Cloves are used as a carminative (Phyllis & James, 2000) and essential oil of clove is also used for dental emergencies and dentistry (Prashar *et al*., 2006). In addition, the cloves are anti-mutagenic, anti-inflammatory, antioxidant, anti-ulcerogenic, anti-thrombotic and anti-parasitic (Miyazawa & Hisama, 2003; Kim *et al*., 1998; Chaieb *et al*., 2007b; Li *et al*., 2005; Srivastava & Malhotra, 1991; Yang *et al*., 2003). Bactericidal or bacteriostatic activity of essential oils, *in vitro* and in food assays, against *Salmonella enterica*, *Escherichia coli* , *Staphylococcus aureus*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and *Candida albicans* strains has been reported (Kim *et al*., 2004). Various *in vitro* studies have used spices as antimicrobials in laboratory media although the levels of spices and their essential oils to inhibit microorganisms in food have been found to be higher than those assays performed using culture media (Uhart *et al*., 2006).

The present study was conducted to evaluate the antibacterial activity of clove oil against food borne bacterial isolates isolated from fruits and vegetable available locally in Chandigarh on different types of media i.e. Nutrient agar, Eosin Methylene Blue agar and Muller Hinton agar.

MATERIALS & METHODS

The total of 5 different fruits and vegetables (capsicum, orange, grapes, potato and tomato) are collected from local market in Chandigarh. The isolation of bacterial and fungal isolates was done using serial dilution agar-plating and spread plate method and then enumerated. After

isolation each bacterial isolate was streaked on nutrient agar media (NAM) and fungal isolate on potato dextrose agar (PDA). All the culture media are autoclaved at 121º C, 15 psi pressure for 15 minutes. The pure cultures were stored and maintained at 4°C in refrigerator. Morphological identification of the bacterial isolates was done using Gram's staining and fungal isolates by lactophenol cotton blue staining. Biochemical characterization for bacterial isolates was done using following biochemical tests: catalase, IMVIC test, hydrogen sulfide production test, urease production, amylase production test were performed. Essential oil of clove (Dabur) was purchased from a local market of Mohali, Punjab. Screening of antibacterial activity of clove oil against food borne pathogens was performed by standard disc diffusion method (Saeed *et al*., 2007) and agar punch well method (Saikumari *et al*., 2016). The disc diffusion method and agar punch well method was done in triplicate and the mean value of the zone of inhibition in mm was calculated. The results were expressed in terms of the diameter of zone of the inhibition. Mean diameter of zone of inhibition and standard deviations were calculated.

RESULTS & DISCUSSION

Ten bacterial isolates mostly food-borne pathogens were isolated from various vegetables available locally e.g., capsicum, orange and potato by serial dilution method. These bacterial isolates were identified as *E.coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus cereus* and *Enterobacter aerogens*. In the present study, the dominant members of the bacteria isolated from spoiled fruits belong to the genera *Bacillus* and *E. coli*. Table 1 shows the viable count of bacterial isolates on various media i.e. Nutrient agar, Eosin Methylene Blue agar and Muller Hinton agar. The maximum number of bacterial colonies was found on EMB agar media. The food associated bacteria isolated from various samples of different spoiled fruits and vegetables identified on the basis of morphological and biochemical characteristics (Table 2).

TABLE 1: Number of bacterial colonies counted on Nutrient agar, Eosin Methylene Blue agar and Muller Hinton agar

	Capsicum			Orange			Potato		
MEDIA	10^{-5}	10^{-6}	10^{-7}	10^{-5}	10^{-6}	10^{-7}	10^{-5}	10^{-6}	10^{-7}
NAM	65.6	40.3	27.6	86.3	60.6	28.3	31	12.6	6.3
EMB	175	122.06	94.05	165.3	108.6	74.6	73	39.3	3.6
MHA	59	35.6	27.3	67	26.6	16.3	19.3	27.6	10.6

TABLE 2: Morphological and biochemical characterization of bacterial isolates

Bacterial Isolate	Shape and Gram's Stainig	Catalyse test	Hydrogen Sulfide Production Test	Urease Test	Citrate Test	Methyl red test	Voges-Proskauer test	Indole test	Amylase
Streptococcus lactis	Coccus; Gram +	-	-	-	-	+	-	-	-
Micrococcus luteus	Coccus; Gram +	-	-	+	+	-	-	-	-
Bacillus sp.	Rod; Gram +	-	-	-	+	-	+	-	+
Enterobacter aerogenes	Rod; Gram -	+	-	-	-	-	+	-	-
Pseudomonas aeruginosa	Rod; Gram -	+	-	-	-	-	-	-	_
Bacillus cereus	Rod; Gram +	-	-	-	+	-	+	-	+
Escherichia coli	Rod;Gram -	+	-	-	+	+	-	+	+
Klebsiella pneumonia	Rod; Gram -	+	-	-	-	-	+	-	-
Staphylococcus aureus	Coccus; Gram +	+	-	-	-	+	+	-	-
Proteus vulgaris	Rod; Gram -	+	+	-	-	+	-	+	-

Chaudhary and Dhaka (2016) have reported *Escherichia coli* (FRb1), *Micrococcus luteus* (FRb2), *Proteus vulgaris* (FRb3), *Enterobacter aerogens* (FRb4), *Bacillus subtilise* (FRb5), *Staphylococcus aureus* (FRb6), *Shigella dysenteriae* (FRb7), *Bacillus cereus*(FRb8), *Klebsiella pneumonia*e (FRb9), *Staphylococcus epidermidis* (FRb10) and *Bacillus megaterium* (FRb11) from different spoiled fruits and the dominant bacteria belong to the genera *Bacillus*. There were four fungal isolates: *Aspergillus niger*, *Pencillium*, *Fusarium* and *Rhizopus* obtained from contaminated capsicum on Potato Dextrose Agar (PDA) media after 5 days of incubation at 37°C. Antimicrobial activity of clove oil against the bacterial strains identified and isolated from fruit and vegetables in our study was qualitatively evaluated by the measurement of inhibition zone by standard disc diffusion method and agar punch well method. The experiments were repeated three times and the results (mm of zone of inhibition) were expressed as mean ± standard deviation. In standard disc diffusion method against all the test bacteria with zone of inhibition ranged from 20mm-34.6mm. Essential oil of clove showed antibacterial activity by Culture VI and culture IX showed maximum zone of inhibition whereas culture I and culture IV covered minimum zone of inhibition respectively. In agar punch well method against all the test bacteria with zone of inhibition ranged from 18mm-30.3mm. Culture VI and culture V showed maximum zone of inhibition whereas culture I and culture IV showed minimum zone of inhibition. The results of this study showed that, *Syzygium aromaticum* (clove oil) had higher inhibitory effect on

Staphylococcus and *Bacillus*. The antibacterial activity of the selected bacterial isolates against clove oil is summarized in Table 3. The results of the present study confirmed that various pathogenic bacteria can be controlled by clove oil. However, further investigations are needed to determine the concentration of essential oils (minimum inhibitory concentration) needed to exhibit antimicrobial activity against food related microorganisms and thus explore their possibility as natural antimicrobial agents for food safety.

TABLE 3: Inhibition zone of Syzygium *aromaticum* on bacterial isolates by disc diffusion method and agar punch well method

Bacterial Isolates	Inhibition zone (mm)	
	Disc diffusion method	Agar punch well method
Culture I *Streptococcus lactis*	20±1.0	18±.57
Culture II *Micrococcus luteus*	24.3±.577	22±1.0
Culture III *Bacillus sp.*	29±1.0	26.6±1.52
Culture IV *Enterobacter aerogenes*	20.6±1.52	20.6±1.52
Culture V *Pseudomonas aeruginosa*	32±1.0	30±1.0
CultureVI *Bacillus cereus*	34.6±1.52	30.3±1.52
CultureVII *Escherichia coli*	32.3±1.1	29.3±1.52
Culture VIII *Klebsiella pneumoniae*	26±1.0	21±1.0
Culture IX *Staphylococcus aureus*	32.6±1.52	28.6±1.54
Culture X *Proteus vulgaris*	28.3±1.52	22.6±1.52

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