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Antibacterial Activities of *Syzygium aromaticum* oil Against Local Clinical Pathogenic Bacteria

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Abstract: *Eighteen bacterial isolates gram negative and gram positive were collected from King Fahd central hospital, Jazan region, Saudi Arabia. Four of these isolates belonging to Pseudomonas aeruginosa, three to Acintobacter baumanni and Klebsiella Pneumonia, two to Methicillin-resistant Staphylococcus aureus (MRSA) and E. coli, and one isolate to Enterobacter sp., E. aerogenes, Staphylococcus capitis and S. epidermis. Bacterial isolates were isolated from different sources e.g., blood, swab, throat, trachea, urine, wound. The antimicrobial activity of four plant oils; cloves (Syzygium aromaticum), ginger (Zingiber officinale), camphor (Cinnamomum camphora), and cinnamon (Cinnamomum verum) were obtained from the local market were evaluated on twelve sensitive and resistant isolates of the collected bacteria. Clove oil showed highest antimicrobial potential on different bacterial isolates (MIC_s ranged from 0.3125 to 3.75 µg/ml). The antimicrobial activity of clove oil was evaluated alone or incombination with 10µg Gentamycin or 5µg Rifampicin, antibiotics on both gram-negative and gram-positive bacteria. Association of antibiotics and clove oil showed synergistic antibacterial activity against tested bacteria.*

Keywords: *Antibiotics, Clove, medical and aromatic plant, gram-positive & gram-negative bacteria, Jizan area*

1. INTRODUCTION

In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant (WHO, 2001, Aibinu *et al.,* 2003; Aibinu *et al*., 2004). The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality (Williams, 2000). Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs (Pretorius *et al.,* 2003, Moreillion *et al.,* 2005).

Aromatic oils are used in many industries, including food preservation, pharmacy, and medicine . They are expected to form new sources of antimicrobial drugs, especially against bacteria(El-gayyar *et al.,* 2001, Raskin *et al.,* 2002, Bankole *et al.,* 2007).

Spices as clove, camphor, cinnamon and ginger have been employed for centuries as food preservatives and as medicinal plants mainly due to its antioxidant and antimicrobial activities. Nowadays, many reports confirm the antibacterial, antifungal, antiviral and anticarcinogenic properties of spice plants. Clove in particular has attracted the attention due to the potent antioxidant and antimicrobial activities standing out among the other spices (Shan *et al.,* 2005)**.** *Syzygium aromaticum* commonly known as clove, is an median size tree from the Mirtaceae family native from the Maluku islands in east Indonesia. For centuries the trade of clove and the search of this valuable spice stimulated the economic development of this Asiatic region (Kamatou *et al.,* 2012).

Clove represents one of the major vegetal sources of phenolic compounds as flavonoids, hidroxibenzoic acids, hidroxicinamic acids and hidroxiphenyl propens (Jirovetz *et al.,* 2006). With regard to the phenolic acids, gallic acid, other gallic acid derivates as hidrolizable tannins. Also, other phenolic acids found in clove are the caffeic, ferulic, elagic and salicylic acids (Shan *et al.,* 2005)**.** The antimicrobial activities of clove have been proved against several bacterial strains e.g., *Escherichia coli* (*E. coli*), *Staphylococcus aureus* and *Bacillus cereus* (Sofia *et al.,* 2007).

The present study was aimed to, (i) collect samples of different pathogenic bacteria from King Fahd central hospital, Jazan region, Saudi Arabia, (ii) determining the in vitro antibacterial activity of four medical and aromatic plants, cloves, camphor, cinnamon and ginger on both gram-negative gram-positive bacteria, (iii) determine the minimum inhibitory concentration (MIC) of the pure clove oil and the minimum

bactericidal concentration (MBC) for the tested bacteria, (IV) determine the synergistic effect of clove oil alone or in-combination with each of Gentamycin or Rifampicin antibiotics against tested pathogenic bacteria.

2. MATERIALS AND METHODS

Collecting pathogenic bacteria:

Eighteen bacterial isolates were supplied by King Fahd central hospital, Jizan region, Saudi Arabia. All isolates whether gram negative or gram positive were pathogenic and isolated from different patients. Twelve sensitive and resistance isolates including; *E. coli*, *Enterobacter* sp., *Klebsiella Pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (MRSA), *S. capitis* and *S. epidermis* were selected for all tests. Culture of these 12 isolates were supplied on selective media and sub-cultured on nutrient agar slants. The slants are preserved at 4°C after incubation for 24-48h at 37°C.

Inoculum preparation:

Fifty ml nutrient broth in 100 ml capacity flask was inoculated with 5 ml bacterial suspension from 24-48h growing slants. The flasks were incubated for 24h and the optical density was measured and the turbidity was adjusted to 0.5 McFarland standard solutions at 600 nm. Colony forming unit was also done on nutrient agar to assure the bacterial count. The inoculum was adjusted to $1x10^8$ cells, cfu before each experiment.

Source of plant oils:

Pure oils of cloves (*Syzygium aromaticum*), ginger (*Zingiber officinale*), camphor (*Cinnamomum camphora*), and cinnamon (*Cinnamomum verum*) were obtained from the local market.

The minimum inhibitory concentration (MIC) determination:

The minimum inhibitory concentration (MIC) of the pure clove oil was determined using the serial dilution technique. Clove oil (50 µl) was added to nutrient broth and inoculated with the bacterial suspension of the sensitive bacterial isolates and incubated for 24-48h at 37°C. At the end of the incubation period the tubes were examined for the presence or absence of growth at the different agents concentration. The least concentration that shows no visible growth was used as MIC for each organism.

The minimum bactericidal concentration (MBC) determination

The minimum bactericidal concentration (MBC) was determined by cultivation of nutrient broth as well as nutrient agar from the tubes that show no growth in MIC determination. The least concentration that shows no growth was used as MBC for each organism.

Determination the synergistic effect of clove oil with antibiotics:

The synergistic effect of clove oil with two antibiotics; 10µg from Gentamycin and 5µg from Rifampicin were tested. The experiment was performed with disk diffusion method. The bacterial isolates were cultivated using pour plate technique. Antibiotics discs were putted on the surface of the inoculated plates either alone or combined with clove oil. The plates were left in refrigerator for three hours to allow diffusion. The plates were incubated for 24-48h at 37°C. The inhibition zone (clear zone) was measured in (mm). Treatments were replicated five times.

Statistical analysis:

Data obtained were statistically analyzed according to SAS software program (SAS, 1997). Comparison among means was made via the least significant difference test (LSD) at ≤ 5% level of probability.

3. RUSTLES

Data in Table (1) showed that eighteen bacterial isolates were collected from King Fahd central hospital, Jazan region, Saudi Arabia. Twelve isolates are gram negative and six are gram positive. Data indicated the presence of four isolates of *Pseudomonas aeruginosa*, three of *Acintobacter baumanni* and *Klebsiella Pneumonia*, two of Methicillin-resistant *Staphylococcus aureus* (MRSA), two of *E. coli* and one isolate of each of, *Enterobacter* sp., *E. aerogenes, Staphylococcus capitis* and *S. epidermis*. All these isolates were isolated from different sources e.g., blood, swab, throat, trachea, urine, wound (Table,1).

Table 1: *Different bacterial genera isolated from different sources, collected from King Fahd central hospital, Jazan region, Saudi Arabia*

No.	Source of isolation	Bacterial isolates	Gram stain reaction
1	Blood	*Acintobacter baumanni*	G+
2	Trachea	*A. baumanni*	G+
3	Wound	*A. baumanni*	G+
4	Urine	*Escherichia coli*	G-
5	Urine	*E. coli*	G-
6	Wound	*Enterobacter* sp.	G-
7	Wound	*E. aerogenes*	G-
8	Throat	*Klebsiella Pneumonia*	G-
9	Wound	*K. Pneumoniae*	G-
10	Wound	*K. Pneumoniae*	G-
11	Trachea	*Pseudomonas aeruginosa*	G-

12	Wound	*P. aeruginosa*	G-
13	Wound	*P. aeruginosa*	G-
14	Wound	*P. aeruginosa*	G-
15	Wound	*Staphylococcus aureus* (MRSA)	G+
16	Swab	*S. aureus* (MRSA)	G+
17	Blood	*S. capitis*	G-
18	Blood	*S. epidermis*	G+

Data pertaining to the antimicrobial potential of the plant oils are presented in Table 2 and Fig. 1, respectively. Camphor, cinnamon, clove and ginger oils presented antimicrobial activity to at least two of the tested bacteria. Clove oil presented the highest activities, it was able to inhibit 9 (75.0 %) types of tested bacteria, followed by ginger oil, which inhibited 3 (25.0%) types of tested bacteria. Meanwhile, both of camphor, cinnamon oils were capable of inhibited 2 (16.7 %) types of tested bacteria (Table, 2 and Fig. 1).

Table 2: *Inhibitory effect of different commercial pure oils on different bacterial genera collected from King Fahd central hospital, Jazan region, KSA*

Bacterial isolates	Camphor	Cinnamon	Clove	Ginger
E. coli	-	-	0.45 ab	-
E. coli	-	-	0.60 b	-
Enterobacter sp.	-	-	0.40 b	-
Klebsiella Pneumonia	-	0.35 a	0.30 a	0.30 a
K. Pneumoniae	0.30 a	-	-	0.30 a
K. Pneumoniae	-	0.30 a	0.40 ab	-
Pseudomonas aeruginosa	-	-	0.45 ab	-
P. aeruginosa	-	-	-	-
Staphylococcus aureus (MRSA)	-	-	0.35 ab	-
S. aureus (MRSA)	0.35 a	-	-	0.35 a
S. capitis	-	-	0.40 ab	-
S. epidermis	-	-	0.35 a	-

Data are averages of 5 replicates. Values, within each column, followed by the same letter(s) are not significantly different at ($P \leq 0.05$). Inhibition zone was measured in (mm).

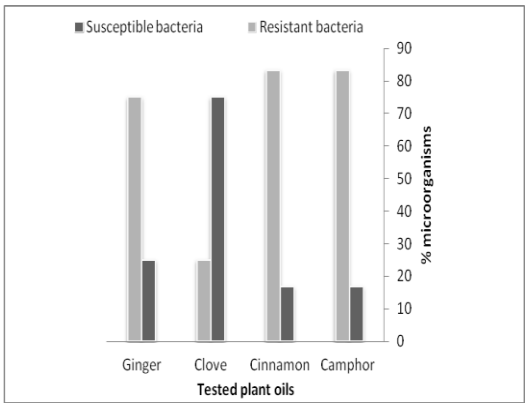

Fig 1: *Antimicrobial activity from plant oils against susceptible and resistant microorganisms*

The minimum inhibitory concentration (MIC) of clove oil was showed in Table (3) and Fig. 2. Results indicated that clove oil showed antibacterial activities at variable degrees against tested bacteria, with MICs values varying from 0.3125 to 3.75 µg/ml. Also, minimal bactericidal concentration (MBC) ranged from 0.625 to 7.5 µg/ml Table (3) and Fig. 2.

Table 3: *The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of clove commercial pure oil on bacteria collected from King Fahd central hospital, Jazan region, KSA*

Bacterial genera	MIC mg/ml	MBC mg/ml
E. coli	3.75	7.5
E. coli	2.5	5.0
Enterobacter sp.	0.625	2.5
Klebsiella Pneumonia	1.875	3.75
K. Pneumoniae	2.5	5.0
K. Pneumoniae	1.25	3.75
Pseudomonas aeruginosa	0.625	0.9375
P. aeruginosa	0.3125	0.625
Staphylococcus aureus (MRSA)	1.25	2.5
S. aureus (MRSA)	0.625	1.25
S. capitis	1.25	2.5
S. epidermis	1.875	2.5

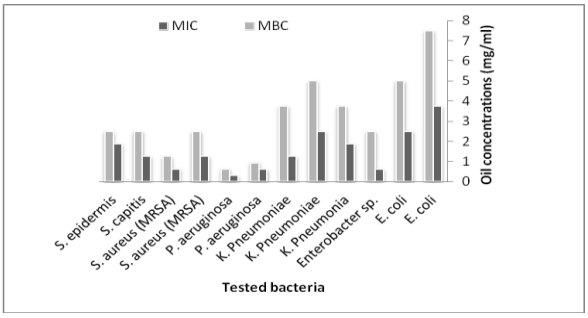

Fig 2: *MIC and MBC of clove commercial pure oil on bacteria collected from King Fahd central hospital, Jazan region, KSA*

Data in Table (4) showed the synergistic effect of clove oil alone or in combination with 10µg Gentamycin or 5µg Rifampicin on the tested bacteria. One isolate of each of *K. Pneumoniae*, *S. capitis* and *S. epidermis* showed resistance against treatment with 10µg Gentamycin alone. Meanwhile, three isolates of *K. Pneumoniae,* two isolates of *E. coli, and P. aeruginosa,* one isolate of each of *Enterobacter* sp. and *S. epidermis* showed resistance against treatment with 5µg Rifampicin alone. The other bacterial isolates were sensitive to both of antibiotics Gentamycin and Rifampicin. Combination between 50µl of clove oil and one of the previous antibiotics resulted in synergistic effect on all tested bacteria (Table,4).

Table 4: *Synergistic effect of clove pure oil alone or with two different antibiotics on bacteria collected from King Fahd central hospital, Jazan region, KSA*

Bacterial genera	Gentamycin only	Oil + Gentamycin	Rifampicin Only	Oil + Rifampicin
E. coli	0.20 a	0.25 a	-	0.40 ab
E. coli	0.20 a	0.25 a	-	0.30 ab
Enterobacter sp.	0.30 ab	0.25 a	-	0.35 ab
Klebsiella Pneumonia	0.35 ab	0.25 a	-	0.50 b
K. Pneumoniae	0.25 a	0.35 ab	-	0.20 a
K. Pneumoniae	-	0.40 b	-	0.30 ab
Pseudomonas aeruginosa	0.20 a	0.25 a	-	0.20 a
P. aeruginosa	0.40 b	0.40 b	-	0.30 ab
Staphylococcus aureus (MRSA)	0.25 a	0.35 ab	0.40a	0.30 ab
S. aureus (MRSA)	0.40 b	0.20 a	0.40 a	0.30 ab
S. capitis	-	0.30 ab	0.35 a	0.25 a
S. epidermis	-	0.40 b	-	0.50 b

The diameter of Inhibition zone was measured in (mm). Gentamycin and Rifampicin were used at concentration 10 and 5µg, respectively. Data are averages of 5 replicates. Values, within each column, followed by the same letter(s) are not significantly different at ($P \leq 0.05$).

4. DISCUSSION

In our work the antimicrobial activity of four plant oils, camphor, cinnamon, clove and ginger were tested against different 12 bacterial isolate. Clove oil inhibited 75.0 % of tested bacteria, followed by ginger oil, with 25.0% and camphor, cinnamon oils with 16.7 % of the tested bacteria. This findings are in agreement with those of other workers (Nascimento *et al.,* 1990; Saxena *et al.,* 1994; Alonso *et al.,* 1995).

The microorganism *E.coli*, which is already known to be multi-resistant to drugs, was also resistant to the plant oils. It was susceptible only to clove. On the other hand, *P. aeruginosa*, had its growth inhibited by clove oil. Such results are very interesting, because this bacterium was isolated from a hospital environment and its control is very difficult by therapeutic means. Studies regarding the mode of action for these plant oils in the bacterial cell should be done (Chandler *et. al.,* 1982; Ellof, 1998; Nascimento *et al.,* 2000).

The minimum inhibitory concentration (MIC) of clove oil has antibacterial activities at variable degrees against tested bacteria, varying from 0.3125 to 3.75 µg/ml on both, gram-negative and gram-positive bacteria. This findings are in agreement with those of other workers (Saxena *et al.,* 1994; Nascimento *et al.,* 2000).

Nascimento *et al.* (2000) reported that clove extracts showed activities in the range (concentrations) from 20 to 250 µg/ml.

The present data showed the synergistic effect of using clove oil (50µl) alone or in combination with 10µg Gentamycin or 5µg Rifampicin on both gram-negative and gram-positive bacteria. This data are agreed with (Chandler *et. al.,* 1982; Carvalho *et al.*, 1988; Bisset, 1994).

Chandler *et. al.* (1982) reported that a synergistic effect was observed for *P. aeruginosa*, which is resistant to 19 different antibiotics. This occurred during the association of antibiotics with extracts from clove, jambolan, pomegranate and thyme.

Bisset (1994) reported that this effect was also observed for *K. pneumoniae* when 20 µg/mL of clove extract was combined to ampicillin. Also, Carvalho *et al.* (1988) reported that the growth of *Proteus* spp. was inhibited when either clove extract (10 µg/mL) or eugenol (5 µg/mL) was combined to tetracycline.

Meanwhile, our data are disagreed with Bhatia and Bajaj (1975) and Cohen, (1992) who reported that no synergetic effect was observed when different concentrations of extracts from lemon balm, clove and eugenol were combined with ampicillin to inhibit the growth of *K. pneumoniae* and *E. aerogenes*. Only the association of thyme (20 µg/mL) with ampicillin was able to cause such an effect.

Data from the literature as well as our results reveal the great potential of plant oils for therapeutic treatment, in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted. Therefore, our results revealed the importance of plant oils when associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health.

REFERENCES

Aibinu, I, Adenipekun, E and Odugbemi, T. 2004. Emergence of quinolone resistance amongst *Escherichia coli* strains isolated from clinical infections in some Lagos State Hospitals in Nigeria. Nigerian J. Health and Biomedical Science 3(2):73-78.

Aibinu, I. E., Ohaegbulam, V. C., Adenipekun, E. A., Ogunsola, F. T., Odugbemi, T. O. and Mee, B. J.

2003. Extended-spectrum beta-lactamase enzymes in clinical isolates of *Enterobacter* species from Lagos, Nigeria. J. Clinical Microbiology 41(5):2197-2200.

Alonso-Paz, E., Cerdeiras, M. P., Fernandez, J., Ferreira, F., Moyna, P., Soubes, M., Vazquez, A., Veros, S., Zunno, L. 1995. Screening of uruguayan medicinal plants for antimicrobial activity. J. Ethnopharmacology 45, 67-70.

Bankole, M. A., Shittu, L. A., Ahmed, T. A., Bankole, M. N., Shittu, R. K. and Kpela, T. 2007. Synergistic antimicrobial activities of phytoestrogens in crude extracts of two sesame species against some common pathogenic microorganisms. Afr. J. Tradit. Complement Altern. Med., 4:427-33.

Bhatia, I. S. and Bajaj, K. L. 1975. Chemical constituents of the seeds and bark of *Syzium cumini*. Planta Med., 28, 347-352, 1975.

Bisset, N. M. 1994. Herbal Drugs and Phytopharmaceuticals. CRC Press, London, 566 pp.

Carvalho, V., Melo, V. M., Aguiar, A. and Matos, F. S. 1988. Toxicity evaluation of medicinal plant extracts by the brine shrump (*Arthenus salina* Leah) biossay. Ciência e Cultura 40, 1109-1111.

Chandler, R. F., Hooper, S. N. and Harvey, M. J. 1982. Ethnobotany and phytochemistry of yarrow, *Achillea millefolium*, Compositae. Econ. Bot., 36, 203-223.

Cohen, M. L. 1992. Epidemiology of drug resistance: implications for a post antimicrobial era. Science 257, 1050-1055.

El-gayyar, M., Draughon, F. A., Golden, D. A. and Mount, J. R. 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J. Food Prot., 64:1019-24.

Ellof, J. N. 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol., 60: 1-6.

Jirovetz, L., Buchbauer, G., Stoilova, I., Stoyanova, A., Krastanov, A. and Schmidt, E. 2006. Chemical composition and antioxidant properties of clove leaf essential oil. J. Agric. Food Chem., 54 (17): 6303-6307.

Kamatou, G. P., Vermaak, I. and Viljoen, A. M. 2012. Eugenol-from the remote maluku islands to the international market place: a review of a remarkable and versatile molecule. Molecules 17(6): 6953-6981.

Moreillion, P., Que, Y. A., and Glauser, M. P. 2005. *Staphylococcus aureus* (Including Staphyloccal Toxic shock). In 'Principles and Practice of Infectious diseases.' (Ed.) Mandell, G.L., Bennett, J. E., Dolin, R. Published by Churchill livingstone Pennyslyvania 6th ed. 2: 2333- 2339.

Nascimento, G. G. F., Locatelli, J., Freitas, P. C. and Silva, G. L. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian J. Microbiology 31:247-256.

Nascimento, S.C., Chiappeta, A. and Lima, R. M. O. C. 1990. Antimicrobial and cytotoxic activities in plants from Pernambuco, Brazil. *Fitoterapia* 61, 353-355.

Pretorius, J. C., Magama S. and Zietsman, P. C. 2003. Growth inhibition of plant pathogenic bacteria and fungi by extracts from selected South African plant species South African J. Botany 20: 188-192.

Raskin, I., Ribnicky, D. M., Komarnytsky, S., Ilic, N., Poulev, A. and Borisjuk, N. 2002. Plants and human health in the twenty-first century. Trends Biotechnol., 20:522-31.

SAS Institute. 1997. SAS/STAT User's Guide.6*th* Edition. SAS Institute Inc., North Carolina, Cury. Inc., 1028 pp.

Saxena, G., McCutcheon, A. R., Farmer, S., Towers, G. H. N., Hancock, R. E. W. 1994. Antimicrobial constituents of *Rhus glabra*. J. Ethnopharmacol., 42, 95-99.

Shan, B., Cai, Y. Z., Sun, M. and Corke, H. 2005. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. J. Agric. Food Chem., 53(20): 7749-7759.

Sofia, P. K., Prasad, R., Vijay, V. K. and Srivastava, A. K. 2007. Evaluation of antibacterial activity of Indian spices against common food borne pathogens. Int. J Food Sci. Technol., 42(8): 910-915.

Williams, R. 2000. Antimicrobial resistance a global threat. Essential Drug Monitor,28-29:1

World Health Organization (WHO). 2001. Traditional medicine. Fact sheet number 134. Revised May, 2003.