Short Communication

Bactericidal effects of essential oils from clove, lavender and geranium on multi-drug resistant isolates of *Pseudomonas aeruginosa*

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Abstract

The inhibitory effects of essential oils including clove, lavender and geranium extracted from Eugenia caryophyllata, Lavandula officinalis and Pelargonium graveolens on multi-drug resistant isolates of Pseudomonas aeruginosa were investigated. The main constituents of clove, lavander and geranium oil were eugenol (80-90%), 1,8-cineol (13%) and citronellol (45%) respectively. Clove had the most effective essential oil against P. aeruginosa. A combination consisting of clove, lavender and geranium oils at a ratio of 3:1:1 showed the most inhibitory effect (32-64 µg/ml) and strong synergy with gentamicin. The essential oils from clove, lavender and geranium exhibited bactericidal activity against multi-drug resistant strains of P. aeruginosa and may be alternatives compounds against these strains in the future.

Keywords: *Essential oils; Clove; Lavender; Geranium; Pseudomonas aeruginosa; Antimicrobial agents.*

Pseudomonas aeruginosa is one of the major causes of serious infections in burn patients, resulting in mortality as high as 50% (Cohen, 1992; Lari *et al*., 2000; Shahcheraghi *et al*., 2003). This bacterium also causes opportunistic infections in different organs leading to diseases such as, bacteremia, and endocarditis. Resistance to different antibiotics is a major therapeutic problem in treatment of infections by this organism. In the last decade, resistance to the new generation of antibiotics within the population of *P. aeruginosa* has increased. The emergence of multi-drug resistant bacteria including *P. aeruginosa* strains has raised the needs for new antimicrobial drugs (Santos *et al*., 1995).

Essential oils are the concentrated, hydrophobic liquids containing volatile aromatic compounds from plants. They possess a wide spectrum of pharmacological activities. The antimicrobial effects of essential oils have been documented and used in herbal medicine in many countries (Schilcher, 1998; Cowan, 1999; Schilcher, 2002; Longbottom *et al*., 2004; Sonboli *et al*., 2005).

Antibacterial effects of hydrous, methanolic and ethanolic extracts of clove, cinnamon, sage, thyme and rosmarinus on Gram-positive and Gram-negative bacteria had previously been investigated (Cowan, 1999). The results showed that all of these plants had antibacterial action on methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* (Cowan, 1999), but they were weakly active against Gram-negative bacteria such as *P. aeruginosa* and enteropathogenic *Escherichia coli* (Shanab *et al*., 2004). With the exception of *P. aeruginosa*, the essential oil of *Grammosciadium platycarpum* with its major components, linalool (70%) and limonene (10%), displayed strong to intermediate antibacterial activity against Gram positive and Gram negative bacteria (Sonboli *et al*., 2005). Similarly, the minimum inhibitory concentration (MIC) for the essential oil of tea tree oil against *P. aeruginosa* was found to be much higher (%2 ≤ MIC < 8%) than that against other bacteria.

Gram-negative bacteria are more resistant to antibiotics than the Gram-positive bacteria due to the permeability barrier provided by the cell wall or to the mem-

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Table 1. Aantibacterial activity and synergy of clove, geranium and lavender oils with gentamicin against different strains of *P. aeruginosa* determined by the disk diffusion method.

Strains	Inhibitory effects of essentials oils and their combinations (millimeters)										Synergy	
	C	G	L	CL 1:1	CLG 2:2:1	CLG 1:1:1	CLG 1:2:2	CLG 3:1:1	CLG 2:1:1	CLG 1:3:1	Gen	Gen+ CLG 3:1:1
ATCC 9027	18	13.6	16	14	14.3	15.3	15	18	16.3	14.3	24	24
513	13	12	12.5	12.5	14.5	12	13.5	13.5	13	15	-	20
514	12.5	10.5	12	13.5	15	13.5	12.5	16	14	15	-	16
515	14.5	10.5	11	13.5	13.5	14.5	13.5	14.5	12	13	22	22
516	11.6	11	11.3	15	11.6	13	13.6	14.3	13	12.3	20	22
589	13.3	10.6	12	13.3	12.3	13.6	12.3	13	12.3	13	-	14
502	12	12	12	13	11.5	15.5	11.5	13	13	12.5	-	16
594	13	11	11.5	13.7	14.7	12.7	11.5	14.2	14	11	-	13
584	12	9.3	11.6	13	13	12.3	11.6	14.6	13	13.5	-	15
581	13	10.5	13.5	13	12.5	13	11.5	14.5	11	13	-	16
586	14.3	12	11.6	14.3	14	14.3	13	13.5	14	14.6	-	14

C: Clove oil, G: Geranium oil, L: Lavander oli, Gen: Gentamicin.

brane accumulation mechanism (Shanab *et al*., 2004). Geranium, clove and lavender possess antimicrobial activities against a wide range of microorganisms (Santos *et al*., 1995; Nascimento *et al*., 2000; Shanab *et al*., 2004). However, their bactericidal effects on *P. aeruginosa* strains have not yet been recognized.

To investigate the *in vitro* bactericidal effect of geranium, clove and lavender oils on *P. aeruginosa*, 10 isolates (isolates number 513-516, 502, 581, 584, 586 and 594) that were cultured from burn patients were used in this study. *P. aeruginosa* type strain ATCC 9027 was used as control in all experiments. The clinical isolates were multi resistant to gentamicin, ciprofloxacin and ceftazidime. The phenotypic characteristics of the clinical isolates have been reported elsewhere (Shahcheraghi *et al*., 2003).

The essential oils were extracted from the appropriate plants by steam distillation (Guenther, 1982). Lavender oil (L) extracted from the flower of Lavandula (*Lavandula officinalis*) is yellowish or yellow-green in colour with a pleasant aroma and contains 1,8-cineol (13%). Geranium oil (G) was extracted from *Pelargonium graveolens*. It is a colorless or green-blue liquid with a floral aroma. The major fractions of this oil are citronellol (45%) and geranial (10-12%) (Guenther, 1982). Clove oil (C) was extracted from the dried flower buds of *Eugenia caryophyllata*. It has a warm, strong, spicy smell and the oil is colorless to pale yellow with a medium to watery viscosity.

Antibacterial activities of the essential oils against *P. aeruginosa* were determined by the disk diffusion and macrobroth dilution assays as recommended by National Committee for Clinical Laboratory Standards (NCCLS, 2000; NCCLS, 2001). The isolates were inoculated onto Mueller-Hinton agar (MHA) and the disks impregnated with the essential oils were placed on the inoculated plates. C, L and G were tested separately and in combinations at different ratios (1:2, 2:1, 1:1, 2:2:1, 1:1:1, 1:2:2, 3:1:1, 2:1:1, 1:3:1). In brief, the surfaces of plates containing the MHA were swabbed with suspension of isolate adjusted to 0.5 McFarland (approximately 10^8 CFU/ml). The McFarland turbidity standard was prepared by adding 0.5 ml of 0.048 mol/l barium chloride (1.173 g $BaCl_2$. $2H_2O$; 1.175% w/v) to 99.5 ml of 0.18 mol/l (0.36 N; 1% v/v) H_2SO_4 (NCCLS, 2001). The blank disks, 6 mm in diameter (Pad-Tan Teb, Tehran, Iran) were impregnated with 20 µl of each essential oil at a concentration of 100 mg/ml. Negative control was prepared using the same solvents (DMSO) employed to dissolve the essential oils. Dimethyl sulfoxide (DMSO) had no antimicrobial effect (Kontoyiannis *et al.,* 2003, Shahidi Bonjar 2004, Baris *et al.,* 2006). All the plates were incubated at 37ºC for 24 h. Antibacterial activity was assessed by measuring the inhibitory zones around the disks (Table 1). The MICs were determined by the macrobroth dilution method using 2-fold dilutions of essential oils ranging from 4 to 512 µg/ml (NCCLS, 2001). The sizes of the inocula were adjusted to 10^5 CFU/ml and inoculated tubes were incubated at 37ºC for 18 h (NCCLS, 2001). The MBCs of the oils were determined by plating 100 µl from each tube used for determining MIC and observed for any growth after 2 days of incubation.

The inhibitory zones of the 10 clinical strains were smaller than those of the reference strain. Shanab and

Table 2. Determination of MIC and MBC of different combination of essential oils on the reference strain (*Pseudomonas aeruginosa* ATCC 9027) by the macrobroth dilution method.

Concentration (µg/ml)	Mixtures of different essential oils at different ratios								
	C	CG 1:1	CL 1:1	CLG 1:1:1	CLG 2:1:1	CLG 2:2:1	CLG 1:3:1	CLG 3:1:1	CLG 1:2:2
MIC	93.75-187.5	93.75-187.5	93.75-187.5	62.5-125	62.5-125	31.25-62.5	31.25-62.5	31.25-62.5	62.5-125
MBC	187.5-375	187.5-375	187.5-375	125-250	125-250	62.5-125	62.5-125	62.5-125	125-250

C: Clove oil, G: Geranium oil, L: Lavander oli, Gen: Gentamicin, MIC: minimum inhibitory concentration, MBC: Minimum bactericidal concentration.

Table 3. Determination of the MIC and MBC of a mixture containing of Clove, Lavander and Geranium (3:1:1) on clinical isolates by the macrobroth dilution method.

Concentrations (µg/ml)	Clinical Strains									
	513	502	514	515	581	586	589	584	594	516
MIC	32-64	32-64	32-64	32-64	32-64	32-64	16-32	32-64	16-32	32-64
MBC	64-128	64-128	64-128	64-128	64-128	64-128	64-128	64-128	64-128	64-128

MIC: minimum inhibitory concentration, MBC: Minimum bactericidal concentration.

colleagues (2004) reported that the ethanolic extract of clove inhibited the growth of *P. aeruginosa* (MIC 781 µg/ml). This was also confirmed by the results of this study. However, in our study the clove oil inhibited the growth of *P. aeruginosa* at concentrations between 93.75 to 187.5 µg/ml. This study also demonstrated that clove oil possessed bactericidal activity at concentrations between 187.5 to 375 µg/ml. Therefore, the clove essential oil extracted by steam distillation showed more activity than ethanolic extraction and may have therapeutic value.

Combination of clove, lavender and geranium at a ratio of 3:1:1 showed the best inhibitory effect on both the reference strain and clinical isolates of *P. aeruginosa* (MIC=32-64 µg/ml). However, differences between the MICs and minimal bactericidal concentrations (MBCs) of different oils were found when they were tested on the reference strain separately (Table 2).

To determine the synergistic effect with gentamicin, the essentials oils were added to the disks containing this antibiotic (gentamicin 10 µg/disk, Himedia Laboratories Pvt Limited, Mumbai, India) (Muroi and Kubo, 1996). The combination of clove, lavender and geranium oils at a ratio of 3:1:1 showed the most inhibitory effect (32-64 µg/ml) and strong synergy with gentamicin (Table 1).

The MBCs of the essential oils (3:1:1) for the reference strain did not differ from those of the clinical isolates and varied from 64-128 µg/ml (Tables 2 and 3).

Lavender oil is more effective against Gram-negative than Gram-positive bacteria. So, the use of lavender and clove oil as herbal drugs may be an alternative choice for the treatment of infections caused by Gram-negative bacteria such as the multi-drug resistant *P. aeruginosa*. Experimental studies with animals are needed to confirm our findings *in vivo*.

Acknowledgements

We thank N. Kazempour, M. Hosseini and Z. Haghighizadeh for their technical assistance, and are very grateful to Mr. Hejazi for his financial support of the project.

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