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Antimicrobial activity of Rosemary, Fennel and Galbanum essential oils against clinical isolates of *Staphylococcus aureus*

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Abstract. The essential oils from rosemary (*Rosmarinus officinalis* L.), galbanum (*Ferula gummosa*), and fennel (*Foeniculum vulgare*) were obtained from Barij Essence Pharmaceutical Company and were analyzed by GC and GC-MS. The essential oils were evaluated for their anti-staphylococcal activities against Methicillin Sensitive (MSSA) and Resistant *S. aureus* (MRSA) by disc diffusion and micro broth dilution assays. GC-MS analysis of oils showed that β-pinene, α-pinene and trans-anethole as the major components of galbanum, rosemary and fennel oils, respectively. The Inhibition Zone diameters (IZ) of essential oils in disc diffusion assay increased in a dose dependent manner and in different concentrations of oils, the IZs were compatible with vancomycin (30 µg). Altogether, antimicrobial evaluations exhibited that galbanum oil had the best antimicrobial activity against MRSA and MSSA, followed by fennel and rosemary oil, respectively.

Keywords: Rosemary; Fennel; Galbanum; Essential oil; Methicillin; *Staphylococcus aureus*.

Introduction

Staphylococcus aureus, a gram positive, non motile, catalase and coagulase positive, facultative anaerobe coccus is a common type of bacteria that normally lives on the skin and nasal passages of healthy people. When it enters the body through a cut or other medical devices, it can cause local or serious infections (Franklin 1998). Methicillin Resistant *S. aureus* (MRSA) has become one of the major causes of nosocomial and community pathogens causing significant morbidity and mortality because there are multi drug resistant pathogens that are resistant to all penicillins, so the option antibiotics for treatment of MRSA infections are limited to antibiotics such as vancomycin, tigecycline, lincozolid and mupirocin (Simor et al. 2007). The patterns of antimicrobial susceptibility of *S. aureus* have been changed worldwide and it has been reported increasingly to be less effective. Development of mupirocin (dos Santos et al. 2007) and vancomycin (Appelbaum 2006) microbial resistance in MRSA has increased in settings with extensive use of these agents. Microbial resistances to conventional antibiotics and adverse effects of these agents have led to find new sources as antimicrobial agents. Medicinal plants have a long history of use as traditional medicines for treatment of different kinds of ailments especially for infectious diseases.

Galbanum, is one of the most important resins from roots and aerial parts of *Ferula gummosa* (Apiaceae) and is one of the most important rangeland products of Iran with high export demand (Nadjafia et al. 2006). Galbanum is used traditionally as food flavor for treatment of some gastrointestinal disorders such as stomach pain, and as antileptic remedy for epilepsy, cholera and as wound healing remedy (Zargari 1995). Galbanum oil can be applied to neck or drunk for improving the memory (Adams et al. 2007).

Foeniculum vulgare (Apiaceae) is a well-known Umbelliferous plant, commonly known as fennel. It is a perennial herb that grows all over the world and is used traditionally from ancient times as carminative, antiseptic, expectorant, digestive and diuretic agents. The seeds of fennel have been used to regulate menstruation, alleviate the symptoms of female climacteric syndrome and dysmenorrheal and increased libido (Albert-Puleo 1980).

Rosmarinus officinalis L. (Labiatae) commonly known as rosemary, traditionally used as antispasmodic and for treatment of dysmenorrhea, respiratory disorders, nervous ailments and to stimulate growth of the hair (Zargari 1995).

The aim of this study was to evaluate the antistaphylococcal activities of rosemary, galbanum and fennel oils against clinical isolates and identifying the chemical composition of the essential oils related to it.

Materials and Methods

Essential oils and identification of chemical compositions of the oils

The essential oils from aerial parts of rosemary, seeds of fennel and the resin of galbanum were prepared from Barij Essence Pharmaceutical Company, Kashan, Iran.

The oil analysis was carried out using GC and GC/MS. The GC apparatus was Agilent technology (HP) 6890 system, capillary column of HP-5MS (60 m × 0.25 mm, film thickness 0.25 µm). The oven temperature program was initiated at 40 °C, held for 1 min then raised up to 230 °C at a rate of 3 °C /min held for 10 min. Helium was used as the carrier gas at a flow rate 1.0 ml/min. The detector and injector temperatures were 250 and 230 °C respectively. GC/MS analysis was conducted on a HP 6890 GC system coupled with 5973 network mass selective detector with a capillary column the same as above, carrier gas helium with flow rate 1 ml/min with a split ratio equal to 1/50, injector and oven temperature programmed was identical to GC. The compounds of the oil were identified by comparison of their retention indices (RI), mass spectra fragmentation with those on the stored Wiley 7n.1 mass computer library, and NIST (National Institute of Standards and Technology)(Adams 2001).

Staphylococcal isolates

Twelve clinical isolates of *S. aureus* cultured from patients and *S. aureus* ATCC 25923 were used in all experiments. Methicillin resistant *S. aureus* directed detected on CHROMagar™ MRSA (CHROMagar Paris, France). Bacterial suspensions were made in Brain Heart Infusion (BHI) broth to concentration of approximately 10^8 CFU/ml using standard routine spectrophometrical method. Subsequent dilutions were prepared from the above suspensions, which were then used in the tests.

Disc diffusion method

The disc diffusion method was employed for determination of antimicrobial activity of essential oil. Briefly, using a sterile cotton swab, above microbial suspensions was spread on the Mueller Hinton Agar (MHA) plates. Sterile paper discs (6 mm in diameter) were impregnated with 10, 15, 20 µl of each oil and were placed on the inoculated plates. After re-

maining at 4 °C for 2 h, plates were incubated for 24 h at 37 °C. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate (NCCLS 2009).

Determination of minimum inhibitory and bactericidal concentrations

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of oil were determined by micro broth dilution assay. The oil was twofold serially diluted with 10% DMSO containing 32-0.0125 µl/ml of oil. These dilutions were prepared in a 96-well micro titter plate. Cation adjusted Muller Hinton broth was used as broth media. After shaking, 100 µl of oil was added to each well. The above microbial suspensions were diluted (1-5 x10^6 CFU/ml) and then 100 µl was added to each well and incubated at 35 °C. MIC was defined as the lowest concentration of oil that inhibits bacteria after 24. MBC value was the first well that showed no growth on Manitol Salt Agar (NCCLS 2009).

Results

Analyses of galbanum oil revealed forty four components which accounted for 100% of the total composition of oil, β-pinene (43.1%) and α-pinene (5.4%) were the main component of essential oil followed by β-cubebene (4.9%), epi-bicyclosesquiphellandrene (4.4%), p-cymene and 4-terpineol (4.1%) (Table 1).

Table1. Chemical composition of *F. gummosa* essential oil ([a]RI: Retention Index).

Compounds	RI[a]	(%)
α- thujene	852	2.5
α- pinene	860	5.4
β- pinene	905	43.1
β- myrcene	915	0.6
3-carene	931	0.6
p-cymene	942	4.1
β- phellandrene	946	0.3
1-limonene	949	0.8
Trans sabinene hydrate	980	1.2
Cis sabinene hydrate	1007	1.2
4-terpineol	1080	4.1
cyclosativene	1276	0.6
α- copaene	1288	0.7
β-elemene	1303	0.6
alloaromadendrene	1334	0.8
Delta selinene	1341	1.7
Epi-bicyclosesquiphellandrene	1351	4.4
soledene	1374	0.6
Gamma-cadinene	1384	4.1
α-amorphene	1385	1.8
Cis calamenene	1387	1.0
Delta cadinene	1390	2.0
eremophilene	1393	0.7
β- bisabolene	1404	0.8
spathulenol	1442	2.7
guaiol	1459	0.9
Cadina-1,4-diene	1476	0.8
Beta cubebene	1506	4.9
bulnesol	1528	1.2
14-norcadin-5-en-4-one isomer A	1532	1.4
viridiflorol	1534	0.6

Thirty three components were identified from rosemary essential oil representing 89.8% of the total oil. The major components were α-pinene (21.5%) and 1,8-cineole (15.2%) followed by verbenone (8.6%), camphor (6.8%) and camphene (6.3%) (Table 2).

Among twenty six identified components of fennel oil, representing 99.9% of total composition, trans-anethole (56.6%), β-thujone (13.2%) were found as the major compounds. p-anisaldehyde (8.7%), fenchone (7.42%) were other main components of fennel oil (Table 3).

The antistaphylococcal activity of essential oils by disc diffusion method exhibited that this effect increased in a dose dependent manner. Inhibition zone diameters of galbanum, fennel and rosemary oils were in compatible with vancomycin (30 µg) at 10, 15, >20 µl of oils, respectively.

Table2. Chemical composition of *R. officinalis* L. essential oil.

Compounds	RI	(%)
tricyclene	844	0.5
α- pinene	863	21.5
camphene	873	6.3
β- pinene	898	3.5
3-octanone	900	0.8
β- myrcene	914	1.6
p-cymene	944	3.0
1,8-cineole	950	15.2
1-limonene	951	2.8
Linalool	1015	2.6
chrysanthenone	1018	1.0
Camphor	1038	6.8
borneol	1069	8.6
4-terpineol	1077	1.5
verbenone	1097	8.6
(+)-2,2,3-trimethylcyclopent-3-ene-1-ethanol	1102	1.0
geraniol	1151	1.8
Borneol acetate	1174	6.1
Trans caryophyllene	1321	1.7
Caryphyllene oxide	1433	1.7

Table3. Chemical composition of *F. vulgare* L. essential oil.

Compounds	RI	(%)
α- pinene	855	0.4
sabinene	892	0.2
β-pinene	894	0.1
β- myrcene	912	0.1
p-cymene	936	0.2
1,8-cineole	942	0.3
1-limonene	948	4.2
fenchone	992	7.42
camphor	1032	0.3
p-allylanisole	1089	3.4
carvone	1122	1.4
m-anisaldehyde	1126	1.4
p-anisaldehyde	1141	8.7
Trans anethole	1185	56.6
β-thujaplicin	1269	13.2
1-m-anisyl-1-propanone	1320	0.2
1-(4-methoxyphenyl)-2-methyl-6-methoxy benzimidazole	2103	0.2
1-propanone,1-(4-methoxy phenyl)	2161	0.7

The diameter of growth inhibition zones ranged from 11.6-34.2 mm at 10-20 µl of galbanum oil with the highest in-

hibition zone value observed 25.6 mm at 10 µl of essential oil.

Fennel oil had antistaphylococcal activity with IZ of 7.2-24.1 mm at 20 µl of essential oil, the highest IZ was related to 2 clinical isolates of *S. aureus* in the ranges of 21.9-24.1 mm. 10 µl of Galbanum oil against under study isolates showed that seven isolates had IZ upper than 15 mm. 15 µl of fennel oil on 13 isolates of *S. aureus* showed that 3 isolates had IZ upper than 15 mm. Rosemary oil had IZs in the ranges of 7.9-15.2 mm. At 20 µl of oil, the IZs of all isolates were lower than that of 15 mm, disc diffusion assay exhibited that galbanum oil had the best antistaphylococcal activity followed by fennel and rosemary oil, respectively (Table 4).

In quantitatively test, the MIC, MBC values of galbanum, fennel, rosemary oils were in the ranges of 8-32, 4-32, 8-32 µl/ml. nine, eight, nine out of 13 isolates of *S. aureus* had the MIC values of 8, 16, 16 µl/ml for galbanum, fennel, rosemary oils, respectively. The almost MBC values for galbanum, fennel, rosemary oils were 16, 32, 32 µl/ml (Table 5).

Discussion

Three tested essential oils exhibited different degrees of antimicrobial activities against clinical isolates of *S. aureus*. The maximum anti-staphylococcal activity was shown by galbanum oil, followed by fennel and rosemary oils. Eftekhar et al (2004) reported the antibacterial activity of 25 µl of galbanum oil with β-pinene (50.1%) and α-pinene (18.3%) as main components by disc diffusion test against *S. aureus* ATCC 25923. Galbanum oil containing α-pinene (14.3%), β-pinene (14.1%) and sabinene (40.1%) inhibited the growth of *S. aureus* at MIC value of 3.125 µl/ml (Abedi et al. 2008). In this study, the chemical composition of galbanum oil is like as Eftekhar et al study (2004) and sabinene was not found in chemical composition of under study galbanum oil.

The antimicrobial activity of galbanum oil can be explained by its main components. β-pinene and α-pinene are bicycle monoterpene hydrocarbon and are precursors of many flavors and fragrances. α-pinene finds in sage, signi-

Table 4. Antistaphylococcal activity of essential oils by disc diffusion method.
(MR=Methicillin Resistance; MS=Methicillin Sensitive; *= millimeter)

		Inhibition Zone Diameters (mm*)									
		Galbanum oil (µl)			Fennel oil (µl)			Rosemary oil (µl)			Vancomycin (µg)
		10	15	20	10	15	20	10	15	20	
S.aureus SA32	MR	12.9±0.1	14.5±0.7	18.4±0.8	10.9±0.1	16.3±0.4	24.1±0.1	11.7±0.4	13.4±0.2	14.1±0.1	17.65±0.21
S.aureus SA3	MR	25.6±0.1	35.5±0.7	34.2±1.7	12.1±0.1	16.2±0.5	21.9±0.2	8.6±1.9	9.9±1.5	12.1±0.1	19.1±0.14
S.aureus SAanimal	MR	16.4±0.6	16.8±0.2	17.5±0.6	10.0±0.6	10.8±0.3	12.3±0.4	7.9±0.1	8.1±0.1	10.1±0.1	18.5±0.14
S.aureus SA4	MR	10±0.3	14.3±0.4	15.7±0.5	8.7±0.1	11.4±0.4	13.1±0.1	8.1±0.4	9.3±0.3	10.7±0.5	17.95±0.35
S.aureus SA26	MR	15.4±0.1	16.4±0.1	20.4±0.0	9.45±0.1	10.2±0.4	12.0±0.0	9.4±0.9	10.3±1.3	11.6±0.5	18.8±0.14
S.aureus SA27	MS	14.6±0.6	18.6±0.4	21.5±0.7	11.1±0.5	12.3±0.4	13.1±0.1	10.0±0.0	11.2±0.1	12.4±0.1	17.95±0.07
S.aureus SA6538	MS	20.2±0.3	23.8±0.3	26.7±0.5	14.4±1.3	14.4±0.6	16.5±0.6	7.8±0.3	8.8±0.3	9.7±0.42	19.1±0.77
S.aureus SA25923	MS	15.8±0.4	19.4±0.2	22.7±0.4	8.7±0.6	10.9±0.1	12.3±0.4	8.5±0.7	8.8±0.3	10.1±0.1	20.2±0.28
S.aureus SA8	MS	11.6±0.5	13.9±0.0	16.2±0.3	13.3±0.1	14.0±0.3	16.1±0.1	8.0±0.1	9.3±0.6	9.5±0.71	19.6±0.28
S.aureus SA6	MR	13.0±0.0	16.3±0.4	17.9±0.1	9.9±0.8	14.1±0.1	16.0±0.1	9.7±1.2	13.3±0.4	15.2±0.3	24.6±0.98
S.aureus SA 31	MR	12.4±0.7	16.4±0.7	17.8±0.4	8.5±0.4	10.9±0.7	12.5±0.3	11.6±0.4	13.2±0.3	14.1±0.1	21.8±0.28
S.aureus SA 33	MS	17.7±0.9	22.9±0.1	25.0±0.1	12.9±1.2	15.6±0.1	18.1±0.1	9.0±0.7	9.9±0.1	10.7±0.4	22.2±0.56
S.aureus SA 34	MR	20.0±0.0	24.2±0.3	26.7±0.5	9.9±0.8	13.4±0.0	14.5±0.1	11.0±0.2	10.9±0.9	12.7±0.6	21.9±0.99

Table 5. The antistaphylococcal activity of essential oils by micro broth dilution assay.
[MIC = Minimal Inhibitory Concentration; MBC=minimal bactericidal cincentration]

	Galbanum oil (µl/ml)		Fennel oil (µl/ml)		Rosemary oil (µl/ml)		Vancomycin (µg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus 25923	16	32	16	32	16	32	1	2
S. aureus SA4	8	8	16	32	8	8	1	2
S. aureus SA8	8	8	16	32	8	8	0.5	0.5
S. aureus SA6	8	8	16	32	8	8	1	2
S. aureus SA3	8	16	16	32	8	16	1	1
S. aureus SA34	16	32	16	32	16	32	1	2
S. aureus SA animal	16	32	16	32	16	32	0.5	1
S. aureus SA 32	16	32	4	8	16	32	1	1
S. aureus SA 33	8	16	8	16	16	32	1	1
S. aureus SA 31	8	16	8	16	16	32	0.5	1
S. aureus SA 27	8	16	8	16	16	32	0.5	0.5
S. aureus SA 26	8	16	8	16	16	32	0.5	0.5
S. aureus SA 6538	8	16	16	32	16	32	1	2

rosemary oils that have been shown to have very different spasmogenic effects (Lis-Blachin et al. 1999) and is able to inhibit significantly the growth and cell viability of gram positive bacteria (Leite et al. 2007). The MIC value of 13.6 µl/ml for α-pinene against *S. aureus* is reported (Pichette et al. 2006). β-pinene is well known monoterpene with antimicrobial activity (Dorman & Deans 2000). So, the antimicrobial activity of galbanum oil could be due to the Pinene-type monoterpene hydrocarbons (α-Pinene & β -Pinene).

Trans-anethole is the most component of sweet fennel oil and reaches 84-90%, in this study the content of fenchone is about 7.4% and the amount of trans-anethole is about 57%. The antimicrobial activity of fennel oil investigated in different studies and the antimicrobial activity of fennel oil has been shown against *S. aureus* (Sagdic & Yasar 2005). Hammer et al (1999) found that fennel oil is active against *S. aureus* at concentration above 1%. Our study showed this oil is effective against *S. aureus* at 4-16 µl/ml. Patra et al. (2002) reported that anethole and its isomer are responsible for antimicrobial activity of fennel oil.

The efflux inhibitory activity of rosemary oil is demonstrated (Oluwatuyi et al. 2004). Major constituents of rosemary oil were α-pinene and 1,8-cineole.

1,8-cineole is a monocyclic terpene alcohol that does not induce autolysis of *S. aureus* but were found to cause leakage. It may permeabilize bacterial membranes and facilitate the entry of others (Carson et al. 2006). The percent of α-pinene in rosemary oil is higher than that of galbanum oil but the antimicrobial activity of rosemary is lower than that of galbanum oil, so, other components in the oil such as 1,8-cineole or β-pinene can affect on antibacterial activity of α-pinene against *S. aureus*.

Among three studied oils, galbanum oil is a suitable case for treatment of infectious diseases that are caused by *S. aureus*, some *in vivo* and clinical studies can be done for demonstration of the antistaphylococcal activity of galbanum oil.

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