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Full Length Research Paper

Antimicrobial effects of rosemary (*Rosmarinus officinalis L.*) essential oils against *Staphylococcus* spp

Eilyad Issabeagloo[1]*, Parviz Kermanizadeh[2], Mohammad Taghizadieh[3] and Reza Forughi[4]

[1]Department of Pharmacology, Medical Sciences Faculty, Tabriz branch, Islamic Azad University, Tabriz, Iran.
[2]Department of Immunology, Medical Sciences Faculty, Tabriz branch, Islamic Azad University, Tabriz, Iran.
[3]Department of Pathology, Medical Sciences Faculty, Tabriz branch, Islamic Azad University, Tabriz, Iran.
[4]Department of Internal Medicine, Medical Sciences Faculty, Tabriz branch, Islamic Azad University, Tabriz, Iran.

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***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. Rosemary, *Rosmarinus officinalis*, is a woody, perennial herb with fragrant, evergreen, needle-like leaves and white, pink, purple or blue flowers, native to the Mediterranean region. It is a member of the mint family Lamiaceae, which includes many other herbs, and is one of two species in the genus *Rosmarinus*. The aim of this study was to evaluate the antimicrobial effects of Rosemary (*R. officinalis* L.) essential oils against *Staphylococcus* spp. Fourteen clinical isolates of *Staphylococcus* were cultured from patients. The disc diffusion method was used for determination of antimicrobial activity of essential oil. Results showed that this inhibitory effect is dose-dependent, to wit, by increasing the concentration of the extract in the culture media, reduction in growth was obviously revealed. In conclusion, it can be stated that rosemary essential oils have inhibitory effect against *Staphylococcus* spp.**

Key words: antimicrobial activity, rosemary (*Rosmarinus officinalis* L.), essential oils, staphylococcus spp.

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., 1996) 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.

Rosemary (*Rosmarinus officinalis* L.) originally grows in southern Europe. Its herb and oil are commonly used as spice and flavoring agents in food processing for its desirable flavor, high antioxidant activity and lately as antimicrobial agent (Lo et al., 2002; Ouattara et al., 1997). Moreno et al. (2006) reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. High percent of the antimicrobial

*Corresponding author. E-mail: Dr.e.issabeagloo@gmail.com. Tel: +989144079927.

Table 1. Chemical composition of *R. officinalis L.* essential oil.

Compound	Retention Index	Percent
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

activity they attributed to carnosic acid and carnosol. It is clear that rosemary extracts have bioactive properties, but their antimicrobial activities have not been deeply characterized. Antimicrobial activities of plant essential oils have been known for centuries, but their strong flavor limited their use in food (Del Campo et al., 2000).

The aim of this study was to evaluation of antimicrobial effects of Rosemary (*R. officinalis* L.) essential oils against *Staphylococcus* spp.

MATERIALS AND METHODS

Bacterial cultures and preparation of rosemary extracts

Fourteen clinical isolates of Staphylococcus cultured from patients and S. aureus ATCC 25923 were used in all experiments. Methicillin resistant *S. aureus* directed detected on CHROMagarTM MRSA (CHROMagar Paris, France). Bacterial suspensions were made in Brain Heart Infusion (BHI) broth to concentration of approximately 108 CFU/ml using standard routine spectro-phometrical method. Subsequent dilutions were prepared from the above suspensions, which were then used in the tests.

Disc diffusion method

The disc diffusion method was used 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 remaining 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).

RESULTS

Chemical composition of *R. officinalis L.* essential oil is showed in Table 1. Biochemical test such as catalase, oxidase, coagulase and OF was carried out to proven the genera and data are showed in the Table 2.

Inhibitory effect of rosemary extract was determined by different concentrations of this herbal extract and results showed that this inhibitory effect is dose-dependent, to wit, by increasing the concentration of the extract in the culture media, reduction in growth was obviously revealed (Table 3).

DISCUSSION AND CONCLUSION

Rosemary emits a kind of pungent, pine-like aroma with a woody, camphorous note. It smells a bit like eucalyptus or tea tree. This aromatic shrub has a scaly bark and dense leathery needle like leaves and tiny pale blue blossoms that are abundant all over. It blooms from about December through to spring and can grow up to five or six feet tall. Classified as a shrub, in France, where they grow wild on the mountains, they are almost a tree. The oil is obtained through steam distillation. It takes about 100 pounds of matter to produce about one pound of oil.

Table 2. Results obtained from different biochemical tests.

Genera	Coagulase test	Hemolysis	Pigmented colonies	Mannitol salt agar	Maltose
Staphylococcus aureus	+	+	+	+	+
S. intermedius	+	+	-	(d)	±
S. hyicus	(d)	-	-	-	-
S. epidermidis	-	(d)	-	-	+
S. saprophyticus	-	-	(d)	(d)	+
S. aureus ssp. anaerobious	+	+	-	0	+
S. capare	-	(d)	-	(d)	(d)
S. gallinarum	-	(d)	(d)	+	+
S. arlettae	-	-	+	+	+
S. lentus	-	-	(d)	+	(d)
S. equorum	-	(d)	-	+	(d)
S. simulans	-	(d)	-	+	±
S. delphini	0	+	-	(+)	+
S. chromogenes	-	-	+	(d)	(d)

d: 11-89% positive, +: 90% and more positive, -: 90% and more negative, 0: unknown.

Table 3. Anti-staphylococcal activity of rosemary essential oils by disc diffusion method.

Genera	Rosemary Inhibition zone diameters (mm*)		
	10%	20%	30%
Staphylococcus aureus	8.76	9.10	10.12
S. intermedius	8.68	9.45	10.32
S. hyicus	9.00	9.05	10.40
S. epidermidis	8.90	8.98	9.52
S. saprophyticus	9.12	9.52	9.86
S. aureus ssp. anaerobious	9.30	9.76	10.03
S. capare	7.92	8.09	9.35
S. gallinarum	8.40	9.03	9.61
S. arlettae	9.02	9.68	9.90
S. lentus	8.93	9.12	10.01
S. equorum	9.63	9.60	10.26
S. simulans	8.23	8.84	9.91
S. delphini	7.98	8.65	9.98
S. chromogenes	8.86	8.97	9.63

It grows all over Spain, North Africa and in France and America. There are different types of Rosemary.

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 (Mahboubi et al., 2011).

Rozman and Jersek (2009) wanted to proof an anti-microbial activity of selected rosemary extracts with two most commonly used methods: disc diffusion method and broth dilution method. With the disc diffusion method they have obtained the inhibition zone and at the lowest concentrations, where no visible bacterial growth was recorded, were assumed as minimal inhibitory concentration values (MIC). They determined MIC values in the ranges from 625 µg extract/ml EtOH to 5000 µg extract/ml EtOH for VivOX 20 and from 312.5 µg extract/ml EtOH do 2500 µg extract/ml EtOH for VivOX 40 in the medium. Also, they have established that the resistance of *Listeria* species against rosemary extracts depends on: selected extract, selected concentration, various species and strain of Listeria. With broth dilution method they have determined minimal bactericidal concentration (MBC), as the concentration giving 0.1% bacterial survival. With this method we have tested two strains of *L. monocytogenes* and in determinate MBC values in the range from 15.63 µg/ml TSB to 98.5 µg/ml TSB for both tested extracts.

Gram-positive bacteria are known to be more susceptible to essential oils than Gram-negative bacteria (Inouye et al., 2001). *P. aeruginosa* was least susceptible to the essential oils. The weak antibacterial activity against Gram-negative bacteria was ascribed to the presence of their cell wall, lip polysaccharide (Nostro et al., 2000). *B. subtilis* was the most susceptible microorganism to the rosemary essential oil. Concerning the activity of pure active compounds, the most susceptible bacteria to thymol was *B. subtilis* (23.0 mm) and the most resistant was *P. aeruginosa* (11.5 mm).

Combinations of galangal with either Rosemary or lemon iron bark showed synergistic antimicrobial activity. Specifically, galangal and rosemary showed synergistic activity against S. aureus and *L. monocytogenes* only, while galangal and lemon iron bark showed synergistic activity against *E. coli* and *S. typhimurium*. Chemical compositions of the extracts were determined by gas chromatographic-mass spectrometric analysis. The major chemical components of the galangal and lemon iron bark extracts were 1'-acetoxy-chavicol acetate (1'ACA) (63.4%) and neral (15.6%), respectively, while 1,8-cineole (26.3%) and camphor (20.3%) were identified as major chemical components of the rosemary extract (Weerakkody et al., 2011).

Santoyo et al. (2005) demonstrate that *S. aureus* was found to be the most sensitive bacteria to the rosemary extracts, whereas the least susceptible was *A. niger*. alpha-Pinene, 1,8-cineole, camphor, verbenone, and borneol standards also showed antimicrobial activity against all the microorganisms tested, borneol being the most effective followed by camphor and verbenone. In that way, it was confirmed that essential oil from Experiment 4, with the best antimicrobial activity, presented the highest quantity of camphor, borneol, and verbenone.

The use of plants to heal diseases, including infectious one, has been extensively applied by people. Data from the literature as well as our results reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to search for new compounds. Once extracted, and before being used in new therapeutic treatments, they should have their toxicity tested *in vivo*. Bioassays (Carvalho et al., 1988; Nascimento et al., 1990) have demonstrated the toxicity of extracts from different plants.

In conclusion, can be state that rosemary essential oils have inhibitory effect against *Staphylococcus* spp.

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