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Study of Chemical Composition and Antimicrobial Properties of Rosemary (*Rosmarinus Officinalis*) Essential Oil on *Staphylococcus Aureus* and *Escherichia Coli in vitro*

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ABSTRACT

Nowadays, with increasing bacterial resistance, finding new antimicrobial agents to replace herbal remedies with less side effects than conventional drugs has provided. The aim of this study was to investigate the chemical composition and antimicrobial properties of Rosemary (Rosmarinus officinalis) essential oil on Staphylococcus aureus and Escherichia coli *in vitro*. In this experimental study, rosemary was prepared from Tabriz city and the essential oil was extracted after drying by water distillation using Clevenger apparatus. The essential oil components were identified by Gas chromatography mass spectrometry (GC-MS). Essential oil of 10, 5, 2.5, 1.25, 0.625 and 0.312% of essential oil was used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Based on the results of GC-MS analysis, 19 compounds were identified, of which 1.8 Cineole and α-pinen had the highest essential oil composition. MIC and MBC of essential oils were obtained on S. aureus (0.625 and 1.25%) and E. coli (1.25 and 2.5%), respectively. The results of this study showed that rosemary essential oil has remarkable antibacterial activity and can be used as a suitable replacement for synthetic antibiotics.

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Introduction

The use of herbal compounds for the treatment of diseases is an old practice in different parts of the world, especially in developed countries. In the past century, there has been a growing focus on medicinal plants with antimicrobial properties that can resolve common problems with the use of antibiotics for reasons such as the increased use of chemical drugs and the increasing resistance of bacteria to these substances [1, 2]. However, most plant species with medicinal properties remain unexplored and there is still much time left to discover new and valuable plant sources [3, 4]. Medicinal plants

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are one of the important sources of antimicrobial agents in different countries [1]. About 60% to 90% of the population in developing countries use herbal remedies [5]. As such, plants can be considered as a source of potentially useful chemicals that are only partially exploited. These potentially useful chemicals can be used not only as medicines but also as unique starting points for the production of pharmaceutical analogues as well as an interesting tool to better understand biological phenomena [6, 7]. Rosemary, scientifically named Rosmarinus officinalis, belongs to the Lamiacea family and is known as a spice and medicinal plant in many countries. It has antibacterial, antioxidant and antifungal effects and is native to the Mediterranean and Asian regions [8, 9]. Rosemary contains oleoresin and tannins, pinene, camphor, bornyl acetate, etc. Rosemary also contains large amounts of salicylates [10, 11]. In traditional medicine, this herb is used for anti-asthmatic, digestive, sedative, relieving, headache, circulatory disorders, visual acuity, anti-rheumatic and irritant effects [12, 13]. Numerous pharmacological effects including antioxidant effect, growth factor stimulation, antimicrobial and antiviral activity and inhibition of hepatotoxicity have been reported for this plant [14-17].The aim of this study was to investigate the antimicrobial properties of Rosemary essential oil on S. aureus and E. coli in laboratory conditions.

Materials and Methods

Extraction and essential oil making

In this *in vitro* study, plant samples were collected from natural areas of Tabriz's greenhouses. After identification and approval of the scientific name by Herbarium Natural Resources of Agriculture Jihad, the samples were dried in a large, convenient space and prepared for grinding. Essential oil extraction was performed using Clevenger apparatus. The essential oil was dehydrated with sodium sulfate and passed through a 0.45 µm microfilter and stored in a dark glass container at 4 ° C until identification and determination of the chemical constituents.

Analysis of essential oil constituents using GC-MS

The constituents of essential oils were identified using Retention indices and mass spectra of the compounds and compared with standard mass spectra and valid references. To do this, the prepared sample of the essential oil was first injected into the gas chromatography apparatus and the most suitable temperature programming of the column was obtained for complete separation of the essential oil compounds. The essential oil was then injected into a gas chromatograph coupled to a mass spectrometer and the mass spectra of the compounds were obtained.

Bacterial strains

Standard strains were used to evaluate the antibacterial effects of rosemary essential oil. These strains, including S. aureus ATCC 25923 and E. coli (ATCC 25922) were obtained from the University of Tehran Microbial Collection. The strains were then identified using culture media and biochemical tests.

Determination of MIC and MBC

One hundred µl of Mueller Hinton Broth was poured into the wells and then 100 µl of essential oil was added to the first well of each row. After mixing the contents of the first well, 100 µl of it was removed and added to the next well. Discard 100 µl of the final well. A control row was assumed for each experiment row (corresponding to one essential oil). The dilution steps of the essential oils were carried out in the same test sequence. Then 100 µl of the bacterial suspension (1.5 106 cfu / ml) was added to each well, but not to the control well. Microdilution method was used to perform this test. Finally, they were incubated at 37 ° C for 24 hours. The presence of turbidity (compared to the control row) indicates bacterial growth and the transparency indicates that the bacteria are not growing. Then, all wells in which bacterial growth was observed were sampled and cultured on MBC plate. Plates were then incubated for 24 hours at 37 ° C. Plates in which bacterial not growth was visible were considered MBC. Each experiment was repeated 5 times to reduce the error of the experiment.

Results

According to the chemical analysis of plant essential oils by GC / MASS, 19 compounds were identified in rosemary that comprised 96.57% of the essential oil. The essential oils were 1.8 Cineole (21.8%), α-pinen (18.7%), Camphor (14.6%), Linalool (13.4%) and Camphene (7.2%), respectively. The results of MIC and MBC of rosemary essential oil by microdilution broth on S. aureus and E. coli are presented in Table 2. The results showed that rosemary essential oil has antimicrobial effects. In general, based on the results of this study, it can be said that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria.

Table 1 Chemical compounds of essential oils of Rosemary

No	Compounds	Percentage of compounds (%)
1.	1,8 Cineole	21.8
2.	α-pinen	18.7
3.	Camphor	14.6
4.	Linalool	13.4
5.	Camphene	7.2
6.	Borneol	5.2
7.	limonene	4.9
8.	Verbenone	3.5
9.	Myrtenole	2.4
10.	Myrcene	2.05
11.	Verbenone	1.02
12.	α-Thujene	0.7
13.	B-Pinen	0.41
14.	Trephine	0.32
15.	3-Carene	0.14
16.	P-Cymenene	0.1
17.	Camphonelal	0.08
18.	Iso-Pinocamphone	0.05
Total Identified Constituents 96.57		

Table 3 MIC and MBC of Rosemary essential oil on E. coli and S. aureus (in percent)

Extract concentration / Bacteria	MIC	MBC
Staphylococcus aureus	0.625	1.25
Escherichia coli	1.25	2.5

Discussion

Rosemary essential oils are a combination of esters, aldehydes, alcohols, ketones and terpenes, which are classified into two groups of major and minor constituents [18]. The main constituents make up about 85% of the essential oils, the quantity and quality of the essential oils vary with the climate, soil composition and age of the plant [19]. In this study, 19 compounds of rosemary essential oil were identified which comprised 96.57%

of the essential oil. The essential oils were 1.8 Cineole (21.8%), α-pinen (18.7%), Camphor (14.6%), Linalool (13.4%) and Camphene (7.2%), respectively. Malakootian and Hatami identified 20 compounds in 2013 with rosemary essential oil analysis, of which 82.99% included 1.8 Cineole, Borneo, -pinen α, Camphor, Linalool, Camphene, limonene and Verbenone [20]. In 2010, Zaouali et al., isolated 25 compounds from the rosemary essential oil analysis. This is inconsistent with the present study because the time and stage of plant picking, plant age, soil type, climate, and the essential oil extraction method are effective on the effective constituents of the plant [21].Okoh et al., in South Africa introduce Verbenone as the main rosemary compound [22]. Linares et al [23], santoyo et al [24] and Angioni et al [25]obtained similar results to the present study. Malakootian and Hatami, by examining non-growth halo at 32 μg of essential oil, showed that non-growth halo was higher than non-growth halo gentamicin, penicillin, streptomycin and erythromycin. MIC and MBC in this study were 3,000 and 3,200, respectively. The time required for the complete destruction of Escherichia coli was 25 minutes [20]. Nascimento et al., Studying the antimicrobial effects of rosemary essential oil on different bacteria, showed that the rate of growth inhibition of this essential oil on S. aureus was 18 mm [26]. In a 2007 study by Fu et al., The antimicrobial effects of rosemary essential oil showed that the growth rate of this essential oil on S. aureus was 18 mm [27]. Other studies have reported the effects of rosemary essential oil on gram-positive S. aureus and B. cereus bacteria [28, 29]. In a study of rosemary essential oils in 2018, ahmady-asbchin and Mostafapour showed that the plant has antibacterial activity against E. coli, S. aureus, S. epidermidis, E. faecalis and P. mirabilis, this property varies depending on the essential oil and the bacterial sex. The highest effect was on P. mirabilis and the least on E faecalis [30]. Soltan Dallal et al. Examined the antimicrobial effects of rosemary essential oil by disk diffusion and dilution methods on methicillin-resistant S. aureus, the diameter of the non-growth halo was 20 mm and MIC and MBC were 40.1 and 81.2 mg/ml, respectively [31]. Comparing these results, it can be said that the effect of rosemary essential oil was more than the extract. Mashreghi M, Momtazi studies of the antimicrobial effects of the alcoholic extract of rosemary on E. coli O157 showed that the extract had little effect in the early stages of bacterial growth and its effects on bacterial growth and proliferation [32]. Antimicrobial effects of rosemary essential oils on bacteria and fungi indicate effective antimicrobial activity of these essential oils. In one study, it was shown that the MIC of rosemary essential oil on Propionibacterium acne was 0.56 mg / ml (14) [24].

Conclusion

Although the present study provides information on the chemical constituents and antibacterial effects of rosemary essential oil, due to time and financial constraints, the effects of all chemicals of this essential oil on different types of microbes have not been possible, Therefore, Researchers need to take a holistic view of this topic. In general, the results of *in vitro* studies showed that the essential oil of this medicinal plant contains various compounds that can have antibacterial activity against E. coli and S. aureus. However, clinical trials on patients after consuming rosemary essential oil are recommended to confirm this data so that it can eventually be made available to patients in the category of medicinal plants.

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