Evaluation of Antibacterial Activity of Alcoholic Extract of Rosemary Leaves against Pathogenic Strains

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**Abstract**

**Background:** Today, the increased use of antibiotics lead to the incidence of resistant strains. We are faced with lack of new antimicrobial drugs that have fewer side effects than antibiotics. Rosemary is a medicinal plant that has many uses in traditional medicine. In this study, ethanol leaves extract of this plant is tested on various pathogens.

**Materials and Methods:** In this experimental study, Rosmarinus officinalis was used to evaluate the antimicrobial effects on pathogens. Ethanol extract of the leaves of this plant, with concentrations of 400, 200, 100 and 50 mg/l were prepared, and antibacterial activities were evaluated by well diffusion method on strains of Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus and Staphylococcus aureus. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined by the microplate method.

**Results:** In this study, ethanol extract of rosemary leaves at concentrations of 400 mg/ml was active against Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus and Escherichia coli. Minimum inhibitory concentration of the extract on the growth of these bacteria from 6.25 mg/ml to 100 mg/ml was change. Also MBC of extract showed range from 12.5 to 200 mg/ml respectively.

**Conclusion:** Result from these finding suggest that, rosemary extract, on all strains, including Staphylococcus aureus, Escherichia coli, Bacillus cereus and Pseudomonas aeruginosa, has an inhibitory effect. Also, the effect of extract, risesed by increasing the concentration.

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Materials and Methods

This experimental study was conducted at Microbiology Research Laboratory, Department of Microbiology, Islamic Azad University of Falavarjan in 2012. The bacterial strains were provided by Microbiological Collection of Tehran Research Center. Herbal samples were collected and identified in cooperation with Herbarium and Herbal Research Center of Isfahan. In each experiment, three iterations were employed to do statistical calculations.

Provision of Bacterial Strains: Bacillus cereus (ATCC: 1247), Staphylococcus aureus (ATCC: 25923), Escherichia coli (ATCC: 25922) and Pseudomonas aeruginosa (ATCC: 1430) that were lyophilized, prepared from Microbial Collection of Tehran Research Center and then revived by standard methods.

Provision of plant samples: The fresh leaves of Rosmarinus officinalis were collected and dried in a place not exposed to sun light. Then the dried leaves were crushed to powder. Fifty gram of leaves were weighted and poured into a sterilized erlenmeyer flask. In the next step, 250 milliliter ethanol (98%) was added so that the herb’s compounds were dissolved. Erlenmeyer flask containing alcohol and herb powder was placed into shaker for 48 hours until the solvent may exert their effect at the temperature of 40°C. Next, rotary was employed to eliminate the solvent. Finally, the Rosemary extracts were preserved in sterilized dishes at refrigerator and to prevent from the light effects, they were wrapped with aluminum covers. Extracts with concentrations of 50, 100, 200 and 400 mg/ml were provided from the extract of Rosmarinus officinallis. The strains of Pseudomonas aeruginosa. Moreover, the concentration of methanol extract, the inhibition zone diameter was measured by millimeter [7, 8].

Determination of MIC: The dilution in microplate method was used to determine Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of different leaves concentration extracts of rosmarinus officinalis. The strains of mentioned pathogenic bacteria were put under a 24 h culture process in Mueller Hinton Broth medium at the temperature of 37°C. Dilution serials of 6.25, 25.2, 50, 100, 200 and 400 mg/ml were provided from the extract and 70 µl of them were added into 96 cells micro plates containing 70 µl bacteria suspension with turbidity of half McFarland. Then the similar tests were done for positive control (medium with bacteria and without extract) and negative control (medium without bacteria). Afterwards, micro plates were heated in a 37°C incubator. The minimum dilution with no seen turbidity was reported as MIC. All tests were repeated three times and the average was presented [9-12].

Determination of MBC: Considering MIC results, the Minimum Bactericidal Concentration was also specified. From all cells in which the growth of bacteria was stopped, plates containing Mueller Hinton Agar were cultured and heated at 37°C for 24 hours. Concentrations without bacteria growth were reported as MBC. All the tests were repeated three times and the results were averaged. Data were analyzed by statistical SPSS-14 Software. To study the significant difference, variance analysis and χ² tests were used and their difference was calculated in a significant level (p≤0.001) [13, 14].

Results

The results of antibacterial activity of Rosmarinus officinalis leaves extracts were shown in table 1. All extracts showed dose dependent activity which increases with increase in concentration (Table 1). Antibiotic chloramphenicol was positive control and DMSO was the negative control. As the table shows, ethanol extract has prevented from the growth of bacteria such as Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus and Staphylococcus aureus. Also, by increasing the concentration of methanol extract, the inhibition zone increased (p≤0.001).

Additionally, the results revealed that contrary to the similar studies, rosemary extract had effect on Pseudomonas aeruginosa. Moreover, the concentration of 400 mg/ml has more effect on Pseudomonas aeruginosa and Staphylococcus aureus. The values of MIC and MBC of ethanol extract of Rosemary leaves has been presented in table 2 against the referred bacteria.

| Table 1. Antibacterial activity of Rosmarinus officinalis leaves extracts against tested bacteria measured in millimeter |
|-----------------|------|------|------|------|------|
| Bacteria        | 50   | 100  | 200  | 400  | Negative | Positive |
| Staphylococcus aureus | 6.25 | 12.5 |
| Bacillus cereus  | 50   | 100  |
| Escherichia coli | 100  | 200  |
| Pseudomonas aeruginosa | 100  | 200  |

| Table 2. MIC and MBC of leaves extracts against tested bacteria (mg/ml) |
|-----------------|------|------|
| Bacteria        | MIC  | MBC  |
| Staphylococcus aureus | 6.25 | 12.5 |
| Bacillus cereus  | 50   | 100  |
| Escherichia coli | 100  | 200  |
| Pseudomonas aeruginosa | 100  | 200  |
The results determined that in tested bacteria, there was a significant difference ($p<0.001$) in terms of sensitivity to ethanol extract. In other words, the most sensitivity was observed in Staphylococcus aureus and the least was seen in Bacillus cereus and Escherichia coli. So, as it shown in this table, MIC is between 6.25 to 100 mg/ml and MBC is between 12.5 to 200 mg/ml ($p<0.001$).

**Discussion**

The results indicated that ethanol extract of Rosemary with concentration about 200 mg/ml has prevented from the growth of Gram-negative bacteria and with concentration between 20 to 100 mg/ml from Gram-positive bacteria. Thus, the research represents the antibacterial effects of this medical herb on Gram-negative and Gram-positive pathogenic bacteria. Growth inhibitory effect of the extract starts from the concentration about 20 mg/ml and by gradual increase of concentration, inhibition zone increases as well. Inhibition zone rosemary ethanolic extract on *Staphylococcus aureus* was between 18 to 22 mm and obtained MIC was about 6.25 mg/ml. It was observed in *Pseudomonas aeruginosa* between 12 to 19 mm. concerning the method of extraction and preventing from using high temperature to decrease the rate of destruction of effective herbal compound, there is a partial difference between these results and the similar studies.

Jarrar et al. examined the effect of ethanol extract alone and with cefuroxime on staphylococcus resistant to methicillin. The achieved MIC was about 0.3 to 3.1 mg/ml which had a significant difference with current studies. The difference may be due to using antibiotic with this herbal extract. The research also determined that using rosemary extract along with cefuroxime would have synergic effects on all resistant bacteria. In a research, Tsai et al. looked at effects of ethanol extract of rosemary and calculated MIC at 1.42 mg/ml. In this research, the extract, prevents well from the growth of oral streptococcus. Having studied the effects of rosemary and clove buds essential oil and extract on Botrytis cinerea, Vesal-Talab et al. reported that in general, ethanol extract of both had no antifungal effect. By fumigation method, clove buds essential oil with concentration of about 600 to 650 ppm had antifungal activity. Rosemary essential oil had such activities in concentration of about 600 to 650 ppm. The results showed that antifungal activities were based on concentration, product application method and the type of plant. Also, the essential oil had higher effect than extract [4-6].

In another studies, Aziza Kamal showed the effects of rosemary essential oil on Gram-positive *Staphylococcus aureus* and *Bacillus cereus* and reported that the effect is higher on Gram-positive bacteria than on *Pseudomonas aeruginosa* and *Escherichia coli*. He stated that according GC-MS, isocarnosol is the biggest compound in rosemary [15-17]. In study of Rozman et al., the effect of rosemary extract on different types of listeria was studied and MIC was specified between 625 to 5000 mg/ml. This research found out that the resistance of listeria depends on rosemary extract, chosen extract; various types of listeria and various concentration of extract [17]. In a research over antimicrobial effects of rosemary essential oil, Fu et al. indicated that the extent of inhibition zone in *Staphylococcus aureus* was 18 mm and MIC was 0.125, which shows the resemblance of these results with current studies [18]. By methods of disk diffusion and dilution in tube, Soltan-Dallal et al. [19], evaluated the antimicrobial effects of rosemary essential oil on *Staphylococcus aureus* resistant to methicillin. The diameter inhibition zone was observed about 20 mm. MIC was 1.40 mg/ml and MBC was 2.8. By comparing these results, we can say that the effect of essential oil is higher than extract and these results are more consistent with Soltan-Dalal’s results. Today, one of the main treatment problems is development of antibiotic resistance, which has attracted special attention. Since antibacterial effects of rosemary essential oil and extract on various types of bacteria have been proved, they are recommended in treatment of infection-induced pathogen bacteria. It is suggested that further research on medicinal plants, would be the promising alternatives for chemical medicines which have unwanted side effects despite of effectiveness.

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**Conflict of Interest**

The authors declare no conflict of interest.

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Prevalence of VIM1 MBL gene

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