Original Article

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# The Antibacterial Effects of Methanol Extract of Ammi majus on Staphylococcus aureus and Escherichia coli

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## ABSTRACT

**BACKGROUND AND OBJECTIVE:** Diversity of plants and the increasing tendency to use them for therapies has increased the significance of screening herbal extracts. Several researches have reported the antibacterial effects of plants. Therefore, the present study aims to investigate the antibacterial effects of methanol extract of Ammi majus.

**METHODS:** After preparing methanol extract (0.5, 1 and 2%), the antibacterial effects of the plant were measured according to minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), disk diffusion method (determining the zone of inhibition) and well diffusion method on Staphylococcus aureus and Escherichia coli.

**FINDINGS:** In well diffusion method, the lowest concentration (0.5%) created a 9±0.000 mm inhibition zone diameter, while the highest concentration (2%) created a 12.3±0.57 mm inhibition zone diameter ($p<0.001$). The photometric tests revealed that the methanol extract of the plant (0.5 to 1%) with mean difference of 0.04 had antibacterial effects on Escherichia coli ($p<0.001$). However, as the concentration increased (2%), adverse non-inhibitory effects could be observed. The tests based on colony counting method demonstrated that Ammi majus extract 0.5, 1 and 2% have antibacterial effect on Staphylococcus aureus ($p<0.001$). However, only the 2% extract had inhibitory effects on Escherichia coli ($p<0.01$).

**CONCLUSION:** Results of the study demonstrated that methanol extract of Ammi majus has more antibacterial effect on Staphylococcus aureus compared with Escherichia coli.

**KEY WORDS:** *Extract, Ammi majus, Staphylococcus aureus, Escherichia coli*.

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## Introduction

Medicinal plants are the primary or sometimes the only treatment agents used for the treatment of many diseases (1-3). Although synthetic antibiotics have played a significant role in treatment of many infectious diseases in recent decades, the problems associated with the incidence of microbial resistance of antibiotics increased the tendency to use herbal medicines (4-6). Ammi majus is an annual gramineous plant and a member of Apiaceae (Umbelliferae) family with several therapeutic uses (7). This is a plant originating in Egypt, particularly the Nile River Valley. This plant can also be found in some provinces of Iran including Kerman, Khorasan, Tehran and Lorestan (7, 8).

It is used as a medicinal and ornamental plant, particularly in Egypt and India (9-11). Staphylococcus aureus is a gram-positive and normal flora bacteria of nose and skin, which can cause nosocomial infections, particularly infections of the skin and its underlying tissue in human (12-14).

Escherichia coli is a gram-negative bacteria of the Enterobacteriaceae family, which can cause infection in urinary tract and constitutes about 90% of bacteriuria in women (15). A study by Selim et al. demonstrated that the coumarin extracted from Ammi majus has antibacterial, antifungal and antiviral properties (8). Duke et al. and Joy et al. also mentioned the antibacterial, antifungal and antiviral properties of this plant (16, 17).

In a study on leaf extracts of this plant, Al-Hadhrami et al. also indicated the antibacterial effects of this plant (18). Considering the importance of this plant in treatment of diseases associated with different types of microorganisms such as bacteria, it is unfortunate that studies carried out in this area are highly limited. Therefore, the present study aims to investigate the antibacterial effects of methanol extract of Ammi majus on Staphylococcus aureus and Escherichia coli.

## Methods

This experimental study was conducted using fruit of Ammi majus plant. Thus, in order to conduct this research, the plant was collected from its natural habitat in Aliabad Henam village, Aleshtar city, Lorestan province (ZAGROS Pharmaceutical Co., herbarium identification code 13594) and was scientifically identified and confirmed by Agricultural Research Center of Lorestan province. In order to prepare methanol extract, 100 g Ammi majus fruit was grinded and powdered and was soaked in 200 cc pure methanol solvent for 72 hours. After 72 hours, the prepared sample passed through filter paper and the solvent was removed in vacuum at the right temperature and using the evaporation system. Then, the obtained methanol extract was prepared at concentrations of 0.5, 1 and 2% for microbial tests.

**Bacteria used:** The strains used in this research included *Staphylococcus aureus* gram-positive bacteria (ATTC25923) and *Escherichia coli* gram-negative bacteria (ATTC25922), each prepared using 0.5 McFarlan suspension in liquid medium (BHI=Brainheartinfusionbroth) (20).

**Disk diffusion method:** Using disk diffusion method and through a paper disc, the sensitivity of microorganisms to antibacterial materials was determined according to the standard method of Kirby-Bauer. First, the bacterial suspension equivalent to 0.5 McFarland was prepared according to the standard method. 3 different concentrations were prepared and placed on the medium next to the flame and under the hood. 40 lambda of the mentioned concentrations (0.5, 1 and 2%) were placed on each disk in 2 steps (20 lambda in each step) and cefotaxime antibiotic disc was used as control (21, 22).

**Well diffusion method:** In this method, wells were created by sterile pasteur pipette next to the flame and under the hood based on the number of desired concentrations under full sterile conditions. 40 lambda of 0.5, 1 and 2% concentrations were placed on wells and for control, DMSO was poured into the middle of the well. Then, the cultured plates were incubated at 37 ºC for 24 hours and the inhibition zone diameter was measured (23).

**Microdilution tests:**

**Photometry method:** This method was performed in a sterile 96-well plate. First, a 0.5 McFarland solution of each microorganism was prepared in two sterile tubes (24, 25); 100 lambda of the desired microorganism with 0.5 McFarland concentration and then 100 lambda of the methanol extract of the plant with the mentioned concentrations were added. On the first day, the optical density was read once by ELISA Microplate Reader and after being kept in bain-marie at 37 ºC for 24 hours, the optical density was read for the second time and the result was compared with the previous day.

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**Colonicount method:** Similar to the previous method, this method was performed in a sterile 96-well plate. 100 lambda of the desired microorganism with 0.5 McFarland concentration and 100 lambda of the methanol extract of the plant with the mentioned concentrations were added.

Then, 4 lambda of the diluted solution (1 : 200 concentration) was cultured in Mueller Hinton Agar Plate for *Staphylococcus aureus* and *Escherichia coli* separately in fully sterile condition and was placed in incubator at 37 ºC using calibrated sterile forceps. In the meantime, the number of colonies grown on the media of the 96-well plate was counted after 24 hours. The same procedure was carried out again the second day and the number of colonies was counted and the results were compared.

After comparing the total number of colonies between these two steps, the lowest concentration of extract in which the microorganisms grew was reported as minimum inhibitory concentration (MIC), and the lowest concentration of extract in which the microorganisms did not grow was reported as minimum bactericidal concentration (MBC).

**Statistical analysis:** After confirming the validity of data, they were analyzed using SPSS 20 and statistical methods of One-way ANOVA with post-hoc Tukey and $p<0.05$ was considered significant.

## Results

Results of disk diffusion test demonstrated that Ammi majus extract at concentrations of 0.5 to 2% had antibacterial effect on Staphylococcus aureus bacteria. The lowest concentration (0.5%) created an inhibition zone diameter of 8.33±0.57 mm, while the highest concentration (2%) created an inhibition zone diameter of 9.33±0.57 mm. Although the mean values were different, the differences were not statistically significant. Nevertheless, different concentrations of this extract did not create zone of inhibition against Escherichia coli bacteria (Fig 1).

Moreover, results obtained from well diffusion method indicated that methanol extract of Ammi majus 0.5 to 2% created zone of inhibition against Staphylococcus aureus bacteria ($p<0.001$); the lowest concentration (0.5%) created a 9±0.000 mm inhibition zone diameter, while the highest concentration (2%) created a 12.3±0.57 mm inhibition zone diameter ($p<0.001$). However, different concentrations of this extract did not have antibacterial effect on Escherichia coli bacteria (Fig 2).

Results obtained from microdilution tests based on photometry method demonstrated that methanol extract of Ammi majus 0.5 to 2% did not have antibacterial effect on Staphylococcus aureus, but had antibacterial effect on Escherichia coli at concentrations of 0.5 to 1% ($p<0.001$). However, as the concentration increased (2%), adverse non-inhibitory effects could be observed (table 1). Results of microdilution tests (Colonicount) indicated that methanol extract of Ammi majus 0.5 to 2% had antibacterial effect on Staphylococcus aureus ($p<0.001$), but had inhibitory effect on Escherichia coli only at a concentration of 2% ($p<0.01$) (table 2).

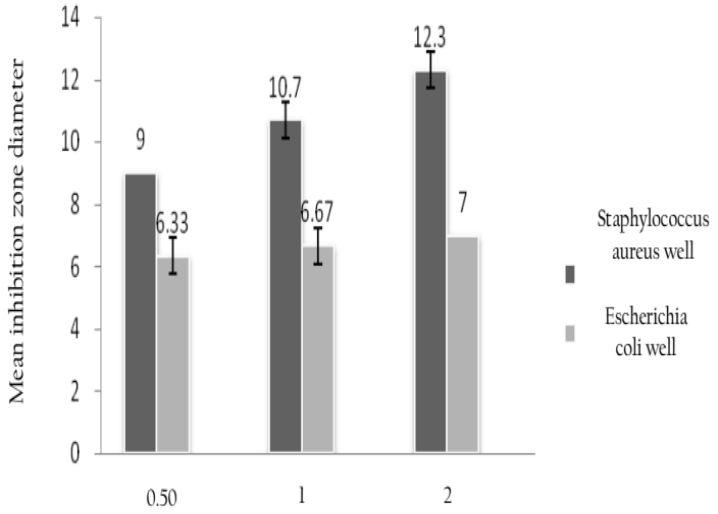


**Figure 1. The mean inhibition zone diameter at different concentrations of methanol extract of Ammi majus on Staphylococcus aureus and Escherichia coli bacteria according to well diffusion method**

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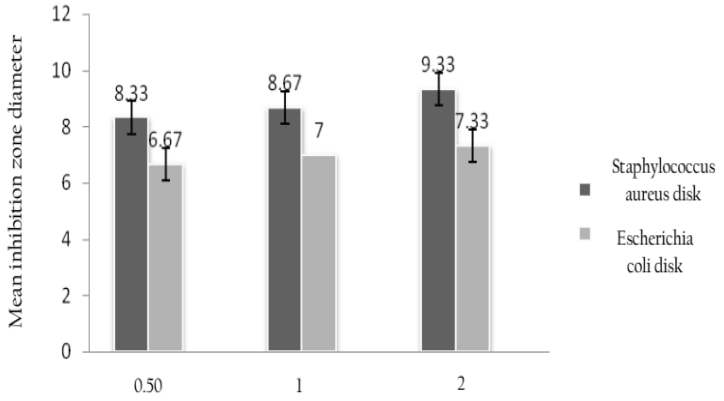


**Figure 2. The mean inhibition zone diameter at different concentrations of methanol extract of Ammi majus on Staphylococcus aureus and Escherichia coli bacteria according to disk diffusion method**

**Table 1. The minimum inhibitory concentration (MIC) of methanol extract of Ammi majus on various microorganisms based on photometry method.**

| Microorganism | Concentration of the extract | Mean | MD | SE | P-value |
|---|---|---|---|---|---|
| Staphylococcus aureus | 0.5 | 0.05183 | 0.03716 | 0.03685 | 0.970 |
| | 1 | 0.05283 | 0.03616 | 0.03685 | 0.975 |
| | 2 | 0.07650 | 0.012500 | 0.03685 | 0.99 |
| Escherichia coli | 0.5 | 0.04583 | 0.04666 | 0.00475 | ** <0.001 |
| | 1 | 0.04367 | 0.04883 | 0.00475 | ** <0.001 |
| | 2 | 0.16433 | - 0.07183 | 0.00475 | ** <0.001 |

**Table 2. The minimum bactericidal concentration (MBC) of methanol extract of Ammi majus on various microorganisms based on ColoniCount method.**

| Microorganism | Concentration of the extract | Mean | MD | SE | P-value |
|---|---|---|---|---|---|
| Staphylococcus aureus | 0.5 | 541666.67 | 525.00000 | 62193.69 | ** <0.001 |
| | 1 | 12500.00 | 941666.66 | 62193.69 | ** <0.001 |
| | 2 | 50000.00 | 1016666.66 | 62193.69 | ** <0.001 |
| Escherichia coli | 0.5 | 1116666.67 | 208333.33 | 84860.99 | 0.244 |
| | 1 | 1133333.33 | 191666.66 | 84860.99 | 0.341 |
| | 2 | 1000000.00 | 325000.00 | 84860.99 | ** <0.010 |

MD: Mean difference of 0.5 McFarlan for different concentrations of methanol extract, ** One-way ANOVA with post-hoc Tukey and significance level ($p<0.05$). P-value is related to the comparison between mean values of different concentrations of methanol extract with mean difference of 0.5 McFarlan.

## Discussion

Results of the present study based on disk diffusion method demonstrated that methanol extract 0.5 to 2% had antibacterial effect on Staphylococcus aureus. However, different concentrations of this extract did not create zone of inhibition against Escherichia coli bacteria. Though, the difference was not statistically significant. Duke et al. also noted the antibacterial, antifungal and antiviral properties of this plant (16). In a study on various gram-positive bacteria such as Streptococcus, Semyari et al. demonstrated that extract of Ammivisnaga plant has antibacterial effects on some species of this bacteria according to disk diffusion method (26). Another study by Jalali et al. on Staphylococcus aureus and Escherichia coli using disk diffusion method demonstrated that the methanol extract of the plant did not create zone of inhibition against Staphylococcus aureus and Escherichia coli and had no antibacterial effect on them even at high concentrations (27). These results were not in line with the results of the present study and it may be due to the

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inadequacy of the active ingredients of this plant compared with Ammi majus. Test results of this study based on well diffusion method indicated that different concentrations of methanol extract created zone of inhibition against Staphylococcus aureus, but different concentrations of the extract had no inhibitory effect on Escherichia coli. In a study about the effect of various extracts of Ammi majus on bacteria using disk diffusion method in vitro, Al-hadidi et al. demonstrated that the ethanolic extract of the plant had inhibitory effect on gram-positive bacteria (Staphylococcus aureus) and the zone of inhibition was observed, while no inhibitory effect was observed in gram-negative bacteria (Escherichia coli) (28). Due to limited number of studies dedicated to the antibacterial effects of this plant in Iran and worldwide, we also focused on similar items on other plants.

Investigating the antibacterial effects of aqueous and alcoholic extract of lavandula stoechas on Staphylococcus aureus, Escherichia coli and Pseudomonas, Khosravi et al. demonstrated that the aqueous and alcoholic extract of this plant had effect on most of the studied bacteria, which is in line with the results of our study (29).

Results of microdilution tests based on photometry method demonstrated that the studied plant had no bacterial effect on Staphylococcus aureus bacteria at concentration of 0.5 to 2% but had antibacterial effect on the gram-negative bacteria (Escherichia coli) at concentrations of 0.5 to 1%. However, adverse non-inhibitory effects could be observed at higher concentration (2%).

In a study entitled "The antibacterial effect of chloroform, ethyl acetate and hydroalcoholic extracts of Scilla persica on gram-negative bacteria (Escherichia coli) and gram-positive bacteria (Staphylococcus aureus) using dilution method", HafezGhoran demonstrated that chloroform and ethyl acetate extracts of Scilla persica had stronger antibacterial effect on Escherichia coli and Staphylococcus aureus, which is not in line with the results of the present study regarding Staphylococcus aureus bacteria (30). One of the reasons for this inconsistency may be related to the type of extract and it is worth noting that because of containing glycoside, the methanol extract used in our study lacks antibacterial properties. Investigating the antibacterial effects of alcoholic extract of sorghum, Sharifi et al. demonstrated that Escherichia coli with the largest inhibition zone diameter was the most sensitive bacteria, that is, the extract showed more inhibitory effect on gram-negative bacteria compared with gram-positive bacteria and the antibacterial property of extract decreased as the concentration decreased, which is similar to the results of the present study (31). The results of microdilution tests based on Colonicount method in the present study demonstrated that methanol extract 0.5 to 2% had antibacterial effect on Staphylococcus aureus, but only the inhibitory effect on Escherichia coli could only be observed at 2% concentration. Abdul-Jalil et al. reported the existence of two flavonoid of this plant's fruit including quercetin and kaempferol (32). Investigating the extract of Ammi majus, Nayebi et al. reported the existence of biggest group of secondary metabolites called terpenoids (33). Therefore, an overview of all these studies concludes that the furanocoumarins, flavonoids and terpenoids in this plant have antibacterial and antifungal properties. Flavonoids, which are hydroxylated phenolic compounds, are observed as a C3-C6 group attached to an aromatic ring and according to the studies, these compounds are produced by plants in response to bacterial infections. Their activity is similar to the mechanisms of action of quinones, probably due to their binding to extracellular proteins, solution and the cell wall of bacteria. Flavonoids with more lipophilic properties can decompose bacterial membranes. The inhibitory activity of terpenoids against bacteria, fungi and viruses is identified but their mechanism of action is not fully recognized yet (34-36).

Results of this research demonstrated that methanol extract of Ammi majus has more antibacterial effect on gram-positive bacteria than gram-negative bacteria. This might be due to the structure of the plasma membrane and cell wall in this type of bacteria (gram-negative), which limits the entrance of the active ingredients of herbal extract into the cell. Therefore, considering the fact that this plant is widely spread in different parts of Iran, more investigations are advised for identification of biological and pharmacological properties of this plant.

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