

The Inhibitory Effect of Ajowan Essential Oil on Bacterial Growth

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Received: April 19, 2014; Revised: May 18, 2014; Accepted: May 18, 2014

Background: *Trachyspermum ammi* or *Carum copticum*, commonly known as Ajowan or Ajwain is a plant used as a spice. Thymol, the major phenolic compound present in Ajowan is known as a strong germicide and anti-spasmodic. It is also used for cleaning the wounds and treating skin infections.

Objectives: Here, we aimed to study the inhibitory effects of Ajowan essential oil on microorganisms like *Escherichia coli* (*E. coli*), *Klebsiella*, and *Staphylococcus aureus* growth.

Material and Methods: Antibacterial activities of Ajowan essential oil (AEO) have been evaluated against two gram negative bacteria; *Klebsiella* and *E. coli* and one gram positive bacteria; *Staphylococcus aureus* (*S. aureus*). Minimum inhibitory concentration (MIC) value was determined against all mentioned bacteria.

Results: The antibacterial activity of AEO was assessed against all selected pathogens and different MIC levels were observed. The essential oil was effective for *S. aureus* with MIC of 1.25 mg/mL, followed by *E. coli* with MIC of 2.5 mg/mL and *Klebsiella* with MIC of 5 mg/mL.

Conclusions: The results of the present study showed that the AEO has antibacterial effects against human pathogens that are resistant to antibiotics. More studies should be performed to demonstrate the detailed mechanism of this antimicrobial effect.

Keywords: Ajowan; Essential Oils; *E. coli*

1. Background

Ajowan or ajwain is a plant growing in India, Pakistan, South East and Near East of Iran, where the seeds are used as a spice. These seeds are used in Asian cooking, especially in India and for baking biscuits, breads and in bean dishes (1, 2). Like caraway, Ajowan seeds are rich in fiber, minerals, vitamins and anti-oxidants (1). Thymol, the major phenolic compound present in Ajowan, has been reported to be a germicide, antispasmodic and antifungal agent (3). It is used for treating skin infections. Ajowan essential oil (AEO) is also used in toothpaste and perfumery. The AEO is an almost colorless to brownish liquid with a characteristic odor and a sharp hot taste (1-3). Ajwain leaves can be crushed and used for skin infections. It is has also been used indigestion and gas relief in Middle East countries like Iran and Egypt. People in these countries, keep Ajwain powder in a thin cloth and smell it frequently, to decreases the pain, in the acute phase of common cold or migraine (2, 3). Ajowan is delicious with green beans and root vegetables. Some people in India, Pakistan and in the South East of Iran add Ajowan seeds to vegetable curries, steamed cabbage, carrots, potato and pumpkin. In Indian cuisine, this seed is usually crushed

in a mortar and then fried in butter to make an aromatic butter sauce, used to flavor vegetables and bean dishes. It is well-known for its medicinal effects, one of the chief being its ability to reduce flatulence. Indians chew the seeds after a meal to aid for digestion. It can also be brewed into tea, to treat colic and diarrhea. It has also important germicidal and fungicidal properties. It helps curing conditions like ringworm and itching and menstrual and post-natal disorders. There are some reports about the effect of plant seeds like Ajowan against infections (1-5).

2. Objectives

In the present study, we investigated the antibacterial activity and minimum inhibitory concentration (MIC) of AEO on some microorganisms, including *E. coli*, *Klebsiella*, and *Staphylococcus aureus* (*S. aureus*).

3. Materials and Methods

This study was conducted in the Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran, after getting the ap-

proval from the Scientific Committee of the Research Center. Before beginning the study, we prepared materials according to what follows.

3.1. Microorganisms and Raw Materials

Ajowan seeds were prepared from a local market. The bacterial strains including *E. coli*, *Klebsiella* and *Staphylococcus* that were resistant to three antibiotics (erythromycin, cefixime, and tetracycline), were purchased from Padtan Teb Company in Iran, Tehran. Drug resistance pattern were as follows; *Klebsiella* was resistant to cefixime and tetracycline, *E. coli* was resistant to erythromycin and tetracycline and *S. aureus* was resistant to erythromycin, cefixime, and tetracycline.

3.2. Ajowan Essential Oil

AEO was extracted by distillation with water, according to Davazdehemami et al. research, 1388. For this purpose, 30 g of dry grain samples that were powdered, was mixed with 400 mL of distilled water and then put into a 500 cc flask and heated for four hours. Heat increases the water vapor pressure and lumps containing essential oils will be broken. Afterwards the extract with water vapor would be placed in a refrigerant. The refrigerant condenses the production and the oil droplets and the water molecules move into two distinct phases. For the essence is lighter than water, it accumulates on the water surface. To collect the oil, water system opens, water exits and the oil remains inside the machine. The essential oil was then accumulated into small bottles. The bottles weight was calculated with laboratory scales, with an accuracy of 0.0001, before and after pouring the essential oil, to determine the oil weight.

3.3. Preparation of a Bacterial Suspension

To prepare a bacterial suspension, first the bacterial sample was inoculated to a steep medium nutrient agar 24 hours before the test, (Merck, Germany). After the bacterial growth, the medium was washed with saline and a concentrated bacterial suspension was obtained. Then the bacterial suspension was poured into a sterile tube, containing normal saline, the turbidity of which was measured with a spectrophotometer at a wave length

of 630 nm. When the turbidity of the above solution reached 0.5 of that of the McFarland solution, dilution was finished. Finally a bacterial suspension was prepared at the concentration of 1.5×10^8 CFU/mL.

3.4. Antibacterial Activity of the Ajowan Essence

Sensitivities of the bacteria with multiple drug resistance, were studied using the broth dilution of one in a round-bottom 96 well plates. The bacteria suspension (the positive control) was added to the first row of house plate medium (the negative control). To the next row of six houses of plates, an amount of 100 mL of Muler Hinton nutrient broth was added. One hundred microliters of the essential oil was added to the concentration of 10 mg/mL of the first well and this action continued until all six wells were poured with concentrations of 1.25, 2.5, 5 and 10. Twenty microliters of the bacterial suspension, equal to 0.5 McFarland, was added to each well. Then the plates were placed at a temperature of 35°C for 24 hours and the opacity by eye. The first opacity that showed the lowest turbidity was determined as the minimum inhibitory concentration (MIC). This experiment was carried out three times and the mean concentration of the three tests, for each well, was determined as the MIC.

3.5. Determination of Minimum Inhibitory Concentration

The concentration of the first well of a sample that did not show any opacity, microbial growth, in the descending serial dilutions (left to right) will be determined as the MIC of the sample. Therefore, all wells after this well will define the microbial growth.

4. Results

An antibacterial activity against all selected pathogens was observed for AEO, with different MIC. The essential oil was effective for *S. aureus* with the MIC of 1.25 mg/mL, followed by, *E. coli* with a MIC of 2.5 mg/mL, and *Klebsiella* with a MIC of 5 (Table 1). MIC is defined as the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism, after a specific incubation time.

Table 1. Effect of Ajowan Essential Oil on the Selected Bacteria^a

<i>E. coli</i>	MIC, mg/mL	<i>Klebsiella</i>	MIC, mg/mL	<i>S. aureus</i>	MIC, mg/mL
1	5	1	2.5	1	2.5
2	5	2	2.5	2	2.5
3	10	3	10	3	2.5
4	10	4	2.5	4	2.5
5	2.5	5	2.5	5	5
6	2.5	6	2.5	6	5
7	2.5	7	2.5	7	2.5
8	5	8	5	8	2.5
9	5	9	5	9	2.5
10	2.5	10	1.25	10	5
11	2.5	11	5	11	5
12	2.5	12	5	12	2.5

^a Abbreviations: MIC: minimum inhibitory concentration.

5. Discussion

Ajowan, one of the aromatic seed spices, is generally used for medicinal purposes in India, Iran, and Middle East countries (1, 3). There are near 16 compounds in Ajowan, but Thymol, the most important and main phenolic compound in Ajowan, has been reported to be a germicide (anti-bacterial and anti-fungal) and an antispasmodic agent (6-8). Also, ethanol and acetone extracts are found to be effective against many bacteria, including *Pseudomonas* species, *E. coli*, *Bacillus subtilis* and *S. aureus* (6, 7). In previous studies the antibacterial activity was assessed, using agar well diffusion method and MIC (6-9). Our results revealed that the bacterial strains growth, including *Escherichia coli*, *Klebsiella* and *S. aureus* that were resistant to two or three antibiotics, (erythromycin, cefixime, and tetracycline) was inhibited by the AEO. Kumar et al. in 2012 showed that five kinds of Ajwain oils had antimicrobial activity on five different bacteria (*Staphylococcus*, *E. coli*, *Proteus*, *Shigella*, and *Pseudomonas*), isolated from urine samples of patients with UTI infection (10). Murthy et al. reported that AEO had considerable inhibitory effects on the mycelial growth and spore germination of the toxigenic fungi *A. ochraceus* (11). Moazeni and colleagues in 2012 showed that 5 mg/mL concentrated AEO killed 100% of protoscolices in 60 minutes. One hundred percent scolicial activity was observed with AEO with concentration of 10 mg/mL, after 10 minutes of exposure (12). Park study showed a good nematocidal activity was achieved with essential oils of Ajowan (13). A report by Gilani in 2013 confirmed the antimicrobial activity of a cream formulated with essential oil of *Trachyspermum ammi* (14). Peruvian study in 2013 showed that the essential oils of Ajowan have a potential botanical insecticide against *Aedes aegypti* mosquito larvae (15). Seo SM et al. also reported the larvicidal activity of Ajowan. Their study evaluated the larvicidal activity of 20 plant essential oils, including Ajowan, against the mosquito *Aedes aegypti*. Results showed that the essential oils of Ajowan had a potential botanical insecticide against *Aedes aegypti* mosquito larvae (16). A study by Huang CC et al. evaluated the antioxidant activities of 25 commonly used essential oils. The recent survey showed the essential oil of ajowan had the best antioxidant activity among the studied essential oils (17). Ajowan is traditionally believed to be a digestive traditional drug in the southern region of India. Dry Ajowan seeds are powdered and soaked in milk and fed to babies. In the North India, it is used as a very effective drug for stomach pain, when taken in a large spoonful with a pinch of salt and a glass of water. It is very effective when swallowed with warm water. Sometimes people eat Ajowan seeds raw. The flavor of raw seeds is hot, fiery, bitter and can leave the mouth slightly numb. When using the whole seeds in cooking, they should be lightly crushed to release the volatile oils, which create the distinctive flavor. Ajowan is also believed to reduce the unwanted effects caused by beans,

when added to bean-based preparations. Medicinally, it has been proven to have different pharmacological activities like antifungal, antimicrobial, antioxidant, cytotoxic, hypolipidemic, antihypertensive, antispasmodic, antilithiasis, diuretic, antitussive, nematocidal, anthelmintic, antifilarial and bronchodilating actions (15-17). Our study also detected the antibacterial effects of AEO on the selected bacteria. It is hoped that in the future we can use this product to treat infections, particularly the resistant ones. The essential oil of *Trachyspermum ammi* is an effective antibacterial agent against human pathogens, even against micro-organisms which are resistant to antibiotics. More studies should be undertaken to show the exact mechanism of its antimicrobial effect.

Acknowledgements

We are grateful to Mrs. Seyyedi Saadati and Nazemi who helped us with their technical support.

Authors' Contributions

Batool Sharifi-Mood, Maliheh Metanat, Mahdiah Shafaghath, Saideh Saidee and Nahid Sepehri equally contributed to designing the study, gathering data, statistical analysis and writing of the manuscript.

Funding/Support

The study was financially supported by the Research Deputy of Zahedan University of Medical Sciences.

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