Antibacterial Activity and Chemical Composition of Ajowan (Carum copticum Benth. & Hook) Essential Oil

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ABSTRACT

Gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS) were employed to determine the chemical composition of essential oil obtained from dry fruits of Carum copticum. Thymol (36.7%), γ-terpinene (36.5%) and β-cymene (21.1%) were found to be the major constituents of the oil. The anti-bacterial activities of the oil were mainly investigated against food poisoning bacteria (Salmonella thyphimurium, Pseudomonas aeruginosa, Entero-pathogenic Escherichia coli, and Staphylococcus aureus) by broth microdilution and agar diffusion methods. The oil exhibited significant anti-bacterial activities against all the examined bacteria. In conclusion, the results of this study showed that the oil of Ajowan is rich in monoterpenes and it may be used as a natural anti-bacterial agent in drug and food industries.

Keywords: Carum copticum, Essential oil, Anti-bacterial activity, Gas Chromatography Mass Spectrometry

INTRODUCTION

During the past centuries, essential oils have been used traditionally in preservation of foods against microbial decay (1). Many of these oils are "generally regarded as safe" (GRAS) and have pleasant odors and taste; therefore, they are widely used in food and cosmetic industries as flavoring and perfume (2). Moreover, essential oils are used safely in herbal medicine as anti-microbial compounds (3). Bacterial food-born illnesses referred as food poisoning are mainly caused by Staphylococcus aureus, Salmonella thyphimurium and, entero-pathogenic Escherichia coli (4). On the other hand, in recent years, resistance to anti-bacterial drugs has increased dramatically. For example, Pseudomonas aeruginosa that is usually considered as one of the main causes of nosocomial infections is resistant to most of the known antibiotics (5). Considering the limited diversity of antibiotics, development of new antimicrobial compounds, especially from natural sources, is of great interest.

Ajowan (Carum copticum Benth. & Hook.) is an annual herbaceous essential oil bearing plant belonging to the Apiaceae family, which grows in India, Iran, and Egypt (6). It has been reported that Ajowan fruit oil has diuretic, carminative, analgesic, anti-dyspnoea and, anti-inflammatory compounds (7). Traditionally, the water extract of Ajowan is widely used to relieve

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the symptoms of flue in children (8, 9). In addition, methanol extract of this plant was reported to have antibacterial activity against multi-drug resistant salmonella typhi (10). The ripening seeds of this plant contain 2-4% essential oil that is rich in monoterpenes like thymol and is mainly used as an antiseptic agent as well as a drug component in medicine (11).

In the present study, the components of C. copticum essential oil were analyzed and examined for its inhibitory activities against some pathogenic and food-born bacteria.

**MATERIALS AND METHODS**

The plant species from which the oil was obtained were collected from Experimental Station Farm of Tarbiat Modares University, Tehran, Iran, and were identified and authenticated by A.R. Khosravi, a plant taxonomist, at Shiraz University, Herbarium, Shiraz, Iran. Voucher specimen (no. 24985) has been deposited in the herbarium.

**Oil Isolation**

The Ajowan seeds were ground and the resulting powder was subjected to hydrodistillation for 3 hours in an all glass Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia (12). The extracted oil samples were dried over anhydrous sodium sulphate and stored in sealed vials at 4°C for gas chromatography (GC) and GC/ mass spectrometry (MS) analysis.

**GC Analysis**

GC analysis was performed, using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was held at 50°C for 5 min and then programmed to 250°C at a rate of 3°C/min. Injector and detector (FID) temperatures were 290°C; helium was used as carrier gas with a linear velocity of 32 cm/s. The percentages were calculated by electronic integration of FID peak areas without the use of response factors correction. Linear retention indices for all components were determined by co-injection of the samples with a solution containing homologous series of C₈-C₂₂ n-alkanes.

**GC/MS analysis**

GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.); oven temperature was 40°C to 240 °C at a rate of 4°C. Transfer line temperature was 260°C. Carrier gas was helium with a linear velocity of 31.5 cm/s, split ratio 1/60. In addition, ionization energy was 70 eV, scan time 1 s, and mass range 40-300 amu. The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds or with the data published in the literature (13). Mass spectra from the literature were also compared (14).

**Antibacterial assay**

Five American Type Culture Collection (ATCC) strains including Staphylococcus aureus (ATCC 29213), Pseudomonas aeruginosa (8821M), Escherichia coli (ATCC 25922), and Salmonella typhimurium (RITCC 1731) were tested by agar diffusion and broth dilution methods. All the tested bacteria were cultured in Mueller-Hinton (MH) broth and incubated at 37°C to reach the turbidity equal to 0.5 McFarland Standard. The number of living bacteria in suspension was measured by culturing 100μl of diluted suspension on MH agar.

Screening the antibacterial activity by agar diffusion method
Table 1. Chemical compositions (%. w/w) of C. copticum essential oil.

<table>
<thead>
<tr>
<th>Components</th>
<th>Retention</th>
<th>Index *</th>
<th>(%)</th>
<th>Identification Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-thujene</td>
<td>932</td>
<td>0.5</td>
<td>MS, RI</td>
<td></td>
</tr>
<tr>
<td>α-pinene</td>
<td>941</td>
<td>0.2</td>
<td>MS, RI, CoI</td>
<td></td>
</tr>
<tr>
<td>Sabinene</td>
<td>981</td>
<td>0.3</td>
<td>MS, RI</td>
<td></td>
</tr>
<tr>
<td>β-pinene</td>
<td>984</td>
<td>2.5</td>
<td>MS, RI, CoI</td>
<td></td>
</tr>
<tr>
<td>α-phylanderene</td>
<td>1000</td>
<td>0.7</td>
<td>MS, RI</td>
<td></td>
</tr>
<tr>
<td>α-terpinene</td>
<td>1022</td>
<td>0.7</td>
<td>MS, RI</td>
<td></td>
</tr>
<tr>
<td>ρ-cymene</td>
<td>1028</td>
<td>21.1</td>
<td>MS, RI, CoI</td>
<td></td>
</tr>
<tr>
<td>β-phylanderene</td>
<td>1035</td>
<td>0.4</td>
<td>MS, RI</td>
<td></td>
</tr>
<tr>
<td>γ-terpinene</td>
<td>1060</td>
<td>36.5</td>
<td>MS, RI, CoI</td>
<td></td>
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<tr>
<td>Terpinene-4-ol</td>
<td>1177</td>
<td>0.01</td>
<td>MS, RI</td>
<td></td>
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<tr>
<td>Thymol</td>
<td>1294</td>
<td>36.7</td>
<td>MS, RI, CoI</td>
<td></td>
</tr>
<tr>
<td>Carvacrol</td>
<td>1306</td>
<td>0.1</td>
<td>MS, RI</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>99.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The retention Kovats indices were determined on DB-5 capillary column. MS= Mass Spectroscopy, RI= Retention Index, CoI= Co injection with authentic compounds.

The agar diffusion method was employed for the screening and determination of antimicrobial activity of the essential oil. 0.1 ml of 0.5 McFarland standard of each of the above-mentioned species was applied to the MH agar with a cotton swab. The plates were allowed to dry for at least 15 min before 10 μl of dissolved essential oil (consisting of 5 μl pure essential oil in the solvent) was added to each well prepared with biopsy punch. A well containing 5 μl solvent was served as control in each plate. The plates were then incubated at 37°C for 24 hours. The diameters of the inhibition zones were measured in millimeters by vernier calipers. All the tests were performed in triplicate.

Determination of minimum inhibitory concentration (MIC)

MICs were determined using the Broth dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS), with some modifications. Briefly, Mueller-Hinton (MH) Broth was supplemented with 0.002% (v/v) tween 80 (Sigma) to enhance dispersion of the Ajowan oil. Geometric dilutions of the essences, ranging from 80.0 to 0.036 μl/ml, were prepared in 1ml MH broth media in the test tubes. Growth controls consisting of MH broth without essence were included for each tested isolate. After the addition of 1 ml of the inoculums (1.5x106) to each tube, they were incubated at 35-37°C for 18-24 hours. In addition, 2 ml of uninoculated MH broth was included as a sterility control and blank. The growth in each tube was compared with that in the control tube. The MIC was the lowest concentration of Ajowan essential oil that resulted in a clear tube. Ten microlitres from each tube was spot-inoculated onto Nutrient Agar (NA) and incubated overnight at 37°C to determine the MBC. The highest dilution that inhibits the bacterial growth on nutrient agar after overnight incubation was taken as MBC. The experiments were performed three times.

RESULTS AND DISCUSSION

The chemical compositions of Carum copticum essential oil are shown in Table 1. Twelve compounds representing 99.7 % of C. copticum essential oil were identified. The major constituents of C. copticum were thymol (36.7%) and its precursors, γ-terpinene (36.5%), and ρ-cymene (21.1%). The oil was also examined for anti-bacterial
activities against 4 standard bacteria by the broth microdilution and agar diffusion methods. The essence exhibited significant antibacterial activities against all examined bacteria by agar diffusion method, except P.aeuroginosa. Minimal inhibitory and bactericidal concentrations of the essential oil against the examined bacteria are presented in Table 2.

In agar diffusion assay, Ajowan showed inhibitory effects against all the examined bacteria except P.aeuroginosa. However, in MIC analysis, the essential oil showed strong inhibitory activity against P.aeuroginosa in comparison to that of the disc diffusion method. These data are in contrast with the results of Bazzaz et al. (15), who reported no antibacterial activity of the oil against P.aeuroginosa. The difference in the results might be due to different constituents of the oil not reported in their work.

Similar to previous studies (7,16), thymol (36.7%) was found to be the major constituent of the oil known as 'Ajwan-ka-phul' (crude thymol), while others reported carvacrol as the major constituent of this oil (17). It has been shown that thymol and its precursors, cymene and terpinene, (18, 19) have strong antimicrobial activities. It has been reported that thymol might induce its antimicrobial action by perturbation of the lipid fraction of the microorganism plasma membrane, resulting in alterations of the membrane permeability and leakage of intracellular materials (20). Although terpinene was the second main constituent identified in the Ajowan oil, no strong antibacterial activity was reported from its gamma isomer (21). P-cymene is another major compound identified in Ajowan oil that is a hydrophobic molecule and causes swelling of the cytoplasmic membrane (21). It is not an effective anti-bacterial agent when used alone (23, 24), however, in combination with other phenolic compounds such as carvacrol, it has shown a great antimicrobial activity by incorporating cymene in the lipid bilayer of bacteria, facilitating the transport of phenolic monoterpenes of EOs across the cytoplasmic membrane (25). On the other hand, some studies have shown that the whole EO has a stronger antibacterial activity than the individual major components (26, 27), demonstrating that the minor constituents are also important to the anti-microbial activity and may have a synergistic influence (21, 28).

Considering the promising inhibitory and bactericidal activity of the examined ESO, it might be used as a natural food preservative as well as antibacterial substance in nosocomial infections. However, further studies are still required to investigate its application in medicine and food industries.

**REFERENCES**


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**Table 2.** Anti-bacterial activity of *Carum copticum* essential oil by broth microdilution and agar diffusion methods.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean diameter of inhibition zones (mm)</th>
<th>MIC ($\text{V/V}_0$)</th>
<th>MBC ($\text{V/V}_0$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S.aureus</em></td>
<td>22</td>
<td>%0.031</td>
<td>%0.031</td>
</tr>
<tr>
<td><em>P.aeuroginosa</em></td>
<td>0</td>
<td>%1</td>
<td>%2</td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>21</td>
<td>%0.031</td>
<td>%0.062</td>
</tr>
<tr>
<td><em>S.typhimurium</em></td>
<td>23</td>
<td>%0.015</td>
<td>%0.031</td>
</tr>
</tbody>
</table>

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