Original Article

Phytochemical Composition and Antibacterial Activity of *Trachyspermum copticum* L. essential oil, East Azerbaijan, Iran

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**Introduction:** The aim of this study was to characterize the chemical composition and antimicrobial activity of *Trachyspermum copticum* essential oil (EO). **Methods:** The chemical composition of seed samples obtained from Mianeh city in East Azerbaijan, was assessed using gas chromatography-mass spectrometry (GC-MS). The antimicrobial activity was evaluated by disc diffusion method against methicillin-resistant *Staphylococcus aureus* (MRSA), other extended-spectrum beta-lactamases (ESBLs) producing, as well as Gram-negative and Gram-positive bacteria. The minimum inhibitory concentration (MIC) value of EO was assessed using agar dilution method. **Results:** Thirteen monoterpenic hydrocarbons (57.6%) and oxygenated monoterpenes (42.4%) compounds were identified in the EO, of which, 3 compounds, including thymol, m-cymene, and γ-terpinene were the major components of the EO with quantities of 41.9, 33.53, and 20.42%, respectively. The EO showed antimicrobial activity against ten microorganisms, especially *Streptococcus sanguis*, *S. aureus* (MRSA strain), and *Klebsiella pneumoniae* (ESBL-producing strain), which was potential better than tetracycline and kanamycin. **Conclusion:** This study confirmed that EO of *T. copticum* has in vitro antimicrobial activity against Gram-negative and Gram-positive bacteria, which has made it an alternative antibacterial agent. J Med Microbiol Infec Dis, 2015, 3 (3-4): 71-74

**Keywords:** Phytochemicals, *Trachyspermum copticum*, Essential Oil.

**INTRODUCTION**

*Staphylococcus aureus* is of great concern in healthcare and community settings, due to involvement in life-threatening infections, and development of resistance to most classes of antimicrobial agents. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a cause of healthcare-associated infections, which had a dramatic increase in number in the 1990s [1], and the recent emergence of MRSA in community-associated infections [2-4] highlights the success of this species as a pathogen and its ability to adapt under pressure from antimicrobial agents.

On the other hand, different reports on extended-spectrum β-lactamases (ESBLs) variants and Metallo-beta-lactamases in the second half of the 1980s and broad geographical distribution of bacteria producing these enzymes, have been considered as another epidemiological phenomenon [5]. Carbapenems are often the last treatment option against ESBL-producing organisms. These organisms have become increasingly resistant to quinolones, aminoglycosides, trimethoprim-sulfamethoxazole, and other antibiotics. Continuous consumption of carbapenems has resulted in the emergence of new classes of Gram-negative bacteria, which is known as superbugs [6].

Multidrug-resistant bacteria have an excessive life-threatening importance, not only in the USA, Europe, and Japan but also in undeveloped countries [7], which have prompted researchers to think of new drugs. Nowadays, due to these problems, new antimicrobial agents and medicinal plants are being investigated as alternatives, especially in countries with the preferred use of these types of drugs, such as Iran. Essential oils (EOs) of medicinal plants contain very potent natural biologically active agents [8].

*Trachyspermum copticum* is an annual plant that grows in Ethiopia, Egypt, and India, and is cultivated in Afghanistan, Pakistan, and Iran, which made it as a target of medicinal plants. Geographic distribution of these species in Iran is in Azerbaijan, Isfahan, Fars, Kerman, Sistan and Baluchestan, Khorasan, and Tehran provinces [9]. These provinces have different ecology, which affects the efficiency of plants. Studies by Khosravi et al. [10] and Hassanshahian et al. [11] showed antifungal and antimicrobial activities of this medicinal plant against clinical isolates, respectively. Thus, the present study was designed to assess antibacterial activity of EO of *T. copticum* (L.) collected from East Azerbaijan province of Iran on Gram-negative and Gram-positive standard strains.

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MATERIAL AND METHODS

Plant materials. Seeds of T. coticum were purchased from markets in Mianeh, East Azerbaijan. The collected materials were air-dried at room temperature (~25°C) in the shade and powdered using a laboratory blender. The studied sample was confirmed as T. coticum by a botanist.

Essential oil extraction. One hundred grams of each sample were mixed with 500 ml of distilled water and subjected to hydrodistillation using a Clevenger-type apparatus for 3 h until total recovery of oil. The preparation of the EO was performed three times, and the obtained oils were dried over sodium sulfate, weighed, and stored at 4°C until use.

Gas-chromatography-mass spectrometry (GC-MS). The obtained EOs were analyzed using an Agilent 6890 gas chromatograph-mass spectrometer (GC/MS) fitted with HP5MS capillary column (30 m×0.25 mm) coupled with an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, Canada). The compounds were identified by matching their recorded mass spectra with the data bank mass spectra (Wiley 7N library).

Strains and growth culture. The antibacterial activity tests included 5 Gram-positive and 5 Gram-negative bacteria acquired from the American Type Culture Collection (ATCC) and the Persian Type Culture Collection (PTCC) including Enterococcus faecalis (ATCC 29212), S. aureus (ATCC25952), S. aureus (ATCC33591) as MRSA, S. aureus (ATCC29213), S. sanguis (PTCC1449), Enterobacter aerogenes (ATCC13048), Klebsiella pneumoniae (ATCC700603) as an extended spectrum β-lactamases producing bacteria, Proteus mirabilis (ATCC43071), and Escherichia coli O157:H7 (purchased from Razi Institute of Iran). These strains were kept at -70°C in Trypticase Soy Broth (TSB) with 20% glycerol, inoculated into TSB and incubated at 35°C. Subsequently, one colony from each culture was subjected to hydrodistillation using a Clevenger-type apparatus for 3 h until total recovery of oil. These strains were kept at 70°C in Trypticase Soy Broth (TSB) with 20% glycerol, and were thymol, m-cymene, and γ-terpinene (41.94, 33.53, and 20.42%), respectively.

Disc diffusion agar and agar dilution methods. In the screening of antibacterial activity of T. coticum EO by disc diffusion method, the greatest inhibition zone (50±3 mm) was against Streptococcus sanguis PTCC 1449, and the lowest MICs were for S. aureus ATCC 25952 and S. aureus ATCC 29213 (both 4 µg/ml, respectively). The results of the antimicrobial assays of EOs of T. coticum are summarized in Table 2.

Table 1. Chemical composition of essential oil of T. coticum collected from East Azerbaijan, Iran

<table>
<thead>
<tr>
<th>P.N</th>
<th>Name</th>
<th>RI</th>
<th>Area%</th>
<th>Case Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-Thujene</td>
<td>928</td>
<td>0.43</td>
<td>2367-85-2</td>
</tr>
<tr>
<td>2</td>
<td>α-Pinene</td>
<td>931</td>
<td>0.4</td>
<td>80-56-8</td>
</tr>
<tr>
<td>3</td>
<td>β-Pinene</td>
<td>961</td>
<td>1.47</td>
<td>99-86-5</td>
</tr>
<tr>
<td>4</td>
<td>β-Myrcene</td>
<td>979</td>
<td>0.7</td>
<td>123-35-3</td>
</tr>
<tr>
<td>5</td>
<td>3-Carene</td>
<td>1005</td>
<td>1</td>
<td>13466-78-9</td>
</tr>
<tr>
<td>6</td>
<td>α-Terpine</td>
<td>1008</td>
<td>0.44</td>
<td>20126-76-5</td>
</tr>
<tr>
<td>7</td>
<td>m-Cymene</td>
<td>1013</td>
<td>33.53</td>
<td>535-77-3</td>
</tr>
<tr>
<td>8</td>
<td>γ-Terpine</td>
<td>1047</td>
<td>20.42</td>
<td>99-85-4</td>
</tr>
<tr>
<td>9</td>
<td>Terpinolene</td>
<td>1078</td>
<td>0.17</td>
<td>586-62-9</td>
</tr>
<tr>
<td>10</td>
<td>4-Terpine</td>
<td>1161</td>
<td>0.25</td>
<td>20126-76-5</td>
</tr>
<tr>
<td>11</td>
<td>Estragole</td>
<td>1177</td>
<td>0.12</td>
<td>140-67-0</td>
</tr>
<tr>
<td>12</td>
<td>Sabinol</td>
<td>1179</td>
<td>0.1</td>
<td>471-16-9</td>
</tr>
<tr>
<td>13</td>
<td>Thymol</td>
<td>1266</td>
<td>41.94</td>
<td>89-83-8</td>
</tr>
</tbody>
</table>

a. Retention indices measured for n-alkanes (c-9 to c-24) on the nonpolar DB-5 column. Tr, traces (<0.1%)
According to the results mentioned above, the growth of all tested microorganisms was inhibited by EO of *T. copticum* seeds. Figure 1 shows the results of the tested microorganisms’ growth in plates containing serial dilutions of EO in 11 Petri dishes (512-0.05 µg/ml) as well as one plate without EO as a growth control.

**DISCUSSION**

The amounts of various compounds of EO of *T. copticum* showed differences in samples taken from different part of Iran. In a study by Mahboubi et al. (2011), the major components of samples from Kashan city, Iran, were reported to be thymol, γ-terpinene, and α-cymene (45.9, 20.6, and 19%, respectively) [14]. Akbarnia et al. (2005) studied 12 samples from different part of Qazvin, Iran, and found that the major components were thymol, γ-terpinene, and p-cymene (41-45, 28-32, and 16-25%, respectively) [15]. In another study by Haghiroalsadat et al. (2011) on *T. copticum* seeds harvested in Yazd province, Iran, it was revealed that thymol (64.9%) and γ-terpinene (11.1%) were the most dominant components of the EO [16]. Rabiey et al. (2014), studied on *T. copticum* seeds harvested in Mashad, Iran, and reported that the most dominant components were thymol (57.18%), p-cymene (22.55%), and γ-terpinene (13.67%) [19]. A glance at the results, shows that the thymol quantity of EO of *T. copticum* obtained from Mashad city [17] was more than those of our study and Yazd, Qazvin, and Kashan [16-18], and γ-terpinene quantity of our study was the same as that of the samples of Mahboubi et al.’s study (2011) in Kashan [14].

Based on the results of the antibacterial effect of EO of *T. copticum*, our hypothesis was documented by growth inhibition zone varied from 25 to 50 mm in the disc diffusion method, and MIC varied from 4 to 32 µg/ml against the studied bacteria in agar dilution method. In a study by Shrivatara et al. (2012), on antimicrobial potential of Ajwain collected from India, it was shown that inhibition zone for *E. coli* MTCC-443, *Bacillus subtilis* MTCC-441, and *S. aureus* MTCC-3160 were 14.8, 13.6, and 9.9 mm, respectively [18].

Aggarwal et al. (2012) studied in Dehradun on 4 species of bacteria obtained from Culture Collection Center, National Culture Laboratory, Pune, India and found that inhibition zone of the oil for *Salmonella typhi*, *E. coli*, *Lactobacillus* and *Bacillus licheniformis* were 40.45, 37.12, 44.54, and 0 mm, respectively [19]. A study by Oroojalian et al. (2010) on EO of *Carcum copticum* showed similar MIC results for *E. coli* O157:H7 and *S. aureus* ATCC 25923.
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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES


