Study of the Chemical Composition of Essential Oils of *Teucrium chamaedrys* L. at the Different Distillation in Mazanderan Province

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Abstract

The composition of the essential oils of *Teucrium chamaedrys* L. belongs to the family Lamiaceae, It is growing wild in the margin of mountainous roads of arid and cold climate of north Iran. In this research, essential oils of *Teucrium chamaedrys* L., extracted and measured at the different location by different methods of distillation and then were analyzed by GC and GC/MS. Investigation and comparison on essential oil constituents of herbs were collected from three localities (Chalus, Galoogah and Gadook). The essential oil yield at the different location from Chalus by water distillation (Clavanger) were (0.06%), and by water & steam distillation (Kyzer & Long) were (0.28%), and by steam distillation were (0.04%), the essential oil yield at the different location from Galoogah by water distillation (Clavanger) were (0.06%), and by water & steam distillation (Kyzer & Long) were (0.05%), and by steam distillation were (0.09%), the essential oil yield at the different location from Gadook by water distillation (Clavanger) were (0.2%), and by water & steam distillation (Kyzer & Long) were (0.16%), and by steam distillation were (0.08%), respectively. Major component identified in sample from Chalus by water distillation (Clavanger) were α-cadinene (15.4%), Z-β-farnesene (9.6%), 1-eicosene (8.2%), and by water & steam distillation (Kyzer & Long) were 1-eicosene (12.8%), α-cadinene (6.3%), benzyl salicylate (5.9%), and by steam distillation were cis-3-hexenyl benzoate (10.9%), (E,Z)-farnesol (10.3%), benzyl salicylate (8.3%). Major component identified in sample from Galoogah by water distillation (Clavanger) were E-α-farnesene (22.9%), α-calacorene (20.2%), α-murrolene (8.0%), and by water & steam distillation (Kyzer & Long) were α-cadinene (30%), E-β-caryophyllene (23.5%), E-α-farnesene (7.2%), and by steam distillation were E-α-farnesene (18.4%), 1-eicosene (15.2%), benzyl salicylate (10%). Major component identified in sample from Gadook by water distillation (Clavanger) were α-murrolene (8.7%), n-heptadecane (7.9%), cis-3-hexenyl benzoate (7.2%), and by steam distillation were α-murrolene (14.4%), cis-3-hexenyl benzoate (11.3%), E-α-farnesene (9.6%), respectively.

Key words: Essential oil, *Teucrium chamaedrys* L., Flowering stage and vegetative stage, GC and GC/MS

Introduction

The genus *Teucrium* (Lamiaceae) is comprised of about 340 species widespread over the world [1]. In the Flora Iranica, this genus is represented by 12 species, of which 3 are endemic [1]. Several *Teucrium* species are used in Iranian folk medicine as medicinal plant [2-5]. These oils are characterized by the presence of sesquiterpenes such as caryophyllene, caryophyllene oxide,
germacrene D, α-humulene, α-muurolene, (E)-β-farnesene and the monoterpene carvacrol. Due to the wide spectrum of biological activities displayed by the essential oils, these compounds are the subject of different researches. The multiple roles of the essential oils and their main components make them natural substances of great importance in several fields such as physiological function of growth, ecological function, development [6], resistance against diseases and insects [7]. They also possess antimicrobial, antiviral, antimycotic, antioxigenic, antiparasitic and insecticidal properties [8-12]. Regarding phytopathogenic viruses, various substances of natural and synthetic origin have been assessed for their antiphytophysical activity [13-16]. The aim of the study was to determine the volatiles of tree locations of grown Teucrium chamaedrys L. in Mazandran province.

Material and Methods

Plant Material

The plant material of Teucrium chamaedrys L. were collected on August 2012 from Mazanderan provience (from different location, Chalus, Galoogah and Gadook) in north of Iran, and were dried in the shade at room temperature. The specimen is deposited in Central Herbarium of Iran (TARI). (see: Holmgren, Index Herbariorum).

Isolation of the Essential oil

100 gr. of dried aerial parts of Teucrium chamaedrys L. were extracted by different methods of distillation: hydro-distillation (Clavanger type), water and steam distillation (Kyzer & Long), and steam distillation. The essential oil yield at the different location from Chalus by water distillation (Clavanger) were (0.06%), and by water & steam distillation (Kyzer & Long) were (0.28%), and by steam distillation were (0.04%), The essential oil yield at the different location from Galoogah by water distillation (Clavanger) were (0.06%), and by water & steam distillation (Kyzer & Long) were (0.05%), and by steam distillation were (0.09%), The essential oil yield at the different location from Gadook by water distillation (Clavanger) were (0.2%), and by water & steam distillation (Kyzer & Long) were (0.16%), and by steam distillation were (0.08%), the quantitative and qualitative analyses of the oils were performed by GC and GC-MS, respectively.

Gas Chromatography

GC analyses were performed using a Shimadzu-9A gas chromatograph equipped with a flame ionization detector, and the percentage of relative amounts were calculated from peak area using a shimadzu C-R4A chromatopac without applying correction factors. The analysis was carried out using a Ph-5 fused-silica column (30m x 0.25 mm, film thickness 0.1μm). The inner surface of the stationary phase material is covered Phenyl DimethylSiloxane 5%. Oven temperature programme were as follow: initial temperature 60 °C to start the final temperature of 210 °C. The initial 3 °C per minute to be added, and then injected into the chamber to a temperature of 280 °C. The carrier gas inlet pressure to the column: helium with a purity of 99.99% of the inlet pressure to the column equal to 1.5 5Kg/cm2 is set.

Gas Chromatography - Mass Spectrometry

The GC/MS unit consisted of a Varian Model 3400 gas chromatograph coupled to a Saturn II ion trap detector was used . The column was same as GC , and the GC conditions were as above. Mass spectrometer conditions were : ionization potential 70 eV; electron multiplier energy 2000 V. The identity of the oil components was established from their GC retention indices, relative to C7-C25 n-alkanes, by comparison of their MS spectra with those reported in the literature [17-19], and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Results and Discussion

Studies showed considerable quantitative and qualitative variations among the major compounds identified by the workers from the different countries which confirm the existence of different chemotypes of Teucrium chamaedrys L. Comparing the chemical constituents identified in the oil analysed with those reported earlier, results showed that the amount of E-β-caryophyllene (23.5%), in our analysed oil was higher than the reported by other workers from Iran by Semnani, et al. [20], on 2005, which showed that the β-caryophyllene (10.5%), available in this region contain higher amount of β-caryophyllene and thus could be used as a rich source of essential oil containing rich amount of β-caryophyllene for low volume high value products.
Fig. 1 A comparison diagram of essential oil from *Teucrium chamaedrys* L. from Chaluss location in Mazandaran province

Fig. 2 A comparison diagram of essential oil from *Teucrium chamaedrys* L. from Galoogah location in Mazandaran province

Fig. 3 A comparison diagram of essential oil from *Teucrium chamaedrys* L. from Gadook location in Mazandaran province
Table 1 Essential oils percentage of *Teucrium chamaedrys* L. at three locations in Mazandaran Province by different methods of distillation

<table>
<thead>
<tr>
<th>Methods of distillation</th>
<th>Chalus</th>
<th>Galoogah</th>
<th>Gadook</th>
</tr>
</thead>
<tbody>
<tr>
<td>First iteration</td>
<td>0.06</td>
<td>0.06</td>
<td>0.2</td>
</tr>
<tr>
<td>Second iteration</td>
<td>0.04</td>
<td>0.08</td>
<td>0.3</td>
</tr>
<tr>
<td>Third iteration</td>
<td>0.08</td>
<td>0.04</td>
<td>0.1</td>
</tr>
<tr>
<td>Mean</td>
<td>0.06</td>
<td>0.04</td>
<td>0.2</td>
</tr>
<tr>
<td>First iteration</td>
<td>0.04</td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
<td>Second iteration</td>
<td>0.07</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>Third iteration</td>
<td>0.09</td>
<td>0.05</td>
<td>0.09</td>
</tr>
<tr>
<td>Mean</td>
<td>0.06</td>
<td>0.05</td>
<td>0.6</td>
</tr>
<tr>
<td>First iteration</td>
<td>0.2</td>
<td>0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>Second iteration</td>
<td>0.17</td>
<td>0.09</td>
<td>0.13</td>
</tr>
<tr>
<td>Third iteration</td>
<td>0.16</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.12</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Essential oils chemical composition of *Teucrium chamaedrys* L. at three location in Mazanderan Province by different methods of distillation

<table>
<thead>
<tr>
<th>Compounds name</th>
<th>R.I.</th>
<th>Chalus</th>
<th>Galoogah</th>
<th>Gadook</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrodistillation</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Clavenger</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Steam distillation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water and Steam distillation (Kyzer &amp; Long)</td>
<td>0.25</td>
<td>0.31</td>
<td>0.28</td>
<td>0.28</td>
</tr>
</tbody>
</table>
In the current research the most important compositions in the essential oil from *Teucrium chamaedrys* L. from Iran by Semnani, *et al*. on 2005, forty-nine components were identified in the this oil.

The major constituents of the essential oil were germacrene D (16.5%), \((Z)\)-\(\beta\)-farnesene (12.2%), \(\beta\)-caryophyllene (10.5%), \(\alpha\)-pinene (9.1%) and \(\delta\)-cadinene (7.4%) [14]. In our research major component identified in sample from Chalus by water distillation (Clavanger) were \(\alpha\)-cadinene (15.4%), \(Z\)-\(\beta\)-farnesene (9.6%), and by water \& steam distillation (Kyzer \& Long) were \(\alpha\)-cadinene (6.3%), and major component identified in sample from Galoogah by water distillation (Clavanger) were \(E\)-\(\alpha\)-farnesene (22.9%), and by water \& steam distillation (Kyzer \& Long) were \(\alpha\)-cadinene (30%), \(E\)-\(\beta\)-caryophyllene (23.5%), \(E\)-\(\alpha\)-farnesene (7.2%), and by steam distillation were \(E\)-\(\alpha\)-farnesene (18.4%), and major component identified in sample from Gadook by steam distillation were \(E\)-\(\alpha\)-farnesene (9.6%).

Kaya, *et al*. from Flora of Turkey by six subspecies reported on 2009, the aerial organs of *T. chamaedrys* L. subsp. *trapezunticum* Rech. f. and the aerial parts were subjected to microdistillation for the isolation of volatiles. The analysis was simultaneously performed by using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The major components were characterized as \(\beta\)-caryophyllene (18%), nonacosane (12%), germacrene D (11%), caryophyllene oxide (7%), and \(\alpha\)-pinene (7%) for subsp. *trapezunticum*, and caryophyllene oxide (23%), \(\alpha\)-pinene (11%), and caryophyllenol II (5%) for subsp. *syspirense* [21].

In our research major component identified in sample from Galoogah by water \& steam distillation (Kyzer \& Long) were \(\alpha\)-cadinene (30%), \(E\)-\(\beta\)-caryophyllene (23.5%), \(E\)-\(\alpha\)-farnesene (7.2%).
The composition of the essential oils of Teucrium chamaedrys L. from Corsica and Sardinia islands were investigated using a combination of gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) after fractionation over column chromatography. Eighty-seven compounds were identified, the main components were β-caryophyllene (29.0% and 27.4%, respectively) and germacrene D (19.4% and 13.5%, respectively), followed by α-humulene (6.8%) and δ-cadinene (5.4%) in the Corsican sample and by Caryophyllene oxide (12.3%) and α-humulene (6.5%) in the Sardinian sample. The study confirms the quantitative variability of the chemical composition of T. chamaedrys oils [22]. In our research major component identified in sample from Chalus by water distillation (Clavanger) were α-cadinene (15.4%), Z-β-farnesene (9.6%), and by water & steam distillation (Kyzer & Long) were α-cadinene (6.3%), and major component identified in sample from Galoogah by water distillation (Clavanger) were E-α-farnesene (22.9%), and by water & steam distillation (Kyzer & Long) were α-cadinene (30%), E-β-caryophyllene (23.5%), E-α-farnesene (7.2%), and by steam distillation were E-α-farnesene and by steam distillation were E-α-farnesene (9.6%). Comparing the results of different studies on essential oil composition of Teucrium chamaedrys L. reveals that their constituents are variable according to their habitat that may be regarded to different chemotypes.

Acknowledgments

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References