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Chemical Composition of the Essential Oil of *Teucrium polium* L. From Iran

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Abstract: This study was designed to examine the chemical composition of essential oil of *Teucrium polium* L. (labiatae). The chemical composition of the essential oil from leaves with young branches of *Teucrium polium* from village in Kerman province, Iran was obtained by hydrodistillation method analyzed by gas chromatography and gas chromatography mass spectrometry. Twenty-eight compounds, accounting for 99.75% of the total oil with 0.75% oil yield were identified. The main constituents of the essential oil were α-pinene (12.52%), linalool (10.63%), Caryophyllene oxide (9.69%), β-pinene (7.09%) and β-caryophyllene (6.98%).

Key words: *Teucrium polium* L. · Essential oil composition · GC/MS

INTRODUCTION

Teucrium is a genus of perennial plants which belongs to the family Lamiaceae (Labiatae) is represented by more than 340 species widespread all around the world and comprises about 12 species in Iran [1, 2]. Common names for this genus include germanders. These species are herbs, shrubs or subshrubs. They are most common in Mediterranean climates and the Middle East. An unusual feature of this genus compared with other members of Lamiaceae is that the flowers completely lack the upper lip of the corolla. Its flowers are small and range from pink to white and its leaves are used in cooking and for medicinal purposes, particularly for the treatment of stomach ailments. This plant is a dwarf, pubescent, aromatic shrub, possessing oval leaves with enrolled margins and dense heads of white flowers. *Teucrium* species are rich in essential oils. Various species of this genus are known for their use in folk medicine. In Iranian folk medicine, *Teucrium polium* is used as anticonvulsant [3] and antispasmodic activity and an ethanolic extract showed anti-inflammatory, antipyretic and antibacterial activities [4, 5] and also is well known for its diurectic, diaphoretic, antihypertensive, antinociceptive and hypolipidemic properties [6-8]. The essential oil of *T. polium* has been widely in other countries studied but yet to now the composition of the essential oil of *T. polium* that grow in Kerman province haven't studied. In the present work we have studied the chemical composition oils of *T.polium* that grow in Kerman province in Iran and then the results were compared with various origins and species in other countries.

MATERIALS AND METHODES

Plant Material and Isolation Procedure: The leaves with young branches of *T. polium* plant were obtained from shrubs grown in a village in Kerman province, Iran at full flowering stage in May 2009. The samples were cleaned in shade condition to prevent hydrolyze of the existing materials and to keep the natural color of the sample fixed. Then they were dried in the temperature of the environment and were powdered and kept at appropriate conditions from the viewpoint of temperature and light until the essential oil taking stage. Afterwards, essential oil was taken from 150 grams of the powdered sample in hydrodistillation method with the help of Clevenger set for three hours . Following the sample oils were dried with anhydrous sodium sulfate and kept in sterile sample tubes in refrigerator. The oil yields were calculated on a dry weight basis as 0.75%.

Gas Chromatography: GC analysis was performed using a model Agilent-6890 gas chromatograph equipped with column DB-5 in 40 meters length, internal diameter of 0.18 mm and film thickness o.25 μm. Oven temperatures was from 60-210 centigrade at a rate of 5 centigrade slope per minute. Injector temperature was 280 centigrade and detector (FID) temperature was 270 centigrade and carrier gas was helium.

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Gas Chromatography/Mass Mass Spectrometry: In order to analyze and identify the combinations forming the essential oil, the Chromatograph Gas set attached to a Mass Spectrometry, Model Shimadzu-QP5050A was used. The conditions of analysis and specifications of the GC/MC set were as follows: Capillary column DB5-MS in 40 meters length, internal diameter of 0.18 mm and layer thickness of 0.18 micro meter, thermal program of oven (5 minutes) in 60 centigrade, then 60-275 centigrade with a 5 centigrade slope per minute, then 10 minutes in 275 centigrade, the temperature of place of injection 280 centigrade, gas conveying Helium, the speed of gas move 0.9 milliliter per minute, the ratio of fission 1 to 43, the rate of injection 0.1 micro liter, temperature of the reservoir of ionization 230 centigrade, ionization mode EI, Ionization energy 70eV. The series of normal Alkans C8-C28 were also injected to the set under the same condition with that of essential oil injection to calculate Restrictive Index (RI) of components of essential oil. The Restrictive Index of components of the sample was calculated by using a computerized program. Finally, the components of essential oil was identified by comparing the mass spectrums obtained with the existing standard mass spectrums at electronic library of Wiley 2000 existing in Absolution software of GC/Ms set and calculation of standard Restrictive Index in accordance with C_8-C_{28} Alkans and comparing them with the existing standard figurers in references [9].

RESULTS AND DISCUSSION

The essential oil was extracted by the hydrodistillation of the dried parts of *Teucrium polium* from village in Kerman province, Iran constituents were analyzed by GC/MS. The oil yields were calculated on a dry weight basis as 0.75% (w/w) of a yellowish oil. The identified combinations in essential oil, restrictive index (RI) and quantitative percentage of the compounds are presented (Table 1). Of the 28 compounds being identified in the essential oil of this plant with 99.75%, the combinations of α-pinene (12.52%), linalool (10.63%), Caryophyllene oxide (9.69%), β-pinene (7.09%) and β-caryophyllene (6.98%) with 46.91 percent constitute the highest percentage of essential oil. The quality and quantity of the materials forming *T. polium* essential oil had some differences and similarities with the cases reported in other regions. The studies of the ingredients of the essential oil of botanical populations with ecological and genetic differences can be of great importance in identifying the variety of essential oil inside the population of specie. It seems that the geographical origin of *T. polium* greatly influences the oil quality. The essential oil of *Teucrium* species has been studied in Iran and in the world. A scientific study of the oil of *T. stocksianum* subsp. *stocksianum* in April 2003 found thirty-eight compounds with 97.7% of the oil were identified. The oil yield obtaind were 0.7% on a dry weight basis. the major component of the oil as Camphene (20.6%), α-Cadinol (19.7%), Myrcene (10.2 %) and Carvacrol (9.9 %) [10]. The studies made have reported the volatile constituents of *T. flavum* leaves from Iran at the full flowering stage in May 2001, eighteen compounds were identified constituting about 99% with 0.2% yield of the oil that major components were β-caryophyllene (30.7%), Germacrene D (21.3 %) and α-humulene (8.4%) [11]. Studies in the Department of Pharmacy, The Medical Sciences University of Shaheed Beheshti Tehran have reported the rate of compounds in

Table 1: Combinations identified in the essential oil of *Teucrium polium* L.

Compound No.	Compound Name	Restrictive Index (RI)	Percentage (%)
1	(E)-2-hexenal	865	0.27
2	α-pinene	927	12.52
3	α-camphene	954	5.73
4	Sabinene	965	0.84
5	1-octen-3-ol	978	2.97
6	3-octanol	982	3.29
7	β-pinene	984	7.09
8	Myrcene	995	1.46
9	ρ-cymene	1025	0.45
10	1,8-cineole	1032	3.60
11	Limonene	1036	1.89
12	camphor	1092	5.21
13	Linalool	1127	10.63
14	α-terpineol	1139	0.33
15	Bornyl acetate	1142	5.34
16	terpinen-4-ol	1198	0.19
17	Carvacrol	1272	5.23
18	β-Myrcene	1296	0.45
19	Camphene	1385	0.27
20	β-caryophyllene	1421	6.98
21	α-humulene	1437	2.75
22	γ-cadinene	1488	3.66
23	Germacrene D	1497	5.03
24	Elemol	1549	1.24
25	Spathulenol	1552	0.21
26	Caryophyllene oxide	1578	9.69
27	α-cadinol	1702	1.68
28	Hexadecanoic acid	1896	0.75
29	Unknown	1965	0.25

The indexes of restrictive have been calculated by injecting the mixture of normal hydrocarbons (C_8-C_{28}) to DB-5 column.

the leaves of *T. orientale*. L. subsp. *orientale* Being collected in ne 2000, from Shiraz, at the time of flowering. Sixty-nine compounds with 96.4% of the total oil were identified. The yield of the oil was 0.03%. The main component were Linalool (17.0%), β-caryophyllene (9.3%) and Caryophyllene oxide (33.5%) [12]. The essential oil of *T. persicum* from Iran was collected in March 2004 from Fars province during flowering stage. Eighty-one components with 93.5% of the total oil were identified. The yield of the oil was 0.01%. The main components of the oil were Caryophyllene oxide (10.6%), α-Pinene (9.4%), geranyl linalool (7.8%), γ-cadinene (7.4%), elemol (6.9%) and α-cadinol (5.5%) [13]. The study volatile constituents of leaves *T. orientale* L. subsp. *taylori* from Iran in June 2007, from Lorestan province, at the time of flowering. Forty compounds were identified constituting about 97.36% of the oil that major components were Linalool (28.60%), Caryophyllene oxide (15.62%), 1,8-cineole (4.5%), 3-octanol (9.55%), β-pinene (8.75%), β-caryophyllene (7.33%) and Germacrene D (4.60 %) [14]. A study on the oil obtained from *T. polium* grown in Iran [15] revealed the presence of sesquiterpenens as major components. Germacrene D (13.2%), β-caryophyllene (18%), spathulenol (10.4%) and bicyclogermacrene (9.0%) being the major components identified.The main and important ingredient studied in different regions of the world in Italia on *T.fruticans* include β-Pinene (21.0%), Germacrene D (18.0 %) β-Myrcene (13.0%) and β-caryophyllene (12.0%) [16]. Such as in species of *T. polium* subsp. *capitatum* major component of the oil were α-Pinene (28.8%), β-Pinene (7.2%) and ρ-Cymene (7.0%) [17]. Chemical composition of the essential oil of the aerial parts of *Teucrium polium* L. grown in Jordan was determined by GC/MS. The oil obtained by hydrodistillation was found to contain 39 components, of which 37 were identified. The major components determined were 8-cedren-13-ol (24.8%), β-caryophllene (8.7%), germacrene D (6.8%) and sabinene (5.2%) [18]. Thirty-seven components were detected in the oil obtained from the aerial parts of Turkish *T. polium* [19]. The major components identified were β-pinene (18.0%), β-caryophyllene (17.8%), α-pinene (12%), caryophyllene oxide (10.0%), myrcene (6.8%), germacrene D (5.3%), limonene (3.5%) and spathulenol (3.3%). An older study [20], from Turkey also, showed the oil of stems and leaves of *T. polium* to consist of β-pinene (10.18%), nerolidol (9.51%), α-pinene (7.68%), alloaromadendrene (7.32%), aromadendrene (6.56%) and sabinol (6.20%) as the major compounds. The oil of *T. polium* growing in Saudi Arabia [21] revealed the presence of 10 terpenoid compounds including the hydrocarbons β-pinene, limonene, α-phellandrene and ä-cadinenes and the alcohols linalool, terpinen-4-ol, guaiol, cedrenol and cedrol and was found to be devoid of esters. Egyptian *T. polium* oil [22] was found to contain myrcene, OC-pinene, menthofuran, ocimene, pulegone and menthone as the main identified components. On the other hand, the oil of *T. polium* sp. *valentinum* endemic to the Iberian Peninsula in Spain [23] was reported to contain α-pinene (15.8%), sabinene (7.2%), β-pinene (11.7%), trans-pinocarveol (4.3%), terpinen-4-ol (4.5%) and β-bisabolene (2.5%) as the major constituents. A scientific study of the oil of *Teucrium polium* ssp. *aurasiacum* from Algeria in April 2003 analyzed by GC/MS. Twenty-one compounds were characterized representing 91.5% of the essential oil and yielded 1.7% of a yellow oil with α-cadinol (46.8%), 3β-hydroxy-α-muurolene (22.5%), α-pinene (9.5%) and β-pinene (8.3%) as the main components [24]. It is the first time that a *T. polium* oil has been found to contain α-pinene (12.52%) at such high percentage in Kerman province. α-pinene was reported as an important constituents of *T. polium* subsp. *Capitatum* (28.8%) [17], *T. polium* sp. *Valentinum* (15.8%) [23], *T. polium* (12%) [19], *T. polium* ssp. *Aurasiacum* (9.5%) [24], *T. persicum* (9.4%) [13] and *T. polium* (7.68%) [20]. Linalool (10.63), has been reported as the major compound of *T. orientale* L. subsp. *Taylori* (28.60) [14] and *T. orientale* L. subsp. *orientale* (17.0%) [12]. Caryophyllene oxide (9.69%) in compared with other regions has been reported as the main compound of the following species: *T. orientale* L. subsp. *orientale* (33.5%) [12], *T. orientale* L. subsp. *Taylori* (15.62%) [14], *T. persicum* (10.6%) [13] and *T. polium* (10.0%) [19]. β-pinene (7.9%) in compared with other regions has been reported as the major compound of the following species: *T. fruticans* (21.0%) [16], *T. polium* (18.0%) [19], *T. polium* sp. *Valentinum* (11.7%) [23], *T. polium* (10.18%) [20], *T. orientale* L. subsp. *Taylori* (8.75%) [14], *Teucrium polium* ssp. *Aurasiacum* (8.3) [24] and *T. polium* subsp. *Capitatum* (7.2%) [17]. β-caryophyllene (6.98%) has been reported as the main constituent of *T. flavum* (30.7%) [11], *T. polium* (17.8%) [19], *T. fruticans* (12.0%) [16], *T.orientale* L. subsp. *orientale* (9.3%) [12], *T. polium* (8.7%) [18] and *T. orientale* L. subsp. *Taylori* (7.33%) [14]. In present study results showed the essential oil of *T. polium*, β-caryophyllene and caryophyllene oxide were reported as the main sesquiterpenes in many other *Teucrium* species, but in this study, in addition to β-caryophyllene and caryophyllene oxide and β-pinene, we also identified α-pinene and linalool as the major compound. The

results of this study could be of interest for further phytochemical and biological investigation of *T. polium* taking into account that α-pinene oil showed marked antimicrobial activity.

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