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Research Article

COMPARISON OF VOLATILE COMPOUNDS IN *TEUCRIUM POLIUM L.* BY HEADSPACE AND HYDRODISTILLATION TECHNIQUES

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ABSTRACT: Aerial parts of *Teucrium polium* were subjected to headspace (HS) and hydro-distillation (HD) techniques after drying, then headspace volatiles and the essential oil were analyzed by GC/MS. Forty-eight and Thirty-nine constituents were identified in hydro-distillation and CombiPAL system which represented 99.7% and 99.8% of the oils, respectively. hydro-distillation method were α-pinene (30.8%), β- pinene (12.0%), myrcene (8.9%), limonene (7.9%), (E)-caryophyllene (5.6%), Germacrene D (6.9%), Bicyclogermacrene (4.5%), α- Eudesmol (2.0), valerianol (3.4%), and 7-epi-α-Eudesmol (3.2%) and the main compounds by CombiPAL techniques were α-pinene (38.8%), β- pinene (15.5%), myrcene (21.0%), limonene (13.1%), and trans- β- ocimene (3.4 %). The Headspace Technique is a new, rapid, simple and eco-friendly method for essential oil analysis of aromatic plants.

Keywords: *Teucrium polium,* CombiPAL system, Headspace, GC/MS, volatile component.

Abbreviation: RI- Retention Indices, GC/MS- Gas chromatography- mass spectrometry, HD- Hydro Distillation, HS- Headspace.

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 (Mozaffarian 1996; Rechinger 1982). Common names for this genus include germanders. These species are herbs, shrubs or sub shrubs. 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, *T. polium* is used as anticonvulsant (Zargari 1992) and antispasmodic activity and ethanolic extract showed anti-inflammatory, antipyretic and antibacterial activities (Tariq et al. 1989; Autore et al. 1984) and also is well known for its diuretic, diaphoretic, antihypertensive, antinociceptive and hypolipidemic properties (Suleiman et al. 1998; Rasekh et al. 2001). The Labiatae (Lamiaceae) is one of the largest and most distinctive families of flowering plants, with about 220 genera and almost 4000 species worldwide (Hedge 1992). Because of the high rate of species diversity and endemism in Labiates, many species are used in traditional and folk medicine in Iran (Naghibi et al. 2005). A wide range of compounds such as terpenoids, iridiods, phenolic compounds and flavonoids have been reported from the members of the family (Richardson 1992).

Teucrium polium L. or poly-germander (Kalpooreh in Farsi) a wild-growing flowering plant belonging to the family Labiatae, has been used as medicinal herb in Iran for over 2000 years as diuretic, antibacterial, antihypertensive, anti-inflammatory, antipyretic, antispasmodic, hypoglycemic (Esmaeili and Yazdanparast 2004; Baluchnejadmojarad et al. 2005). *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 medicine (Zargari 1992). Of 28 compounds being identified in the essential oil of this plant with 99.75%, the combination of α-pinene (12.52%), linalool (10.63%), caryophyllen oxide (6.69%), β- pinene (7.09%), and caryophyllene (6.98%) with 46.91% percent constitue the highest percentage of essential oil. A study on oil obtained from *Teucrium polium* grown in Iran revealed the presence of sesquiterpenes as major components oil (Moghtader 2009). Different studies showed that the chemical compositions depend on many parameters such as harvesting time, extraction technique, the geographical origin of the plant, and the part of the plant analyzed, (Najafian et al. 2012; Fellah et al. 2005). Techniques commonly used to extract the volatile components include steam distillation, hydro distillation, dynamic and static headspace, supercritical fluid extraction and solvent extraction Headspace sampling for gas chromatographic analysis has many advantages. (Fakhraei et al. 2005). The goal of this study was to investigate the composition of volatile components of *Teucrium polium* by HD and CombiPAL system (Headspace) Techniques from Iran.

MATERIAL AND METHODS

Plant material

Samples of *T. polium* were collected from Post-e-Chenar, near Sarvestan in Fars province (South of Iran). The plant specimen (No: 14428) was identified by the herbarium of Fars Research Center for Agriculture and Natural Resources, Shiraz, Iran. A voucher specimen of the plant is deposited in the herbarium of the Research Center for Agriculture and Natural Resources, Shiraz, Iran.

Hydro distillation essential oil extraction

The aerial parts were air-dried at ambient temperature in the shade. The dried samples of *T. polium* were subjected to hydro-distillation using an all glass Clevenger-type apparatus for 3 hours, to extract essential oils, according to the method outlined by the European Pharmacopoeia (Anonymous 1997). The essential oils were separated from the aqueous layer, dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (4°C) before gas chromatography-mass spectrometric (GC/MS) analysis.

Headspace volatiles extraction

Up to 3 gr of each *T. polium* dried sample was crushed and placed in 20 mL headspace vial, and immediately sealed with silicone rubber septa and aluminum caps. The vials were then transferred to the headspace tray. The headspace proceeded on the CombiPAL System which was provided with headspace auto-sampler, heater and agitator. The vial was heated up to 80° C and retained for 20 minutes while being agitated; the temperature of the sampling needle and transmission lines' temperature was 85° C.

Identification of the oil components by GC/MS

GC Analysis was carried out using an Agilent-technology chromatograph with HP-5 column (30m ×0.32 mm i.d. × 0.25 µm). Oven temperature was performed as follows: 60° C to 210° C at 3°/min; 210° C to 240° C at 20 °/min and hold for 8.5 min, injector temperature 280° C; detector temperature, 290° C; carrier gas, N_2 (1 ml/min); split ratio of 1:50. GC-MS analysis was carried out using a with Agilent 7890 operating at 70 eV ionization energy, equipped with a HP-5 MS capillary column (phenyl methyl siloxane, 30m × 0.25 mm i.d× 25µm) with He as the carrier gas and split ratio 1:50. Retention indices were determined using retention times of n-alkanes that were injected after the essential oil under the same chromatographic conditions. The retention indices for all components were determined according to the method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library data published mass spectra data (Adams 2007; Adams and Yanke 2007; McLafferty 1989; Joulain et al. 2001).

RESULTS AND DISCUSSION

The results obtained by HD and CombiPAL techniques are presented in Table 1 and 2.

Table 1. The essential oil Chemical compositions of *Teucrium polium* by HD method.

No.	Compounds	RI[a]	Area,%
1	α-thujene	9.23	0.2
2	α-pinene	9.31	30.8
3	Camphene	9.45	0.3
4	Sabinene	9.70	0.1
5	β-pinene	9.74	12.0
6	Myrcene	9.87	8.9
7	α-phellandrene	10.03	0.05
8	α-terpinene	10.14	0.03
9	p-Cymene	10.21	0.03
10	limonene	10.25	7.9
11	1,8-cineole	10.28	0.1
12	cis–β-ocimene	10.33	0.2
13	trans-β-ocimene	10.43	1.6
14	γ-terpinene	10.55	0.04
15	Terpinolene	10.85	0.1
16	Linalool	10.97	0.1
17	α-campholenal	11.24	0.1
18	trans-pinocarveol	11.35	0.2
19	cis-p-Mentha-2,8-dien-1-ol	11.41	0.6
20	Pinocarvone	11.59	0.1
21	Boeneol	11.63	0.1
22	Terpinene-4-ol	11.74	0.04
23	α-terpineol	11.87	0.1
24	Myrtenal	11.93	0.3
25	Verbenone	12.07	0.03
26	Carvone	12.40	0.01
27	Bornyl acetate	12.82	0.3
28	α-Copaene	13.72	1.0
29	β-Bourbonene	13.81	0.08
30	β-Elemene	13.88	0.4
31	(E)-caryophyllene	14.16	5.6
32	γ-Elemene	14.30	0.4
33	α-Guaiene	14.35	0.5
34	α-humulene	14.49	0.5
35	Germacrene D	14.77	6.9
36	Bicyclogermacrene	14.92	4.5
37	β-Bisabolene	15.02	0.5
38	(Z)-α-Bisabolene	15.04	0.3
39	7-epi-α-Selinene	15.13	0.6
40	δ-cadinene	15.20	0.9
41	α-Agarofuran	15.41	0.2
42	Germacrene B	15.52	0.9
43	Spathulenol	15.73	1.0
44	10-epi-γ-Eudesmol	16.15	1.1
45	α-Eudesmol	16.45	2.0
46	Valerianol	16.48	3.4
47	7-epi-α-Eudesmol	16.53	3.2
48	Eudesm-7(11)-en-4-ol	16.85	1.2

RI[a], retention indices

Forty-eight compounds were identified using hydro-distilled method which comprised 99.7% of the essential oils and Thirty-nine compounds were identified with Headspace Techniques that comprised 99.8 % of the oils. The main essential oil compounds of *Teucrium polium* by HD method were α-pinene (30.8%), β- pinene (12.0%), Myrcene (8.9%), Limonene (7.9%), (E)-caryophyllene (5.6%), Germacrene D (6.9%), Bicyclogermacrene (4.5%), α-Eudesmol (2.0), Valerianol (3.4%), and 7-epi-α-Eudesmol (3.2%).Fig-1

Table 2. Headspace volatile chemical Compositions (%) of *Teucrium polium*

No.		Compound	RI[a]	Area,%
1		α-thujene	9.24	0.4
2		α-pinene	9.32	38.8
3		Camphene	9.46	0.4
4		Sabinene	9.71	0.3
5		β-pinene	9.75	15.5
6		Myrcene	9.89	21.0
7		α-phellandrene	10.04	0.06
8		α-terpinene	10.15	0.05
9		p-Cymene	10.22	0.04
10		limonene	10.26	13.1
11		1,8-cineole	10.29	0.1
12		cis–β-ocimene	10.34	0.6
13		trans-β-ocimene	10.44	3.4
14		γ-terpinene	10.55	0.05
15		Terpinolene	10.86	0.1
16		1-Octen-3-yl acetate	11.09	0.01
17		α-campholenal	11.24	0.05
18		allo-Ocimene	11.26	0.01
19		trans-pinocarveol	11.36	0.04
20		cis-p-Mentha-2,8-dien-1-ol	11.42	0.1
21		Pinocarvone	11.60	0.04
22		Boeneol	11.63	0.01
23		Terpinene-4-ol	11.74	0.004
24		α-terpineol	11.88	0.02
25		Myrtenal	11.94	0.05
26		Nerol	12.25	0.01
27		Bornyl acetate	12.83	0.04
28		α-Copaene	13.72	1.1
29		β-Bourbonene	13.82	0.02
30		β-Elemene	13.89	0.08
31		(E)-caryophyllene	14.16	1.6
32		α-Guaiene	14.35	0.4
33		α-humulene	14.50	0.1
34		Germacrene D	14.77	1.1
35		Bicyclogermacrene	14.93	0.5
36		β-Bisabolene	15.02	0.4
37		(Z)-α-Bisabolene	15.05	0.2
38		Cubebol	15.13	0.02
39		δ-cadinene	15.20	0.05

RI[a], retention indices

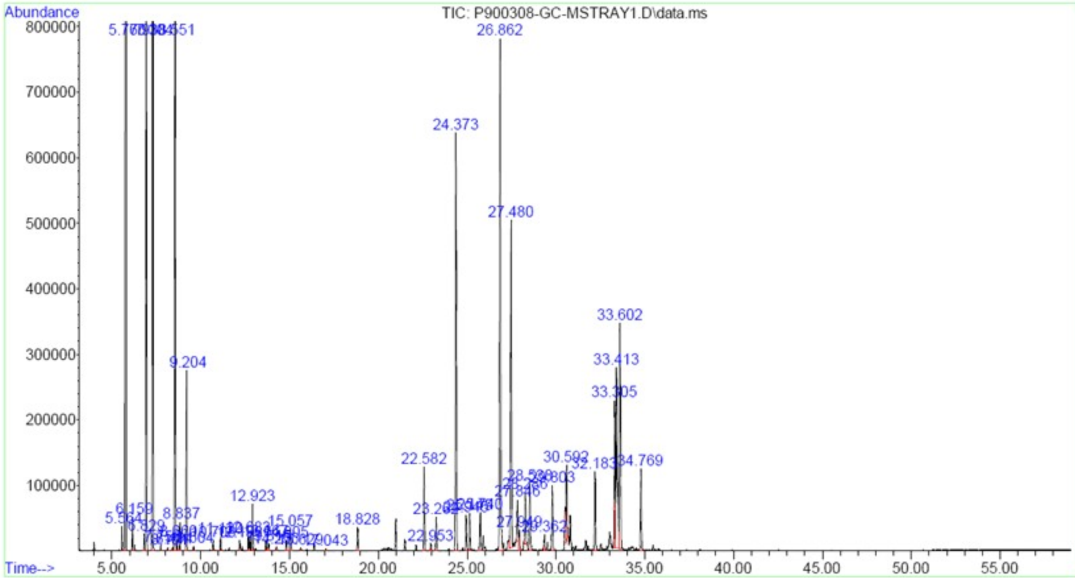

Fig1. Gas Chromatograph of *Teucrium polium* by HS (Headspace) method.

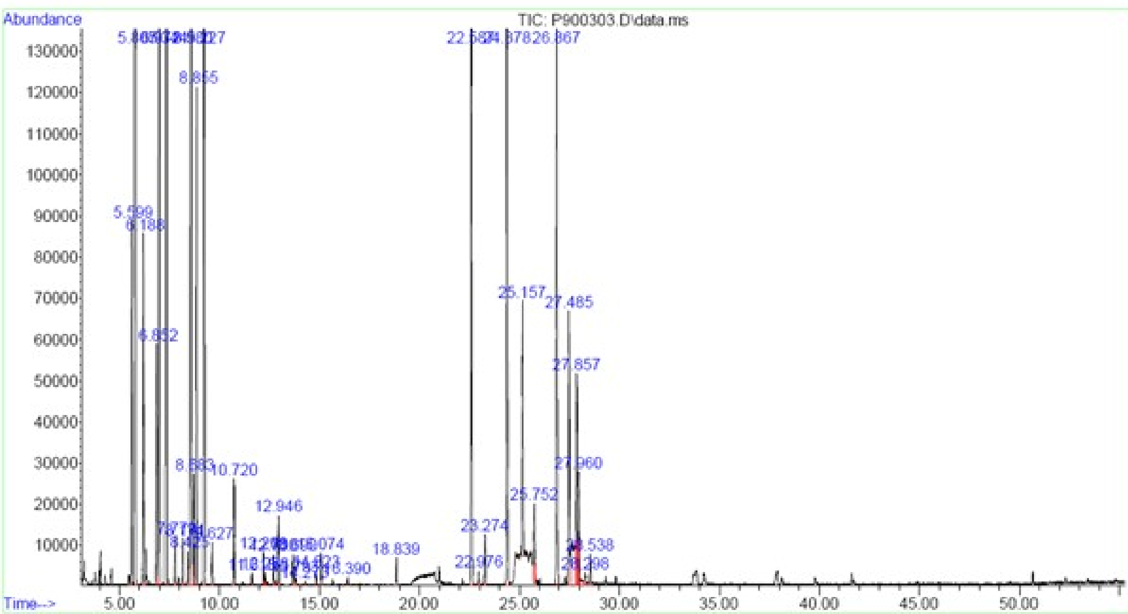

Fig2. Gas Chromatograph of *Teucrium polium* by HD method.

Additionally in 2009 Moghtader has found that 28 compounds being identified in the essential oil of this plant with 99.75%, the combination of α-pinene (12.52%), linalool (10.63%), caryophyllen oxide (6.69%), β- pinene (7.09%), and caryophyllene (6.98%) with 46.91% percent constitue the highest percentage of essential oil. Moreover main important essential oil compounds obtained by CombiPAL techniques were α-pinene (38.8%), β- pinene (15.5%), myrcene (21.0%), limonene (13.1%), and trans- β- ocimene (3.4 %). Fig-2.

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CONCLUSION

Our results showed that the number of components were different in these two methods. The GC/MS analysis results of the samples, extracted by the first method (hydro distillation), led to identification of 48 compounds among which Twelve of them were sesquiterpenes (15.42%) while by the second method (headspace SPME), Thirty-nine compounds were identified that 99.17% (35 compounds) of them were monoterpenes. It could be concluded that more percentage of sesquiterpenes in HD method was because of the presence of water, and more time in comparision with HS method so HS SPME could be the appropriate method for identifying lighter components.

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