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Extraction and Identification of Essential Oil Components from *Tanacetum polycephalum Subsp. duderanum* from two Habitats in Iran (Semnan and Firouzkouh) by Water Distillation and GC and GC/MS

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Abstract

The genus *Tanacetum L.* belongs to the *Asteraceae* family and *Anthemideae* tribe is one of the most important medicinal plants that contains 26 species in Iran, 12 of them are endemic. This paper reports the essential oil composition of *Tanacetum polycephalum subsp. duderanum* from two habitats in Semnan Province and Firouzkouh in Iran. Essential oil extracted by water distillation on Plant from flowers, leaves and stems were collected on May 2015. The yields of essential oils by water distillation from Semnan flower were 1.1%, leaf 0.6% and stem 0.33% and from Firouzkouh on flower were 0.51%, leaf 0.83 and stem 0.22%, respectively. Main components obtained from water distillation from Semnan on flower were camphor 53.63%, Limonene 7.85%, camphene 7.61%, 1,8-cineole 4.55%, on leaf were camphor 53.38%, 1,8-cineole 12.92%, cis-sabinene hydrate acetate 4.14% and 5-methyl-3-heptanone 4.09% and on stem were camphor 60.89%, cis-sabinene hydrate acetate 6.51%, 1,8-cineole 5.45%, and from Firouzkouh on flower were camphor 23.30%, 1,8-cineole 13.59%, cis-sabinene hydrate acetate 13.01%, camphene 12.46%, on leaf were 1,8-cineole 27.46%, cis-sabinene hydrate acetate 17.87%, camphor 17.06%, camphene 9.89%, on stem were camphor 33.94%, 1,8-cineole 21.41, cis-sabinene hydrate acetate 13.99%, camphene 6.06%, respectively.

Keywords: *Tanacetum polycephalum subsp. duderanum; Essential Oil; Water Distillation*

Introduction

The Asteraceae is the largest plant family. The family comprises more than 1600 genera and 23000 species [1,2].

Its many genera and species, its worldwide distribution and the fact that it comprises many useful plants have made it the subject of many karyological studies [3]. Many karyological and cytological studies have been performed in the Asteraceae [4-7]. The native flora of Iran comprises about 8000 angiosperm species. The genus *Tanacetum* (L.), formerly *Pyrethrum* (Zinn.), is a large, poorly defined classification group in the Asteraceae (Composite) containing polymorph species, many of which have applications as herbal medicines [8]. *Tanacetum* is one of the largest genera of the family Asteraceae, containing 250 - 500 taxa depending on the opinions of the authors who have studied the genus [9-17]. It is distributed throughout the Northern Hemisphere, with very few representatives (not more than 10 species) in the Southern Hemisphere. It seems that the genus is a polyphyletic complex and should be revised carefully regarding its

species and subgenus [3, 18, 19]. The genus *Tanacetum* belongs to the tribe Anthemideae of the Composite and comprises only one species of herbaceous plant, *Tanacetum polycephalum* subsp. *polycephalum* Syn.: *Tanacetum polycephalum* Schultz-Bip. subsp. *farsicum* Podl. [20]. Anthemideae- systematic review. In: Heywood, V.H., Harborne, J.B. and Its distribution pattern in Iran, in the rangelands of Zagros and Alborz Mountains and also in North Khorasan in the northeast of Iran [21]. The species of *Tanacetum* genus is a valuable medicinal plant. The flora of Iran comprises 26 species of *Tanacetum*, of which 12 are endemic [22,23]. *Tanacetum polycephalum* is used in folk medicine to treat many disorders [24], therefore, it seems interesting to investigate its biological activity and chemical analysis. Essential oils are a complex mixture of natural compounds, mainly monoterpenes, sesquiterpenes and their oxygenated derivatives, Asteraceae is a valuable source of essential oil-containing plants and there are many reports on the volatile constituents of the oils of these plants [25,26]. It traditionally been used as a spicy additive for food. In cosmetics and as herbal remedies, due to existence of biologically active compounds, this species is known as a good treatment for infectious disease [27]. The genus is rich in essential oils, bitter substances and sesquiterpene lactones [28]. These oils have been shown to possess antibacterial [29], and antioxidant activity [30], Flavonoids [31], and insecticidal effects [32]. Some members of the genus *Tanacetum* has traditionally been used in balsams, cosmetics, dyes, insecticides, medicines and have been found to act as a preservative and are used in herbal remedies. According some published study, the oils and extracts of members of the *Tanacetum* genus exhibit anti-inflammatory, antibacterial and antifungal [33]. These species have traditionally been used as a spicy additive for food, in cosmetics and as herbal remedies due to their biologically active compounds and many infectious diseases are known to be treated with herbal remedies throughout the history of mankind [34, 35, 36, 37]. Previous chemical investigations on different species of *Tanacetum* L. have been shown the presence of acetylenes [38], sesquiterpene lactones and essential oils [39-41]. The essential oil of *Tanacetum fruticulosum* C.B.Clarke (A synonym of *Tanacetum gracile* Hook.f. and Thomson) from Iran was investigated by GC, GC/MS and NMR spectroscopy. Some new farnesyl derivatives were identified by their NMR spectra [41]. Mirahmadi., *et al*. 2013 [42], reported on *Tanacetum polycephalum subsp*. *duderanum*, from Iran in total, 26 components representing about 98.4 % of its composition were identified. Artemisia alcohol (42.7 %), camphor (22.0 %), yomogi alcohol (14.0 %), camphene (4.8 %), 1,8-cineole (4.7 %) were the main components of the essential oil. Sefidkon., *et al*. 2007 [43], reported on the aerial parts of 8 populations *Tanacetum Polycephalum,* four populations from *T. polycephalum* Schultz-Bip. subsp. *duderanum* (Boiss.) Podl. were investigated. Hydro-distilled volatile oils from the aerial parts of eight samples of *T. polycephalum*, were investigated mainly by a combination of GC and GC/MS. The main components of the essential oils were as follow: *T. polycephalum* Schultz- Bip. subsp. *duderanum*: α-thujone (11.0% - 43.4%), camphor (8.0% - 18.8%), β-thujone (3.4% - 15.1%) and borneole (8.3% - 23.0%). Main components and their percentage varied by ecological parameters. Rezaee., *et al*. 2012 [44], studied on chemical composition of the essential oil of three *Tanacetum* L. species from north-west of Iran, on three species *Tanacetum angulatum* Willd. *Tanacetum canacens* DC. and *Tanacetum pinnatum* Boiss. growing wild in Iran. Plant flowers and leaves were collected from different locations of north- west of Iran. Samples were hydro-distilled to produce the oils in the yields *(v/w)* of 0.4% for leaves and 0.02% for flowers for both *T. angulatum* and *T. canacens*, collected from Azerbaijan province (Tabriz), in of 0.05% for leaves and 0.2% for flowers *T. pinnatum* from Zanjan province (Zanjan). Main oil components of *T angulatum* Wild. identified by GCIMS for leaves were 1,8-cineole (75.3%), camphor (8.1%) and for flowers were 1,8-cineole (66.0%), camphor (9.0%). For *T. canacens,* main oil components of leaves were 1,8-cineole (25.3%), α- calacorene (7.9%) and for flowers were n-eicosane (19.7%), α- calacorene (13.3%). Main oil constituents of *T. pinnatum* leaves were camphor (24.2%), o-calacorene (13.3%), and for flowers were germacrene B (33.0%), n-eicosane (10.5%). The aim of this study was investigation and comparison of essential oil content and composition of *Tanacetum polycephalum subsp. duderanum* from two habitats in Semnan Province and Firouzkouh in Iran.

Experimental Methods

Plant Material

The plant of *Tanacetum polycephalum subsp. duderanum* from two habitats in Iran (Semnan and Firouzkouh) center of Iran, Samples were collected by M. Golipour on 1 May 2015 and identity of the plant was determined by V. Mozaffarian in Iranian Botanical Garden (IBG).

The samples were extracted by water distillation from flowers, leaves and stem Plant. The yields of essences with method of water distillation from Semnan were flower 1.1%, leaf 0.6% and stem 0.33% and from Firouzkouh were flower 0.51%, leaf 0.83 and stem 0.22%, then samples were analyzed by GC and GC/MS.

Gas chromatography

GC analyses were performed using a gas chromatography, Ultera Fast Module –GC, made in Italia. Profile column machine brand Ph-5 capillary column, manufactured by Shimadzu with Length of 30 mm and an inner diameter of 1/0 mm thick 25/0 mm, the inner surface of the stationary phase material is covered Phenyl Dimethyl Siloxane 5%. Column temperature program: 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 5/1 kilogram per square centimeter 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 2000V.

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 and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co-injection with standards available in the laboratories [45-47].

Results and Discussion

Essential oil composition of *Tanacetum polycephalum subsp. duderanum* from two habitats in Semnan Province and Firouzkouh in Iran table 1.

		Firoz Koh			Semennan		
Compounds name	**R.I.**	**Flower**	**Leaf**	**Stem**	**Flower**	**Leaf**	**Stem**
2- heptanone	882	0.48					
α- thujene	931		0.21	0.14			
α- pinene	936	0.56	0.47	0.28	0.43	0.37	
5-methyl-3-heptanone	943	1.97	1.53	1.15		4.09	1.37
Camphene	961	12.46	9.89	6.06	7.61		3.09
Sabinene	973	0.47	0.40	0.32	0.20	0.50	0.16
p-mentha-1(7),8-diene	1005	1.18	1.01	0.74	1.22	1.03	0.50
δ- 3- carene	1013		0.21	0.23			0.22
α- terpinene	1039	0.62	0.40	0.38	0.25	0.29	0.17
p- cymene	1049	1.61	1.80	1.23		0.44	0.35
Limonene	1053	2.19	0.99	1.05	7.85	3.84	2.08
1,8-cineole	1060	13.59	27.46	21.41	4.55	12.92	5.45
Artemisia alcohol	1079	0.79	0.76	0.78	0.37	0.57	0.62
Trans-sabienene hydrate	1096			1.54		0.97	0.74
6-camphenol	1106	0.36		0.21	0.2	0.25	0.24

β-thujone	1111	2.19	0.25	0.17	0.56	0.27	0.23
Chrysanthenone	1127		1.55	1.11	0.20	0.52	0.52
p-menth-3-en-8-ol	1148	0.74	1.08	0.77	1.48	0.83	1.21
Trans-β- dihydro terpineol	1159				0.31	0.33	0.71
Santolinyl actate	1170	0.41	0.39	0.50	0.62	0.55	1.26
Terpin -4-ol	1173				0.69		
Camphor	1180	23.30	17.06	33.94	53.63	53.38	60.89
Octanol acetate	1211	0.66	0.81	0.91	1.18	1.29	1.38
Cis-sabinene hydrate acetate	1222	13.01	17.87	13.99	2.86	4.14	6.51
Neo-iso-dihydro carveol	1226	0.91	0.82	1.00	0.80	0.98	1.57
Trans-crysanthenyl acetate	1236	1.62	1.24	1.00	0.81	0.74	0.92
Cuminyl aldehyde	1241	0.32	0.25	0.42	0.58	0.54	0.76
Heptyl isobutyrate	1247		0.25	0.23			0.22
Trans-sabinene hydrate acetate	1258	0.74	0.58	0.61	0.54	0.52	0.81
Cis-chrysanthenyl acetate	1273	6.31	2.92	3.55	1.15	0.93	1.61
bornyl acetate	1284	0.38	0.21	0.26	2.31	1.21	1.34
2-ethyl- isomenthone	1289	1.42	1.13	1.24	0.60	0.46	0.59
neo-verbanol acetate	1317	5.80	3.87	2.75	1.71	1.05	1.28
α- terpinyl acetate	1349	1.24			0.21		
α- copaene	1374	0.80	0.41	0.51	0.17		
γ – cadinene	1514	0.62					
α- cadinene	1534			0.20			1.12
Caryophyllene alcohol	1567	1.00	0.22				
Spathulenol	1575	0.69					
Tetradecanal	1613				0.31		
β- eudesmol	1647		0.40	0.41	0.15		0.54
Ar-turmerone	1664						0.21
(Z, E)- farnesol	1687		0.48	0.29			
(Z, Z)-farnesol	1712		0.38	0.25			0.47
(z)- nuciferol	1731	0.55	0.21	0.16	0.57	0.28	0.52
Isoamyl dodecanoate	1862	0.37					

Table 1: *Essential oil composition of Tanacetum polycephalum subsp. duderanum from two habitats in Iran (Semnan and Firouzkouh) of Iran by water distillation.*

Some earlier works have been reported on the essential oils of various *Tanacetum* L. species [28,48,49].

The essential oil of *Tanacetum polycephalum subsp. duderanum* from two habitats in Semnan Province and Firouzkouh in Iran table 1. Essential oil extracted by water distillation from Plant on flowers, leaves and stem were collected on May 2015. The yields of essential oils by water distillation from Semnan from flower were 1.1%, leaf 0.6% and stem 0.33% and from Firouzkouh from flower were 0.51%, leaf 0.83 and stem 0.22%, respectively. Main components obtained from water distillation from Semnan on flower were camphor 53.63%, Limonene 7.85%, camphene 7.61%, 1,8-cineole 4.55%, on leaf were camphor 53.38%, 1,8-cineole 12.92%, cis-sabinene hydrate acetate

4.14% and 5-methyl-3-heptanone 4.09% and on stem were camphor 60.89%, cis-sabinene hydrate acetate 6.51%, 1,8-cineole 5.45%, and from Firouzkouh on flower were camphor 23.30%, 1,8-cineole 13.59%, cis-sabinene hydrate acetate 13.01%, camphene 12.46%, on leaf were 1,8-cineole 27.46%, cis-sabinene hydrate acetate 17.87%, camphor 17.06%, camphene 9.89%, on stem were camphor 33.94%, 1,8-cineole 21.41, cis-sabinene hydrate acetate 13.99%, camphene 6.06%, respectively.

Conclusion

A previous report by Mirahmadi., *et al*. 2013 [42], indicated that only 26 components representing about 98.4 % of its composition were identified present in essential oil of *Tanacetum polycephalum subsp. duderanum*, from Iran. Artemisia alcohol (42.7 %), camphor (22.0 %), yomogi alcohol (14.0 %), camphene (4.8 %), 1,8-cineole (4.7 %) were the main components of the essential oil. Which our components on camphor, camphene and 1,8-cineole are similar but with different percentage which can due to essential oil composition is a very complex one also because it is influenced by different factors such as climate, extraction methods, etc.

Rezaee., *et al*. 2012 [50], reported on essential oil composition of *Tanacetum angulatum* Willd. *Tanacetum canacens* DC. and *Tanacetum pinnatum* Boiss. growing wild in Iran. Plant flowers and leaves were collected from different locations of North- West of Iran. Samples were hydro-distilled to produce the oils in the yields (v/w) of 0.4% for leaves and 0.02% for flowers for both *T. angulatum* Willd and *T. canacens* DC., collected from Azerbaijan province (Tabriz), in of 0.05% for leaves and 0.2% for flowers *T. pinnatum* from Zanjan province (Zanjan) Main oil components of *T. angulatum* Wild. identified by GC/MS for leaves were camphor (8.1%) and for flowers were, camphor (9.0%). Main oil constituents of *T. pinnatum* leaves were camphor (24.2%).

Also, Sefidkon., *et al*. 2007 [43], reported on the aerial parts of 8 populations *Tanacetum Polycephalum,* four populations from *T. polycephalum* Schultz-Bip. subsp. *duderanum* (Boiss.) Podl. were investigated. Hydro-distilled volatile oils from the aerial parts of eight samples of *T. polycephalum*, were investigated mainly by a combination of GC and GC/MS. The main components of the essential oils were as follow: *T. polycephalum* Schultz- Bip. subsp. *duderanum*: α-thujone (11.0% - 43.4%), camphor (8.0% -1 8.8%), β-thujone (3.4% - 15.1%) and borneole (8.3% - 23.0%). Main components and their percentage varied by ecological parameters. These quantitative and qualitative differences between the present results and other researches could be ascribed to the harvest time, geographical origins and environmental factors.

With compare our work with Sefidkon., *et al*. 2007 [43], only in camphor are similar but with different percentage other components are different which can due to it is influenced by different factors such as climate, extraction methods, etc.

Nezhadali., *et al*. 2009 [27], reported on Chemical composition of the essential oils from the flower of *Tanacetum polycephalum subsp. duderanum* as a herbal plant in Iran, The chemical composition of the essential oil obtained by hydro-distillation from flower of *Tanacetum polycephalum subsp. duderanum* the dried plant in full flowering stage is 2.74%, and was analyzed by GC/MS and 23 compounds constituting 80.41% of the oil were identified. The major components were, cis-Chrysanthenyl acetate (27.18%), Thuj-3-en-10-al (10.70%), Linalool (9.72%), Linalool butyrate (8.80%), Carvacrol (5.64%), Geranyl acetate (5.44%), Menthyl isovalerate (2.94%), 1,8-Cineole (1.93), α- Pinene (1.61%) and Bornyl acetate (1.27%).

The comparing of the chemical compounds in the essential oil of *Tanacetum Polycephalum Subsp. duderanum* flower of our study with Nezhadali., *et al*. 2009 [27], show that most of the compounds in the oils of this flower are varied by ecological parameters. These quantitative and qualitative differences between the present results and other researches could be ascribed to the harvest time, geographical origins and environmental factors.

Main components obtained from water distillation from Semnan on flower were camphor 53.63%, and on leaf were camphor 53.38%, and on stem were camphor 60.89%, which Camphor has a wide variety of topical uses due to its antibacterial, antifungal, and anti-inflammatory properties. It can be used to treat skin conditions, improve respiratory function, and relieve pain.

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