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Essential Oil Variability Of Eight Populations Of *Tanacetum Polycephalum* From Iran

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ABSTRACT

Tanacetum polycephalum from compositae family consist of some subspecies in Iran. In this research, with the aim of phytochemical investigation, the aerial parts of 8 populations of this aromatic plant were collected, from different localities, and three subspecies were characterized. Four populations from *Tanacetum polycephalum* Schultz-Bip. subsp. *argyrophyllum* (C.Koch.) Podl., two populations from *T.polycephalum* Schultz-Bip. subsp. *polycephalum* and two populations of *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. *argyrophyllum*: camphor (3.1%-52.0%), 1,8-cineole (18.2%-36.3%), pinocarvone (0.0%-31.4%), α-pinene (3.2%-9.5%) and p-cymene (0.5%-10.4%). *T.polycephalum* Schultz-Bip. subsp. *polycephalum*: camphor (15%-37.3%), 1,8-cineole (7.2%-8.8%), borneol (7.4%-9.4%) and β-thujone (0.7%-7.2%). *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.

KEYWORDS

Polycephalum;
Compositae;
Phytochemical variability;
Camphor;
1,8-cineole;
Thujone;
Iran.

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INTRODUCTION

The genus *Tanacetum* (syn. *Chrysanthemum*), belonging to Composite family, with Ca. 200 species, is distributed over Europe and west Asia. It presents 26 species in Iran, 12 of them are endemic[1]. Among them, *T.polycephalum* Schultz-Bip consists of 7 subspecies named *T.polycephalum* subsp. *agryrophylum* (C.Koch.) Podl., *T.polycephalum* subsp. *azerbaidjanicum* Podl., *T.polycephalum* subsp. *duderanum* (Boiss.) Podl., *T.polycephalum* subsp. *farsicum* Podl., *T.polycephalum* subsp. *heterophyllum* (Boiss.) Podl., *T.polycephalum* subsp. *junesarense* (Bornm.) Podl., *T.polycephalum* subsp. polycephalum[1].

Tanacetum parthenium has been used since ancient times for the variety of medicinal proposes, and recently has gained considerable prominence due to its ability to alleviate the symptoms of migraine[2], arthritis and psoriasis[3], and to inhibit blood platelet aggregation[4]. Parthenolide and a number of related sesquiterpene lactones are considered to be responsible for these activities.

Pervious chemical investigations on different species of *Tanacetum* have shown the presence of acetylenes[5], flavonoids[6], sesquiterpene lactones[7] and essential oils[8-11].

The oils obtained by steam distillation of the flowers and leaves of *Tanacetum polycephalum* Schultz Bip. ssp. *heterophyllum* growing wild in Iran have been investigated, previously[12]. The main constituents of the oil of the flowers were camphor (59.1%), camphene (14.9%) and 1,8-cineole (10.1%), whereas the leaves oil comprised mainly camphor (53.5%), bornyl acetate (12.1%) and camphene (10.9%).

The extract of the aerial parts of *T.polycephalum* was reported to contain nerolidol derivatives, derived lactones and also germacranolids[7].

The aim of this study was investigation and comparison of essential oil content and composition of three subspecies of *T.polycephalum*, in different locality of Iran, that due to our searches have not been the subjects of previous study.

MATERIALS AND METHODS

Plant material

The aerial parts of three subspecies of *Tanacetum polycephalum* were collected from three different provinces in northwestern (West Azarbayejan), western (Hamedan) and North (Tehran) of Iran, in the flowering stage, TABLE 1. The voucher specimens have been deposited in the national herbarium of Iran (TARI).

Isolation procedure

Air-dried aerial parts of the plants (50-70g) were subjected to hydro-distillation for 3h using a Clevenger-type apparatus to produce oils in yields presented in TABLE 1. The oils were dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (2°C) before analysis.

Gas chromatography

GC analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m×0.25mm i.d., film thickness 0.25μm).

TABLE 1: Materials used in this study and oil yields obtained from *T.polycephalum*

Sample Code	Locality	Subsp.	Oil Yield (%)
T_1	Iran, Hamedan, Galeh-bor Assad-abad, 2280 m (TARI).	Polycephalum	0.14
T_2	Iran, Hamedan, Alvand Mountain, Ganjnameh, 2260 m (TARI).	Polycephalum	0.33
T_3	Iran, Tehran, Tochal, 2420-2450 m (TARI).	Duderanum	0.69
T_4	Iran, Tehran, Tochal, 2920-2950 m (TARI).	Duderanum	0.12
T_5	Iran, West Azarbayejan, Takab, Mayeen bolagh, Blooz area, 2200-2240 m(TARI).	Argyrophyllum	0.47
T_6	Iran, West Azarbayejan, Takab, Takht Soleiman, Dandi to Zanjan Road, 2370-2385 m, southern and western south aspect (TARI).	Argyrophyllum	0.26
T_7	Iran, West Azarbayejan, Takab, Takht Soleiman, Dandi to Zanjan Road, 2370-2385 m, western north aspect (TARI).	Argyrophyllum	0.28
T_8	Iran, West Azarbayejan, Khouy, Ghatour Valley, 2860 m (TARI).	Argyrophyllum	0.18

Oven temperature was held at 50°C for 5 min and then programmed to 250°C at a rate of 3°C/min.

Injector and detector (FID) temperature were 290°C; helium was used as carrier gas with a linear velocity of 32 cm/s.

Gas chromatography-mass spectrometry

GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d.); Oven temperature was 40°C to 240°C at a rate of 4°C, transfer line temperature 260°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60 , Ionization energy 70 eV; scan time 1 s ; mass Range 40-300 amu.

Identification of components

The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature[13,14].

RESULTS AND DISCUSSION

The oils isolated by hydro-distillation from the aerial parts of eight population of *Tanacetum polycephalum* were found to be yellow liquids and obtained in yields of 0.12% to 0.69% (w/w) based on dry weights. The highest oil yield obtained from T_3 (Tehran Province, Tochal, 2420-2450 m) and the lowest from T_4 (Tehran Province, Tochal, 2920-2950 m). It seams increasing the height decreases the oil yield.

The chemical composition of the *T. polycephalum* oils can be seen in TABLE 2. The components are listed in order of their elution on the DB-5 column. In the oils of *T.polycephalum* Schultz-Bip. subsp. *polycephalum* (T_1 and T_2), 23 and 17 compounds were identified, respectively. The major components of the oil of T_1 were found to be cis-β-terpineol (24.4%), camphor (15.0%), trans-sabinyl acetate (13.0%), borneol (7.4%), 1,8-cineole (7.2%) and bornyl acetate (6.5%). The main components of the oil of T_2 were camphor (37.3%), camphene (10.8%), borneol (9.4%), 1,8-cineole (8.8%), β-thujone (7.2%) and α-pinene (6.9%). The other differences between the oil compositions of these two populations have been shown in TABLE 2. Although these two oils have some common major components, but cis-β-terpineol, trans-sabinyl acetate and bornyl acetate that were present in the T_1 oil as main constituents, and also some minor components were not found in the oil of T_2. These differences could be related to different ecological situation of two populations. The Galeh-bor Assad-abad is a semi-dried region with means of 353 mm raining, while Alvand montain is semi-wet and cold region with means of 502 mm raining.

In the oils of *T.polycephalum* Schultz-Bip. subsp. duderanum (Boiss.) Podl. (T_3 and T_4), 15 and 20 compounds were identified, respectively. The major components of the oil of T_3 were α-thujone (43.4%), borneol (23.0%) and camphor (8.0%) and those of T_4 were camphor (18.8%), β-thujone (15.1%), α-thujone (11.0%), borneol (8.3%) and pinocarvone (8.1%). It seems the high percentage of α- and β-thujone is characteristic for subsp. duderanum. Comparison of the oil composition of T_3 (2420-2450m) with T_4 (2920-2950m), showed by increasing the height, not only the oil yield was decreased but also the percentage of α-thujone and borneol was decreased and camphor and β-thujone were increased.

In the oils of four samples of *Tanacetum polycephalum* Schultz-Bip. subsp. *argyrophyllum* (C. Koch.) Podl. (T_5, T_6, T_7 and T_8), 18, 18, 23 and 13 compound were identified, respectively. The major components of these samples were as follow:

T_5: pinocarvone (31.4%), 1,8-cineole (18.5%), camphor (18.5%), α-pinene (9.5%) and bornyl acetate (5.9%).

T_6: 1,8-cineole (18.2%), pinocarvone (13.4%), cis-p-menth-2-en-1-ol (9.5%), 1-terpineol (8.2%), and p-cymene (6.6%).

T_7: 1,8-cineole (36.3%), terpinen-4-ol (5.8%) and p-cymene (5.7%).

T_8: camphor (52.0%), p-cymene (10.4%), α-pinene (8.6%) and β-thujone (7.7%).

Although 1,8-cineole was one of the main component of the oils of T_5, T_6 and T_7, but this compound was not found in the oil of T_8. Instead of, the highest percentage of camphor was found in the oil of T_8. It seems the subsp. *argyrophyllum* shows more variability in its oil in different regions. Presence of

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TABLE 2 : Percentage composition of the oils of 8 populations of *Tanacetum polycephalum*

No	Compound	RI	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
1	Santolina triene	906	0.6	-	1.4	2.8	-	0.6	0.4	0.5
2	Tricyclene	924	-	-	-	0.5	0.6	-	-	-
3	α-thujene	930	-	0.5	1.0	2.7	0.8	-	0.2	2.7
4	α-pinene	938	1.0	6.9	1.8	3.0	9.5	3.2	5.6	8.6
5	Camphene	952	2.8	10.8	-	2.6	-	-	0.8	1.5
6	Sabinene	975	-	3.8	2.1	3.4	1.5	0.5	2.1	1.2
7	β-pinene	980	0.5	-	-	-	0.4	2.0	3.1	-
8	α-phellandrene	1004	-	0.6	-	1.1	-	-	-	-
9	1,4-cineole	1014	-	-	-	-	-	-	0.3	-
10	α-terpinene	1017	-	0.6	1.6	3.1	-	-	-	0.7
11	p-cymene	1025	2.4	2.4	5.0	3.5	0.5	6.6	5.7	10.4
12	Limonene	1030	-	-	-	-	-	2.5	0.7	-
13	1,8-cineole	1033	7.2	8.8	1.1		18.5	18.2	36.3	-
14	γ-terpinene	1061	0.6	0.6	1.7	2.3	1.0	1.0	1.2	0.8
15	Artemisia alcohol	1080	-	-	-	0.5	-	-	-	-
16	Linalool	1098	2.4	-	-	-	-	-	1.4	-
17	α-thujone	1101	-	1.6	43.4	11.0	-	-	-	0.9
18	β-thujone	1113	0.7	7.2	3.4	15.1	-	-	-	7.7
19	Myrcenol	1117	-	-	-	0.8	-	-	-	-
20	Cis p-menth-2-en-1-ol	1120	1.7	-	-	-	1.1	9.5	0.5	-
21	1-terpineol	1132	-	-	-	-	-	8.2	1.1	-
22	Camphor	1141	15.0	37.3	8.0	18.8	18.5	3.1	4.8	52.0
23	Trans-β-terpineol	1144	24.4	-	-	-	-	-	-	-
24	Pinocarvone	1160	-	0.5	0.5	8.1	31.4	13.4	-	2.3
25	Borneol	1165	7.4	9.4	23.0	8.3	0.7	3.3	1.3	-
26	Terpinen-4-ol	1177	1.9	0.5	1.7	3.5	2.4	4.7	5.8	1.7
27	p-cymen-8-ol	1183	0.8	-	-	-	-	-	-	-
28	α-terpineol	1189	0.7	-	-	-	-	2.6	3.8	-
29	Cis-piperitol	1191	0.9	1.7	-	0.9	0.9	3.5	0.7	-
30	Trans-piperitol	1203	-	2.5	-	-	0.8	5.2	-	-
31	Cuminyl aldehyde	1241	0.6	-	-	-	-	-	-	-
32	Piperitone	1250	1.6	-	-	-	-	-	-	-
33	Bornyl acetate	1284	6.5	-	3.5	2.5	5.9	-	-	-
34	Trans sabinyl acetate	1290	13.0	-	-	-	-	-	-	-
35	α-terpinyl acetate	1348	-	-	-	-	-	-	1.4	-
36	Geranyl acetate	1381	-	-	-	-	-	-	0.5	-
37	Germacrene D	1478	0.8	-	-	-	1.2	-	1.3	-
38	Bicyclogermacrene	1492	2.1	-	-	-	1.6	1.9	2.5	-
39	Total	-								

RI = Retention Index; t = trace = less than 0.05%

5.9% bornyl acetate in the oil of T_5, 13.4% and 31.4% pinocarvone in the oil of T_6 and T_5, esters like geranyl acetate and α-terpinyl acetate in the oil of T_7 and 7.7% β-thujone in the oil of T_8 is characteristic for each oil. Other differences are shown in TABLE 2.

The high differences in the oil composition of the subspecies of *Tanacetum polycephalum* from different regions showed this plant affected by ecological

factors intensively. For example, the locality of samples T_6 and T_7 are similar and just the gradient is different (TABLE 1), but the oil compositions are different more than that expected.

Comparing these results with the oils of other *Tanacetum* species[11,12] showed some similarity between subspecies. α-thujone, that was the main compound in the oil of *T.polycephalum* subsp. *duderanum*, was reported to be the major constituent of *Tanacetum argyrophyllum* var. *argyrophyllum* leaf (52.0%) and flower (63.0%) oils. Caryophyllene oxide (13%) and α-thujone (12%) were found as the main constituents of the oil of *Tanacetum argenteum* subsp. *canum* var. *canum*. Borneol (28%), 1,8-cineole (12%) and bornyl acetate (10%) were the major constituents of *T.praeteritum* subsp. *praeteritum*. In the oil of subsp. *massicyticum*, α-thujone (51%) and β-thujone (10%) were characterized as the main constituents. Camphor was also the main component of the oil of *T.polycephalum* subsp. *heterophyllum*[11].

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