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Original Research Article

Comparison of the volatile oils of *Artemisia tournefortiana* Reichenb. obtained by two different methods of extraction

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ABSTRACT

The essential oils obtained by hydrodistillation and solvent free microwave extraction from the aerial parts of *Artemisia tournefortiana* were analyzed by GC and GC/MS. Forty-six components representing 82.3% of the hydrodistilled oil and thirty-nine components representing 81.0% of the solvent free microwave extraction oil of the plant were identified. The main components of the water-distilled oils were (*Z*)-β-farnesene (34.2%) and nonadecane (8.1%), whereas in the microwave extraction method 2-propenoic acid, 2-ethyl hexyl ester (30.0%) and spathulenol (19.5%) were the major constituents. The water-distilled oil of *Artemisia tournefortiana* was rich with regard to sesquiterpenes (64.5%), whereas the solvent free microwave extraction oil was rich with regard to sesquiterpene hydrocarbons (40.0%) and non-terpenoid compounds (39.3%) and the monoterpene hydrocarbon fraction was relatively small, representing 1.7% of the total oil. According to this study, the composition of the two oils showed significant differences in the contents of the main components.

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1. Introduction

Artemisia (family Asteraceae) is a genus of small herbs or shrubs found in northern temperate regions of the world. Thirty-four species of this genus are found in Iran, of which two are endemic, namely *A.melanolepis* Boiss. and *A. kermansis* Podl (Rechinger, 1963; Mozaffarian, 1998). The plants of the genus *Artemisia* have always been of great botanical and pharmaceutical interest, particularly due to their use in the liqueur-making industry (Sacco et al., 1983). Furthermore, *A. annua* has gained a considerable attention because of its antimalarial activity which can be attributed to the presence of artemisinin in its aerial parts (Woerdenbag et al., 1990). This plant is of economic significance for its essential oils that are sometimes used in fragrances, perfumery, and cosmetic product (Libbey and Sturtz, 1989; Lawrence, 1990). *A. monosperma* is a perennial fragrant plant which has been reputed in folk medicine as an anti-spasmodic and anthelmintic agent. It has also been used in disorders associated with hypertension (Khafagy et al., 1971). *Artemisia austriaca* and *A. spicigera* are odorous herbs that have been used as antiseptic and stomachic remedies in folk medicine (Baytop, 1963). In the literature, some other *Artemisia* species involving *Artemisia sieberi*, *Artemisia annua* L., and *Artemisia absinthium* have been studied.

The genus *Artemisia* has been chemically investigated, and some valuable acetylenic compounds (Bohlmann et al., 1973), flavonoids (Wollenweber et al., 1992), coumarins (Rybalko et al., 1976), and terpenoids, especially sesquiterpene lactones (Rustaiyan et al., 1987; Rustaiyan et al., 1989a; Rustaiyan et al., 1989b; Marco et al., 1993a) have been reported in their corresponding organic extracts. In this regard, phytochemical studies on *A. chamaemelifolia* revealed the presence of numerous sesquiterpene acids (Trendafilova-Savkova et al., 2003), tricyclic sesquiterpenes (Marco et al., 1996), and coumarins (Bandyukova and Konovalova, 1970), whereas a methanolic extract of the aerial parts of *A. turcomanica*

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afforded two new germacrenolides and several known sesquiterpene lactones (Marco et al., 1993b). In addition, the methanolic extract from the aerial parts of *A. tournefortiana* has shown three eudesmanolide derivatives (Sanz and Marco, 1990; Talzhanov et al., 2007).

The chemical profiles of the essential oils from different *Artemisia* species have been characterized by several authors (Khazraei-Alizadeh and Rustaiyan, 2001; Morteza-Semnani and Akbarzadeh, 2005; Dob and Benabdelkader, 2006; Nematollahi et al., 2006; Firouznia et al., 2007; Rustaiyan et al., 2009; Haider et al., 2010; Padalia et al., 2011; Sharopov and Setzer, 2011; Nekoei et al., 2012; Mohammadhosseini et al., 2016; Zanousi et al., 2016; Mohammadhosseini, 2017).

The plants belonging to the genus *Artemisia* has always been of great botanical and pharmaceutical interest from ancient times (Rustaiyan and Masoudi, 2011). They have also found impressive impacts in traditional medicines for the treatment of a variety of diseases and disorders. For instance, the leaf of *A. douglasiana* has been shown to be an efficacious complementary herbal treatment against chronic bladder infection in paralyzed persons. *A. austriaca* and *A. spicigera* are odorous herbs that are used as antiseptics and stomachic in folk medicine (Güvenalp et al., 1998). The herb of *A. vestita* has been widely used in traditional Tibetan and Chinese medicine for a broad array of inflammatory diseases, such as rheumatoid arthritis, contact dermatitis and sepsis (Sun et al., 2006).

In this work, the comparison of hydrodistillation (HD) and solvent free microwave extraction (SFME) methods for the extraction and subsequent analysis of the volatile oils of *A. tournefortiana* Reichenb was investigated for the first time.

2. Experimental

2.1. Plant material

The aerial parts of *A. tournefortiana* were collected during the flowering stage in the Northen Khorassan Province, Iran, in July 2015. An IBRC number (IBRC P1000632) has been deposited at the Iranian Biological Resource Center, Tehran, Iran. The map of sampling area and *A. tournefortiana* photo is shown in Fig. 1 (a and b), respectively.

2.2. Isolation of the essential oils

2.2.1. Hydrodistillation (HD)

The air-dried aerial parts of *A. tournefortiana* were subjected to water distillation using a Clevenger-type apparatus for 3 h. The obtained essential oils were dried over anhydrous sodium sulfate and stored at 4 °C after filtration until tested and analyzed. The yield was found to be 0.2% (w/w).

Fig. 1. (a) The map of sampling area and (b) the photo of the *A. tournefortiana*.

2.2.2. Practical details of solvent free microwave extraction (SFME) set up

Solvent-free microwave extraction was performed in a Milestone ETHOS 1600 batch reactor, which is a multimode microwave reactor operating at 2455 MHz with a maximum delivered power of 1000 W, variable in 10 W increments. The dimensions of the PTFE-coated cavity were 35×35×35 cm.

During the experiment, some key parameters such as time, temperature, pressure, and power were controlled using the "easy-wave" software package. Accordingly, the temperature was monitored with the aid of a shielded thermocouple (ATC-300) which was directly inserted into the sample container.

In a typical SFME procedure, 250-g portions of the air-dried aerial parts of *A. tournefortiana* were moistened prior to the extraction by soaking in water for 1 h, then the excess water was drained off. After this step, the moistened plant was placed in a reactor without the addition of any solvent or water. The obtained essential oil was collected, dried with anhydrous sodium sulfate, and stored at 0 °C until being used. The yield was found to be 0.08% (w/w).

2.3. Gas chromatography (GC)

GC analysis was performed on a Shimadzu 15A gas chromatograph equipped with a spilt/spiltless (ratio 1:30), injector (250 °C) and a flame ionization detector

(250 °C). N_2 was used as carrier gas (1 mL/min) and the capillary column used was DB-5 (50 m×0.2 mm, film thickness 0.32 μm). The column temperature was kept at 60 °C for 3 min and then heated to 220 °C with a 5 °C/min rate and kept constant at 220 °C for 5 min. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

2.4. Gas chromatography-mass spectrometry (GC/MS)

GC/MS analysis was performed using a Hewlett-Packard 5973 with an HP-5MS column (30 m×0.25 mm, film thickness 0.25 μm). The column temperature was kept at 60 °C for 3 min. and programmed to 220 °C at a rate of 5 °C/min. and kept constant at 220 °C for 5 min. The injector and GC/MS interphase were maintained at 270 °C. The flow-rate of helium as a carrier gas was exactly set at 1 mL/min. The split ratio was 1/50. All the MS spectra were taken at 70 eV over the mass range (m/z) 45-465 amu at a speed of 2.8 scan/s. The temperature of the ion source and transfer line were regulated at 250 °C and 280 °C, respectively.

2.5. Identification of components

The retention indices for all the components were determined according to the Van Den Dool method, using *n*-alkanes as standards (Kulisic et al., 2004). The compounds were identified (RRI, DB-5) by comparison with data reported in the literature and by conformity of their MS with either the Wiley library or with published MS (Adams, 2007).

3. Results and Discussion

3.1. Chemical profile of the essential oils of *A. tournefortiana* using HD and SFME techniques

The identified volatile components and their relative percentages from the aerial parts of *A. tournefortiana* obtained by HD and SFME are given in Table 1 and Table 2, respectively. The components of each profile have been listed in order of their elution on the DB-5 column.

As shown in these tables, about 82.3% (46 components) of the water-distilled oil and 81.0% (39 components) in the SFME oil of *A. tournefortiana* were identified.

The water-distilled oil of the plant consisted of five monoterpene hydrocarbons (3.4%), four oxygenated monoterpenes (2.0%), fourteen sesquiterpene hydrocarbons (48.5%), twelve oxygenated sesquiterpenes (16.0%), three diterpenes (1.5%), and eight non-terpenoid compounds (10.9%). The main components of the oil were (*Z*)-β-farnesene (34.2%), nonadecane (8.1%), caryophyllene oxide (3.0%), β-caryophyllene (2.6%), and limonene (2.4%). Accordingly, the water-distilled oil of the plant was rich with regard to sesquiterpene hydrocarbons (64.5%).

The oil isolated through SFME approach from the aerial parts of the plant (*A. tournefortiana*) consisted of three oxygenated monoterpenes (1.7%), six sesquiterpene hydrocarbons (10.3%), twelve oxygenated sesquiterpenes (29.7%), and eighteen non-terpenoid compounds (39.3%). According to our systematic characterization, 2-propenoic acid, 2-ethyl hexyl ester (30.0%), spathulenol (19.5%), caryophyllene oxide (3.9%), bicyclogermacrene (3.4%), and germacrene D (3.1%) were the major constituents of this oil.

The oil of the plant was rich with regard to sesquiterpene hydrocarbons (40.0%), and non-terpenoid compounds (39.3%), while the monoterpene fraction was relatively low, only representing 1.7% of the total oil. According to these results, the composition of the two oils shows significant differences for the contents of the main components.

3.2. Compositions of essential oils of other *Artemisia* species

Some of our earlier works have been reported on the oils of various *Artemisia* species. The volatile oil of the *A. santolina* was found to contain lavandulol (37.2%), 1,8-cineole (15.9%) and linalool (13.6%) as major constituents and the oil of *A. gypsacea* was characterized by higher amounts of 1,8-cineole (36.5%) and β-thujone (28.4%) (Rustaiyan et al., 2000a).

The major constituents of the oils of *A. aucheri* and *A. deserti* were camphor (44.0% and 45.5%) and 1,8-cineole (14.3% and 16.7%), respectively (Rustaiyan et al., 2000b; Mohammadpoor et al., 2002).

The dominant compounds in the oil of *A. biennis* from Iran, were camphor (24.6%), artemisia ketone (11.4%) and α-pinene (10.2%) (Nematollahi et al., 2006). The oils obtained from the aerial parts of *A. persica* from Iran were rich in (*Z*)-ocimenone (39.6%), ascaridole (16.0%) and α-terpinene (10.0%), whereas those of the leaf were predominately involving *cis*-sabinene hydrate (38.8%) and terpinolene (13.3%). The flower oil was characterized by higher amounts of *cis*-sabinene hydrate (41.2%) and ethyl-2-nonynoate (24.4%). β-Cedren-9-one (76.7%) was the predominant compound in the root oil (Mirjalili et al., 2006). Davanone (40.1%, 32.3%, and 12.6%) was the main constituent in the oils separated from the stem, leaf and aerial parts of the *A. ciniformis*.

The flower, leaf, and stem oils of *A. incana* were rich in α-thujone (28.7%, 28.1%, and 22.2%), 1,8-cineole (20.5%, 22.1% and 25.5%) and camphor (10.5%, 10.1%, and 12.6%), respectively (Rustaiyan et al., 2007).

The oils of the *A. kermanensis* and *A. kopetdaghensis*

Table 1

Percentage composition of the essential oil of *A. tournefortiana* obtained by hydrodistillation.

NO.	Compounds	Class	Chemical formula	Hydrodistillation	
				RI[*]	Percentage (%)
1	Myrcene	M.H.[a]	$C_{10}H_{16}$	991	0.2
2	*p*-Cymene	M.H.	$C_{10}H_{14}$	1026	0.2
3	Limonene	M.H.	$C_{10}H_{16}$	1031	2.4
4	γ-Terpinene	M.H.	$C_{10}H_{16}$	1062	0.4
5	Terpinolene	M.H.	$C_{10}H_{16}$	1088	0.2
6	*iso* Pentyl 2-methyl butanoate	N.E.[b]	$C_{10}H_{20}O_2$	1099	0.3
7	*trans*-Rose oxide	O.M.[c]	$C_{10}H_{18}O$	1127	0.2
8	Camphor	O.M.	$C_{10}H_{16}O$	1143	0.6
9	Estragole	O.M.	$C_{10}H_{12}O$	1195	0.7
10	Citronellol	O.M.	$C_{10}H_{20}O$	1228	0.2
11	Dihydro carvyl acetate	O.M.	$C_{12}H_{20}O_2$	1235	1.0
12	α-Copaene	S.H.[d]	$C_{15}H_{24}$	1376	1.8
13	β-Bourbonene	S.H.	$C_{15}H_{24}$	1384	1.7
14	Tetradecane	N.H.[e]	$C_{14}H_{30}$	1399	0.3
15	β-Caryophyllene	S.H.	$C_{15}H_{24}$	1418	**2.6**
16	β-Gurjunene	S.H.	$C_{15}H_{24}$	1432	0.3
17	Neryl acetone	O.M.	$C_{13}H_{22}O$	1434	0.5
18	(Z)-β-Farnesene	S.H.	$C_{15}H_{24}$	1443	**34.2**
19	*ar*-Curcumene	S.H.	$C_{15}H_{22}$	1483	1.3
20	(E)-β-Ionone	N.K.[f]	$C_{13}H_{20}O$	1485	0.4
21	*cis*-β-Guaiene	S.H.	$C_{15}H_{24}$	1490	0.8
22	α-Selinene	S.H.	$C_{15}H_{24}$	1494	0.4
23	*trans*-β-Guaiene	S.H.	$C_{15}H_{24}$	1500	1.6
24	(*E*,*E*)-α-Farnesene	S.H.	$C_{15}H_{24}$	1508	0.8
25	γ-Cadinene	S.H.	$C_{15}H_{24}$	1513	0.6
26	δ-Cadinene	S.H.	$C_{15}H_{24}$	1524	2.0
27	α-Cadinene	S.H.	$C_{15}H_{24}$	1534	0.2
28	α-Calacorene	S.H.	$C_{15}H_{20}$	1542	0.2
29	(*E*)-Nerolidol	O.S.[g]	$C_{15}H_{26}O$	1564	0.3
30	*trans*-Sesquisabinene hydrate	O.S.	$C_{15}H_{26}O$	1580	0.2
31	Dendrolasin	O.S.	$C_{15}H_{22}O$	1574	2.4
32	Spathulenol	O.S.	$C_{15}H_{24}O$	1576	2.4
33	Caryophyllene oxide	O.S.	$C_{15}H_{24}O$	1581	**3.0**
34	Viridiflorol	O.S.	$C_{15}H_{26}O$	1590	0.4
35	γ-Eudesmol	O.S.	$C_{15}H_{26}O$	1630	2.0
36	α-Bisabolol	O.S.	$C_{15}H_{26}O$	1683	2.4
37	Aristolone	O.S.	$C_{15}H_{22}O$	1756	1.1
38	β-Bisabolenal	O.S.	$C_{15}H_{22}O$	1764	0.4
39	(*E*,*E*)-Farnesyl acetate	O.S.	$C_{17}H_{28}O_2$	1843	0.9
40	Nonadecane	N.H.	$C_{19}H_{40}$	1900	**8.1**
41	Phytol	O.D.[h]	$C_{20}H_{40}O$	1949	0.3
42	6-(*p*-tolyl)-2-Methyl-2-heptenol	N.A.[i]	$C_{15}H_{22}O$	1956	0.5
43	Eicosane	N.H	$C_{20}H_{42}$	2000	0.3
44	Kaurene	D.H.[j]	$C_{20}H_{32}$	2034	1.0
45	Henicosane	N.H.	$C_{21}H_{44}$	2100	0.3
46	(*E*)-Phytol acetate	O.D.	$C_{22}H_{42}O_2$	2221	0.2
		Total			**82.3**

[a] M.H.: Monoterpene hydrocarbons
[b] N.E.: Non-terpenoid ester
[c] O.M.: Oxygenated monoterpenes
[d] S.H.: Sesquiterpene hydrocarbons
[e] N.H.: Non terpenoid hydrocarbons
[f] N.K.: Non terpenoid ketone
[g] O.S.: Oxygenated sesquiterpenes
[h] O.D.: Oxygenated diterpenes
[i] N.A.: Non-terpenoid aromatic ester
[j] D.H.: Diterpene hydrocarbon
[*] RI: Relative retention indices as determined on a DB-5 column using the homogenous series of *n*-alkanes (C_9-C_{26}).

Table 2

Percentage composition of the essential oil of *A. tournefortiana* obtained by solvent free microwave extraction.

NO.	**Compounds**	**Class**	**Chemical formula**	**Solvent free microwave extraction (SFME)**	
				RI*	**Percentage (%)**
1	2,4-Dimethyl hexane	N.H.[a]	C_8H_{18}	822	0.3
2	Propyl cyclopentane	N.H.	C_8H_{16}	831	0.4
3	*cis*-1-Ethyl-3-methyl cyclopentane	N.H.	C_8H_{16}	842	0.2
4	4-Methyl Octane	N.H.	C_9H_{20}	860	0.2
5	Nonane	N.H.	C_9H_{20}	899	0.2
6	5-Methyl nonane	N.H.	$C_{10}H_{22}$	961	0.2
7	2,6-Dimethyl octane	N.H.	$C_{10}H_{22}$	972	0.2
8	1-Decene	N.H.	$C_{10}H_{20}$	991	0.4
9	Decane	N.H.	$C_{10}H_{20}$	999	3.4
10	Undecane	N.H.	$C_{11}H_{24}$	1099	0.3
11	Borneol	O.M.[b]	$C_{10}H_{18}O$	1165	0.4
12	Dodecane	N.H.	$C_{12}H_{26}$	1199	0.8
13	2-Propenoic acid, 2-ethylhexyl ester	N.E.[c]	$C_{11}H_{20}O_2$	1232	**30.0**
14	(*E*)-Anethole	O.M.	$C_{10}H_{12}O$	1283	0.1
15	Bornyl acetate	O.M.	$C_{12}H_{20}O_2$	1285	0.2
16	Piperitenone oxide	O.M.	$C_{10}H_{14}O_2$	1363	1.1
17	Tetradecane	N.H.	$C_{14}H_{30}$	1399	0.7
18	β-Caryophyllene	S.H.[d]	$C_{15}H_{24}$	1418	1.3
19	Neryl acetone	O.M.	$C_{13}H_{22}O$	1434	0.1
20	(*Z*)-β-Farnesene	S.H.	$C_{15}H_{24}$	1443	2.2
21	α-Humulene	S.H.	$C_{15}H_{24}$	1454	0.2
22	Cedr-8-en-13-ol	O.S.[e]	$C_{15}H_{24}O$	1470	0.3
23	Germacrene D	S.H.	$C_{15}H_{24}$	1480	**3.1**
24	β-Selinene	S.H.	$C_{15}H_{24}$	1485	0.1
25	Bicyclogermacrene	S.H.	$C_{15}H_{24}$	1494	**3.4**
26	(*Z*)-Nerolidol	O.S.	$C_{15}H_{26}O$	1534	0.4
27	Spathulenol	O.S.	$C_{15}H_{24}O$	1576	**19.5**
28	Caryophyllene oxide	O.S.	$C_{15}H_{24}O$	1581	**3.9**
29	Thujopsene-2-β-ol	O.S.	$C_{15}H_{26}O$	1583	0.2
30	α-Acorenol	O.S.	$C_{15}H_{26}O$	1620	1.0
31	γ-Eudesmol	O.S.	$C_{15}H_{26}O$	1630	2.1
32	β-Acorenol	O.S.	$C_{15}H_{26}O$	1634	1.1
33	Selin-11-en-4-α-ol	O.S.	$C_{15}H_{26}O$	1652	0.4
34	α-Bisabolol	O.S.	$C_{15}H_{26}O$	1683	0.5
35	Octadecane	N.H.	$C_{18}H_{38}$	1800	0.4
36	(*Z,E*)-Farnesyl acetate	O.S.	$C_{17}H_{28}O_2$	1818	0.2
37	6,10,14-Trimethyl-2-pentadecanone	N.K.[f]	$C_{18}H_{36}O$	1848	1.1
38	1,2-Benzenedicarboxylic acid,butyl octyl ester	N.A.[g]	$C_{20}H_{30}O_4$	1862	0.1
39	Eicosane	N.H.	$C_{20}H_{42}$	2000	0.3
	Total				**81.0**

[a] N.H.: Non-terpenoid hydrocarbons

[b] O.M.: Oxygenated monoterpenes

[c] N.E.: Non terpenoid ester

[d] S.H.: Sesquiterpene hydrocarbons

[e] O.S.: Oxygenated sesquiterpenes

[f] N.K.: Non-terpenoid ketone

[g] N.A.: Non-terpenoid aromatic ester

*RI: Relative retention indices as determined on a DB-5 column using the homogenous series of n-alkanes (C_9-C_{26}).

were rich in davanone (21.4% and 59.1%), respectively. In the oil of *A. haussknechtii*, 1,8-cineole (16.5%), camphor (14.2%) and artemisia ketone (10.5%) were found to be the major constituents (Rustaiyan et al., 2009).

α-Pinene (23.9%, 23.0% and 29.2%) and spathulenol (23.9%, 15.8%, and 29.2%) had the highest frequencies in the flower, leaf, and stem oils of *A. campestris* (Kazemi et al., 2009).

On the other hand, menthyl acetate (26.5%, 22.0%, 20.5%, and 20.5%) and (*Z*)-nerolidol (20.8%, 26.3%, 14.7%, and 18.1%) were the main constituents in the aerial parts, stem, leaf, and flower oils of *A. chamaemelifolia*, respectively. Meanwhile, 1,8-cineole (15.5%), spathulenol (15.2%), camphor (14.8%), santolina alcohol (14.6%) and *trans*-β-terpineol (11.6%) were the major constituents in the oil from the aerial parts of *A. turcomanica* (Masoudi et al., 2012).

The leaf of *A. douglasiana* has a potential application of herbal treatment for chronic bladder infection in paraplegic people. The leaf oil has been analyzed by GC/MS and the major components were found to be camphor (29.0%), artemisia ketone (26.0%), artemisia alcohol (13.0%), α-thujone (10.0%), 1,8-cineole (8.0%), and hexanal (5.0%).The leaf oil and its major components showed limited antimicrobial activity *in vitro*. Therefore, it is somewhat unclear whether the oil exerts a direct antimicrobial effect *in vivo*, or plays some stimulant roles on host defenses (Setzer et al., 2004).

4. Concluding remarks

The aim of the current study was to compare the chemical compositions of the essential oils of *A. tournefortiana* which obtained by classical hydrodistillation and solvent free microwave extraction approaches. The obtained essential oils using both methods were analyzed by GC and GC/MS approaches. Forty-six components representing 82.3% of the water-distillation oil and thirty-nine components representing 81.0% of solvent free microwave extraction oil of the plant were identified. The main components of the water-distilled oil were (*Z*)-β-farnesene (34.2%) and nonadecane (8.1%), whereas using the microwave extraction method, 2-propenoic acid, 2-ethyl hexyl ester (30.0%) and spathulenol (19.5%) were the major constituents. According to these results, the chemical profiles of the two oils show significant differences in the total contents of the main constituent components. This study revealed that in the essential oils from the aerial parts of *A. tournefortiana* using the conventional-distillation and solvent free microwave extraction, sesquiterpene hydrocarbons and non-terpene hydrocarbons were the most abundant representative groups of natural compounds, respectively.

Conflict of interest

The authors declare that there is no conflict of interest.

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