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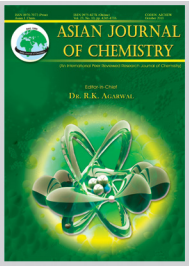

Composition of Essential Oil of *Artemisia absinthium* by Three Different Extraction Methods: Hydrodistillation, Solvent-Free Microwave Extraction & Headspace Solid-Phase Microextraction

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Traditional hydrodistillation, solvent-free microwave extraction and headspace solid-phase microextraction methods have been compared and evaluated for their effectiveness in the isolation of essential oils of stems, leaves and fruits of *Artemisia absinthium* L. and then, the essential oils were analyzed by a combination of GC and GC/MS. A total of 72 components were identified accounting for 95.57-100 % of the oil composition. In hydrodistillation method, 16, 21 and 31 components were identified in the stem, leaf and fruit, which represented 100, 98.74 and 97.97 % of the total composition of the oil, respectively. In solvent-free microwave extraction method, 27, 23 and 20 components were identified in the stem, leaf and fruit, which represented 98.25, 99.70 and 99.99 % of the total composition of the oil, respectively. In headspace solid-phase microextraction method, 37, 37 and 31 components were identified in the stem, leaf and fruit, which represented 99.35, 98.69 and 95.57 % of the total composition of the oil, respectively. In these methods, β-thujone, 1,8-cineole, *cis*-chrysanthenol, sabinene and α-phellandrene were the major compounds in the oils of different organs. This *Artemisia* was rich in monoterpens. The highest percent monoterpene hydrocarbons and oxygenated monoterpens were in the fruit in case of the headspace-solvent-free microwave extraction method, in the amount of 48.58 and 72.71 %, respectively. Statistically differences were obtained in the compositions and the chemical class distribution.

Key Words: *Artemisia absinthium*, Extraction methods, Essential oil, Green technology.

INTRODUCTION

Due to the increasing consumer demand for more natural foods, the abuse of toxic synthetic food substances and the increasing microbial resistance of pathogenic microorganisms against antibiotics, natural substances isolated from plants are considered as promising sources of food preservative[1-3].

The genus *Artemisia* belongs to a useful group of aromatic and medicinal plants comprising about 300 species found in the northern hemisphere[4]. In Iran, the genus *Artemisia* of the family compositae is represented by 34 species, including *Artemisia absinthium*[5,6]. *Artemisia absinthium* L. is an aromatic plant of the family *Asteraceae*, subfamily *Asteroideae*, tribe Anthemideae and is know by the common names wormwood (UK), absinthe (France), wermut (Germany) and afsantine (Iran)[7-9]. The plant grows in North and East of Iran and also is very common in Turkey[10,11]. *Artemisia absinthium* oil has been used widely as pharmaceuticals, flawouring, antifungal and antimicrobial agents in the food industry. The essential oil of aromatic herbs is traditionally obtained by hydrodistillation[12,13]. Chemical analysis of *A. absinthium* has shown that its volatile oil is rich in thujone (α and β), which has been earlier reported as an anthelmintic[4,14]. In other research, analysis of the chemical composition *A. absinthium* oils extracted from plants grown in USA showed β-thujone (17.5-42.3 %) and *cis*-sabinyl acetate (15.1-53.4 %) as the main components[15]. The chemical composition, antimicrobial and antioxidant activities of *A. absinthium* oil from Turkey also, was investigated. The results showed chamazulene (17.8 %), nuciferol butanoate (8.2 %), nuciferol propionate (5.1 %) and caryophyllene oxide (4.3 %) as the main components in *A. absinthium* oil. The oils had inhibitory effects on the growth of bacteria and fungi tested and also showed moderate to weak antioxidant activities, respectively[16,17]. Various novel techniques have been developed for the extraction of natural products from plants in order to shorten the extraction time, decrease the solvent consumption, increase the extraction yield and enhance the quality of extraction. Among them, the solvent-free microwave extraction (SFME) and more recently, the headspace solid-phase microextraction (HS-SPME) have been considered as alternatives for the

extraction of essential oil or volatile compounds from aromatic plants[18]. Solvent-free microwave extraction is a technique which combines microwave heating with dry distillation at atmospheric pressure for the isolation and concentration of the essential oils in plant materials. In SFME method, there is no need to add any solvent or water if fresh plant material is used. If dry plant material is used, the sample is rehydrated by soaking in water for some time and then draining off the excess water[19-21]. Solvent-free microwave extraction has been used to obtained essential oils from three different spices (ajowan, cumin and star anise[22], three different aromatic herbs (basil, garden mint and thyme)[23] and cardamom seed[24]. Microwave heating has been recently used for the isolation and analysis of essential oils[22-24]. In other technique, a modern alternative to traditional sample preparation technology has been introduced solid-phase microextraction (SPME). This technique is a extraction method developed by Pawliszyn and coworkers[25,26]. To facilitate HS-SPME, it is essential to have target analytes transferred from the sample matrices into the headspace. When HS-SPME is used for samples for which a strong association between native analytes and sample matrix exists, heating may be required to enhance the release of analytes into the headspace phase. For solid samples such as traditional Chinese medicines, heating can enhance the analyte concentrations in the headspace[27-29]. Moreover, an increase in the sample temperature is generally beneficial in speeding the achievement of extraction equilibrium[30]. Headspace-solid-phase microextraction coupled to GC/MS has been shown to be a simple and solvent-free method for the analysis of essential oils in plant materials[31,32]. In other studies[28,30,33,34], HS-SPME was successfully developed for the analysis of essential oils in traditional Chinese medicines. In this study, the chemical compositions of the essential oils of the stem, leaf and fruit of *A. absinthium* for the first time obtained by HS-SPME and SFME. The hydrodistillation (HD) was studied and compared. All experimental results were also statistically evaluated by two-way analysis of variance.

EXPERIMENTAL

Stems, leaves and fruits of *A. absinthium* were collected in September 2010 from the Kojur of Nowshahr, province of Mazandaran, northern Iran. Voucher specimens have been deposited at the herbarium of the department of farmacognosy, faculity of pharmacy, Tehran university of Medical science, Tehran,Iran.

Hydrodistillation apparatus and methods: The dried stems (100 g), leaves (100 g) and fruits (80 g) were separately subjected to hydrodistillation using a Clevenger-type apparatus according to the European pharmacopea[35] for 4 h. The essential oils were collected, dried under anhydrous sulphate and stored at 4 ºC until used. Essential oil yield was expressed in terms of the weight of the oil collected per gram of dry plant material. The yields were (0.35, 0.75 and 0.70 %) (w/w) respectively.

Solvent-free microwave extraction apparatus and methods: The microwave oven used for SFME was a Milestone srl operating at 2450 MHz. The dimensions of the interior cavity of the oven were 29 cm × 37 cm × 40 cm. A Clevenger system outside the microwave cavity condensed the distillate continuously. Condensed water was refluxed to the extraction vessel in order to provide uniform condition of temperature and humidity for extraction. For SFME, 50 g of dried organs *A. absinthium* were soaked in 800 mL distilled water at room temperature (25 ºC) for 1h in order to hydrate the external layers of the plant material performed at atmospheric pressure, the stem, leaf and fruit of were heated using an optimize fixed power of 800w for optimize time 0.5 h without added any solvent or water. The chemical compositions of essential oils isolated were analyzed by GC/MS. The yields of this methods were (0.40, 0.85 and 0.80 %) (w/w) respectively.

Headspace solid-phase microextraction: A manual SPME holder and 75 micro meter PDMS-CAR fiber from Supelco (Bellefonte, USA) were used for the SPME procedure. The fiber was condition at 250 ºC for 0.5 h in GC injector. 1 g of powdered sample were placed in 20 mL sample vials sealed with septum-type caps from supelco (Bellefonte, USA) and heated for 15 min at 70 ºC. After this time the SPME needle was pierced the septum, the PDMS fiber was extended through the needle and exposed to the head-space above the sample for 15 min. After an optimize extraction time (15 min), the fiber was drown into the needle and then the needle was removed from the septum and inserted directly on to the injection port of the GC. The desorption of analytes from the fiber coating was performed by heating the fiber in the split less (250 ºC) injection port at for 3 min.

GC and GC/MS analysis: GC analysis were performed on a Shimadzu 15A gas chromatograph equipped with a spilt/spiltless (ratio 1:30), injector (250 ºC) and a flame ionization detector (250 ºC). Nitrogen 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 pin). The column temperature was kept at 60 ºC for 3 min and then heated to 220 ºC with a 5 ºC/min rate and kept constant at 220 ºC for 5 min. Relative percentage amounts were calculated from peak area using a CR5 SHIMADSU CR PACK without the use of correction factors. GC/MS analysis were performed using a Hewlett-Packard 5973 with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60 ºC for 3 min and programmed to 220 ºC at a rate of 5 ºC/min and kept constant at 220 ºC for 5 min. The flow rate of helium as carrier gas was 1 mL/min, final temperature 230 ºC and detector temperature 250 ºC; MS were taken at 70 eV (E1), electron multiplier voltage 1800 eV; mass range, 30 to 350 amu; scan time and 2 scan/sec.

Identification of components: The components of the oil were identified by comparison of their mass spectra with those of the M.S 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[36]. The retention indices were calculated for all volatile constituents using a homologous series of C9 to C18 *n*-alkanes.

Statistical analysis: Percentage of the major components of the essential oils in three different organs and methods were statistically evaluated by two-way analysis of variance (ANOVA). The analysis were carried out using SPSS package software (version 18).

RESULTS AND DISCUSSION

The volatile components obtained from *A. absinthium* are listed in Table-1 in which the percentage and retention indices of the component are given. A total of 72 components were identified accounting for 95.57-100 % of the oil composition. In hydrodistillation method, 16, 21 and 31 components were identified in the stem, leaf and fruit oils, which represented 100, 98.74 and 97.97 % of the total compositions of the oils, respectively. In the stem, leaf and fruit, β-thujone (40.91 %, 36.44 %, 24.27 %), 1.8-cineole (14.09 %, 13.58 %, 8.10 %), sabinene (10.05 %, 9.78 %, 8.35 %) and *cis*-chrysanthenol (8.92 %, 8.87 %, 18.92 %) were the major components, respectively. In solvent-free microwave extraction method, 27, 23 and 20 components were identified in the stem, leaf and fruit oils, making up 98.25, 99.70 and 99.99 % of the total compositions of the oils, respectively. In the stem, leaf and fruit, β-thujone (22.39 %, 32.07 %, 31.77 %), 1.8-cineole (9.93 %, 14.81 %, 11.36 %), sabinene (6.54 %, 11.75 %, 10.25 %) and *cis*-chrysanthenol (23.06 %, 12.81 %, 20.63 %) were the major components, respectively. In headspace solid-phase microextraction method, 37, 37 and 31 components were identified in the stem, leaf and fruit oils, which represented 99.35, 98.69 and 95.57 % of the total composition of the oils, respectively. In the stem, leaf and fruit β-thujone (17.63 %, 17.20 %, 18.95 %), 1.8-cineole (12.01 %, 12.46 %, 8.41 %), sabinene (8.87 %, 11.62 %, 13.87 %), α-phellandrene (10.87 %, 9.48 %, 9.33 %) and β-pinene (5.72 %, 15.50 %, 10.02 %) were the major components, respectively.

The chemical class distribution of the essential oils components of the plant is reported in Table-1. The compounds were separated into four classes, which were monoterpene hydrocarbons, oxygenated monoterpens, sesquiterpene hydrocarbons and oxygenated sesquiterpens. As it can be seen in Table-1, in the stem. leaf and fruit oils, monoterpens were higher than sesquiterpens and oxygenated monoterpene components were the major constituents of the essential oils; except in HS-SPME method, which monoterpene hydrocarbons was slightly higher than the oxygenated monoterpens. The total amount of the monoterpene fractions in the oils of organs

TABLE-1
YIELDS, EXTRACTION TIME AND CHEMICAL COMPOSITION OF *A. absinthium* ESSENTIAL OILS OBTAINED BY HYDRODISTILLATION (HD), SOLVENT-FREE MICROWAVE EXTRACTION (SFME) AND HEADSPACE SOLID-PHASE MICROEXTRACTION (HS-SPME)

Compounds[a]	Rt[b]	Contents (%)								
		Stem			Leaf			Fruit		
		HD	SFME	HS-SFME	HD	SFME	HS-SFME	HD	SFME	HS-SFME
α-Thujene	930	-	-	-	-	-	0.83	0.26	-	-
α-Pinene	939	1.59	2.39	3.03	1.85	3.62	5.79	4.41	2.83	5.02
Benzaldehyde	960	-	-	-	-	-	0.95	-	-	-
Sabinene	975	**10.05**	**6.54**	**8.87**	**9.78**	**11.75**	**11.62**	**8.35**	**10.25**	**13.87**
β-Pinene	979	6.29	5.66	**5.72**	6.28	7.37	**15.50**	8.46	6.52	**10.02**
Myrcene	991	-	0.45	9.46	0.56	-	-	0.33	0.17	4.05
α-Phellandrene	1003	2.79	7.85	**10.87**	2.84	6.17	**9.48**	2.20	4.20	**9.33**
α-Terpinene	1017	-	-	-	-	-	0.72	-	-	-
p-Cymene	1025	0.73	1.82	3.49	1.11	0.37	2.26	2.62	0.75	4.25
Limonene	1029	-	-	1.68	-	-	-	0.17	-	1.00
1,8-Cineole	1031	**14.09**	**9.93**	**12.01**	**13.58**	**14.81**	**12.46**	**8.10**	**11.36**	**8.41**
(Z)-β-Ocymene	1037	-	-	2.39	-	-	1.34	-	-	1.04
γ-Terpinene	1060	-	-	0.54	-	-	0.68	0.37	-	-
Artemisia ketone	1062	-	-	-	-	0.32	-	-	-	-
cis-Sabinene hydrate	1070	-	0.35	-	0.54	0.73	0.24	0.37	0.57	-
Linalool	1097	-	-	-	0.64	0.25	0.80	0.73	0.34	0.56
trans-Sabinene hydrate	1098	-	-	-	-	0.28	-	0.24	-	-
Nonanal	1101	-	-	1.05	-	-	-	-	-	-
α-Thujone	1102	1.93	0.90	5.68	2.21	3.00	6.63	1.77	1.94	1.82
β-Thujone	1114	**40.91**	**22.39**	**17.63**	**36.44**	**32.07**	**17.20**	**24.27**	**31.77**	**18.95**
iso-3-Thujanol	1138	1.01	0.69	1.14	1.26	1.37	1.29	2.30	1.81	0.51
Camphor	1146	-	-	-	-	0.16	-	-	-	-
Isopulegol	1150	-	-	0.93	-	-	0.77	0.19	-	-
neiso-3-Thujanol	1152	-	-	-	-	-	-	0.21	-	-
***cis*-Chrysanthenol**	1164	**8.92**	**23.06**	**3.03**	**8.87**	**12.81**	**2.47**	**18.92**	**20.63**	**4.35**
Δ-Terpineol	1166	-	-	-	-	0.15	-	-	-	-
Borneol	1169	-	-	0.29	-	-	-	-	-	-
4-Terpineol	1177	0.91	-	-	1.12	0.16	-	2.41	0.35	-
α-Terpineol	1189	1.70	0.95	0.99	1.84	1.56	0.97	1.11	0.91	0.40
trans-Carveol	1217	0.77	2.05	-	1.05	1.28	0.19	3.67	3.03	0.63
Hexyl-2-methyl butanoate	1236	-	-	0.37	-	-	0.21	-	-	-
α-Citral	1238	-	-	-	-	-	0.13	-	-	-
Cuminal	1242	-	-	0.33	-	-	0.33	-	-	0.58
Geraniol	1253	-	-	-	-	-	-	0.31	-	-

cis-Chrysanthenyl acetate	1265	-	0.95	0.38	-	-	-	0.46	-	-
Geranial	1267	-	-	0.29	-	-	0.25	-	-	-
Bornyl acetate	1289	-	0.44	-	-	-	-	-	-	-
Thymol	1310	-	-	-	-	-	0.24	-	-	-
Eugenol	1359	-	-	-	-	-	0.17	-	-	
Geranyl acetate	1381	-	-	0.24	-	-	-	-	-	-
α-Cubebene	1388	-	-	0.36	-	-	-	-	-	-
B-Elemene	1391	-	-	0.53	-	-	0.54	-	-	-
Calarene	1401	-	-	-	-	-	-	-	-	0.53
α-Gurjunene	1410	-	-	0.35	-	-	-	-	-	-
α-Cadrene	1412	-	-	0.26	-	-	0.34	-	-	-
β-Caryophyllene	1419	-	-	0.47	-	-	0.13	-	-	-
β-Copaene	1432	-	-	0.21	-	-	0.28	-	-	-
β-Gurjunene	1434	-	-	-	-	-	-	-	-	0.40
Cadina-1,4-diene	1440	-	-	-	-	-	-	-	-	0.28
Aromadendrene	1441	-	-	-	-	-	-	-	-	0.38
(z)-β-Farnesene	1443	-	-	0.29	-	-	-	-	-	2.02
γ-Gurjunene	1477	-	-	0.58	-	-	0.25	-	-	-
β-Cadinene	1477	-	-	0.36	-	-	0.19	-	-	0.35
Cadina-1,6-diene	1477	-	-	-	-	-	-	-	-	0.43
Geranyl propanoate	1478	1.28	-	0.71	1.63	-	-	-	-	-
γ-Muurolene	1480	-	-	-	-	-	0.31	-	-	1.32
Germacrene D	1485	-	1.06	-	-	-	-	0.49	-	-
β-Selinene	1490	-	1.27	2.86	0.44	0.12	1.67	0.64	0.19	1.89
γ-Cadinene	1514	-	-	0.73	-	-	0.50	-	-	0.80
Δ-Cadinene	1523	-	-	0.46	-	-	0.24	-	-	0.46
α-Calacorene	1546	-	-	-	-	-	-	0.83	-	-
Elemol	1550	-	1.10	-	-	-	-	-	-	-
Geranyl butanoate	1564	-	-	-	-	-	-	0.21	-	0.24
Spathulenol	1578	-	0.69	-	-	-	-	0.24	-	-
Caryophellene oxide	1583	-	0.90	-	-	-	-	-	-	-
Anozol	1591	-	-	-	0.17	-	-	-	-	-
10-epi-γ-Eudesma	1624	-	1.05	-	-	0.28	-	-	-	-
γ-Eudesmol	1632	-	0.76	-	-	-	-	-	0.28	-
epi-α-Cadinol	1640	3.00	2.55	0.77	3.20	0.30	0.72	2.59	0.77	1.34
α-Cadinol	1654	-	0.80	-	-	-	-	-	-	-
α-Bisabolol	1684	-	0.69	-	-	-	-	-	-	-
Chamazulene	1732	4.03	0.96	-	3.33	0.77	-	0.74	1.32	0.34
Total		100.00	98.25	99.35	98.74	99.70	98.69	97.97	99.99	95.57
Group components										
Monoterpene hydrocarbons		21.45	24.71	46.05	22.42	29.28	48.22	27.17	24.72	48.58
Oxygen-containing monoterpens		71.52	61.71	44.02	69.35	68.95	45.30	65.27	72.71	36.45
Sesquiterpene hydrocarbons		4.03	3.29	7.46	3.77	0.89	4.45	2.70	1.51	9.20
Oxygen-containing sesquiterpens		3.00	8.54	0.77	3.20	0.58	0.72	2.83	1.05	1.34
Extraction time (min)		240	30	15	240	30	15	240	30	15
Yield (%, w/w-dry basis)		0.35	0.40	-	0.75	0.85	-	0.70	0.80	-

[a]Compounds presented in order of elution from the HP-5MS capillary column.
[b]Kovat's retention index to *n*-alkans on the HP-5MS capillary column.

of *A. absinthium* (21.45-72.71 %) were higher than sesquiterpene fractions (0.58-9.20 %).

Among the nine samples, the fruit oil in the HS-SPME method had the highest monoterpene hydrocarbons (48.58 %) and sesquiterpene hydrocarbons (9.20 %) and in the SFME method had the highest oxygenated monoterpens (72.71 %), but oxygenated sesquiterpens were the major constituents for oil of stem in the SFME method, in the amount of 8.54 %. Percentage of the major components of the essential oils in organs and methods also, were statistically evaluated by two-way analysis of variance (ANOVA). The results were shown in Table-2 and Fig. 1. In can be seen that were found significant difference in α-phelandrene compound ($p < 0.01$) and in *cis*-chrysanthenol compound ($0.01 < p < 0.05$) in methods, but were not found significant difference in organs. On the other hand, the chemical class distribution of the essential oil components were statistically evaluated. The results were shown in Table-3 and Fig. 2. It can be seen that were found significant difference in monoterpene hydrocarbons ($p < 0.01$), oxygenated monoterpens ($p < 0.01$) and sesquiterpene hydrocarbons ($0.01 < p < 0.05$) in methods, but were not found significant difference in organs. Previous studies showed that myrcene (10.80 %), β-thujone (10.10 %) and *trans*-sabinyl acetate (26.40 %) were as the major components of the essential oil of *A. absinthium*[37]. β-Pinene and β-thujone are also found in the oil of *A. absinthium*[14,38]. Sabinyl acetate, one of the major compounds characterized in the oils of *Artemisia absinthium* in different geographical origin[39]. In other research, SPME method was employed to analyze volatile organic compounds, which α-terpinene, limonene, β-terpinene and

TABLE-2
RESULTS OF TWO-WAY ANALYSIS OF VARIANCE (ANOVA) FOR THE MAIN COMPONENTS IN ORGANS AND METHODS

Compounds	Factor	F	Sig	
Sabinene	Organs	1.463	0.334	n.s
	methods	0.971	0.453	n.s
β-Pinene	Organs	1.495	0.328	n.s
	methods	1.792	0.378	n.s
1,8-Cineole	Organs	3.633	0.126	n.s
	methods	0.265	0.780	n.s
α-Phellandrene	Organs	3.981	0.112	n.s
	methods	56.904	0.001	**
cis-Chrysanthenol	Organs	2.138	0.234	n.s
	methods	11.981	0.020	*
β-Thujone	Organs	0.201	0.826	n.s
	methods	4.156	0.106	n.s

n.s: No significant difference *Significance difference (0.01 < p < 0.05); **Significance difference (p < 0.01)

TABLE-3
RESULTS OF TWO-WAY ANALYSIS OF VARIANCE (ANOVA) FOR THE MAIN COMPONENTS IN ORGANS AND METHODS

Chemical class	Factor	F	Sig	
Monoterpene hedrocarbons	Organs	2.609	0.188	n.s
	methods	286.68	0.00005	**
Oxygenated monoterpens	Organs	0.259	0.784	n.s
	methods	24.408	0.006	**
Sesquiterpene hydrocarbons	Organs	1.189	0.393	n.s
	methods	8.458	0.037	*
Oxygenated sesquiterpenes	Organs	0.894	0.478	n.s
	methods	0.749	0.529	n.s

n.s: No significant difference *Significance difference(0.01 < p < 0.05); **Significance difference (p < 0.01).

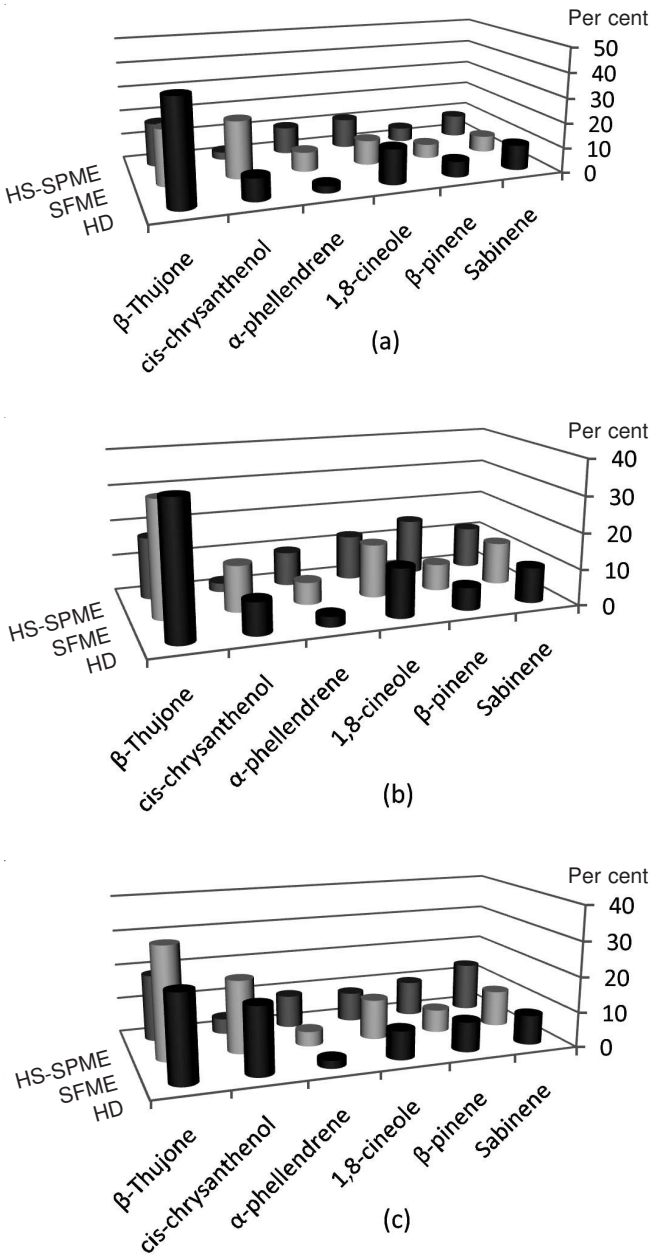

Fig. 1. Percentage of the major compounds of essential oils of *A. absinthium* in different methods. a: stem, b: leaf, c: fruit

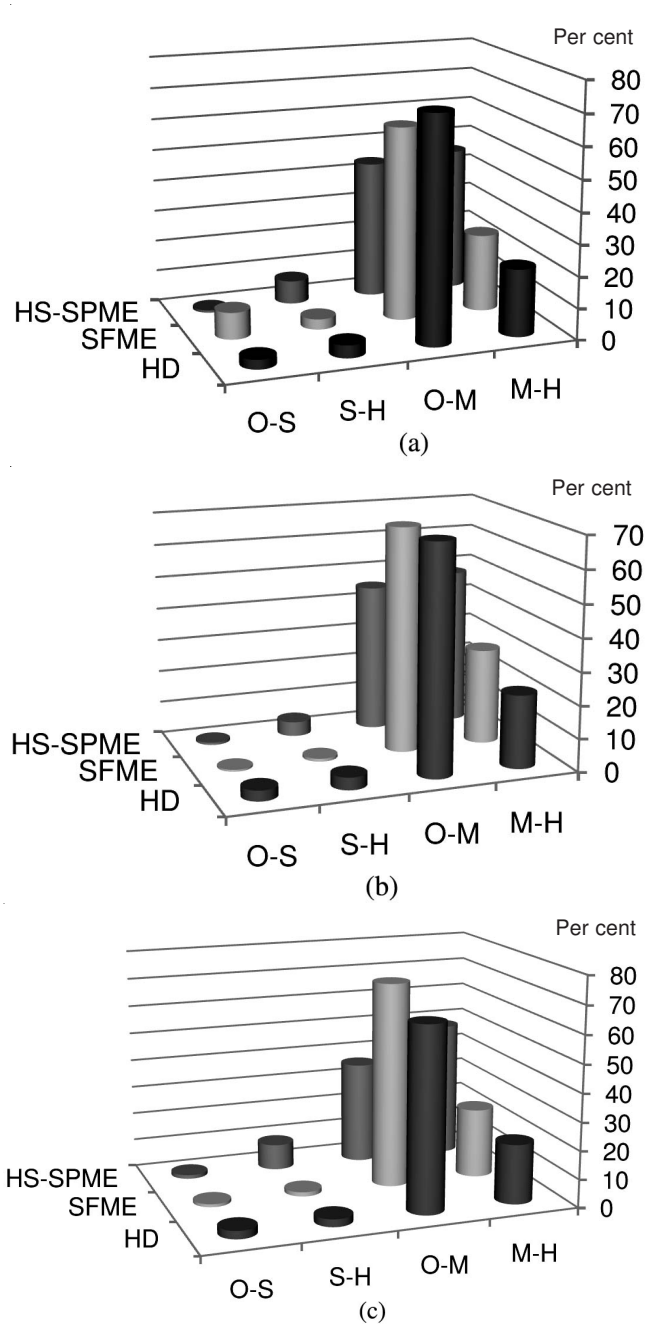

Fig. 2. Perecentege of chemical class of composition of *A. absinthium* in different methods (M-H: monoterpene hxdrocarbons, O-M: oxygenated monoterpens, S-H: sesquiterpene hydrocarbons O-M: oxygenated sesquiterpens); a: stem, b: leaf, c: fruit

terpinolene were the main components[40]. Pinene derivatives were found in the oil of some *Artemisia* species, for example, α-pinene was found in the oil of *A. annua*[41] and *A. biennis*[14], β-pinene was found in the oil of *A. absinthium*[42], *A. scoparia*[43] and *A. campestris*[44,45] and γ-terpinene was found in the oil of *A. scoparia*[46] and *A. afra jacq*[47].

Conclution

Seventhy-two components were identified accounting for 95.57-100 % of the oil composition. Hydrodistillation (HD), solvent-free microwave extraction (SFME) and headspace solid-phase microextraction (HS-SPME) methods used for analysis of the components of *Artemisia absinthium*. The results showed that, in comparison with the hydrodistillation (HD) and solvent-free microwave extraction (SFME) which are time consuming and needs large amounts of botanical material, the headspace solid-phase microextraction method offers advantages such as: (1) short extraction time (15 min against 4 h for hydrodistillation and 0.5 h for SFME), (2) more compounds isolated and identified (105 compounds against 68 compounds for hydrodistillation and 70 compounds for SFME in organs), (3) does not needs large amount of plant material (1 g against 100 g for hydrodistillation and 50 g for SFME), (4) simplicity. On the other hand, percentage of the major components and the chemical class distribution of the essential oils were statistically evaluated. The results showed that, significant differences were found in this study.

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