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Study of the volatile compounds in *Artemisia absinthium* from Iran using HS/SPME/GC/MS

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ABSTRACT

The aerial parts of the plant Artemisia absinthium were collected in July 2010 from Binaloud mountain (Khorasan Razavi Province of Iran). It is air dried in a shadow place. A headspace solid-phase micro-extraction (HS-SPME) method followed by gas chromatography–mass spectrometry (GC/MS) was used for the analysis of volatile compounds in the leaves of Artemisia absinthium. The dried plant was powdered and the aroma compounds of a 0.05g were isolated by HS-SPME fiber in 15 min. The chemical compounds of the plant were investigated by gas chromatography mass spectrometry (GC/MS). A total of 72 constituents, representing more than 97% of the volatiles were identified. The main compounds, were camphor (14.83%) , p-cymene (10.35%), isoledene (8.52%), caryophyllene (6.92%), isopulegol acetate (6.09%), hysterol (5.64%), isocaryophillene (5.53%), diisoamylene (5.09%), β-farnesene (3.94%) and cyclohexane,2,4 -diisopropyl-1,1 -dimethyl (3.07%). This herbal medicine traditionally uses as anthelmintic, antibacterial, antifungal, insect repellent, narcotic and digestive. Consequently, this fast and simple method can be used for the analysis of the volatile compounds emitted from Artemisia absinthium.

Keywords: *Artemisia absinthium*; GC/MS; SPME; camphor; *p*-cymene.

INTRODUCTION

The genus *Artemisia*, belonging to the family *Compositae* (*Asteraceae*). *Artemisia absinthium* is an aromatic plant of the family *Asteraceae*, subfamily *Asteroideae*, tribe *Anthemideae* and is known by the common names wormwood. *Artemisia absinthium* (wormwood) is a aromatic, perennial small shrub distributed in Europe and Asia [1]. The essential oil of this plant originating from different countries has been the subject of previous investigations [2]. At present, *A. absinthium* is commonly used in food industry in the preparation of aperitives, bitters and spirits[3].

The herb is native to warm Mediterranean countries, usually found growing in dry waste places such as roadsides, preferring a nitrogen-rich stony and hence loose soil. Wormwood has been naturalized in northeastern North America, North and West Asia and Africa. The plant`s essential oil and bitter principles underlie its medicinal and commercial significance[4].

Different chemotypes were reported in literature [5-9] and the main components were found to differ in relation to the plants origin. Extraction methods such as Soxhlet extraction and liquid-liquid extraction are routinely being used in laboratories throughout the world. Unfortunately, these methods are generally time consuming and sometimes require large amounts of toxic and expensive organic solvents. Considering the limitations of these sampling techniques, headspace solid-phase micro-extraction (HS-SPME) emerges as an attractive alternative [3]. The technique has been reported to be relatively inexpensive, solventless, fast, reproducible, and simple procedure[10-13].

The aim of this research was to develop the HS/SPME method in combination with GC/MS, for the analysis of the volatile compounds from *Artemisia absinthium* plant. In our present work, the chemical composition of *Artemisia absinthium* identified by using aerial part of the dried plant. *Artemisia absinthium* were collected in July 2010 from Binaloud mountain (Khorasan Razavi Province of Iran). After one week drying the plant in shadow place, we used the dried plant for our experiments.

METHODS AND MATERIALS

Plant Material and SPME Fiber

Aerial parts of wild growing *Artemisia absinthium* were collected in July 2010 from Binaloud mountain (Khorasan Razavi Province of Iran).The plant was identified in Herbarium of Payame Noor University, Mashhad and a Voucher specimens (PNUMH19) was deposited. It is air dried in a shadow place. The SPME manual holder and a 50µm poly(dimethyl siloxane)-divinylbenzene (PDMS-DVB) fiber , were purchased from Supelco(USA) .The SPME fiber was conditioned as recommended at some degrees below the fiber maximum temperature before it was used for the first time . Before the first daily analysis , the fiber were conditioned for 5 min at 250°C in the GC injector.

HS-SPME Extraction

The sample was ground to a fine powder and in each experiment 50 mg of it was used . The sample placed in a vial , which is sealed with a septum-type cap. The sample preparation (HS-SPME) in the presence of water as a solvent was provided at 50°C. In the experiments after transfer of the sample to glass vial , 1ml of double distilled water was added to it . The vial was put in a aluminum block over a hot plate / magnetic stirrer device and samples were heated and stirred for l0 min at 50°C. Thermal desorption of analyte from the fiber in the GC injection port for 5min and GC/MS analysis were done simultaneously.

GC/MS Analysis

The GC/MS analysis was carried out on a Shimadzu GC/MS model QP5050 . The capillary column was DB-5(30x 0.2mm,film tickness 0.32µm). The operating conditions were as follows, carrier gas , helium with a flow rate at 1.7ml/min, column injector and detector temperatures ,

were 250°C and 285°C, respectively. The ionization potential was 70 ev. The initial temperature of column was 60°C(held 1min) and then heated to 140°C with a 3°C/min rate and then heated to 250°C with 50°C/min and kept constant for 3 min. Identification of components in the sample was based on the retention index(RI), similarity index (SI) , National Institute of Standards and Technology (NIST) MS spectral library and literature survey . The relative percentage of the oil coustituents was calculated from GC peak areas . The parameters of sample preparation and analysis like adsorption, desorption, sample amount and volume of water were used the amounts that reported in the litereatures.[9-10]

RESULTS AND DISCUSSION

The sample preparation time was 15min (10 and 5min for adsorption and desorption, respectively). Also, the total analysis time including sample preparation and GC/MS run was less than 1h. The chromatogram of HS-SPME analysis of *Artemisia absinthium* is shown in Fig. 1. The qualitative and quantitative analytical results are shown in Table1. A total of components were identified by GC/MS, representing 97.58% of the volatile components in the head space of the sample.

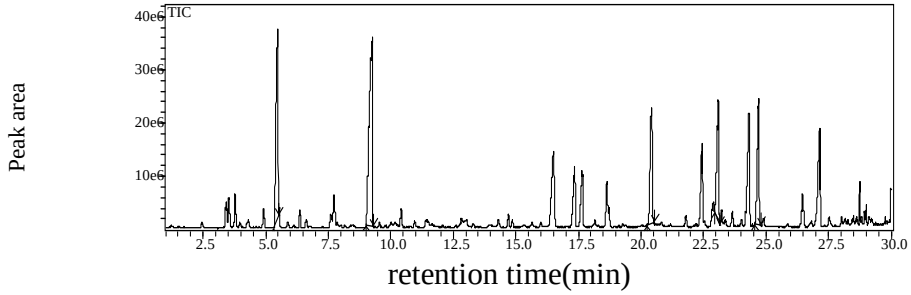

Figure 1: The chromatogram of *Artemisia absinthium* from Binaloud obtained by HS-SPME/GC/MS.

Table 1. **The variation of essential oil compositions of *Artemisia absinthium* collected from Binaloud mountain, and identified by GC/MS.**

NO.	RI*	Compound name	Cont.%	NO.	RI	Compound name	Cont.%
1	807	hexanal	0.19	37	1277	geranial	0.33
2	946	α-pinene	0.97	38	1280	Isopulegol acetate	6.09
3	960	camphene	1.25	39	1284	*trans*-carvone oxide	0.1
4	968	benzaldehyde	0.16	40	1291	pipertone oxide	0.5
5	984	artemiseole	0.05	41	1292	isobornyl acetate	0.24
6	987	*β*-pinene	0.42	42	1302	perilla alconol	0.03
7	998	myrcene	0.04	43	1337	diisoamylene	5.09
8	1012	*α*-phellandrene	0.7	44	1357	cyclohexane,2,5-diisopropyl-1,1-dimetyl	3.07
9	1015	2,4-hepta dienal,(*E*,*E*)	0.05	45	1367	cyclohexane,2,5-diisopropyl-2,3-dimetyl	2.64
10	1018	caren(delta-3)	1.08	46	1379	α-ylangene	0.36
11	1033	p-cymene	10.35	47	1380	isoledene	8.52
12	1059	hyacinthin	0.21	48	1383	*α*-copaene	2.5
13	1064	lilac alehyde	0.12	49	1388	isobornyl propionate	0.1
14	1069	*γ-terpinen*	0.59	50	1397	1-(adamantly-1)pentanol-1	0.05

15	1115	linalool	1.44	51	1398	*β*-elemene	0.08
16	1115	thujone	0.07	52	1407	(+)-sativen	0.02
17	1116	camphenol	0.07	53	1418	caryophyllene	6.92
18	1118	*n*-amyl isovalerate	0.11	54	1422	cedrene	0.07
19	1120	thymol	0.22	55	1436	thujopsene-13	0.05
20	1146	*trans*-pinocaveol	0.59	56	1440	*γ-elemene*	0.64
21	1147	sabinol	0.49	57	1447	*β*-humulene	0.56
22	1150	camphor	14.83	58	1457	*β*-farnesene	3.94
23	1151	*cis,beta*-terpineol	0.27	59	1461	*α*-humulene	0.47
24	1170	terpineol,*z-beta*	0.57	60	1487	germacrene D	0.11
25	1172	borneol	0.43	61	1494	neoisothujyl alcohol	0.06
26	1173	ethyl cyclohexa propionate	0.32	62	1515	*α*-farnesene	0.34
27	1184	4-terpineol	0.69	63	1521	*β*-lonone	0.88
28	1190	*p*-cymen-8-ol	0.11	64	1531	*Δ*-cadinene	0.30
29	1196	*α*-terpineol	0.25	65	1535	hysterol	5.64
30	1211	verbenone	0.19	66	1590	globulol	0.38
31	1214	(*E*)-3(10)-caren-4-ol	0.09	67	1592	isocitronellol	1.52
32	1230	*β*-cyclocitral	0.06	68	1607	isocaryophillene	5.53
33	1245	*p*-cumic aldehyde	0.63	69	1627	ethyl chrysanthemate	0.46
34	1256	thymoquinon	0.15	70	1647	*α*-bisabolene epoxide	0.37
35	1259	*cis*-myrtanol	0.09	71	1649	cubenol	0.08
36	1262	geraniol	0.34	72	1656	*β*-eudesmol	1.35
				total			**97.58**

*retention index

The major components of *Artemisia absinthium* were (Table 1) camphor (14.83%) , *p*-cymene (10.35%), isoledene (8.52%), caryophyllene (6.92%), isopulegol acetate (6.09%), hysterol (5.64%), isocaryophillene (5.53%), diisoamylene (5.09%), *β*-farnesene (3.94%) and cyclohexane,2,4-diisopropyl-1,1-dimethyl (3.07%).These results are in the most cases agreement with the components that obtained from aerial parts of the plant in previous studies with hydrodistilation method [1-2]. On the other hand the percent of the chemical compounds are not the same. These behaviors can be related to the type of the plant, aerial or flower parts and also the geographical regions of the plant growing places. Camphor, *p*-cymene and isoledone make about 34% of the volatile compounds in the herbal plant. Figs. 2-4 show the peaks of camphor, *p*-cymene and isoledone that obtained from mass spectrometer. A comparison between the percentages of some main components in the head space of the sample is shown in Fig.5. As it is shown in Fig.5, non-oxygenated terpenes is the most components in the volatile components of the plant.

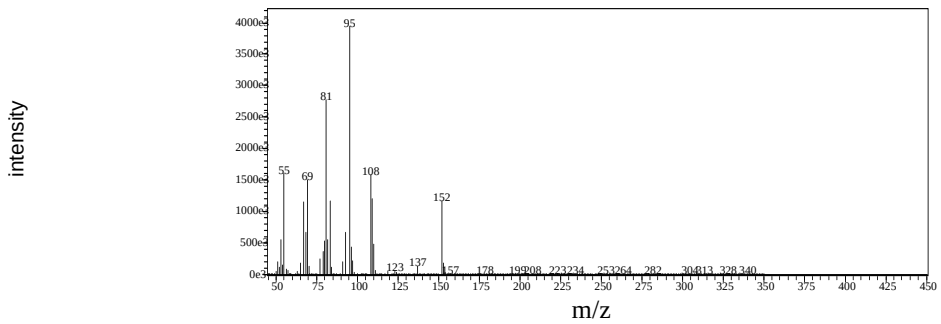

Figure 2: The peak of camphor obtained by the mass spectrometer.

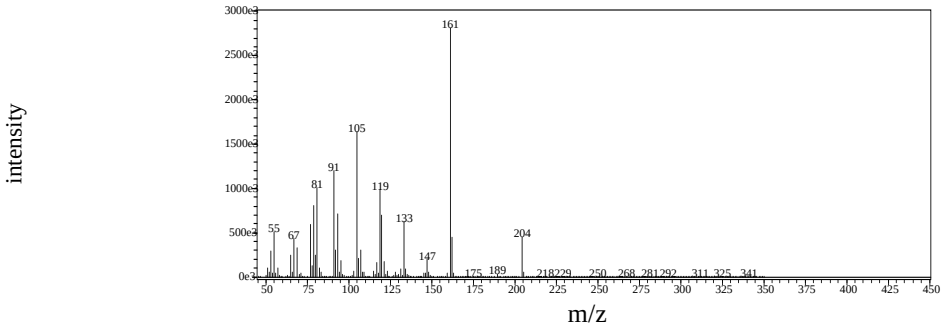

Figure 3: The peak of *p-cymene* obtained by the mass spectrometer.

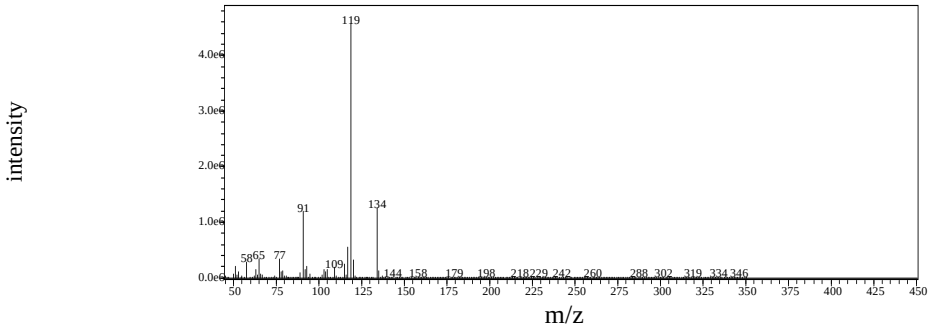

Figure 4: The peak of isoledene obtained by the mass spectrometer.

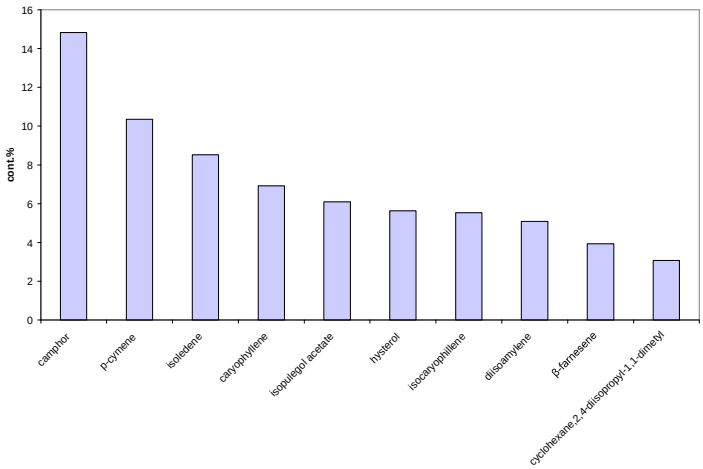

Figure 5: **The variation of main constituents in volatile components of *Artemisia absinthium*. from Binaloud mounain, Iran**.

Acknowledements

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