Advanced Herbal Medicine, 2018; 4(3): 55-63

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Study of phytochemical characteristics *Artemisia persica* Boiss in Ilam Province

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Received: 04/June/2017 Accepted: 09/August/2017

ABSTRACT

Background and aims: The genus Artemisia is one of the largest and most widely distributed of the nearly 100 genera in the tribe Anthemideae of the Asteraceae (Compositae). 34 species of Artemisia have been reported in Iran. Several secondary metabolites characterize the chemical composition of the genus Artemisia. Therefore, the current study aimed to phytochemical Characteristics of Artemisia persica Boiss collected from the field and provenance of Ilam.

Methods: The aerial parts of plants were collected from Ilam field and Kabirkooh mountain. After extraction of Artemisinin, the analysis was performed with an HPLC system. Extraction of essential oil it was done by hydro distilled. Phytocomponents identified in Artemisia persica Boiss essential oils by GC/MS system.

Results: The essential oil yield was reported in Kabirkooh mountain and field 0.92 % and 0.6%, respectively. The major oil compounds of samples of Artemisia persica Boiss collected were included: α-Pinene, 1,8-Cineole, (Z)-Sabinene hydrate, (E)-Pinocarveol, Pinocarvone, Artedouglasia oxide C, Laciniata furanone E, Artedouglasia oxide D, Artedouglasia oxide B. The Artemisinin was 2.7 ppm in the Kabirkooh mountain sample. However the field sample had 1.5 ppm.

Conclusion: *To achieve the appropriate level of the target compounds it is important to considered an appropriate place for sampling.*

Keywords: Artemisia persica Boiss, essential oil, Artemisinin, phytochemical

Original article

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INTRODUCTION

The genus Artemisia is one of the largest and most widely distributed of the nearly 100 genera in the tribe Anthemideae of the Asteraceae (Compositae) (Mucciarelli and Maffei, 2002). 34 species of Artemisia have been reported in Iran (Mozaffarian, 2008). Chemical composition and biological activities of Artemisia spp essential oils has been reported recently (Lopes-Lutz et al, 2008).

Sixty constituents were identified in essential oil from aerial parts, leaves, flowers and roots of Artemisia persica Boiss from Iran. The oils were extracted by hydrodistillation and the composition of the oils were analyzed by a combination of GC and GC/MS (Rustaiyan and Faridchehr, 2014).

There are several secondary metabolites characterize in the chemical composition of the genus Artemisia. A survey of the literature indicates that almost all classes of compounds are present in the genus, with particular reference to terpenoids and flavonoids (Rabie et al, 2012). "Sefidkon et al (2013) evaluated the antimicrobial effect of the essential oil of Artemisia spicigera against Gram positive and Gram negative bacteria. Their results indicated that all bacteria except Pseudomonas aeruginosa have the oil produced inhibition zone more than 20 mm.

Artemisinin is a sesquiterpene compound which has been isolated from Aretemisia annua for the first time (Arab et al, 2006). Previous studies have been reported the treatment effects of Artemisinin and its derivatives in of Malaria, Hepatitis B, various types of Cancer, especially Blood Cancer, and Leishmaniasis (Efferth et al, 2001; Lai and Singh, 2006; Romero et al, 2006).

Due to the effect of geographical location, altitude and climate on the yield and composition of volatile oils (Mojarrab et al, 2012) the current study aimed to evaluated the phytochemical characteristics of Artemisia persica Boiss collected from Kabirkooh mountain and Ilam field.

METHODS

Geographical Profile the region

The geographical coordinates of the places where they took samples are shown in table 1.

Plant material

The aerial parts of plants were collected from Ilam field and Kabirkooh mountain. Plant material was identified by Ramin Agriculture and Natural Resources University of Khuzestan and

transported to Analytical chemistry laboratory for study of phytochemical characteristics.

Table 1. Geographical Profile the region

Region Name	Latitude	Longitude	Height
Ilam	33º 36′	46º 36′	1427 m
Kabirkooh mountain	33º 09′	47º 24′	3050 m

Preparation of Extracts Artemisinin

Approximately, 5 g of each plant sample was weighed accurately and macerated with 250 mL of n-hexane at room temperature for 2 days using a laboratory shaker. Then, the n-hexane phases were filtrated and evaporated under vacuum until dryness. Them residue was dissolved again in 100 mL of n-hexane and the n-hexane phase was washed in a separatory funnel with 2 % NaOH solution to getrid of the impurity, which is soluble in NaOH. After abandoning the alkali solution present in the lower layer, the upper solution was washed with distilled water several times until it was neutralized. The extract, obtained after distillation under vacuum at 45 ºC in rotary evaporator, was dissolved with 95% ethanol and then filtrated in 250 mL measuring flask. Then, 10 mL of filter liquor was transferred into a 100 mL measuring flask. 40 mL of 0.2 % NaOH solution was added in the flask, and then, let it react at 50 ºC for 30 min. After that, 0.08 mol/L acetic acid solution was filled up to the mark (Hao, et al, 2002).

HPLC analysis

The analysis was performed with an HPLC system consisting of an HPLC Knuer smartline series quaternary pump with degasser and a photodiode array detector. The Nucleodur C18 column (5 µm; 250 mm x 4.6 mm), at 30 °C. The system was controlled and data analysis was performed with clarity system. All the calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas. A mobile phase consisting of formic acid (% 0.2 v/v): acetonitrile (50:50) by isocratic elution was chosen to achieve maximum separation and sensitivity. Flow rate was 1.0 mL/min. Column temperature was set at 30 °C. The samples were detected at 254 nm using

Table 2. The yield essential oil *Artemisia persica Boiss*

Location	yield essential oil (%)
Field	0.6
Kabirkooh mountain	0.92

photodiode array detector (Erdemoglu et al, 2007).

Extraction of essential oil

100 gm of cleaned and dried plant material was powdered using metal mortar and pestle and placed in a round bottom flask fitted with condenser hydro distilled for 3 hrs at atmospheric pressure and constant temperature. The strongly aromatic oil was separated from the water layer using n-Hexane and the solvent was removed by boiling.

GC-Mass analysis

The essential oils of Artemisia persica Boiss were analyzed using a Agilent GC (7890) MS (5975) gas chromatography-mass spectrometer (GC-MS) fused with a capillary column of HP-5 MS (30 m × 0.25 mm i.d., 0.25 µm film thickness) with ionization potential of 70 ev. Helium was used as carrier gas at a constant flow of 0.8 ml/min and an injection volume of 1µl was employed, injector temperature 290°C; Ion-source temperature 280°C. The oven temperature was programmed from 50°C (isothermal for 5 min), with an increase of 3°C/min, to 240°C and with an increase of 15°C/min, to 280°C held for 10 min. Identified by comparison of their mass spectra with the Wiley 7n and National Institute of Standards and Technology (NIST5.0) libraries. Kovats indices of components were obtained by the aid of standard n-alkanes (C8–C20) injection, under the same chromatographic conditions (Adams, 2001).

RESULTS

Essential oil yield was calculated by determining the percentage of moisture to the dry weight of each sample at the time of extraction (table 2). The retention times and chemical composition of phytocomponents in Artemisia persica Boiss essential oil are presented in table3.

The essential oil yield was reported in Kabirkooh mountain and field 0.92 % and 0.6%, respectively. The major oil compounds of samples of Artemisia persica Boiss collected were

Table 3. Phytocomponents identified in *Artemisia persica Boiss* essential oils

Component	KI	Component %	
		Field	Kabirkooh mountain
α-Thujene	0930	0.08	0.10
α-Pinene	0939	6.66	3.43
Camphene	0954	0.33	0.3
Sabinene	0975	0.3	0.08
ß-Pinene	0979	0.25	0.17
Myrcene	0991	-	0.08
α-Terpinene	1017	0.18	-
***p*-Cymene**	1026	1.8	1.6
1,8-Cineole	1031	5.9	6.3
γ-Terpinene	1060	0.45	0.28
(Z)-Sabinene hydrate	1070	23.12	24.1
(E)-Arbusculone	1071	0.1	-
(E)-Pinocarveol	1139	9.2	10.3
(E)-Verbenol	1141	0.13	0.12
Pinocarvone	1165	7.2	7.9
Borneol	1169	0.9	0.75
Terpineol-4-ol	1177	0.31	0.34
***p*-Cymen -8-ol**	1183	-	0.1
Myrtenal	1196	2.14	2.06
Verbenone	1205	0.09	0.15
(E)-Carveol	1217	0.5	0.41
(E)-Pinocarvyl acetate	1298	0.8	0.92
Phenyl ethyl 3-methyl butanoate	1491	0.05	-
Artedouglasia oxide C	1524	11.9	12.04
Laciniata furanone G	1529	1.37	1.30
Laciniata furanone F	1533	1.86	2.41
Laciniata furanone E	1542	10.08	10.3
Laciniata furanone H	1550	2.08	2.15
Artedouglasia oxide D	1561	4.54	5.15
Artedouglasia oxide B	1582	4.82	5.22

included:: α-Pinene, 1,8-Cineole, (Z)-Sabinene hydrate, (E)-Pinocarveol, Pinocarvone, Artedouglasia oxide C, Laciniata furanone E, Artedouglasia oxide D, Artedouglasia oxide B. The α-Terpinene, (E)-Arbusculone, and Phenyl ethyl 3-methyl butanoate combination was only existed in field and sample, while, Myrcene and p-Cymen -8-ol combination was only existed in Kabirkooh mountain samples.

Artemisinin chromatogram is presented in Figure 1. The isolation of Artemisinin peak chromatogram of the s Artemisinin in extract samples is presented in Figure 2. Artemisinin levels were calculated for each sample and presented in Table 4.

Location	Artemisinin (ppm)
Field	1.5
Kabirkooh mountain	2.7

The results showed that this concentration is different in samples collected from the field and Kabirkooh mountain Ilam province. The Artemisinin was 2.7 ppm in the Kabirkooh mountain sample. However the field sample had 1.5 ppm.

DISCUSSION

Due to chemical analysis of essential oil Artemisia persica Boiss main combination of this essential oil includes α-Pinene, 1,8-Cineole, (Z)-Sabinene hydrate, (E)-Pinocarveol, Pinocarvone, Artedouglasia oxide C, Laciniata furanone E, Artedouglasia oxide D, Artedouglasia oxide B. We found that α-Terpinene, (E)-Arbusculone, and Phenyl ethyl 3-methyl butanoate combination was only existed in field and sample, while, Myrcene and p-Cymen -8-ol combination was only existed in Kabirkooh mountain samples. Others studies showed component was 80% monoterpenes and sesquiterpene, but the very difference was the percentage and type compounds (Bicchi et al, 1985; Sadeghpour et al, 2004; Mirjalili et al, 2006) . This may be due to season changes, growth stage, collection time of planting, climate conditions and plant growth place (Mahbubi et al, 2007; Mirjalili et al, 2006). Although the productions of secondary metabolites are controlled by genes, but their production will be significantly affected by environmental conditions. Physical and chemical properties of soil, micronutrients and

macronutrients are most important factors (Palevitch, 1987).

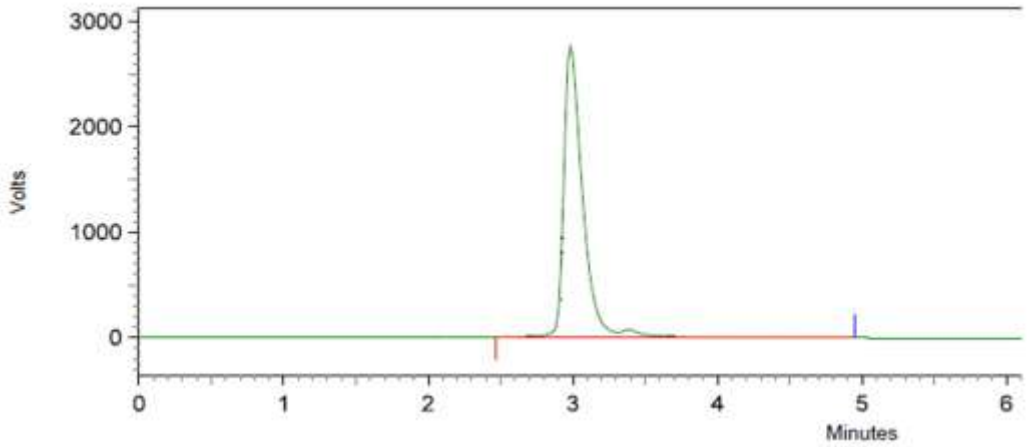

Figure 1. Chromatogram of Artemisinin standard

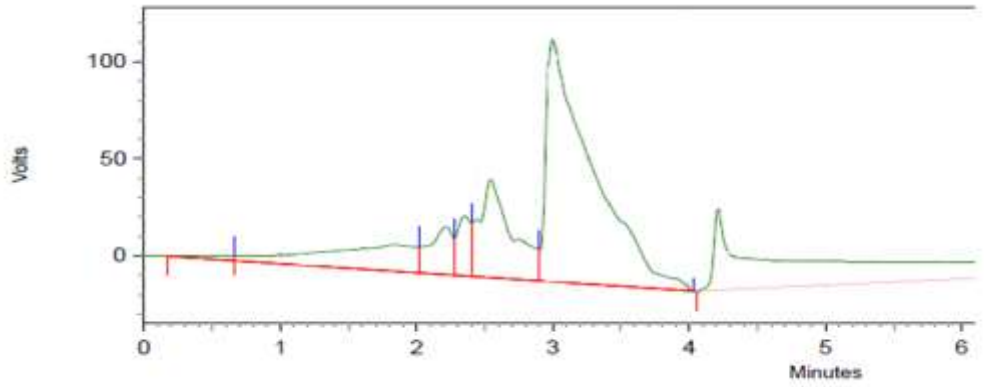

Figure 2. Chromatogram of Artemisinin in extract sample

The results of the present study showed that the Artemisinin level were higher in the Kabirkooh mountain leaves sample. However the field sample have the lower levels of the Artemisinin. It seems that Artemisinin has been influenced by the growing conditions. Environmental factors play an important role in the production and accumulation of secondary metabolites in medicinal plants. Temperature, precipitation, light intensity, and the above height of sea level are the most important environmental factors affecting the accumulation of secondary metabolites (Kostova and Dinchev, 2005).

CONCLUSIONS

Temperature, precipitation, light intensity, and the above height of sea level are the most important environmental factors affecting the accumulation of secondary metabolites, to achieve the appropriate level of the target compounds it is important to considered an appropriate place for sampling.

AUTHORS' CONTRIBUTIONS

The authors declare that they have no conflict of interests.

CONFLICT OF INTEREST

Authors have declared that no conflicts of interest exist.

ACKNOWLEDGEMENT

The authors are grateful to thank those researchers who generated useful findings for this study.

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