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Advances in Bioresearch

ORIGINAL ARTICLE

Identification of Components in essence oil of *Prangos ferulacea* using different Extraction methods

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ABSTRACT
Aromatic and pharmaceutical plants are widely used in different industries in order to obtain valuable components. In the present study the components present in the essence of Prangos ferulacea was fully investigated. These essences are sensitive to gathering method. The effect of different technique of extraction including hydraulic distillation (HD) and head space-soild phase micro extraction (HS-SPME) on the quality of the produced essence were inspected and their components were recognized using gas chromatography and mass spectroscopy. The essence gathered by HD method was included25 different components which made about 90.35% of the total product. On the other hand, HS-SPME method could gather 24 components making about 99.18% of total volume of essence. Additionally, the percentage of oxygenate and non-oxygenate components were also determined using HD and HS-SPME methods.
***Keywords**: Prangos ferulacea, Extraction, Essence oil*

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INTRODUCTION

The *Prangos ferulacea* has been used as a conventional medicine in Iran to cure digestion disorders. It was also known as an herb removing ache and inflammation between the public [1]. This herb is also used as a cure for timpanists, strong laxative of stomach, anti-blowing, alleviating nerve pains, anti-inflammation, antivirus, anti-parasite, anti-fungus and antibacterial [2-3]. Recently, some investigations revealed the effect of different species of this herb as antivirus of HIV [4].The *Prangos ferulacea* is a rich source of anti-oxidant and some researches claimed that this property surpluses vitamin E agents [5]. There are 15 different species of *Prangos ferulacea* in Iran which five of them are native [6]. This herb needs humidity for growing in addition to cold weather and frosting in a complete life cycle. Therefore, it can be found in snowy places with considerable length of cold weather. The herb reproduction is better in clayey soils and soil texture and structure has a great effect on the level of major and minor expansion of the root [7-8].The herb gets sprout in last March and grows gradually till the end of April. Afterwards, it enters into reproduction phase and the fruits are ready in mid-May. The seeds can be gathered at the time of withering by July [9-10]. *Pachymerus acacia* is known as the major pest of *Prangos ferulacea* which is from Bruchidae family and hatches on the leaves of the herb [11]. The plant is a major source of anti-oxidants [12]. Accordingly, in the following research different components of the herb were extracted using HD and HS-SPME methods by means of GC and GC-MS devices. Finally, the components obtained from two methods were compared together.

MATERIALS AND METHODS

Materials and instruments

In this research, chemical materials like anhydrite sodium sulfate, normal hexane and standard oleic acid were purchased from Merck Company. Also, Gas chromatography apparatus (GC, model HP-6890) and Mass spectroscopy (Model HP-5973, USA) were used for analysis of components. SPME fiber assembly excluding solid phase and SPME fiber holder made by SUPELCO (USA).Electric mill for herbaceous parts grinding, if necessary; this mill is needed for SPME method and the model is Ikawerke M20 (Germany).Characterisation of the used fibres in the SPME method is displayed in Table 1.

Table 1. Characterisation of the used fiber in the SPME method

Type of fiber	(carboxen / poly dimethyl siloxane , CAR / PDMS)
Adsorbent thickness	75 micrometer
Type of adsorbent connections	strongly network
Color	Black

Experimental procedure

Hydraulic distillation (HD)

The essence oil was gathered using Clevenger instrument. 100 g of dried herb was grinded and the essence was gathered in the instrument for 3.5 hour. The operation performed using anhydrite sodium sulfate. The produced oil was conserved in a dim and closed container till analyses.The chromatography results of essence oil obtaining from *Prangos ferulacea* using HD method is showed in Figure 1.

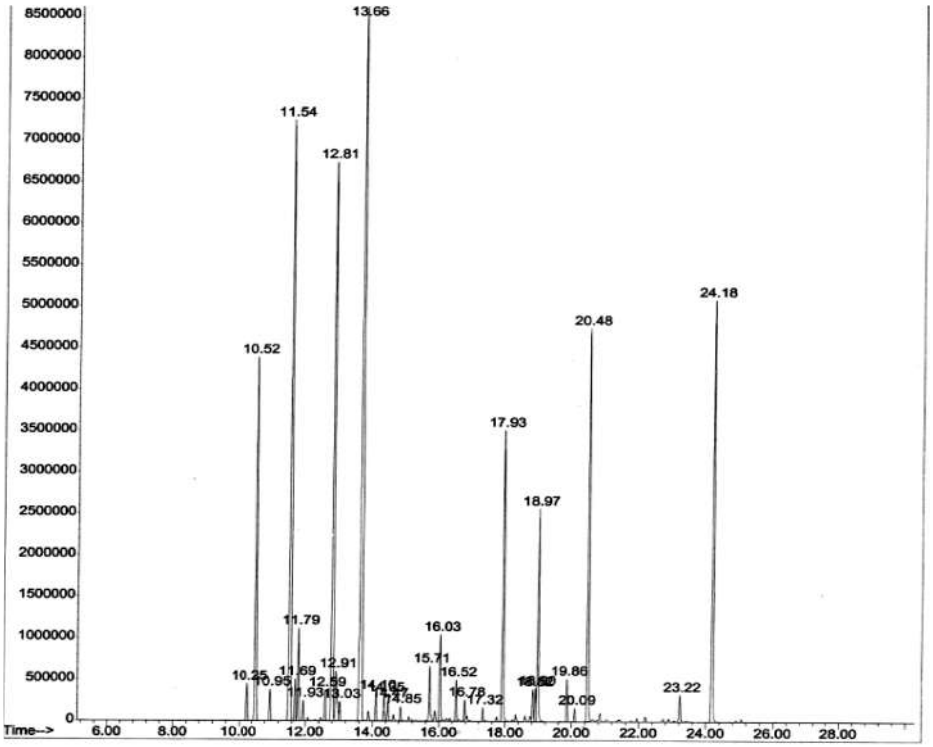


Figure 1- The chromatography results of essence oil obtaining from *Prangos ferulacea* using HD method

HS-SPME method

The method carried out using minimum amount of herb powder (1g) without using any solvent. The circulating bath model mLw8 made by mLwHU was used in this experiment which had the ability of temperature control during extraction process. The fiber assembly was kept in a 10 ml glass container and the fiber holder attached to the container. The glass container was inside the circulating bath to reach the bath temperature. For heat desorption of pulled components on the fiber, the injection performed immediately into the GC-MS instrument. This study tests the suitability of the Carboxen–polydi-methylsiloxane (CAR–PDMS) fiber.The SPME device and CAR–PDMS (75mm) were used as fibers used in this study were purchased from internal standards (I.S.s). (Results are shown in table 1).The fibers ereMeSEt and Et S were supplied by Aldrich conditioned by inserting them into the GC system injector at 28^0C for 30 min and they were immediately used to prevent contamination. Before the extraction with the fiber, the sample vials were equilibrated for 30 min at 25^oC. After- wards, the stainless steel needle in

which the fiber is housed was pushed through the vial septum, allowing the fiber to be exposed to the headspace of the sample for 30 min. Then, the fiber was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption at 300 C for 1min.

Separation and identification of components in essence oils

As the components present in essence oils are known as volatile and semi-volatile oils, therefore GC-MS method was applied for separation and identification of the components. The result spectrums were compared with standard mass spectrum of Adams (Adams, R.P., 2004). In order to confirm the identified components of standard mass spectrum, Quatz deterrence index was applied. Firstly, the Alkanes of C_8-C_{25} were injected into GC-MS and deterrence time for each Alkane was measured using KI=100n when 'n' is the number of carbons in related Alkane. Quatz deterrence index of essence oils were calculated using the following equation:

$$KI=100n + 100\left(\frac{t_x - t_n}{t_{n+1} - t_n}\right)$$

After dewatering the produced oil, the oil was diluted using normal hexane (Merck) with the proportion of 1 to 10 and then injected into GC-MS.

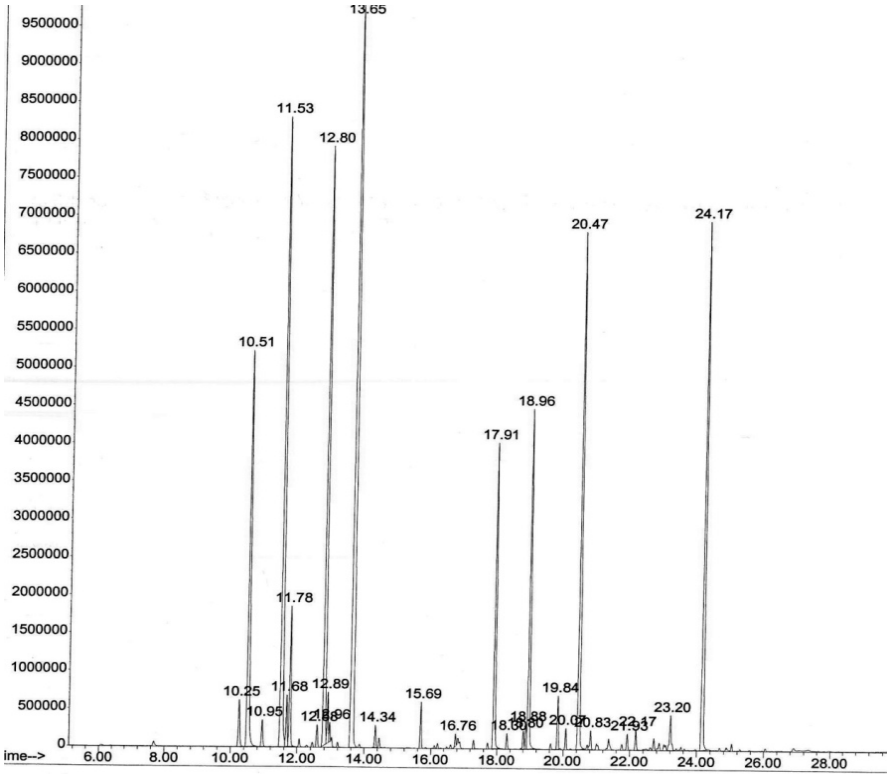
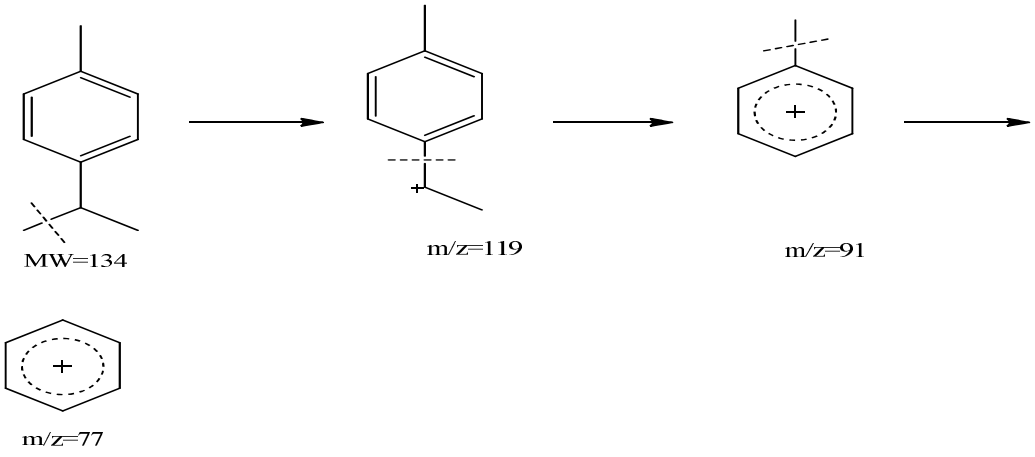


Figure 2- The chromatography results of essence oil obtaining from*Prangosferulacea*using HS-SPME method



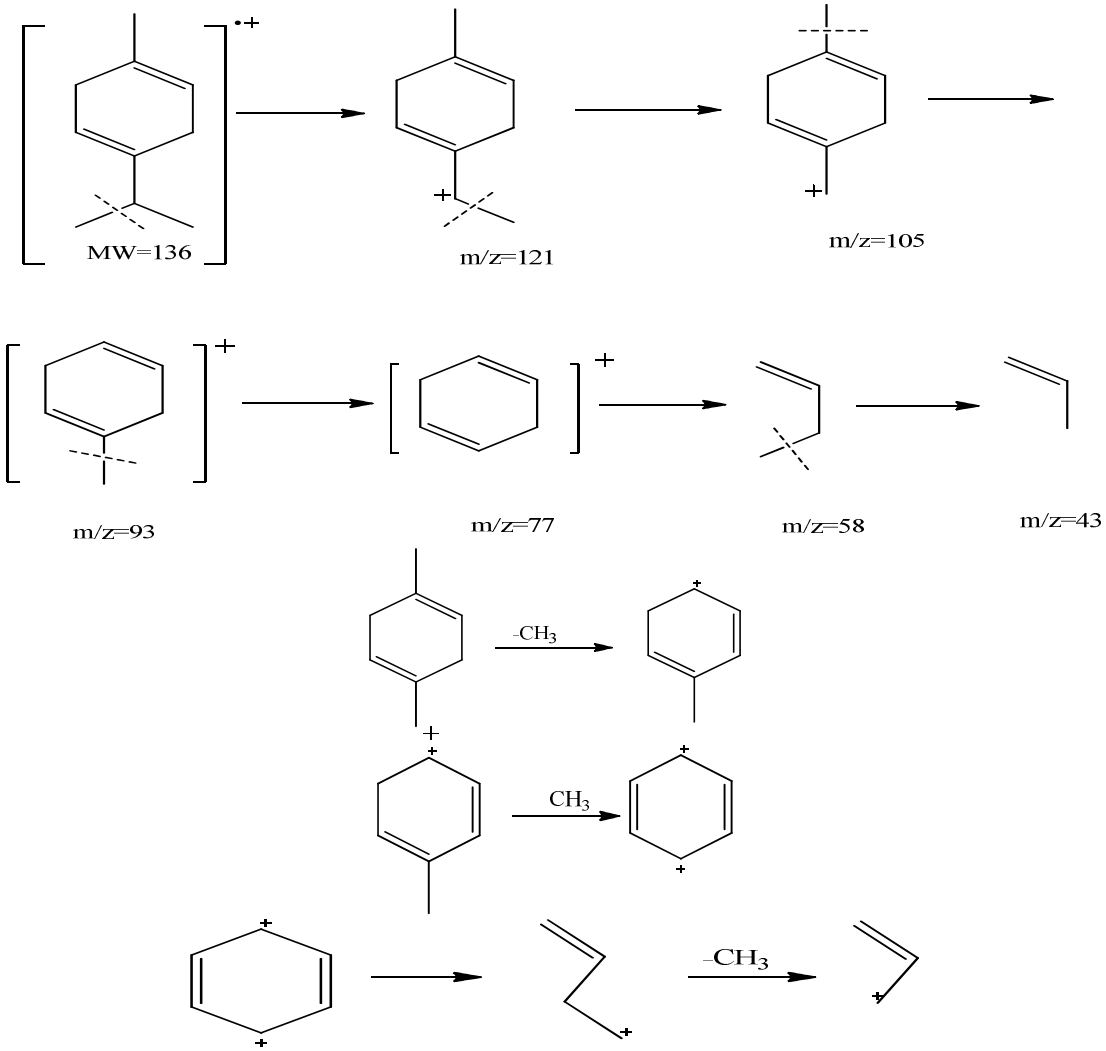


Figure 3 :Analysis results of the extraction and identification of compounds in essence oil *Prangos ferulacea*

RESULTS AND DISCUSSION

The results extraction and identification of components derived from the essence oil of *Prangos ferulacea*

Elimination of methyl group at the first stage leads to formation of a stable phase of carbo-cation benzyl. Thereafter, the ethylene group is removed and a tropylium ion which is more stable than carbo-cation benzyl is produced known with a peak index equal to 91 on the graph. Acetylene removal from tropylium gives a peak equal to 65. There is another possibility that deletion of a methylene group from this ion proves it to be an aromatic component (peak equal to 77). This removal of methyl group causes fabrication of stable carbo-cation benzyl. At the next stage, methyl removal gives an allyliccarbo-cation. After rearrangement inside molecular structure, another conjugated alkene gives an allyliccarbo-cation. Methyl group deletion causes reproduction of dienecarbo-cation. On both sides, there is allyliccarbo-cation. In fact, there are components stable on both sides and another removal of methyl group leads to reproduction of stable allyliccarbo-cation. The components present in essence oil extracted from *Prangosferulacea* obtained from different extraction methods (HD, HS-SPME) are shown in Table 2 with the percentage of components and related Quatz index.

Table 2- The components of essence oil *Prangos ferulacea* using HD and SPME-HS methods

Compound	KI	HD (%)	HS-SPME (%) (60˚C)
- Thujeneα	930	0.66	0.88
Pineneα–	939	7.75	8.48
Camphenene	954	0.58	0.48
Sabinene	975	15.19	14.86
Pineneβ–	979	0.72	0.87
Myrcene	991	1.62	2.33
Terpinenα–	1017	0.53	0.37
Para-cymene	1025	13.55	12.91
Limonen	1029	1.35	0.78
1,8-cineole	1031	0.34	-
Ocimene(z-beta)	1037	-	0.24
γ- Terpinene	1060	21.4	20.71
Artemisia alcohol	1084	0.48	-
-terpineoleneα	1089	0.45	0.34
Citronellal	1153	1.05	0.69
Thujene4ol	1169	1.59	-
γ - Therpineol	1199	0.72	-
Fenchy acetate(endo)	1220	0.33	-
Citronellol	1226	0.23	-
Cuminal	1242	6.11	-
Anethol(z)	1253	-	5.56
Geranial	1267	-	0.23
Bornyl acetate	1289	0.6	0.31
γ – Therpinental	1291	4.19	6.31
Acetanisol(meta)	1299	-	0.34
Citronelly acetate	1353	0.73	0.77
Nery acetate	1362	0.22	0.31
Geranyl acetate	1381	-	10.24
Franesense(z)beta	1443	-	0.23
Isobornyl-3-methyl	1524	0.54	0.74
Bornylangelate	1566	9.42	10.2
Total		90.35	99.18

Study on components in essence oil of *Prangos ferulacea*

As it is shown in Table 2, in essence oil of the herb obtained from hydraulic distillation (HD), there are 25 components which make 90.35% of the whole essence. This essence contains 66.59% Hydrocarbonicmonoterpene, 12.25% Oxygenated monoterpene and 9.96% Oxygenated sesquiterpene. The main components of essence oil were: sabinene (15.19%), para cymene (13.55%), gamma Terpinene (21.40%) and bornylangelate (9.42%).On the otherhand, using HS-SPME method extracted 99.18% of the whole essence that contained 24 components. This essence holds 63.25% Hydrocarbonicmonoterpene, 12.79% Oxygenated monoterpene and 10.94% Oxygenated sesquiterpene. The main components of essence oil were: sabinene (14.86%), para cymene (12.91%), gamma Terpinene (20.71%), geranyl acetate (10.24%) and bornylangelate (9.42%).

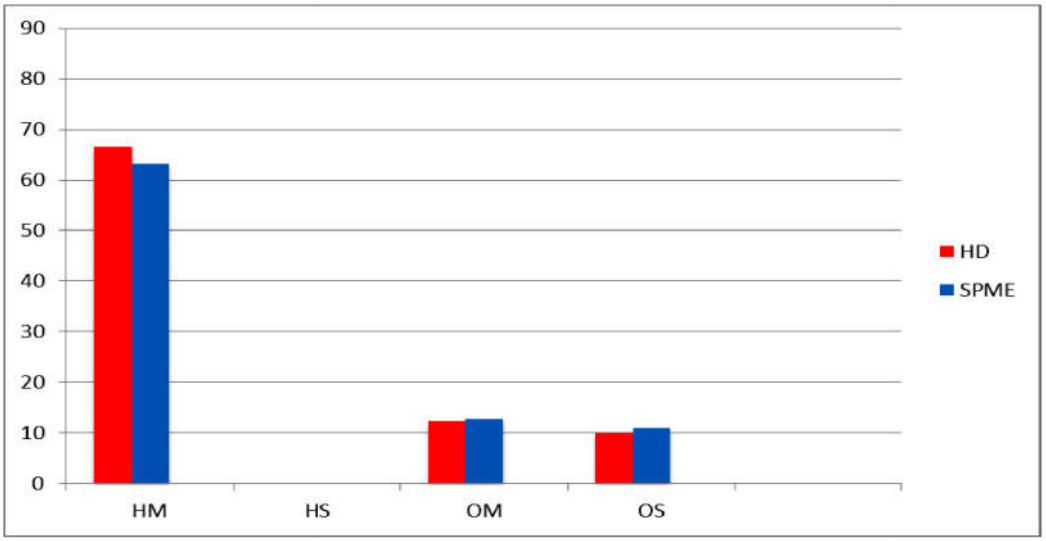


Figure 4- The percentage of different component groups of the essence oil of *Polylophium involucratum* using HD and HS-SPME methods

Figure 4 shows the percentage of different component groups of essence oil extracted from the herb using HD and HS-SPME methods. In Figures 4 and 5, the vertical axis shows the percentage of components and horizontal axis shows Hydrocarbonicmonoterpenes, Hydrocarbonicsesquiterpenes, Oxygenated monoterpenes and Oxygenated sesquiterpenes and other components. Figure 5 shows the percentage of oxygenated and non-oxygenated components present in *Prangos ferulacea* essence oil with both test methods.

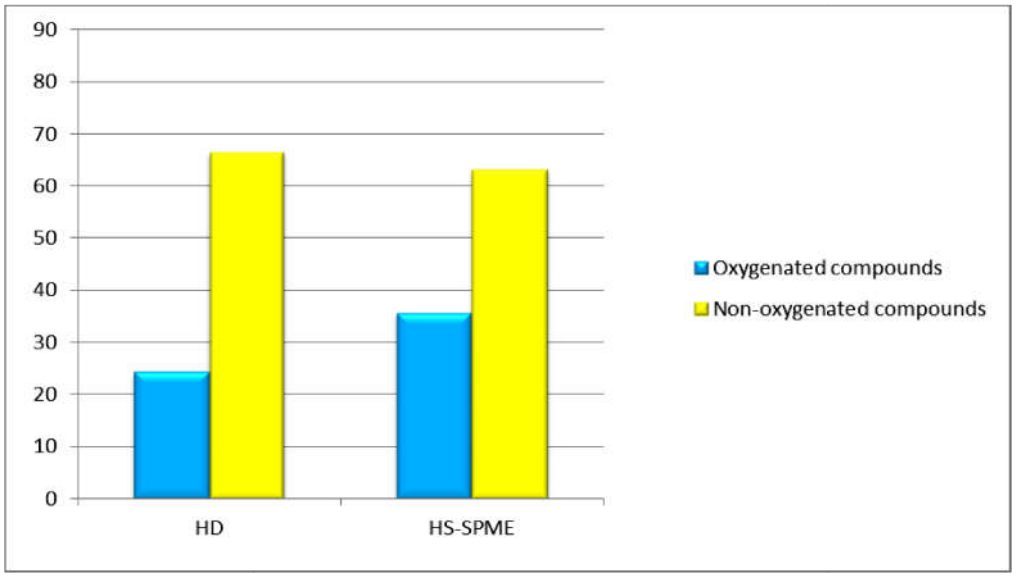


Figure 5- The percentage of oxygenate and non-oxygenate components in the essence oil of *Prangos ferulacea* using HD and HS-SPME methods

CONCLUSION

Hydraulic distillation (HD) is a common method of essence oil extraction method from aromatic and pharmaceutical herbs applicable in industrial usage; since it has no chemical pollution. But in this technique components sensitive to heat will destroy and this affects the quality of essence oil. A considerable amount of sample is required and it takes a long time to extract the oil. In contrast, HS-SPME method is a fast and simple method which does not need solvent and small quantity of the sample is required for analysis. This method can be easily used for volatile components of aromatic and pharmaceutical herbs. Generally, HS-SPME method and extraction with microwave without solvents help essence oil extraction without high energy consumption and long analysis time. Besides, small amount of the sample is enough. The results of this research confirm the similarity between the extracted

components from HD and HS-SPME methods; but relative difference exists between the results. The oxygenated components of essence oil from SPME method are higher in quantity compared with HD method.

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