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Comparison of the components of the volatile oils from leaves of *Ziziphus jujuba* extracted by changing the solvent system and the separation methods

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Quantity and quality of the components of volatile oils are highly dependent on the conditions of extraction. In this study, the volatile components of the leaves of *Ziziphus jujuba* in the flowering period were investigated by changing the extraction solvent and the method of extraction. At first, the volatile components were extracted by changing the type of the extraction solvent in the simultaneous distillation-extraction (SDE) method. Then, the effects of the extraction method on the quality and quantity of the extracted compounds were studied using the hexane solvent via two methods of SDE and percolation. The extracted volatile oils were separated and identified using GC/MS and GC/FID. The extraction method itself, proved to have the most effect on the number and type of the compounds of the volatile oils. Also the mean of molecular weight and solubility of the compounds of the extracted volatile oils in water, were so different from one another.

Keywords: *Ziziphus jujuba*; Separation method; Volatile oil; Simultaneous distillation-extraction; Cold percolation method

INTRODUCTION

Aromatic plants have been known for a very long time and the use of them in the food and perfume industries have a long history [1].

Ziziphus is a genus of the family Rhamnaceae which consists of about 40 species and is a small spiny shrub, distributed in warm-temperate zones and subtropical regions throughout the world [2].

In Iran, *Ziziphus* is mostly found in the central regions and Khorasan and Golestan provinces.

The drupe and the flesh of *Ziziphus* contain its most medicinal properties, also the leaves have shown healing properties. The infusion of the leaves is usually gargled to treat sore throat, bleeding gums and joint pain [3-5].

Kurihara *et al*. [6] extracted the saponin, ziziphin, from the dried leaves of *Z. jujuba*. Leaves of *Z. jujuba* due to the existence of the active substance of ziziphin, can suppress the sweet taste sensation in flies (Pharma regina), rats and hamsters [6].

In a study conducted by Shirdel and Mirbadalzadeh [7] it was determined that the ethanolic extract of leaves of *Z. jujuba* has a hypoglycemic effect in diabetes mellitus patients and its effect is similar to that of glibenclamide [7].

The anti-allergic activity of the water extract of leaves of *Z. jujuba* was studied by measuring its inhibitory effect on the activation of hyaluronidase (bovine testes) *in vitro;* and *Z. jujuba* proved to have strong anti-allergic activity [7].

El Husseiny and El Kholy [8] evaluated insecticidal properties of different extracts of the leaves of *Z. jujuba*. They showed that Petroleum ether extract of the leaves of *Z. jujuba* is able to reduce the population dynamics of *Culex pipiens*; either directly through larval kill, or indirectly through its latent effects expressed in reduction of egg hatchability, inhibition of adult emergence, interruption of life stages and the effect that it has on sex ratio [8].

Due to the fact that plant extracts usually are a combination of various types of bioactive compounds, their separation, identification and characterization of those bioactive compounds still remain a big challenge in the way of the processes [9].

In the traditional methods of extracting natural compounds from plants such as water or steam distillation and extraction with organic solvents such as soaking, there are some disadvantages such as loss of volatile components, degradation of compounds and remaining toxic solvents [10, 11]. Due to the increasing usage of natural compounds in the recent years, the effects of different extraction methods and types of solvent on the quality and quantity of volatile compounds, extracted from the leaves of *Z. jujuba* during the flowering season were studied.

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MATERIALS AND METHODS

Materials

Leaves of *Z. jujuba* were collected from one of the central provinces of Iran (Qom province) during the flowering period in 2014. The plant materials were authenticated by the Department of Botany of Islamic Azad University, Qom Branch.

Samples were dried and subsequently ground in a blender to obtain fine powder. All reagents and chemicals used in this study were from Merck or Sigma Companies.

Extraction methods

Extraction by SDE method

The volatile oils of *Z. jujuba* were extracted from dried leaves samples via hydro-distillation, using the simultaneous distillation-extraction (SDE) method [12]. Also the organic solvents of n-hexane and ethyl acetate were used in the extraction process. The extraction process took 2 h. Then the solvent was removed and the solution was dried over anhydrous sodium sulfate. The extracted oils were stored at 4°C in a sealed vial until analyzed.

Extraction by cold percolation method

The plant extracts were prepared by the cold percolation method. Ten grams of dried powder were added to 300 ml of solvent in a conical flask and the mixture was kept for 48 hours with intermittent shaking. After that, the extract was filtered through Whatman No. 1 filter paper; then the solvent was removed using a rotary evaporator and subsequently dried, until a constant weight of each extract was obtained. The residues were stored at 4°C for further use [13].

Gas chromatography analysis

Analytical gas chromatography of the volatile oils was carried out using a Hewlett-Packard 5975B series gas chromatograph with Agilent HP-5 capillary column (30 m×0.25 mm, f.t 0.25 µm); carrier gas He; split ratio 1:10 and using a flame ionization detector. The column temperature was adjusted at 50°C which was unchanging for 10 min and was programmed to rise up to 240°C at a rate of 4°C/min and then stay constant at that temperature for 15 min. GC/MS was performed on an HP 5975B with a Hewlett–Packard 5973 quadrupole detector, on capillary column HP-5 (30 m×0.25 mm; f.t 0.25 µm).

The MS was operated at 70 eV ionization energy. Retention indices were calculated using retention times of n-alkanes that were injected after the volatile oil at the same chromatographic conditions. Quantitative data were obtained from the electronic integration of the FID peak areas.

The components of the oils were identified by comparing their mass spectra and kovats indexes with Wiley library and published books, data bases available and credible websites [14].

RESULTS AND DISCUSSION

In this study, qualitative and quantitative characteristics of the components of the volatile oil of the leaves of *Z. jujuba* in the flowering season were evaluated after changing certain parameters such as the extraction solvent and the extraction method.

At first the components of the volatile oils were extracted by changing the type of extraction solvent in the SDE method. Ethyl acetate and hexane as the polar and aprotic solvents, with different dipole moments were used (D_{Hexane} =0.08, $D_{Ethyl\ acetate}$ =1.88).

In the second segment, by using the hexane solvent but changing the extraction method from

SDE to cold percolation, the effects of the extraction method on the quality and quantity of the extracted compounds were studied.

Other important matters to take into consideration are the type and number of the extracted compounds. The compounds separated using the hexane solvent and the SDE method have a noticeable difference compared to the compounds separated by the percolation method; that is to say Eugenol, trans-β-Ionone, α-Farnesene and 2-Hexenal are some of the main compounds in the SDE method which were not separated in the percolation method. Likewise α- Pinene, Linoleic acid and Diisooctyl adipate were some of the separated constituents of the percolation method which were not obtained in the SDE method.

Some compounds such as palmitic acid and phytol, exist in both of the separation methods but their percentages are different. From the SDE method while using the hexane solvent, 34 compounds were obtained; but this number for the ethyl acetate solvent with the same method is 30 (Tables 1 & 2). When the SDE method and the hexane solvent were used, 34 volatile compounds were extracted while only 11 volatile compounds were obtained from the percolation method with the same solvent. As it can be seen, in separation of compounds by the SDE method, there is only a little difference in the number of extracted compounds after changing the solvent but, when the separation method is changed, this difference is much greater.

It was previously reported that volatile oil content of medicinal plants is influenced by the extraction method. Gavahian and his colleague demonstrated that essential oils obtained by hydrodistillation and steam distillation, were almost similar in their physical properties and chemical compositions [15] but Xie et al. showed that among the three extraction methods, the solvent extraction method could extract compounds with low volatility and high molecular weight. They proved that both of the methods of headspace solid-phase microextraction and steam distillation, could extract volatile components [16].

In order to study the extracted compounds, parameters such as solubility in water and the boiling point were used. According to the results demonstrated in Table 3, the mean of solubility values of the extracted compounds in water (in 20°C) by the SDE method when using the ethyl acetate solvent, was 2414.01 ppm; while this value for the hexane solvent was 882.22 ppm.

Table 1. Chemical composition of the volatile oils of the leaves of *Z .jujuba* extracted by the SDE method

No	Components	extracted solvent: Ethyl acetate [a] (%)	extracted solvent: Hexane [b] (%)	RI[c]
1	Octane	-	9.10	≤ 800
2	n-Hexanal	2.45	-	800
3	(E)-2-Hexenal	26.31	11.26	852
4	Xylene	1.38	-	870
5	6-Methyl-5-hepten-2-one	-	1.80	991
6	α-Toluenol	0.96	-	1044
7	cis-Linalool oxide	-	1.04	1092
8	trans-Linalool oxide	0.67	-	1093
9	Terpinolene	-	2.26	1104
10	(3E)-6-Methyl-3,5-heptadien-2-one	-	0.61	1110
11	Naphthalene	14.21	-	1191
12	Fenchol	-	0.95	1197
13	α-Terpineol	1.40	-	1198
14	Dodecane	1.70	-	1200
15	Tridecane	1.34	-	1300
16	Eugenol	-	9.58	1367
17	Cyclohexane	-	5.00	1396
18	Tetradecane	2.01	-	1401
19	Dihydropseudoionone	-	1.67	1456
20	*trans*-β-Ionone	2.83	5.55	1493
21	Pentadecane	2.26	-	1501
22	α-Farnesene	-	6.13	1511
23	δ-Cadinene	-	6.31	1528
24	Dihydroactinolide	1.88	1.27	1540
25	E-Nerolidol	1.27	2.85	1569
26	(E)-3-Eicosene	1.05	-	1593
27	Hexadecane	2.17	-	1601
28	Benzophenone	1.86	-	1639
29	Benzyl benzoate	3.60	-	1776
30	Hexahydrofarnesyl acetone	1.33	-	1849
31	1-Butyl 2-isobutyl phthalate	-	0.56	1968
32	Diisobutyl phthalate	2.20	0.92	1877
33	Dibutyl phthalate	2.74	-	1972
34	Palmitic acid	3.28	2.55	1980
35	Phytol	9.57	16.63	2118
36	Diisooctyl adipate	1.23	-	2400
Total		89.70	86.04	

[a]Compounds extracted with hexane by the SDE method; [b]Compounds extracted with hexane by the percolation method
[c]RI: Relative retention indices to C8–C24 n-alkanes on HP-5 MS column

Table 2. Chemical composition of the hexane extract of the volatile oils of the leaves of *Z .jujuba* extracted by the SDE and Percolation methods

No	Components	SDE method [a](%)	Percolation method [b](%)	RI[c]
1	Octane	9.10	-	≤ 800
2	(E)-2-Hexenal	11.26	-	852
3	6-Methyl-5-hepten-2-one	1.80	-	991
4	cis-Linalool oxide	1.04	-	1092
5	Terpinolene	2.26	-	1104
6	(3E)-6-Methyl-3,5-heptadien-2-one	0.61	-	1110
7	Fenchol	0.95		1197
8	Eugenol	9.58	-	1367
9	Cyclohexane	5.00	-	1396
10	Dihydropseudoionone	1.67	-	1456
11	trans-β-Ionone	5.55	-	1493
12	α-Farnesene	6.13	-	1511
13	δ-Cadinene	6.31	-	1528
14	Dihydroactinolide	1.27	2.08	1540
15	E-Nerolidol	2.85	-	1569
16	α-Pinene	-	7.90	1841
17	Z-11-Tetradecenoic acid	-	1.47	1848
18	1-Butyl 2-isobutyl phthalate	0.56	-	1968
19	Diisobutyl phthalate	0.92	-	1877
20	Dibutyl phthalate	-	3.63	1972
21	Palmitic acid	2.55	18.42	1980
22	Phytol	16.63	31.70	2118
23	Linoleic acid	-	13.54	2154
24	Diisooctyl adipate	-	8.03	2400
25	Pentacosane	-	3.68	2495
Total		86.04	90.45	

[a]Compounds extracted with hexane by the SDE method; [b]Compounds extracted with hexane by the percolation method; [c]RI: Relative retention indices to C8–C24 n-alkanes on HP-5 MS column

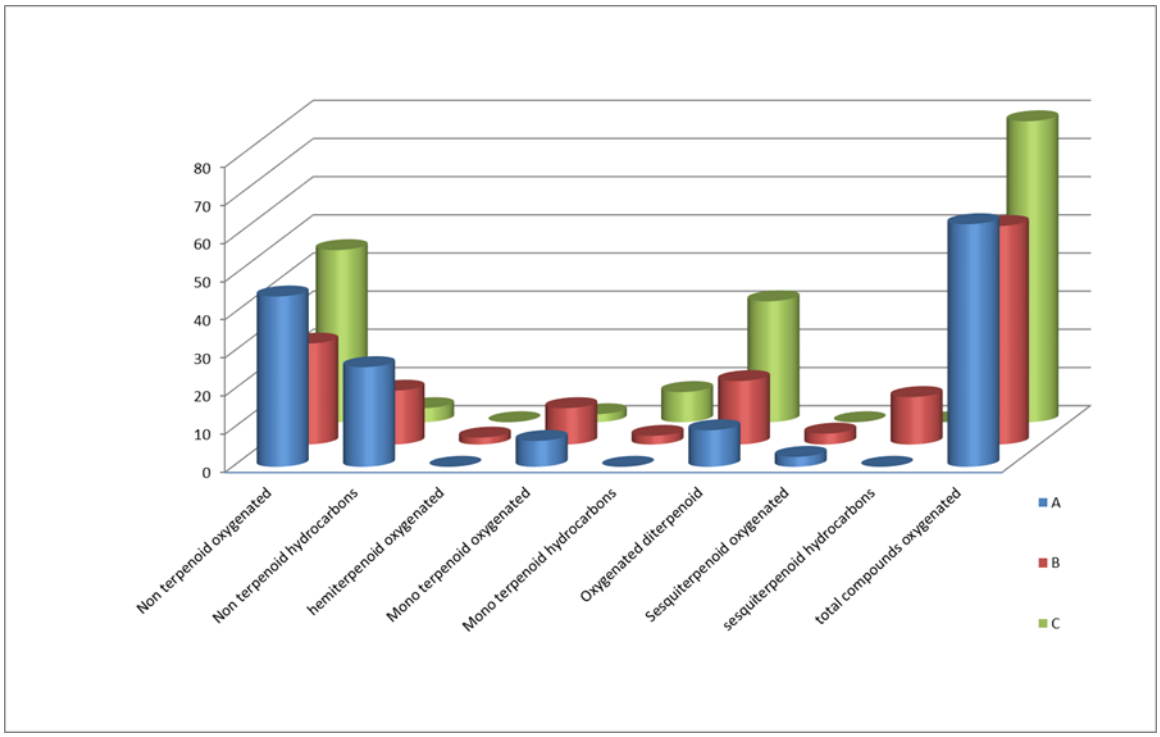

Fig. 1. The category of the compounds extracted from the volatile oils of the leaves of *Z .jujuba* by the SDE and Percolation methods

A: Compounds extracted with Ethyl acetate by SDE method

B: Compounds extracted with Hexane by SDE method

C: Volatile compounds extracted with Hexane by percolation method

Table 3. Comparison of the compounds extracted from the volatile oils from the leaves of *Z .jujuba* by the SDE and percolation methods using three measured parameters

Parameter measured	A	B	C
Average molecular weight	203.15	188.61	265.94
Average solubility in water (In 25 ºC , ppm)	2414.01	882.22	68.63
Average boiling point (In 760 mm Hg)	260.86	237.23	284.11

A: Compounds extracted with ethyl acetate by SDE method;
B: Compounds extracted with hexane by SDE method;
C: Volatile compounds extracted with hexane by percolation method.

The mean of the boiling points of the extracted compounds for the ethyl acetate was 260.86°C and the mean of the boiling points of the extracted compounds for the hexane solvent was 237.23°C.

The results showed that the boiling point and solubility of the extracted molecules in water have a direct relationship with the dipole moment of the extraction solvent. It can be concluded that the ethyl acetate solvent in comparison with the hexane solvent, has extracted compounds with higher dipole moment values. This result is compatible with the existing difference between the numbers of extracted oxygenated compounds (Figure 1).

The second segment of this study investigated the extraction methods by which the volatile components were extracted by the hexane solvent using the two methods of SDE and percolation and then, the results of these methods were compared to each other.

According to Table 3, the mean solubility of the extracted compounds in water (in 20°C) using the percolation method was 68.63 ppm; while this value for the SDE method was 882.22 ppm. Also, the mean molecular weight of the compounds extracted by the percolation method was 265.94 u and for the SDE method it was 188.61 u. These results clearly confirm the ability of the SDE method in separating compounds with higher dipole moments and lesser molecular weight compared to the percolation method.

CONCLUSIONS

Due to the fact that volatile oils are used in different industries, finding the best extraction methods in order to improve their quality and also, to obtain the most suitable chemical compounds for any particular application is crucial. According to the changes in quality and quantity of the volatile oils that can be observed in tables 1-3 and figure 1, it can be concluded that the SDE method can separate compounds with higher dipole moment but lesser molecular weight in comparison with the percolation method. Also as it was expected, these results reveal this fact that the compounds separated by the ethyl acetate solvent are more polar than the compounds separated by the hexane solvent.

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СРАВНЯВАНЕ НА КОМПОНЕНТИТЕ НА ЛЕТЛИВИ МАСЛА ОТ ЛИСТАТА НА *Ziziphus jujuba* ЧРЕЗ ПРОМЯНА НА РАЗТВОРИТЕЛЯ И МЕТОДА НА РАЗДЕЛЯНЕ

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(Резюме)

Количествата и качествата на компонентите на летливите масла силно зависят от условията на екстракция. В настоящата работа ние изследвахме летливите компоненти в листата на *Ziziphus jujuba* в периода на цъфтеж, променяйки екстрагента и метода на екстракция. Най-напред, ние екстрахирахме летливите компоненти чрез промяна на разтворителя чрез едновременна дестилация и екстракция (SDE); във втората част, използвайки хексан като разтворител по два метода (SDE и перколация), ние изследвахме ефекта на метода на екстракция върху количеството и качеството на екстрахираните съединения. Екстрахираните масла бяха разделени и идентифицирани чрез GC/MS и GC/FID. Най-голяма разлика се наблюдава, когато методът на екстракция бе променен по начин, при който броят и типът на съединенията драстично се променя. Също така средната молекулна маса и средната разтворимост на компонентите на екстрахираните летливи масла са различни.