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Separation, Identification and Determination of Volatile Compounds of *Ziziphora persica Bunge* Using HS-SPME/GC-MS

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***Abstract*—The aerial parts of the plant *Ziziphora persica* were collected on May 2007 from Babaaman (North Khorassan Province of Iran). The chemical compounds of the plant were isolated by hydro distillation (HD) and solid phase microextraction (HS-SPME) fiber. A total of 22 constituents, representing more than 98% of the oil were identified by gas chromatograph/mass spectrometry (GC/MS). The most presented compounds of the essential oil and HS-SPME (in the optimum conditions) analysis for aerial parts of *Z. persica* were Pulegone, menthone, 1,8- cineole, beta-pinene , p-myrcene, alpha-pinene, camphene, sabinene and gama-terpinene . In the most cases it was found a highly corrolation for presented compounds that, isolated by HS or HS-SPME methods.**

***Index Terms*—Fiber, GC/MS, HD, Pulegone , SPME, , *Z.Persica*,**

I. Introduction

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries.[1-4] *Ziziphora persica* is an edible medicinal plant, which is widely distributed in Iran, and leaves, flowers and stems are frequently used as wild vegetable or additive in foods to offer aroma and flavor. In Iranian folk medicine, *Ziziphora* species has been also used as infusions for various purposes such as sedative, stomachic and carminative among others. The genus *Ziziphora* L. belongs to the Lamiaceae family consists of four species (*Z. clinopodioides*, *Z. capitata*, *Z. persica* and *Z. tenuior*) that widespread all over Iran.[5] *Ziziphora* with the common Persian name "*kakuti-e kuhi*" comprised nine subspecies native to Iran. The composition and antibacterial and antioxidant activity of the essential oil and various extracts of *Ziziphora* were reported.[6]

Solid-phase microextraction was first introduced by Arthur and Pawliszyn.[7] It is a simple, rapid, solventless sample-pre-treatment technique that can automatically perform sampling, clean-up, concentration, derivatization and introduction to the chromatograph.[8] The major constituents of *Z. s*peices have been reported are pulegone, isomenthone, limonene, 1,8-cineol and piperitenone.[6] Here, it is reports the composition of the essential oil and volatile compounds of *Z. persica Bung*e from Iran using HD and HDHS-SPME.

II. Experimental

A. Plant Material and Hydrodistillation

Aerial parts of wild growing *Zizphora persica* were collected from Babaaman of Bojnourd (Northern Khorassan Province of Iran) on May 2007. It is air dried in a shadow place. The dried plant was powdered and the essential of it was isolated by hydrodistillation for 4h. The oil was dried over anhydrous sodium sulfate and stored at 4 ^{0}C.

B. HS-SPME procedure

A manual SPME holder and three different fibers, polydimethylsiloxane (PDMS), divinylbenzene/carboxen/polydimethylsiloxane(DVB/CAR/PDMS), and fiber carbowax/polyethylene glycol (CW/PEG) from Supelco (Bellefonte, USA), were used for the SPME procedure. All the fibers were conditioned prior to use by insertion into the GC injector at 250 ^{0}C for 0.5 h for PDMS, at 270 ◦C for 1 h for DVB/CAR/PDMS , and 240 ^{0}C for 0.5 h for CW/PEG . The powdered samples were placed in 10 ml sample vials sealed with septum-type caps from Supelco (Bellefonte, USA). For each extraction, after the SPME needle pierces the septum, the fiber was extended through the needle and exposed to the headspace above a sample 0.05g under a temperature (20-55 ^{0}C). After an extraction time (5-30 min), the fiber was with drawn into the needle, and then the needle was removed from the septum and inserted directly onto the injection port of the GC. The desorption of analytes from the fiber coating was performed by heating the fiber in the injection port at 250 ^{0}C for 2-10 min. At last the analytes were transferred directly onto the chromatographic column for analysis.

C. Essential Oil and HS-SPME Analysis

The essential oil and volatile compounds in the head space of the sample vial were analyzed by GC/MS. Determination of the oil percent in the plant obtained (0.84 w/w%). Identification of components in the oil was based on (RI), NIST computer library and literature survey. The relative percentage of the oil constituents was calculated

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from GC peak areas. All of HS-SPME experiments were done for three times and the results are mean of them. The relative standard deviations were found about 7% for the HS-SPME experiments. The results for HD method is the average of duplicate runs.

III. Results and Discussion

A. HD Method

HD study for the chemical compositions of essential oils of *Z. persica* growing in North Korassan, Iran(Babaaman Bojnourd) , was conducted. The yield of oil is quiet low, about 0.84 w/w%. The oil is yellow with odor characteristic of plant. The hydrodistillation time for 60g of dried *Z. persica* is about 4h. The total ion chromatogram (TIC) retention time for the plant was found about 30 min. The most compositions of the oil were isolated around the first 20 min of the analysis procedure and conditions. The qualitative and quantitative analytical results are shown in Table1. A total of 22 components were identified by GC/MS, representing 98.61% of the components in the HD method.

B. HS-SPME Method

C. SPME Fibers

Three types of fibers (PDMS 100 µm, DVB/CAR/PDMS 2Cm 50/30 µm , and CW/PEG 60 µm) were used to evaluate the effect of fiber types on the extraction of volatile compounds in *Z. persica*. Fig. 1 shows the total peak areas of the obtained compounds by the three types of fibers. DVB/CAR/PDMS fiber achieved higher extraction of the analytes than the other fibers.This suggested that the retention ability of the DVB/CAR/PDMS fiber for the volatile compounds in this herbal plant is much stronger than the rest two fibers. As shown in Fig.1, the fibers with a mediumpolar coating appeared to be more efficient for the extraction of *Z.persica* compounds. It probably resulted from the fact that most of the analytes in the sample are of medium polarity. The polarity of the two fibers were supposed to be in the order of PDMS< DVB–CAR–PDMS. In this case, the fiber DVB–CAR–PDMS has higher extraction ability than the others. On the basis of the above results, the DVB–CAR–PDMS fiber was selected for the extraction of the volatile compounds in this medicinal plant.

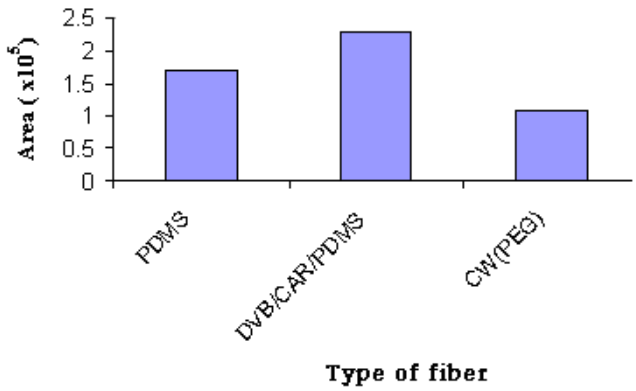

Fig.1. Effect of type of fiber on the total peak areas of all the obtained compounds from Z.persica.

D. Extraction Temperature

The extraction temperature had a significant influence on the extraction because it can influence the distribution coefficients of the compounds between the sample and the headspace and between the headspace and the fiber. The extraction temperature was varied from 20 to 55 ^{0}C. It was found that the total peak areas increased steadily with the temperature. The results of extraction temperature effect on the total peak area of all the obtained compounds shown in Fig. 2. From these results, the temperature of 50 ◦C was finally used for the present work.

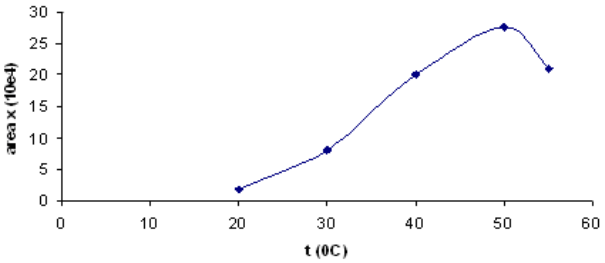

Fig.2. The results of temperature optimization for HS-SPME analysis of Z.persica.

E. NaCl Amount

In general, the addition of salts led to an increase of the extraction yield because of the salting-out effect . Salting out increased the ionic strength of the aqueous solution and, in this way, could decrease the solubility of organic analytes; thus, partitioning the volatile flavor compounds from the aqueous solution to the headspace and the fiber coating was improved . The effect of salt amount depends on type of analyte. In this study, there was found no effect of NaCl on the results of HS-SPME for *Z. Persica* analysis (data not shown). Therefore distilled water was used for the rest of extractions without addition of NaCl to the sample solution

F. Extraction Time

The extraction time varying from 5 to 30 min was investigated and the results are shown in Fig. 3. Fig.3, shows the effect of extraction time on the total peak areas of all the obtained compounds from *Z. persica*. The profile for the total peak area shows a highest total peak area at 10 min. So, the extraction time of 10 min was finally selected for the analysis.

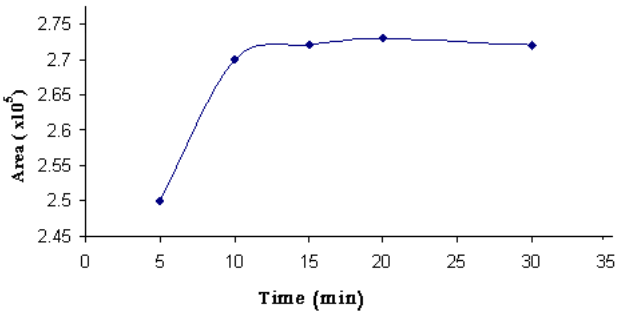

Fig. 3. The result of adsorption time for HS-SPME analysis of *Z.persica*.

G. *Desorption Time*

Desorption time in the injection port was investigated in the range of 2–10 min while keeping the fiber at the same injection. The profile for the total peak area shows an almost complete desorption of the compounds at 4 min. The effects of desorption time on the total peak area and individual peak areas are shown in Fig. 4. The profile for the total peak area shows an almost complete desorption of the compounds at 4 min. Taking into consideration that a longer desorption time may hurt the fiber lifetime, the desorption time of 4 min was employed for the purpose.

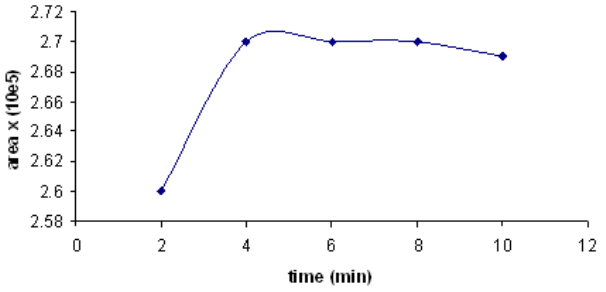

Fig. 4. The result of desorption time for HS-SPME analysis of Z.persica.

The most presented compounds of the essential oil and HS-SPME analysis in aerial parts of Z. persica were Pulegone ، menthone ،1,8- cineole ،beta-pinene , p-myrcene, alpha-pinene, camphene, sabinene and gama-terpinene (see Table 1).

According to the litrature studies major component of the plant is pulegone . The changes in the essential oil components for the same plant in different places might have arisen from several differences (like as climate, seasonal , geographical), as mentioned in the literature. Fig.5, shows there is no any major differences between HD and HS-SPME methods for the results. So, the peresent rapid and easy method can be used for the analysis of *Z. persica* plant.

TABLE 1. VARIATION OF ESSENTIAL OIL COMPOSITION OF *Z. PERSICA*, COLLECTED FROM NORTH KORASSAN (BOJNOURD), IRAN AND ANALYZED BY HS-SPME/GC/MS AND HYDRODISTILLATION/GC/MS.

Compound	RI[a]	% with SPME	% with HD
Alpha-pinene*	933	0.12	0.10
Camphene	955	0.15	0.08
Sabinene	974	0.10	0.14
Beta-pinene	986	4.3	3.88
p-myrecene	990	0.52	0.58
Beta-cymene	1022	0.43	0.55
1,8-cineol	1033	13.2	11.67
Trans-ocimene	1060	0.14	0.12
Gama-terpinene	1060	0.70	0.61
Isomenthol	1110	0.27	0.11
Linalool	1133	0.07	0.02
Isolimonene	1154	0.31	0.22
Isomenthone	1200	0.06	-
(+)- pulegone	1222	77.3	78.14
Cyclohexanol	1280	0.07	-
Menthone	1296	1.1	1.32
Menthol acetate	1270	0.12	0.09
Thymol	1294	0.17	0.23
Piperitone	1317	0.11	0.10
Rosefuran	1348	-	0.18
Piperitenone	1368	0.11	0.05
Germacrene**	1478	0.18	0.42
Total		99.71	98.61

[a]Retention Index
*Composition with the lowest retention time
**Composition with the highest retention time

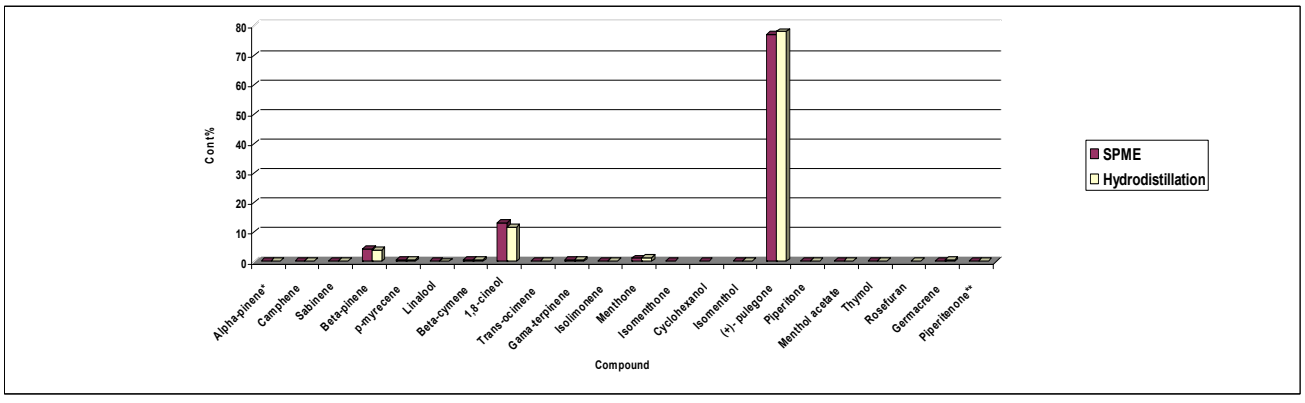

Fig. 5. The results of HS-SPME and HD-GC/MS analysis of Z.persica.

IV. CONCLUSSION

In the present work, analysis of volatile compounds of *Z. persica* using HD and HS- SPME was successfully performed. The results show (Fig.5) that isolation, extraction and concentration of essential oil in *Z. persica* can be done by HD–SPME. Twenty-two compounds were identified in the herbal plant using the proposed method. The experimental results demonstrate that HS-SPME is a simple, rapid and solvent-free method for determination of essential oils in *Z. persica*.

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