GC/MS ANALYSIS OF VOLATILE ORGANIC COMPOUNDS OF *SATUREJA BACHTIARICA BUNG* USING US-HS-SPME AND NANO SCALE INJECTION TECHNIQUES

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The gas chromatography–mass spectrometry (GC–MS) and   Ultrasonic assist with headspace solid phase microextraction (US-HS-SPME) were developed for the analysis of essential oil compounds in dry *Satureja bachtiarica bung* aerial parts. The essential oil extracted from the aerial parts of the plant was analyzed by nano scale injection to a GC/MS system. As a result, 21 constituents, representing   99.68 % of the oil, were identified.   The highest extraction efficiency was achieved with a 100 μm polydimethylsiloxane (PDMS) fiber. Different experimental parameters such as the type of coating used for the fibers, sonication time, extraction time, temperature, and desorption time were optimized.   The major components by this method are as   carvacrol (64.31٪),cis-jasmone (15.19   ٪), p-cymene(3.19, β-bisabolene(5.31٪) and geranyl acetone(5.00٪) Compared to conventional technique, HD the advantages of the proposed method are: short extraction time and high extraction efficiency. All experimental results show that the proposed method is an alternative tool for fast analysis of essential oils in dry aerial parts of *Satureja bachtiarica bung*   for the first time

1. Introduction

The genus Satureja belongs to the family Lamiaceae , subfamily Nepetoideae and the tribe Mentheae . One of the diagnostic characteristics of the subfamily Nepetoideae is that its representatives contain more than 0.5% of essential oil [1-2]. It has been reported that there are marked differences between and within the subspecies of Satureja essential oil composition [3]. During recent years, antiviral [4], antinociceptive and antiinflammatory [5], antibacterial and antifungal [6-8], antispasmodic and antidiarrhea [9] and vasodilatory [10] effects have been reported for different species of Satureja growing in different parts of world. Satureja (Labiatae) species are present in mountainous areas in Iran, mainly in western and northern parts. S. intermedia C. A. Mey is a rare species endemic of Iran. S. mutica Fisch & C. A. Mey is a highly aromatic species growing on calcareous rocks in northeastern Iran. S. macrantha C. A. Mey. is a small shrub distributed in northwestern and western parts of Iran [11]. There are two important famous species of Satureja used as culinary herbs: Satureja hortensis L. and Satureja montana L. The main constituents of the essential oil of S. hortensis and S. montana are the phenols, carvacrol and thymol, as well as p-cymene, β-caryophyllene, linalool and other terpenoids [12]. Due to these various usages of Satureja species or their oils, we were interested in studying essential oil contents and compositions of Satureja species in Iran. The essential oil compositions of four satureja species of Iran, S. khuzistanica Jamzad [13], S. bachtiarica Bunge [14], S. spicigera (C. Koch) Boiss. [15] and S. sahandica Bornm. [16] were reported previously. The major components of S. khuzistanica were p-cymene (39.6%) and carvacrol (29.6%), while those of S. bachtiarica were thymol (44.5%) and γ-terpinene (23.9%). The main constituents of the essential oil of S.

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spicigera were thymol (35.1%), p-cymene (22.1%) and γ-terpinene (13.7%). The main constituents of the essential oils of eight populations of S. sahandica were thymol (19.6%–41.7%), p-cymene (32.5%–54.9%) and γ-terpinene (1.0%–12.8%). A wide variety of analytical methods is used to extract the volatile compounds from plant material. Techniques commonly used to extract the essential oils include steam distillation, hydrodistillation, dynamic and static headspace, supercriticalfluid extraction and solvent extraction [17-20].In 1990, Arthur and Pawliszyn [21] introduced a completely solvent-less method, which was termed solid-phase microextraction (SPME), in which a fused silica fiber coated with a stationary phase is exposed to the sample or its headspace and the target analytes partition from the sample matrix to the fiber coating [22]. After extracting for a set period of time, the fiber is transferred to the heated injection port of a GC (or GC–MS) for analysis. The method has been applied widely in recent years to the determination of the volatile chemical components of plants and flowers [23-25]. Solid-phase microextraction (SPME) has received growing interest from many areas. SPME integrates sampling, extraction, concentration and sample introduction into a single uninterrupted process, resulting in high sample throughput. This process has important advantages over conventional extraction techniques due to its ease of use, being rather rapid, portable and solvent-free. In the last years, interest towards the technique has been increased, and a great number of applications have been developed [26-29].

Literature searches indicated that the oil of *S. bachtiarica*, have not been the subject of the Ultrasonic assist with headspace solid phase microextraction (UA-HS-SPME in previous studies.

2. Experimental

2.1 Chemicals and reagents

Helium, 99.999%, used as carrier gas, was purchased from Roham Gas Company (Tehran, Iran). The alkane mixture consisting of the C_8-C_{26} alkanes (concentration of 40 mg/mL in hexane) was purchased from Fluka. All other chemicals were of the highest purity available from Merck or Fluka. Doubly distilled deionized water was used.

2.2 Instruments and GC/MS operating conditions and procedure

In the work, for the first time, Satureja bachterica bung was collected fron around Iylam area and identified by Prof. Dr. N. Akbari of Agriculture faculty, Lorestan University, Khoramabad south-west of Iran, at an altitude of 1100 m in june 2009. The aerial parts of the plant were dried at room temperature by spreading them on clean aluminum foil in the laboratory. 10-g portions of air-dried sample were ground to a coarse powder using a household coffee grinder. The ground samples were stored in nylon bags and placed in a refrigerator prior to analysis. Extraction of the volatiles from the plant sample using SPME fibers was achieved by placing 0.5 g of ground sample into a 40-ml vial to which 500 µl double-distilled water was added as a matrix modifier, the vial was then vigorously shaken by hand to ensure homogeneous dispersal of the spiked water. The sample vial was then placed into an ultra-sonicator and incubated for 15 min to allow the volatiles to equilibrate between the headspace and sample matrix, during which time the sample was heated to 70 ºC. The actual SPME extraction of volatile compounds was accomplished by incubation with a polydimethylsiloxane (PDMS) fiber at 70 ºC for 40 min. Ultrasonic irradiation (18 kHz, 450 W) was applied by means of a PFO100 5RS Series ultrasonicator (Italy) equipped with a water bath in which the extraction vials were placed. The samples were sonicated to create stress in the sample matrix to facilitate the release of the analytes, and control the temperature during the extraction process.

The alkane mixture (C_6-C_{28}), 40 mg/mL in hexane) was used for the calculation of retention indices (RIs). Loading the alkane mixture onto the fiber was carried out using a 5-min head space extraction from the 10-ml SPME vial including 1 ml double-distilled water spiked with 10 µl of the above-mentioned mixture.

2.3. GC–MS analysis

Gas chromatography was performed with a Shimadzu model GC-17A (Kyoto, Japan) instrument equipped with a Shimadzu Quadropole-MS (QMS) model QP5050 detector. Separation was achieved using a 30 m × 0.25 mm I.D capillary fused silica column 6 coated with a 0.25 μm film of DB5-MS (5% Phenyl-95% Polydimethyl Siloxane), and a split/splitless injector with a 1 mm internal diameter glass liner. Ten-, 20- and 40-milliliter sample vials sealed with PTFE coated silicone septa (Supelco) were used for extraction. SPME fibers with PDMS (100 μm, non-bonded) coating, were used as commercial fibers for the extraction of analytes. The fibers were handled using a manual SPME fiber holder provided by Supelco (Bellefonte, PA, USA). Analytes extracted onto the fiber were injected into the injection port of the GC system. A 1.0-nL (1.0 μL of sample diluted in 1000 ml of n-hexane, v/v) volume of sample was injected onto the column in the splitless mode [24-26]. The fiber was kept in the injection port for additional 2 min after injection to ensure the complete desorption of the compounds from the fiber. Every 10 analyses on a GC run were carried out in the presence of the fiber but without sampling to assure complete desorption. The injector was set at 220 ºC. The carrier gas was helium and flowed at a rate of 1 ml/min. The GC was operated in a splitless mode. The column temperature was initially set at 40 ºC and increased to 200 ºC at a rate of 4 ºC/min, and remain at 200 ºC for 1 min, resulting in a total GC run time of 41 min. The temperature of the ion source was kept at 220 ºC, and the transfer line temperature at 250 ºC. The mass fragments were collected in the range from m/z 40 to 450 with an acquisition rate of 1000 to provide a satisfactory number of points per peak for effective spectral resolution. The ionization energy of 70 eV and the detector voltage of 1700 V were applied to the QMS detector.

2.4. Qualitative and quantitative analyses

Most constituents were identified by gas chromatography through comparison of their retention indices (RIs) with those of the literature [30] and [31] or with those of authentic compounds available in our laboratories. The retention indices (RIs) were determined in relation to a homologous series of n-alkenes (C_6-C_{28}), under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature [32]. Component relative concentrations were calculated based on GC peak areas without using correction factors.

3. Results and discussion

In order to provide a complete peak separation of extracted compounds, some preliminary SPME-GC/MS experiments were performed using ground Satureja bachterica bung samples utilizing a PDMS fiber. From different recorded chromatograms, it become clear that the best GC program was as which mentioned previously (the column initial temperature: 40 ºC, with a rate of 4 ºC/min increased to 200 ºC, and remain at 200 ºC for 1 min). The optimal conditions were as fiber's coating type: PDMS, sonication time: 15 min, extraction time: 40 min, extraction temperature: 70 ºC, desorption time: 2 min, and water content: 500 μl per 0.5 g of ground sample. The use of a simplex optimization method was of paramount importance in order to select the best working conditions of interrelated variables.

A number of SPME fibers of different polarity and coating thickness are commercially available and have been used for extraction of the volatile compounds in medicinal plants [33-34]. Among the fibers, PDMS or PDMS-based mixed fibers are most commonly used. Among them, PDMS is a non-polar coating that has been known to perform very effectively for a wide range of mostly non-polar and semi-polar analysts. Hence, the fibers with PDMS coating were used throughout this research   the presence of a little content of water in the matrix of plant samples, as modifier, improves the release of volatile compounds and subsequently increases their concentrations in the headspace of sample [33]. Occupation of active sites on sample matrix by

water molecules helps the analysts to release from their native chemical or physical conjunctions with matrix and enter to gaseous headspace phase [34].

In order to get access to the absolute mass percentage of the identified compounds, the essential oil of *Satureja bachtiarica bung* was analyzed after extraction by hydro-distillation (HD). Our experience showed that SPME could not give the exact mass percentage of the constituents of volatile compounds in comparison with HD [14] , [28], due to the limited load capacity of micro-scale fibers especially for main components [26]. However, SPME is capable to analyze the volatiles with the least extraction time, sample amount, and sample preparation steps in addition to, significant ability of trapping and extracting of compounds which are more volatile [27-29]. The oil isolated by hydro-distillation from the aerial part of the plant was obtained in 0.67% w/w yield. The composition of the oil of the *Satureja bachtiarica bung* species is listed in Table 1.

Twenty five constituents, representing 99.68% of the total components in the oil of *Satureja bachtiarica bung* was characterized carvacrol (64.31٪),cis-jasmone (15.19 ٪), p-cymene(3.19, β-bisabolene(5.31٪) and geranyl acetone(5.00٪) .While monoterpens consisted of 5.68%, oxygenated monoterpenes consisted of 86.49%, sesquiterpenes consisted of 6.98% and oxygenated sesquiterpenes consisted of 0.78% of the oil.

Table 1: Composition of bioactive   components of volatile oil of aerial parts of Satureja bachtiarica bung using SPME method

No	Compounds	RT	RI_{std}	RI_{cal}	Area(٪)
1	α-Thujene	10.966	930	934	0.15
2	α-Pinene	11.255	939	942	0.12
3	β-Myrcene	13.431	991	1003	0.42
4	α-Terpinene	14.737	1017	1033	0.33
5	p-Cymene	15.1	1025	1041	3.19
6	γ-Terpinene	16.406	1060	1071	1.00
7	Cis-Sabinene Hydrate	17.132	1070	1088	0.28
8	Terpinolene	17.567	1089	1097	0.14
9	Linalool	18.148	1097	1112	0.53
10	4-Terpineol	21.703	1177	1202	0.77
11	Thymol methyl ether	23.734	1235	1256	0.39
12	Cis-Jasmone	24.61	1393	1280	15.19
13	Carvacrol	26.78	1299	1339	54.04
14	Eugenol	29.32	1359	1411	0.3
15	δ-Elemene	30.34	1338	1442	0.06
16	Geranyl acetone	30.7	1455	1452	5.00
17	α-Humulene	31.35	1455	1472	0.57
18	α -Curcumene	31.5	1481	1476	0.05
20	β-Selinene	31.93	1490	1489	t
21	α-Farnesene	33.17	1506	1527	0.31
22	β -Bisabolene	33.48	1506	1537	5.31
23	α-Bisabolene	34.47	1507	1568	0.62
24	Caryophyllene oxide	36.36	1583	1629	0.67
25	β-Eudesmol	36.79	1651	1643	0.11
	total				89.58
Mono terpene					5.63
Oxygenated Monoterpene					76.28
Sesquiterpene					6.92
Oxygenated Sesquiterpene					0.78
Diterepen					0.0
Oxygenated diterepen					0.0

RT: retention time**; RI**retention indeces in elution order; t=less than 0.05(٪)

4. Conclusion

The ultrasonic assist with headspace solid phase microextraction technique showed the highest extraction efficiency with a 100 μm polydimethylsiloxane (PDMS) fiber and it was found that the oxygenated sesquiterpene and oxygenated monoterpene fractions decreased but the amount of sesquiterpenes and monoterpenes increased. Thus, the experimental parameters such as extraction time, ultra sound effects can be optimized for the particular aim of SPME, to obtain a high yield of essential oil, or to obtain essential oils of differing composition.

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