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CHEMICAL CHARACTERIZATION OF VOLATILE ORGANIC COMPONENTS OF *SALVIA OFFICINALIS* USING ULTRASONIC-ASSISTED HEAD SPACE SOLID-PHASE MICROEXTRACTION AND HYDRO-DISTILLATION EXTRACTION METHODS

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ABSTRACT

The paper takes up a comparative study concerning volatile oils of Salvia officinalis. The study wascarried out in the presence of Ultrasonic assisted with headspace solid phase microextraction (UA-HS-SPME) accompanied with gas chromatography - mass spectrometry (GC/MS) as well as using hydrodistillation to analyze volatile compounds in the oils Salvia officinalis. The highest extraction efficiency was achieved with a 100 μm polydimethylsiloxane (PDMS) fiber. Different experimental parameters such as fiber's coating type, sonication time, extraction time and temperature, and desorption time were investigated. As a result, 14 compounds with the HD and UA-HS-SPME-GC/MS method were investigated. Comparison of the UA -HS- SPME and the commonly used distillation method (HD) showed that the UA -HS- SPME method is simpler and requires much less sample amount, shorter extraction time and lower temperature in addition to high ability of trapping and extraction of volatile and thermo-sensitive compounds. The major components used by HD and UA -HS- SPME methods are as follow: Linalool (5.31 %, 7.44 %) Butyl benzoate (7.82 %, 5.42 %), n-Hexyl benzoate (29.17 %, 40.21 %) and Benzyl benzoate (42.92 %, 24.14 %), respectively.

Keywords: *ultrasonic, microextraction, hydro-distillation, Salvia officinalis.*

INTRODUCTION

Fifty-eight species of the genus Salvia (Lamiaceae) are found in Iran, seventeen of which are endemic [1-2]. Several species of Salvia have been reported to exhibit antibacterial, estrogenic, antioxidant and anti- tumor activities [3-6] and are used in the treatment of Eczema, psoriasis and tuberculosis [7]. In contrast to the oils of many *Salvia* species [8-13], a literature search did not reveal any references to previous work on the oil of this plant [8-19]. Solid-phase microextraction (SPME) uses a fine rod (fused silica or metal) with a polymeric coating to extract organic compounds from their matrix and directly transfer them into the injector of a gas chromatograph for thermal desorption and analysis. It is a growing sample preparation technique and an attractive alternative to classical extraction methods that reduces solvent usage and exposure, disposal costs and extraction time for sample separation and concentration purposes. SPME has attracted increasing attention in the field of environmental and food analysis for its simplicity, efficiency, good precision, low detection limit, and ease of automation [20]. It has been used for many applications, including the

determination of substituted benzene compounds [21], caffeine in beverages [22], volatile organic compounds in water [23], polyaromatic hydrocarbons [24], polychlorinated biphenyls [25], chlorinated hydrocarbons [26], phenols [27], pesticides [28], fatty acids [29], nitrosamines in sausages [30], volatile and semi-volatile sulphur compounds in beer and Cheddar cheese [31] , as well as volatile compounds in rice samples [32]. Ultrasound is one of the present energies for accelerating of the extraction processes and an attractive alternative for stirring and shaking in different extraction modes [33]. It is well known that the ultrasonic waves cause the formation of tiny bubbles in the matrix subjected to fast adiabatic compressions and expansions, named cavitation. The cavitation gives rise to high temperatures and pressures within the tiny 5 bubbles with minimal effect on the temperature of the overall system [34]. Cavitation favours penetration and transport at the interface between a fluid phase and a solid matrix, subjected to ultrasound energy and cavitation. On the other hand, the rigidity of the solid sample matrices limits the shaking or stirring of them in general extraction methods, especially with fragile fibers, in SPME extractions. Thus, this research was aimed at the development of an approach for ultrasonic assisted headspace solid-phase microextraction (UA-HS-SPME) in combination with gas chromatography–mass spectrometry (GC/MS) for the analysis of volatile compounds in dry *Salvia officinalis* medicinal plant. To the best of our knowledge, no related publications are available for the purpose.

EXPERIMENTAL

Chemicals and Reagents

Helium, 99.999 %, used as carrier gas, was purchased from Roham Gas Company (Tehran, Iran). Alkane mixture consisting of the C_8-C_{20} 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

Instruments and GC/MS Operating Conditions and procedure

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 performed using a 30 m X 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, twenty and fourthly 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 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 in the injection port of the GC system using 9 mm silicon septa. The fiber was kept in the injection port for additional 2 min after injection for a complete desorption of the compounds from the fiber. Every 10 analyses a GC run was done with the fiber without sampling to assure complete desorption. The injector was set at 220°C. The carrier gas was helium at a flow rate of 1ml/min. GC was operated in a splitless mode. The column temperature was initially set at 40°C and increased to 200°C at a rate of 4°C/min, and remained at 200°C for 1 min, resulting in a total GC run time of 41 min. The ion source temperature was kept at 220°C, and the transfer line temperature at 250°C. The mass fragments were collected in the range from m/z 35 to 450 with an acquisition rate of 1000 to provide satisfactory number of points per peak for the effective spectral resolution. Ionization energy of 70 Ev and the detector voltage of 1700 V were applied for the MS detector. 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 was placed. Sonication was applied to create stress in the sample matrix for better releasing of the analytes, and to control of the temperature during the extraction process.

Conventional Clevenger or Hydro-distillation apparatus and procedure

100 g aerial parts of the plant *Salvia officinalis* were submitted to hydro-distillation with a Clevenger-type apparatus according to the European Pharmacopoeia, and extracted with 2 l of water for 2 h (until no more essential oil was obtained) to produce oil in 0.67 % w/w yield. The essential oil was collected, dried un-

der anhydrous sodium sulphate and stored at 4°C until used. Extractions were performed at least three times, and the mean values were reported to produce oil in 0.67 % w/w yield

Ultrasonic-Assisted Head Space Solid-Phase Microextraction

Salvia officinalis was collected from around Drood area (south west of Iran) and identified by Prof. Dr. N. Akbari of Agriculture faculty, Lorestan University, Khoramabad south-west of Iran, at an altitude of 1270 m in June 2008. The aerial parts of the plant were dried in room temperature by spreading them on a clean aluminum foil in the laboratory. 10 g portions of air-dried sample were subjected to a household coffee grinder in order to turn them into a coarse powder. The ground samples were stored in nylon bags and placed in a refrigerator until the time when the analysis became possible. For extraction of volatiles of the plant sample using SPME fibers, 0.5 g of ground sample was placed in a 40 ml vial, 500 µl double-distilled water, as matrix modifier, was added and the vial was vigorously shaken toughly by hand for homogeneous dispersing of the spiked water. The sample vial was then placed into an ultra-sonicator and a 15 min incubation time was applied for equilibration of volatiles between the headspace and sample matrix, during which the sample was heated to 70°C. The actual SPME extraction of volatile compounds was accomplished using a 40 min period at 70°C with a PDMS fiber. Alkane mixture (C_8-C_{26}), 40 mg/mL in hexane) was used for calculation of retention indices (RIs). Loading of the alkane mixture on fiber was carried out by 5 min head space extraction from a 10 ml SPME vial including 1 ml double-distilled water spiked with 10 µl of the above-mentioned mixture.

Qualitative and quantitative analyses

Most constituents were identified by gas chromatography through comparison of their retention indices (RIs) with those from the literature [39,40] or with those of authentic compounds available in our laboratories. The retention indices (RIs) were determined in relation to a homologous series of n-alkanes (C_8–C_{24}) 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 the literature [40,41]. Component relative concentrations were calculated based on GC peak areas without using correction factors.

RESULTS AND DISCUSSION

In order to provide a complete peak separation of extracted compounds, some preliminary SPME-GC/MS experiments were performed using ground *Salvia officinalis* samples and a PDMS fiber. From different recorded chromatograms, it became clear that the best GC program was the one mentioned previously (column initial temperature: 40°C, with a rate of 4°C/min increased to 200°C, and keeping at 200°C for 1 min). The experimental parameters such as fiber's coating type, sonication time, extraction time and temperature, desorption time, and water content of sample were optimized using previously mentioned experience [35] of our research group on comparison of HS-SPME and HS-SDME for chemical characteristics of *Satureja Khuzestanica* and *Satureja Rechingeri,* performed in Lorestan University. The optimization of the parameters was accomplished by using the simplex method. The optimal conditions were as fiber's coating type: PDMS, sonication time 15 min, extraction time 40 min, extraction temperature 70°C, 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 for the 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 [36,37]. 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 analytes. Hence, the fibers with PDMS coating were used throughout this research. The presence of a small amount 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 the sample [33]. Occupation of the active sites on the sample matrix by water molecules helps the analytes to be released from their native chemical or physical conjunctions with matrix

and enter gaseous the headspace phase [32]. In order to get access to the absolute mass percentage of the identified compounds, the essential oil of *Salvia officinalis* 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, due to the limited load capacity of micro-scale fibers especially for main components [34]. 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 [32]. The oil isolated by hydro-distillation from the aerial part of the plant was obtained in 0.89 % w/w yield. The composition of the oil of the *Salvia* species is listed in Table 1, in which the percentage and retention indices of components are given.

Fourteen constituents, representing 94.9 % of the total components in the oil of *Salvia officinalis*, were characterized: Linalool (5.31 %, 7.44 %), Butyl benzoate (7.82 %, 5.42 %), n-Hexyl benzoate (29.17 %, 40.21 %) and Benzyl benzoate (42.92 %, 24.14 %) respectively as the main compounds. Monoterpenes and sesquiterpenes are shown in Table 2 and Fig. 1.

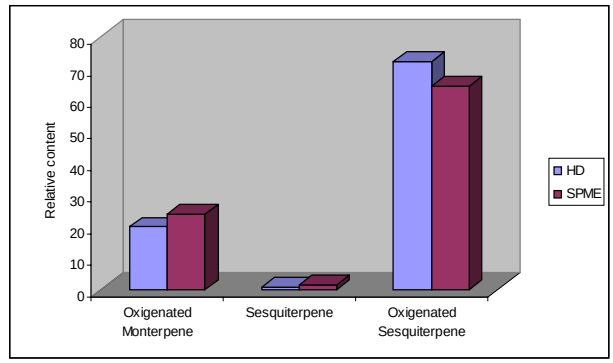

Fig. 1. Class of extracted compounds by HD (1) and by UA-HS-SPME (2) methods.

Previous investigation on oils of the *Salvia* genus showed the oils of S. sahendica, S. lereifolia and S. multicaulis were also dominated by monoterpenes. On

Table 1. Composition of the essential oil of *Salvia officinalis* by UA-HS-SPME RT: Retention Time, RI: Kovats Constant.

No.	Compound	RI_{cal}	Relative content (%) HD	SPME
1	Linalool	1107	5.31	7.44
2	Hexyl isobutyrate	1147	0.70	1.55
3	n-Heptyl propionate	1203	0.17	0.54
4	Octyl acetate	1210	0.54	0.70
5	Hexyl-2-methylbutyrate	1236	1.60	1.89
6	Hexyl Isovalerate	1243	2.42	4.20
7	Butyl benzoate	1393	7.82	5.47
8	Benzyl isovalerate	1406	0.50	0.63
9	Amyl benzoate	1458	0.66	1.42
10	Germacrene D	1496	0.27	0.70
11	(Z,E)-α-Farnesene	1504	0.49	0.90
12	n-Hexyl benzoate	1610	29.17	40.21
13	n-Heptadecane	1699	0.23	3.21
14	Benzyl benzoate	1777	42.92	24.14

Table 2. Comparison of Compounds extracted by UA-HS-SPME and HD methods.

Terpene	Relative content (%) HD	SPME
Oxygenated Monterpene	19.72	23.84
Sesquiterpene	0.76	1.60
Oxygenated Sesquiterpene	72.09	64.35
Others	0.23	3.21

the other hand, the oils of S. aethiopis and S. hypoleuca contain mainly sesquiterpenes [15-18]. The variations in chemical composition could be due to the nature of the soil, the amount of sunlight, temperature variations and the occurrence of chemotypes [38].

CONCLUSIONS

In conclusion the proposed HS-SPME-GC/MS method provided an effective combination to perform asa rapid analytical method for the direct profiling of the medicinal plants' volatile compounds without manipulation of samples. A large number of compounds can be identified in the headspace above various plant samples using this system. The collected volatile profiles can be eventually used for classification of plants for medicinal, agricultural, or marketing proposes. Although, SPME is a powerful method for chemical screening of many multi-component samples, such as foods and plants, due to limited capacity of fiber coating, a simultaneously exhaustive analysis such as HD, is needed to acquire the absolute weight percentage of constituents. In the present study, we could identify 14 compounds by both methods.

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