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Short Communication

Single-Drop Microextraction Using Two Fast Methods: Microwave and Heat for the Determination of the Volatile Compounds from *Grammosciadium platycarpum* Boiss and Hausskn

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Abstract

Background and Aim: Single drop micro-extraction (SDME, solvent phase micro-extraction, liquid–liquid micro extraction, etc.) is less well-known and is based on a traditional liquid–liquid extraction (LLE) technique, but utilizes only a few microliters of organic solvent as the extracting phase.

Materials and Methods: A gas chromatography–mass spectrometry (GC–MS) based approach has been proposed in the present study in order to specify the essential oil constituents in *Grammosciadium platycarpum* Boiss. This method was developed following the microwave assisted headspace single-drop micro-extraction (MH-SDME).

Results: The optimization was carried out for the MH-SDME parameters that included the microwave power, the sample weight, the nature of the extracting solvent, the extraction time, as well as the volume of the micro-drop.

Conclusion: Functioning of the approach proposed here was compared with the traditional hydrodistillation (HD) and single-drop micro-extraction (SDME). In order to identify the volatile compounds in *G. platycarpum*, the MH -SDME and HD -SDME methods were successfully used in this study. The use of these methods led to the identification of 31 compounds in *G. platycarpum*. In comparison with HD and HD -SDME approaches, MH-HS-SDME is a basic, quick and economical approach for analyzing the essential oils in *G. platycarpum*.

Keywords: Single-drop microextraction, *Grammosciadium platycarpum* Boiss, Headspace, Microwave

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Introduction

Grammosciadium platycarpum Boiss & Hausskn (Apiaceae) is a glabrous perennial categorized as one of the three local species of the genus *Grammosciadium* DC in Iran (1). This plant is plentifully found in mountains, and particularly grows in the Sahand Mountain hills located in Iran (2). It is sold as a vegetable, which could be added to foods. It is found in local markets in spring. According to the research works carried out on *Grammosciadium platycarpum* Boiss & Hausskn, particularly on the essential oil in its aerial parts, this plant contains various terpenoid compounds. These studies indicated

the close relationships between this species and the taxonomically related species *G. scabridum* (3). According to the traditional beliefs, the use of the aerial parts of *G. platycarpum* influences the function of the kidneys, causing diuresis (4-6).

The microwave-assisted followed by headspace single-drop micro-extraction (MH-SDME) was used in the present study. MH-SDME was utilized for isolating, extracting, and concentrating the essential oil of the plant. The plant was analyzed using GC–MS. Using this developed method, the parameters of MH-SDME, microwave power, extracting solvent, the weight of the sample, extraction time, and micro-drop volume were investigated. An ordinary HD method was used for essential oil extraction from the same species for indicating the feasibility of this approach.

Materials and Methods

Reagents and Materials

G. platycarpum leaves were gathered during June, 2018, in Northern Iran. The *G. platycarpum* leaves were firstly ground in order to obtain the fine powder. The particle size was 120 meshes. Subsequently, the sample was prepared for the research. Dodecane, hexadecane, heptadecane, and n-Heptane were purchased from Merck Co. (Darmstadt, Germany). We used a microwave oven produced by the Samsung Company (Korea) to conduct this research. The maximum power delivered by the oven was 900W.

Gas Chromatography–Mass Spectrometry

The mass spectrometer used in this work was an HP 6890 N GC system jointed to an HP MSD5973 N quadruple mass spectrometer. The compositions were extracted, and then were detached using an HP-5MS capillary column (30 m 0.25 mm i.d., 0.25 µm film thicknesses). The samples were distilled and extracted using split injection. The ratio of 50:1 was used for it. The starting temperature in the column oven was 40 increasing to 160 ºC at 4 ºC/min. Then, at 10 ºC/min, it was programmed to rise to 260 ºC. 240 and 230 ºC was set as the temperature for the injection and ion source. Helium with the flow rate of 1.1 mL/min served as the carrier gas. The ionizing energy was 70 eV. The full-scan mass spectra with the scan range of 40–550 amu provided the research data. The retention indexes under the programmed temperature conditions for n-alkanes (C6–C24) and the oil on an HP-5MS column with the identical conditions were calculated for identifying the volatile oil components. The mass spectra of the separate constituents were compared with those reported in the library and the internal reference mass spectra in order to identify them. The comparison of the retention indexes with the indexes of the original compounds or the indexes reported in the literature confirmed the identification. The Kovats' retention indexes were calculated for all the components. To this end, the gas chromatograms were used by interpolating between bracketing *n*-alkanes.

Essential Oils Isolation

The room temperature was used for drying the *G. platycarpum* plant. The plant was in the shadow for 70 hours. The *G. platycarpum* (100 g) leaves, which were dried under the room temperature, were ground. Then, the hydro-distillation was applied to them for 3 hours. To this end, a Clevenger-type device suggested by British Pharmacopeia was used. In short, the plant was placed in the water in an immersion way, and it was heated until it boiled. After boiling, the essential oil and the water vapor were evaporated. As the final stage, the vapor was gathered using a condenser. The separation of the water and essential oil was carried out after cooling down the condensed material.

Identification of Essential oil Constituents

The retention indexes and mass spectra of the essential oil constituents were compared with the retention indexes and mass spectra reported in Wiley 7 N (Wiley, New York, NY, USA) computer library in order to identify these constituents. This computer library was developed using original materials. They could also be compared with the authentic compounds and approved by the comparison of their retention indexes. The Kovats' retention indexes were calculated for all the components. To this end, the gas chromatograms were used by interpolating between bracketing *n*-alkanes. The corresponding series of *n*-alkanes (C-6 to C-24) served as the standards.

Simultaneous Hydrodistillation-Static Headspace Liquid Micro Extraction-Gas Chromatography–Mass Spectrometry

The extraction process is composed of three stages as follows: aqueous sample mixture, headspace, and organic micro-drop acceptor. In the present research, a round-bottom flask (25 ml) was used for immersing the

plant (2.5 gram) in the dried and crushed state. To immerse the plant, 3 ml water was poured into the flask. Subsequently, it was warmed until it boiled by a heating mantle. The *n*-heptadecane was used as the internal standard. In order to take 3 µL of it (containing 200-ppm *n*-hexadecane), an appropriate hygienic micro-syringe was used. Then, the syringe needle was inserted into the hydrodistilling plant at the headspace through a septum. After 2 minutes, as the optimal duration, the plunger was taken out and the micro drop was put in the syringe. The needle was taken out of the headspace, and its content was added to the GC system. Finally, the relative peak areas related to the components were calculated corresponding to the internal standard (*n*-hexadecane).

MH–SDME of Essential Oil

A microwave oven was used for the purpose of heating. The highest power delivered by the oven was 900 W (model of GE614ST/GE614 W, provided by Samsung Co., Korea). The process described below was followed for the extraction of MH–SDME. 2.5 gram of the plant was placed in a round bottom flask (25 mL) having 1 mL water. When the condenser was assembled, a 10 µL micro-syringe (Hamilton, USA) was injected into the sample in the headspace. The micro-syringe contained n-heptadecane (3 µL) as the extracting solvent, and n-hexadecane acting as the internal standard (volume ratio = 200:1). The syringe plunger dejected, and a micro drop of the extracting solvent was suspended in the needle tip. Subsequently, the plant was placed in the microwave oven to be heated. Following the (2.5 min) extraction, the plunger was taken out. Then, the micro drop was placed back to the micro-syringe. Lastly, the micro drop was injected in the GC–MS injection port directly.

Results and Discussion

The volatile components were isolated, extracted, and concentrated within one stage. Selection of the solvent, the volume of the solvent, extraction duration, power of microwave, and sample mass may influence the MH-SDME extraction efficiency. Thus, these factors were also studied. The extracting solvent nature, the volume of the solvent, sample mass, and extraction time were also studied for HD-SDME method. It is necessary to select the appropriate extracting solvent. Various types of solvents with varying polarities and different boiling points were investigated for MH-SDME and HD-SDME methods. The solvents included n-dodecane, n-hexadecane, n-heptane, nitrobenzene, and n-heptadecane. Figure 1 indicates the comparison of the relative peak areas calculated for five solvents. N-heptadecane showed the minimum overlap, the highest signals, and the best extraction efficiency. Overlap of the solvent peaks with some of the oil components was observed in n-dodecane. Heptane showed high volatility property at high temperatures, and thus it was approximately disappeared. With the addition of the hexadecane to the heptadecane (1: 200 (v/v) ratio), the mixture was used as the internal standard.

Table 1: Experimental conditions used and results obtained for the SDME experiments performed in the simplex optimization procedure.

Exp. no	Sample weight (g)	Droplet volume(µl)	Extraction time (min)	Temperature (^{0}C)
1	2	2	30	70
2	2	2	30	75
3	2	3	30	70
4	2	2	35	70
5	3	2	30	70
6 (Refl.[a])	2.5	2.5	33	73
7 (Refl.)	2.75	2	34	74
8 (Refl.)	3.1	2	29	76
9 (Refl.)	2.5	2.5	26	72

[a] Reflection

Table 2: Experimental conditions used and results obtained for the MH- SDME experiments performed in the simplex optimization procedure.

Exp. no	Sample weight (g)	Droplet volume(µl)	Extraction time (min)	Microwave power(W)
1	2	2	2	180
2	2	2	2	300
3	2	3	2	180
4	2	2	3	180
5	3	2	2	180
6 (Refl.[a])	2.5	3	2.5	300
7 (Refl.)	2.8	2	3	300
8 (Refl.)	3.1	3	2	450
9(Refl.)	3	3	3	450
10(Refl.)	3	3	3	300

[a] Reflection

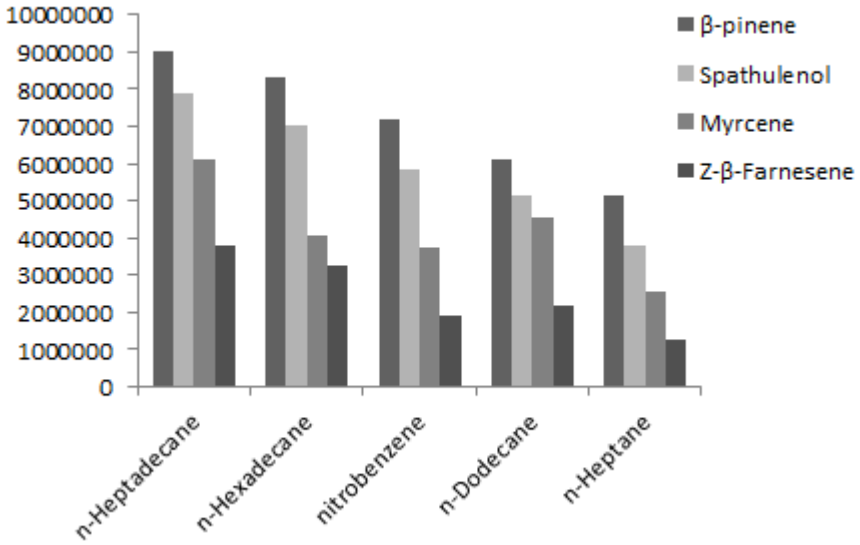

Figure 1. Effect of solvents on the extraction efficiency.

Optimization of MH-SDME and HD-SDME

The parameters that influence the efficiency of the extraction in MH-SDME and HD-SDME approaches were optimized using a simple method. When a simplex approach similar to this one is used, the experiments required for achieving the highest extraction efficacy would be essentially reduced. During the optimizing process, the relative areas of four major peaks in the GC-MS chromatogram were controlled. In the proposed approach, the designed number of experiments was (n + 1). n denotes the parameters affecting the efficiency of the extraction in SDME approach. Moreover, the worst response situations were indicated. This process was repeated until no improvement could be observed in the response. The modification was carried out in some of the reflections if necessary. Table 1, Table 2, Figure 2, and Figure 3 indicate the conditions of the primary experiments and the next experiments for MH-SDME and HD-SDME in summary.

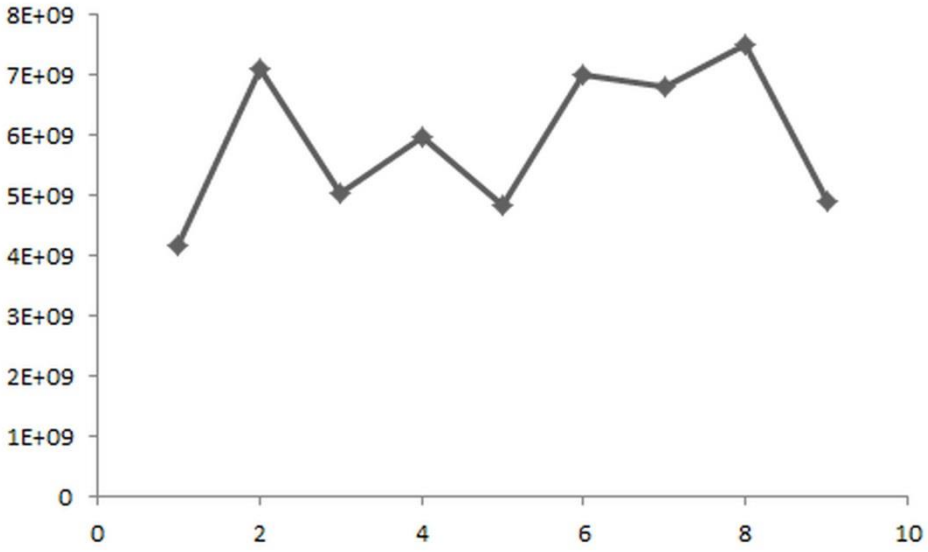

Figure 2. The response (sum area of four main peaks) for the designed experiments mentioned in Table 1.

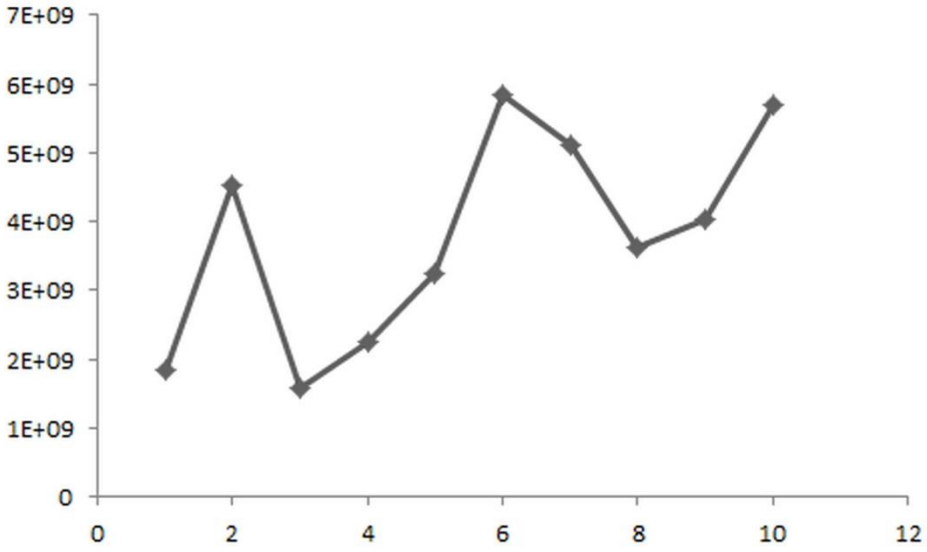

Figure 3. The response (sum area of four main peaks) for the designed experiments mentioned in Table 2.

HD-SDME and MH-SDME of G. Platycarpum

The peak areas corresponding to the total peak area for the ordinary hydro-distillation, MH-SDME, and HD-SDME approaches were calculated for obtaining the constituents of *G. platycarpum* oil and their percentages, MH-SDME, and HD-SDME approaches, which have been given in Table 3.

The extraction process, which is the same for MH-SDME and HD-SDME, involves the achievement of the volatile compounds through steaming, causing a solvent phase. In this case, the extractions should have a majority of higher volatility compounds regardless of the higher speed of MH-SDME compared with HD-SDME. In the hydro-distillation approach, the volatile oil is isolated from *G. platycarpum* within a long time (3 hours), while it was a rapid process in the MH-

Table 3: Constituents of the oil of *Grammosciadium platycarpum* Boiss.

No	Compounds	RI	(HD) Area%	SDME Area%	MH-SDME Area%
1	α-Thujene	929	0.10	0.11	0.06
2	α-Pinene	938	0.10	0.08	0.03
3	Camphene	951	0.05	0.03	0
4	Sabinene	978	0.20	0.14	0.04
5	β-pinene	980	3.15	2.84	2.25
6	Myrcene	998	1.75	1.51	1.04
7	α-Terpinene	1022	0.61	0.63	0.43
8	p-cymene	1026	1.06	1.00	0.84
9	1,8-cineole	1030	0.23	0.34	0.22
10	Z- β -Ocimene	1039	1.65	1.27	1.12
11	γ-Terpinene	1062	0.46	0.29	0.37
12	Terpinolene	1080	0.08	0.03	0
13	Cis Linalool oxide	1085	0.15	0.12	0.06
14	linalool	1102	50.34	46.86	41.51
15	α-Terpineol	1190	3.40	2.89	1.94
16	Geraniol	1255	1.48	1.27	1.21
17	Bornyl acetate	1279	0.13	0.10	0.05
18	α-Copaene	1381	0.06	0.08	0.03
19	E-caryophyllene	1426	2.74	2.55	1.87
20	Z-β-Farnesene	1440	1.12	1.17	1.10
21	α-humulene	1452	5.69	5.68	4.35
22	(9-epi-E) Caryophyllen	1463	1.31	1.12	1.14
23	γ –Gurjunene	1472	1. 22	1.08	0.76
24	Bicyclogermacrene	1494	0.06	0.05	0
25	E,E-α-Farnesene	1500	3.83	3.24	2.86
26	β-bisabolene	1513	0.63	0.58	0.41
27	γ-cadinene	1524	1.10	1.07	0.79
28	γ-Elemol	1545	1.83	1.33	0.88
29	Spathulenol	1572	2.52	2.51	1.64
30	Caryophyllene oxide	1580	0.24	0.12	0.10
31	Humulene epoxide	1607	2.81	2.44	2.14

SDME method. After isolation, the isolated volatile compounds were derived and concentrated at the same time using a suspended micro drop (total time of 2.5 minutes). In the HD -SDME method, one step was used for the completion of the isolation, extraction, and concentration (total time of 29 min minutes). Furthermore, a very low amount of the sample (3.1 g) was required in the MH -SDME method. Also, the required amount of the extraction solvent (3 µL) was low.

Conclusion

In order to identify the volatile compounds in *G. platycarpum*, the MH -SDME and HD -SDME methods were carried out successfully in the present study. The use of these methods led to the identification of 31 compounds in *G. platycarpum*. In comparison with HD and HD -SDME approaches, MH-HS-SDME is a basic, quick, and economical approach for analyzing the essential oils in *G. platycarpum*.

Acknowledgment

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

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