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Comparison of Hydrodistillation, Microwave Hydrodistillation and Solvent Free Microwave Methods in analysis of the essential oils from aerial parts of *Haplophyllum robustum* Bge. By GC/MS method.

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ABSTRACT

The genus *Haplophyllum*, which belongs to the Rutaceae family, includes 18 species in Iran. In this study, after collecting plant material, botanical identification and suitable drying, the essential oils from aerial parts of *Haplophyllum robustum* Bge. were extracted by three different isolation techniques, conventional hydrodistillation (HD), microwave-assisted hydrodistillation (MAHD) and solvent free microwave extraction (SFME) and analyzed by gas chromatography and gas chromatography coupled to mass spectrometry. The main components of three essential oils were sabinene (28.9%-31.5%), β- phellandrene (11.0%-14.6%), 1,8- cineole (7.2%-11.0%), 3,5- dimethoxy toluene (5.1%-14.2%), β- pinene (7.3%-8.9%) and terpinene-4-ol (5.8%-6.2%). Higher amounts of oxygenated monoterpenes such as 1,8-cineole, Camphor and terpinene-4-ol were present in the oil of MAHD and SFME (21.1% and 21.0%, respectively) in comparison with HD (19.0%). However, HD oil contained more monoterpene hydrocarbons such as sabinene, β- phellandrene, β- pinene (72.6 %) than SFME and MAHD extracted oils (67.9% and 62.8%, respectively). MAHD and SFME offered substantial advantages over conventional HD. A similar extraction yield was achieved at significantly shorter extraction time when using MAHD and SFME instead of HD. Also microwave irradiation significantly reduced the extraction time and did not adversely influence the composition of the essential oil. MAHD and SFME is also more environment-friendly than HD. Compared with many solvent extraction techniques, such as Soxhlet, solvent extraction, and accelerated solvent extraction, MAHD and SFME is a modern, green, and rapid approach.

Key words: *Haplophyllum robustum*, Essential oil, Hydrodistillation, Microwave-assisted hydrodistillation, Solvent free microwave extraction

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1. INTRODUCTION

Aromatic plants have been known for a very long time and the use of them in the food and perfume industry have a long history. Among the aromatic plant species, the genus *Haplophyllum* (Rutaceae) consists more than 22 species in the world. This genus is present in central and eastern areas of Asia. Among the 18 species present in Iran, 9 species are endemic. Several *Haplophyllum* species are known locally as "Sodaby" where they overlap western and south-eastern parts of Iran. Among them, *Haplophyllum robustum* Bge. which has the widest usage, is distributed in Kerman, Sistan and Khorasan Province of Iran (Mozaffarian, 1996). In the literature, there are many studies about the chemical composition and various activities of essential oil of *Haplophyllum* species (Onifade *et al.*, 2008, Javidnia *et al.*, 2009, Saglam *et al.*, 2001, Inigo *et al.*, 2002). In terms of *H. robustum* there was published two papers about its chemical constituents (Masoudi *et al.*, 2004, Rahimi-Nasrabadi *et al.*, 2009). Essential oils (EOs, also called volatile or ethereal oils) are aromatic oily liquids obtained from plant materials by physical means. The constituents of an essential oil may be classified into two principal groups: (a) hydrocarbons (terpenes, sesquiterpenes and diterpenes); (b) oxygenated compounds derived from these hydrocarbons including alcohols, aldehydes, esters, kethons, phenols, oxides, etc (Heath, 1978). Essential oils are widely used in the cosmetic industry, most especially, in the production of various cologne waters, bathing lotions, hair lotions, shampoos, and as components of disinfectants and insecticides (Boelens, 1985). Plant essential oils have been used for thousands of years for food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin, 1997, Reynolds, 1996). The essential oil of aromatic herbs is traditionally obtained by hydrodistillation. However, this technique has been controversial for subsequent determination of the oil chemical composition because of the possible transformation of aroma-active compounds by heat, steam, and pH (Jiménez-Carmona, 1999). Losses and degradation of some volatile compounds due to long extraction times, degradation of unsaturated or ester compounds through thermal or hydrolytic effects are the principal disadvantages of this extraction method (Khajeh *et al.*, 2004, Tuan *et al.*,1997). Thus, developing an alternative rapid, sensitive, safe, and energy conserving extraction technique is highly desirable. There is an increased demand for new extraction techniques, amenable to automation, with shortened extraction times and reduced organic solvent consumption, preventing pollution and reducing sample preparation costs. Recently, much attention has been given to the application of microwave dielectric heating in many various processes in the chemical and food industry in order to replace the conventional extractive methods. Extraction processes performed under the action of microwave selective heating (Chemat *et al.*, 2006). Driven by reducing energy and waste water or solvent, advances in microwave extraction have resulted in a number of techniques such as microwave-assisted solvent extraction (MASE) (Ganzler *et al.*,1986, Lettelier *et al.*, 1999) vacuum microwave hydrodistillation (VMHD), compressed air microwave distillation (CAMD) (Craveiro *et al.*, 1989), microwave hydrodiffusion and gravity (MHG) (Abert*et al.*, 2008, Bousbia *et al.*, 2009)-Vian and microwave-accelerated steam distillation (MASD) (Chemat *et al.*,2006, Sahraoui *et al.*,2008). Microwave-assisted hydrodistillation (MAHD) method also is a more recent technique used to recover volatile components (fadeh *et al.*, 2011, Wang *et al.*, 2009, Golmakani *et al.*, 2008, Rezvanpanah *et al.*, 2008, Phutdhawong *et al.*, 2007, Iriti *et al.*, 2006). In this method, plant material placed in a Clevenger apparatus is heated inside a microwave oven for a short period of time to extract the essential oil. Heat is produced by microwave energy. The sample reaches its boiling point very rapidly, leading to a very short extraction or distillation time (Ramil *et al.*, 2003, Tomaniová *et al.*,1998). The efficiency of MAHD is strongly dependent on the dielectric constant of water and the matrix (Brachet *et al.*,2002). Another extraction method in this area is solvent free microwave extraction (SFME). This technique is based on the combination of low microwave heating and distillation is performed at atmospheric pressure without

addition of water or any solvent. SFME appears to be particularly attractive for the isolation of essential oil from *Rosmarinus officinalis* L., *Origanum Glandulosum*, *Elletaria cardamomum* L. and *Cuminum cyminum* L. (Lucchesi *et al.*, 2007, Okoh *et al.*, 2010, Bendahou *et al.*, 2008, Wang *et al.*, 2006). Some of the advantages of this method over HD includes, rapidity in attaining the extraction temperature of 100˚C for the first essential oil droplet, high yield of essential oil, lower energy requirement and high purity of the oil extracted using SFME (Lucchesi *et al.*, 2004). Since the *Haplophyllum robustum* oil has been used widely as pharmaceuticals, it is necessary to find the most suitable method for the improvement of the quality of *H. robustum* oil. In this study, to our knowledge, we investigated for the first time, the chemical composition of *Haplophyllum robustum* extract and oil obtained by MAHD and SFME, respectively. The chemical composition of *H. robustum* oil obtained by hydrodistillation (HD) was also studied and compared. Therefore, the comparison of the three techniques in terms of isolation times, yields and composition were reported.

2. EXPERIMENTAL

2.1. Plant material

Fresh aerial parts of *H. robustum* Bge. were collected in Baghin region of Kerman city (south-eastern Iran) in June 2011 during the flowering period. The herbs were then dried under ambient conditions (30–40˚C) for three days on a large screened tray. Certified species was then kept in a dark and cold room until used shortly after that for the experiments.

2.2. Conventional Clevenger or Hydrodistillation apparatus and procedure (HD)

100 g of dried aerial parts of *H. robustum* were submitted to hydrodistillation with a Clevenger-type apparatus (Clevenger, 1928, Maisonneuve, 1996) according to the European Pharmacopoeia, and extracted with 2l. of water for 180min (until no more essential oil was obtained). The essential oil was collected, dried under anhydrous sodium sulphate yielding 0.50% and stored at 4˚C until used.

2.3. Solvent free microwave extraction (SFME)

Solvent free microwave extraction was carried out with a Milestone MAO20-A apparatus. This is a multimode microwave reactor 2.45 GHz with a maximum delivered power of 1000 W variable in 10 W increments. Temperature was monitored by an external Infrared (IR) sensor. Based on a relatively simple principle, this method involves placing dried aerial parts of *H. robustum* in the microwave reactor, without any added solvent or water. The internal heating of the in situ water within the *H. robustum* distends the oil glands and sacs and leads to rupture of the glands and oleiferous receptacles. This process thus frees essential oil, which is evaporated by the in situ water of the plant material. A cooling system outside the microwave oven condenses the distillate continuously. Excess water was refluxed to the extraction vessel in order to restore the water to the plant material. In a typical SFME procedure performed at atmospheric pressure, 50 g of dried aerial parts *H. robustum* were placed into the reactor without addition of water or any solvent and the microwave oven was operated at 600 W power levels. The exhaustive extraction of the essential oil was obtained in 15 min. The essential oil was collected, dried over anhydrous sodium sulphate yielding 0.54% and stored at 4˚C until used.

2.4. Microwave-assisted hydrodistillation (MAHD)

MAHD was carried out in a similar manner as the one explained for SFME. 100g of dried aerial parts of *H. robustum* were hydrodistilled with 500 mL of water by microwave energy at 600 W. The extraction oil was performed at atmospheric pressure for 30 min. The essential oils were dried over anhydrous sodium sulphate yielding 0.63% and stored in the dark at 4˚C until analysis.

2.5. Gas Chromatography/Mass Spectrometry (GC/MS)

Essential oil composition was determined by gas chromatography coupled to mass spectrometry (GC – MS) analysis on a Hewlett-Packard 6890 gas chromatograph coupled to a 5973A mass spectrometer. The column was HP5MS (30 m ×0.25 mm×0.25µm filmthickness) with helium as carrier gas. GC oven temprature was kept at 60˚C for 5 min and programmed to 250˚C at a rate of 5˚C/mim, and then kept constant at 250˚C for 5min. MS wera taken at 70 eV. Alkans were used as reference points in the calculation of relative retention indices (RRI).

2.6. Gas chromatography

A Hewlett-Packard 6890 GC system was used for gas chromatography analysis, fitted with a fused-silica capillary column with a polar stationary phase HP5MS (30m×0.25mm×0.25µm film thickness). The column temperature progress from 60 to 280 ˚C at 2 ˚Cmin^{-1}. Injection was performed at 250˚C in the split less mode; 1µL of sample was injected. A flow rate of 0.3 ml/min carrier gas (N2) was used. Flame ionization detection was performed at 320 ˚C.

2.7. Qualitative and quantitative analyses

Most constituents were identified by comparison of their GC Kovats retention indices (RI), determined with reference to a homologous series of C_5–C_{28} *n*-alkanes and with those of authentic standards available in the authors' laboratory. Identification was confirmed when possible by comparison of their mass spectral fragmentation patterns with those stored in the MS database (National Institute of Standards and Technology, Adams and Wiley libraries) and with mass spectra literature data (Adams, 1995). Component relative concentrations were obtained directly from GC peak areas obtained with GC-FID.

3. RESULTS AND DISCUSSION

Essential oils from aerial parts of *Haplophyllum robustum* Bge. obtained by conventional hydrodistillation (HD), microwave-assisted hydrodistillation (MAHD) and solvent free microwave extraction (SFME). The oils were investigated by capillary GC and GC/MS. In total, 17 constituents were identified. Among them, 11 were Monoterpene hydrocarbons, 5 were Oxygenated monoterpenes and only one compound was non-terpenoid. All these compounds were characterized by comparing their mass spectra and their retention indices with those of our own library. The retention indices of the oils, yields, extraction times, extract constituents and their relative percentages are listed in Table 1. Sabinene, β-phellandrene, 1,8- cineole, 3,5- dimethoxy toluene, β- pinene and terpinene-4-ol were the main components in the essential oil extracted from *H. robustum* but the relative amounts differed for the three extraction methods. Seventeen compounds were identified in the hydrodistilled oil which accounted for 96.7% of the total oil composition. This oil was dominated by monoterpenoids such as α-pinene, sabinene, β- phellandrene, β-pinene, 1,8 cineole, linalool, camphor and Terpinene-4-ol. Eleven constituents representing 98.1% of total MAHD oil have been identified. However, 11 compounds were

identified from the SFME oil which accounted for 99.2% of the total oil composition. This oil was also dominated by monoterpenoids. Sabinene, a monoterpene hydrocarbon, is the most abundant component present at 31.5%, 28.9% and 29.2%, respectively for HD, MAHD and SFME. In the same way, β-phellandrene, which is also a monoterpene hydrocarbon amounted to 11.0%, 12.2% and 14.6% in the HD oil, MAHD and SFME oils, respectively. Both MAHD oil and SFME extract contained the highest percentage of 1,8- cineole (10.8% and 11.0%, respectively) and 3,5- Dimethoxy toluene (14.2 and 10.3, respectively), while these two compounds, only amounted for 7.2% and 5.1%, respectively, in the HD oil. Terpinene-4-ol, an oxygenated monoterpene, is also the major component in the oils extracted by these three methods with equivalent amounts of 5.8%, 6.2% and 6.1% from HD, MAHD and SFME, respectively. There are five components in the oil were extracted with HD, such as camphene, β-myrcene, *p*-cymene, α-terpinolene and linalool that are not present in the oil were extracted with MAHD and SFME methods. The HD oil investigated in this study is qualitatively in agreement with Masoudi et al. (2004) who analyzed the essential oil from aerial parts of *H. robustum* and reported that sabinene (30.5%) and β- pinene (18.2%) were the main constituents in HD oil. But according to Rahimi-Nasrabadi et al. [7] 1,8- cineole (38.1%), myrcene (10.69%), α- pinene (8.46%) and 4- terpineol (6.96%) were identified as the main components in the essential oils from leaves and flowering aerial parts of *H. robustum*. Whereas they were detected, in the present study as 1,8- cineole (7.2%), myrcene (2.5%), α-pinene (4.6%) and 4-terpineol was not detected. Differences in the essential oil composition of *H. robustum* could be attributed to climatic effects on the plants that are growing in different habitats. A critical observation of the oil compositions revealed that Higher amounts of oxygenated monoterpenes such as 1,8-cineole, Camphor and terpinene-4-ol were present in the essential oils isolated by MAHD and SFME (21.1% and 21.0%, respectively) in comparison with the oil extracted by HD (19.0%). This difference in the essential oil composition of *H. robustum* could be attributed to the hay absorption of microwave by polar compounds in MAHD and SFME methods more than in HD extraction. This supports the hypothesis that the nature and composition of essential oils may vary depending on the methods of extraction used (Lucchesi *et al.,* 2004, Riela *et al.,* 2008). Microwave irradiation highly accelerated the extraction process, but without causing considerable changes in the volatile oil composition, a phenomenon already described by Pare and Belanger(Pare *et al.,* 1997) According to Presti et al. (2005) the method of essential oil extraction affects their chemical compositions and biological activities. Recently, some studies (Lucchesi *et al.,* 2004, Okoh *et al.,* 2010) showed that MAHD and SFME oils were more active against microorganisms than the oil obtained through HD. This may be partly due to the fact that the microwave extracted oil and these clases of compounds have been proved to possess strong antibacterial and antifungal activities (Presti *et al.,* 2005, Deba *et al.,* 2008, Sokmen *et al.,* 2003, Sandri *et al.,* 2007). However, HD oil contained more monoterpene hydrocarbons such as sabinene, β- phellandrene, β- pinene (72.6%) than SFME and MAHD extracted oils (67.9% and 62.8%, respectively). Concerning the comparison of the three techniques in terms of isolation times and yields, both microwave extraction and distillation were clearly fast (15 and 30 min), while 3 h were required for hydrodistillation (reference method). Indeed, water with a high dielectric constant absorb the radiation from the microwaves resulting in arise in the temperature more rapidly than that in HD. Higher temperature causes an easier degradation of plant cells and consequently a shorter extraction time can be achieved (Käufmann *et al.,* 2001). It is interesting to note that distillation time of 30 min with MAHD and 15 min with SFME provided oil yield comparable to that obtained after 180 min by means of HD (0.63%, 0.54% and 0.50%, respectively). These results indicated a substantial saving of time and energy in the extraction procedure.

4. CONCLUSION

Microwave-assisted hydrodistillation (MAHD) and solvent free microwave extraction (SFME) techniques have been compared with the conventional hydrodistillation (HD) method, for the extraction of essential oil from aerial parts of *H. robustum*. This microwave extraction methods offers important advantages over traditional hydrodistillation, namely; water and solvent free process (in SFME method), shorter extraction times (30 min for MAHD and 15 min for SFME against 3 h for hydrodistillation); better yields (0.54% for MAHD and 0.63% for SFME against 0.50% for HD); environmentally friendly; lower cost; and the possibility for a better reproduction of natural aroma of the *Haplophyllum robustum* Bge. essential oil than the hydrodistilled essential oil.

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Table1. The composition of *Haplophyllum robustum* Bge.

No	Compound	RI	HD(%)	MAHD(%)	SFME(%)
1	α- Thujene	930	2.1	1.7	2.2
2	α- Pinene	941	4.6	2.2	2.6
3	Camphene	958	2.4	-	-
4	Sabinene	976	31.5	28.9	29.2
5	β- pinene	971	7.6	7.3	8.9
6	β- Myrcene	989	2.5	-	-
7	α- phellandrene	1010	1.3	1.7	1.5
8	α- Terpinene	1022	3.4	3.6	3.5
9	*p*-Cymene	1030	0.9	-	-
10	β- phellandrene	1038	11.0	12.2	14.6
11	1,8- Cineole	1054	7.2	10.8	11.0
12	γ-Terpinene	1068	5.3	5.2	5.4
13	α- Terpinolene	1093	0.4	-	-
14	Linalool	1132	1.8	-	-
15	Camphor	1144	3.8	4.1	3.9
16	Terpinene-4-ol	1187	5.8	6.2	6.1
17	3,5- Dimethoxy toluene	1263	5.1	14.2	10.3
	Total(%)		96.7	98.1	99.2
	Extraction time(min)		180	30	15
	Oil yield(W/W%)		0.50	0.63	0.54
	Monoterpene hydrocarbons		72.6	62.8	67.9
	Oxygenated monoterpenes		19.0	21.1	21.0
	non-terpenoid compounds		5.1	14.2	10.3

Volatile oils obtained by hydrodistillation, solvent free microwave extraction and microwave-assisted hydrodistillation.