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Superheated water extraction, steam distillation and Soxhlet extraction of essential oils of *Origanum onites*

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Abstract Superheated water extraction (SWE) at various temperatures (100, 125, 150 and 175°C), steam distillation, and Soxhlet extraction were compared in the extraction of essential oils from two samples of the plant *Origanum onites*, one cultivated, the other wild. C18 solid-phase extraction was used to elute the essential oils from the SWE aqueous extract. The compositions of the extracted essential oils obtained from all three methods were then characterized by comprehensive GC×GC/time-of-flight mass spectrometry (TOF/MS). The highest essential oil yields were obtained by using SWE at 150°C with a flow rate of 2 mL min^{-1} and a pressure of 60 bar for 30 min: these were 3.76 and 4.11% for wild and cultivated *O. onites* samples, respectively, expressed as a percentage of 100 g of dry (leaf) matter. The yields obtained using SWE at 150°C were slightly higher than those from conventional methods. Steam distillation was performed for 3 h, and Soxhlet extraction was completed in 12 h. The major compounds found were borneol, terpinen-4-ol and carvacrol.

Keywords Superheated water extraction · Steam distillation · Soxhlet extraction · Gas chromatography–mass spectrometry

Introduction

The family *Labiatae* (syn. *Lamiaceae*) consists of numerous species including aromatic plants such as *Thymus*, *Origanum*, *Thymbra*, *Coridothymus* and *Satureja*. These plants are traditionally used in natural remedies for asthma, indigestion, headaches and rheumatism [1]. *Origanum* species are used as powerful disinfectants, flavouring agents, in perfumes and in scenting soaps [2]. *Oreganum* oils are mainly used in perfume compositions and in seasoning mixtures for Italian, Spanish, Greek and Turkish cuisine [3]. Dried *Oreganum* leaves and essential oils are used by the flavouring industry in various liqueur formulations, tomato sauces, condiments, in baked goods such as pizzas and in salad dressings [4].

The genus *Origanum* L. comprises 49 taxae belonging to 10 different sections and is characterized by a large morphological and chemical diversity. Most *Origanum* species (ca. 75%) are found exclusively in the eastern Mediterranean subregion [5]. The essential oils of *Origanum* species have proven to be antibacterial [6], antifungal [7] and antioxidant [8] activities. *O. onites* is a perennial species with woody stems and can be distinguished from other *Origanum* species by it's one-lipped calyces. It is found growing in the western and southern coastal areas of Turkey and in the southern Greek mainland and islands [9].

Currently, the most common methods used for the isolation of essential oils from natural products are steam distillation and solvent extraction. However, the loss of some volatile compounds, low extraction efficiency, degradation of unsaturated compounds through thermal or hydrolytic effects and toxic solvent residue in the extract may be encountered with these extraction methods [10]. Lately, more efficient extraction methods such as supercritical fluid extraction (SFE) [3] have been used for the isolation of organic compounds from various plants.

Superheated (or subcritical) water extraction (SWE) has been used as a technique for extraction from solid samples in a number of recent studies [11–15]. The term 'superheated water' is used to denote the region of the condensed phase which occurs between 100°C and the critical point (374°C). The pressures required to maintain a condensed state of water are moderate (i.e. 15 bar at 200°C and 85 bar at 300°C). Previous workers [11–15] have reported that superheated water is a useful alter-

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native method for the extraction of essential oils because it enables rapid extraction and the use of low working temperatures. This avoids the loss and degradation of volatile and thermolabile compounds. Additional positive aspects of the use of SWE are its simplicity, low cost and no undesirable environmental effects.

Contemporary high-resolution separation of volatile and semi-volatile compounds of essential oils normally employs the technique of capillary gas chromatography/mass spectrometry. However, the complex nature of these samples results in extended gas chromatography run times. In the past few years, comprehensive two-dimensional gas chromatography (GC×GC) has been shown to be an extremely powerful technique for the analysis of essential oils [16, 17]. The coupling of comprehensive GC to time-of-flight mass spectrometry (TOF/MS) is very recent. High TOF/MS acquisition rates offer a superior separation power [18, 19].

The aim of this study was to investigate the separation of essential oils from the leaves of two samples of *O. onites* from different sources using SWE, steam distillation and Soxhlet extraction, and then to compare the resulting compositions and quantities.

Materials and methods

Materials

Origanum onites leaves were collected in June 2003 in Denizli, western Turkey, from two different stations. One sample was from plants growing wild in the Ortaca mountains, and the other was from a crop in the village of Gözler which has a fairly new, but thriving, industry in the cultivation of *O. onites* and is about 30 km from Ortaca. Leaves of each were separated from the branches and air-dried before being stored in separate polyethylene bags according to source until extraction which was completed within 1 month of collection.

Hexadecane (as an internal standard) was provided by Aldrich (Gillingham, Dorset, UK). Hexane, methanol, ethyl acetate and water were of HPLC grade, supplied by Fisher Scientific (Loughborough, UK). DSC-18 solid-phase extraction (SPE) cartridges (500 mg/3-mL tubes) were purchased from Supelco (Gillingham, Dorset, UK).

Superheated water extraction

A detailed description of the laboratory-built SWE apparatus has been given elsewhere [15]. The water was purged with nitrogen to remove dissolved oxygen prior to the extraction. Deoxygenated water was used in an HPLC pump programmed for a constant flow of 2 mL min^{-1}. A Carlo Erba series 4200 GC oven heated the extraction system. A 3-m-long pre-heated coil (0.76-mm i.d.×1.6-mm o.d.) was used to equilibrate the water to the desired temperatures. A 10.4-mL extraction cell (Keystone Scientific, Bellefonte, Pa., USA) equipped with a 0.5-μm frit both at the inlet and outlet was connected to a 1-m cooling loop (in iced water) outside of the oven. A pressure control valve was placed between the cooling loop and the collection vial.

SWE was performed using 1.5 g of air-dried *O. onites* leaves, an extraction cell which contained a stainless steel filter and glass wool at both ends, a 2 mL min^{-1} flow rate, temperatures of 100–175°C, a pressure of around 60 bar and 30 min of extraction time. A DSC-18 solid-phase extraction (SPE) cartridge was used to re-extract the analytes with hexane/ethyl acetate (9:1). The DSC-18 cartridge was first washed using 4 mL of methanol followed by 4 mL of water. Next, the sample was loaded at an approximately 2 mL min^{-1} flow rate and washed with 4 mL of water. The cartridge was then dried for 5 min by means of a vacuum and a further 5 min using a nitrogen gas flow. Following the drying process, the DSC-18 cartridge was eluted with 4 mL of a hexane/ethyl acetate (9:1) mixture. The collected eluent was concentrated under a nitrogen stream to a volume of 0.5 mL. An appropriate amount of hexadecane was added into the concentrate as an internal standard. The mixture (1 μL) was directly injected into the GC×GC/TOF/MS.

Soxhlet extraction

Soxhlet extraction of 1.5 g of the air-dried plant material was carried out using 50 mL hexane for 12 h (a total of 72 cycles). Hexane was chosen as the Soxhlet extraction solvent due to the mostly non-polar nature of the essential oil of *O. onites*. The Soxhlet extracts were evaporated with nitrogen until they reached 0.5 mL in volume. This chromatography-ready extract was stored at 0–4°C until analysis.

Steam distillation

Air-dried leaves were subjected to steam distillation in a Clavenger apparatus for 3 h. The essential oils were dried over anhydrous sodium sulfate and stored in a dark glass bottle at 0–4°C until analysis.

Chromatographic analysis

The GC×GC/TOF/MS system consisted of an HP 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph and a Pegasus III TOF-MS (LECO, St. Joseph, Mich., USA). The first column was a non-polar DB5 (10 m×0.18-mm i.d.×0.18-μm film thickness) and the second column a polar DB17 (1.6 m×0.18-mm i.d.×0.18-μm film thickness). Both columns were purchased from J&W Scientific (Folsom, CA, USA). The columns were connected by means of a press-fit connector. The second dimension column was installed in a

separate oven, which was maintained in the main GC oven. Using the separate oven provides a more flexible system, since it allows fine-tuning of the retention in the second column by using a higher or lower temperature relative to the first dimension column. In this particular system, the need to use a two-oven system was driven by detector stability considerations, requiring accurate and stable control of the secondary column temperature. This temperature control of both ovens enabled more rapid and higher-resolution separations to be carried out.

This system does not require any valving or switching facilities. The modulator is the key to the performance of the GC×GC experiment. Cryogenic modulation was performed using a jet-type modulator which was installed at the top of the second dimension column. This consists of two hot and two cold jets with the nozzles providing the cold jets mounted orthogonal to the hot jets. Nitrogen gas is cooled by heat exchange through copper tubing immersed in liquid nitrogen outside the GC system and delivered through vacuum-insulated tubing to the cold jets, which provide two continuous jets of approximately 10 L min^{-1} of cold nitrogen gas. The modulation time was 10 s. When the hot downstream pulse is fired the analytes are effectively injected into the second dimension column. A detailed description of the apparatus is given elsewhere [17].

Helium was used as a carrier gas. The initial temperature of the first dimension column was 35°C for 30 s and the subsequent temperature program was a heating rate of 5°C min^{-1} until 250°C was reached. The initial temperature of the second dimension column was 50°C for 30 s and a 5°C min^{-1} heating rate was used until 265°C. Peak identification was made using TOF/MS with electron impact ionization. The mass spectrometer used a push plate frequency of 5 kHz, with transient spectra averaging to give unit-resolved mass spectra between 45 and 350 amu at a rate of 50 spectra s^{-1}. Mass spectra were compared against the NIST 1998 (National Institute of Standards and Technology, Gaithersburg, Md.) mass spectral library.

Results and discussion

The separation of compounds from the aqueous extract obtained by SWE is a critical stage. Solid-phase extraction has been found to be a better technique than liquid–liquid extraction for the removal of compounds from the aqueous environment of SWE [15]. Rovio et al. [12] and Ozel et al. [15] reported that essential oils were successfully removed from an aqueous extract by SPE by using C-18 as a packing material.

The essential oils from the leaves of two *O. onites* samples were obtained using SWE, steam distillation and Soxhlet extraction. Optimization of SWE conditions has been studied elsewhere [14, 15]. Ayala et al. [14] discovered that a temperature of 125°C, 2-MPa pressure and a 1 mL min^{-1} flow rate is optimum for SWE of edible essential oil. Ozel et al. [15] also optimized the SWE of *Thymbra spicata* using a temperature of 150°C, a pressure of 60 bar, a flow rate of 2 mL min^{-1} and 30 min of extraction time. Both workers also discovered that temperature is more critical than other parameters. Various temperatures (100–175°C) were investigated using a 2 mL min^{-1} water flow rate, a pressure of around 60 bar and 30 min of extraction time to understand how temperature would affect the extraction yields. Tables 1 and 2 show the SWE results of the *O. onites* leaves obtained from Ortaca and Gözler, respectively. Table 3 lists the percentage of essential oil extracted from the two *O. onites* samples using the SWE, steam distillation and Soxhlet techniques. The yields of essential oils from the *O. onites* leaves (Ortaca) were 2.35, 3.48, 3.76 and 3.16% at 100, 125, 150 and 175°C, respectively. Those of the Gözler leaves were 3.12, 3.97, 4.11 and 3.77% at 100, 125, 150 and 175°C, respectively. The yields obtained using SWE (from both samples) at 150°C (30 min) were very similar to those produced by steam distillation (3 h) and Soxhlet extraction (12 h). Although the composition of the essential oils were similar, the cultivated *O. onites* leaves (Gözler) contained slightly more essential oil than those growing wild in the Ortaca mountains. This was the case for all investigated temperatures. The yields are given as a percentage weight based per 100 g of dried leaves: these are the mean of four experiments, and the relative standard deviation was in a range of 1–9%.

The SWE yields from both samples increased with temperatures up to 150°C. However, a further rise in temperature to 175°C resulted in a small decrease in both yields. At 175°C, the superheated water extracts were dark brown. Furfural and 3-furaldehyde, found in higher quantities in both samples at 175°C, may be browning reaction products. Thus, at this higher temperature, essential oil yields may have decreased due to burning of the sample. Similar effects were seen in both samples (Tables 1 and 2). Degradation at 175°C was also found by Kubatova et al. [13] during extractions from savory and peppermint and Ozel et al. [15] during extractions from *Thymbra spicata* using SWE.

Tables 1 and 2 list the compounds identified in each *O. onites* sample together with their retention times and relative abundances. It can be seen that the change in temperature resulted in a slight change of components and composition of the extracted essential oils. The total number of components found increased with temperature from 100 to 175°C. However, the overall amount of essential oil recovered decreased at 175°C. The composition of oil from both samples was found to be similar with a few minor differences. Carvacrol was the main component of both (between 68.21 and 80.17%). Borneol, terpinen-4-ol, 2-caren-10-al, linalool, (*Z*)-α-terpineol, thymol and *o*-cymene were found to be the other major compounds from the wild-growing Ortaca sample. However, linalool and *o*-cymene were not found to be major compounds in the cultivated Gözler sample.

Essential oils normally contain a complex mixture of organic compounds. They are largely composed of a range of saturated or partly unsaturated cyclic and linear molecules of relatively low molecular mass, and within this range a variety of hydrocarbons and oxygenated compounds occur. Conventional one-dimensional gas chromatography generally does not provide sufficient separation for complex mixtures. Since essential oils contain numerous components, it is possible that some components can obscure the analytes of interest [20]. Comprehensive GC×GC/TOF/MS was used to analyse essential oils of *O. onites* leaves in this study. The first-dimensional separation is based on separation by boiling point in a non-polar column. The second-dimensional separation is based on separation by polarity using a polar column. The inclusion of the latter makes this an overall two-dimensional chromatogram. As can be appreciated from Tables 1 and 2, there are some components which can only be separated on the second column. For example, even if the first dimensions of α-phellandrene (430, 4.49), *o*-cymene (430, 485) and undecane (560, 3.10), linalool (560, 5.15) are the same, they have different second dimensions which make their separation from each other and therefore their recognition possible.

Monoterpene compounds are less valuable than oxygenated compounds as they contribute to the fragrance of the oil only in a minor way. It can be seen in Tables 1, 2 and 4, that the essential oil from both the *O. onites* samples contains only very small amounts of monoterpenes (α-thujene, α-pinene, camphene and decane) and has higher concentrations of oxygenated compounds (carvacrol, terpinen-4-ol, borneol and thymol). This makes the essential oil more valuable. It should be noted that the amounts of monoterpenes and oxygenated compounds found were similar using all the three extraction methods. Gounaris et al. [5] extracted oils from a sample of *O. onites* from another location (Crete, Greece) by steam distillation and found a higher concentration of monoterpenes and a lower concentra-

Table 1 Compounds, retention times and percentage compositions of *O. onites* (Ortaca) found at various temperatures using superheated water extraction

Compound[a]	^1t_R[b] (s)	^2t_R[b] (s)	Percentage[c]			
			100°C	125°C	150°C	175°C
Ethyl propionate	100	7.54	ND	0.07	0.14	0.08
Tropilidene	120	8.08	0.01	0.08	0.08	0.04
2-Hexanal	210	8.64	ND	0.04	0.04	0.05
Furfural	240	4.84	ND	ND	ND	0.17
3-Furaldehyde	260	4.62	ND	ND	0.02	0.36
α-Thujene	290	3.26	ND	0.01	ND	ND
α-Pinene	300	4.28	0.12	0.09	0.08	0.07
Camphene	310	3.70	0.08	0.04	0.01	ND
1-Octen-3-ol	370	4.16	0.28	0.31	0.34	0.31
Decane	390	2.88	0.01	0.01	0.01	ND
3-Octanol	400	4.04	0.12	0.14	0.10	0.11
α-Phellandrene	430	4.49	ND	0.01	0.12	0.13
o-Cymene	430	4.85	2.50	1.87	1.75	1.88
Eucalyptol	440	4.80	ND	0.09	0.12	0.16
Limonene	520	6.88	0.01	ND	0.01	ND
Terpinolene	530	4.70	0.22	0.18	0.19	0.15
(*Z*)-α-Terpineol	540	5.96	3.57	3.02	2.96	3.12
γ-Terpinene	550	6.10	0.66	0.47	0.37	0.51
Undecane	560	3.10	0.27	0.16	0.18	0.15
Linalool	560	5.15	2.54	2.01	2.15	2.27
cis-Sabinenehydrate	570	5.40	ND	ND	0.04	0.02
Pinocarveol	610	6.14	ND	ND	ND	0.01
Camphore	620	7.42	0.02	0.10	0.17	0.16
Borneol	660	6.32	2.1	4.91	5.12	4.88
Terpinen-4-ol	680	5.96	1.2	3.71	5.02	4.57
Carene	700	3.32	ND	ND	ND	0.02
Dodecane	710	3.48	ND	0.01	0.01	0.02
α-Terpineol	720	6.74	0.17	0.42	0.57	0.66
cis-Piperitol	730	6.06	ND	ND	0.01	0.01
(*E*)-3-Caren-2-ol	740	6.80	ND	0.02	0.03	0.03
l-Carvone	790	7.54	ND	0.10	0.12	0.14
Linalyl anthranilate	810	5.24	ND	ND	ND	0.02
Thymol	920	6.57	3.58	2.09	1.86	1.71
Carvacrol	950	6.82	78.08	73.27	70.66	68.21
Caryophyllene	1,040	5.58	ND	ND	0.02	0.04
2-Caren-10-al	1,090	5.62	0.11	2.17	3.86	4.01
α-Bisabolene	1,180	5.26	ND	ND	0.02	0.03
Caryophyllene oxide	1,270	7.32	ND	ND	0.05	ND
Eicosane	1,370	3.56	ND	ND	ND	0.11
α-Cadinol	1,380	5.26	ND	0.01	0.01	0.02
Unknown			4.35	4.59	3.75	5.42

[a]As identified by GC×GC/TOF/MS software; names according to NIST mass spectral library

[b] ^1t_R and ^2t_R are the retention times in the first and second dimension, respectively (in the case of multiple identification, the retention time of the best spectral matching peak is shown. If component is present in more than one temperature application, retention times were given for 150°C; all first-dimension retention times were within ±10 s, and second-dimension retention times were within ±0.12 s)

[c]Percentage of each component is calculated as peak area of analyte divided by peak area of total ion chromatogram *ND* not detected

Table 2 Compounds, retention times and percentage compositions of *O. onites* (Gözler) found at various temperatures using superheated water extraction

Compound[a]	^1t_R[b] (s)	^2t_R[b] (s)	Percentage[c]			
			100°C	125°C	150°C	175°C
Ethyl propionate	100	7.54	ND	ND	0.01	0.01
Tropilidene	120	8.08	ND	0.01	0.03	0.03
2-Hexanal	210	8.64	0.01	0.04	0.04	0.03
Furfural	240	4.84	ND	ND	0.02	0.14
3-Furaldehyde	260	4.62	ND	ND	0.01	0.21
α-Thujene	290	3.26	ND	ND	ND	0.01
α-Pinene	300	4.28	0.01	0.04	0.07	0.08
Camphene	310	3.70	0.11	0.17	0.15	0.06
1-Octen-3-ol	370	4.16	0.18	0.41	0.49	0.36
Decane	390	2.88	ND	ND	0.02	0.01
3-Octanol	400	4.04	ND	0.02	0.05	0.07
α-Phellandrene	430	4.49	ND	ND	0.01	ND
o-Cymene	430	4.85	0.41	0.48	0.60	0.51
Eucalyptol	440	4.80	ND	0.04	0.09	0.09
Limonene	520	6.88	ND	ND	0.01	0.02
Terpinolene	530	4.70	0.01	0.05	0.09	0.08
(*Z*)-α-Terpineol	540	5.96	3.02	2.88	2.51	2.60
γ-Terpinene	550	6.10	0.40	0.36	0.49	0.57
Undecane	560	3.10	ND	ND	0.02	0.01
Linalool	560	5.15	0.12	0.33	0.28	0.29
cis-Sabinenehydrate	570	5.40	ND	0.01	0.09	0.15
Pinocarveol	610	6.14	ND	ND	ND	0.01
Camphore	620	7.42	0.22	0.28	0.34	0.36
Borneol	660	6.32	4.20	3.39	3.48	3.71
Terpinen-4-ol	680	5.96	2.37	4.88	5.17	5.28
Carene	700	3.32	ND	ND	ND	0.01
Dodecane	710	3.48	ND	ND	0.02	0.01
α-Terpineol	720	6.74	0.81	0.86	0.72	0.60
cis-Piperitol	730	6.06	ND	ND	0.05	0.11
(*E*)-3-Caren-2-ol	740	6.80	ND	0.01	0.08	0.04
3-Thujen-2-one	780	7.42	ND	ND	ND	ND
l-Carvone	790	7.54	0.19	0.37	0.71	0.92
Linalyl anthranilate	810	5.24	ND	ND	ND	0.01
Thymol	920	6.57	3.2	2.88	2.46	3.11
Carvacrol	950	6.82	80.17	77.16	72.84	70.15
Caryophyllene	1,040	5.58	ND	ND	0.01	0.08
2-Caren-10-al	1,090	5.62	0.15	2.17	4.22	3.99
α-Bisabolene	1,180	5.26	ND	0.02	0.19	0.25
Caryophyllene oxide	1,270	7.32	ND	0.01	0.08	0.07
Eicosane	1,370	3.56	ND	ND	ND	0.14
α-Cadinol	1,380	5.26	ND	ND	0.02	0.07
Unknown			4.42	3.13	3.72	5.74

[a] As identified by GCxGC/TOF/MS software; names according to NIST mass spectral library

[b] ^1t_R and ^2t_R are the retention times in the first and second dimension respectively (in the case of multiple identification, the retention time of the best spectral matching peak is shown. If component is present in more than one temperature application, retention times were given for 150°C; all first-dimension retention times were within ±10 s, and second-dimension retention times were within ±0.12 s)

[c] Percentage of each component is calculated as peak area of analyte divided by peak area of total ion chromatogram *ND* not detected

tion of the valuable oxygenated compounds. This variation is unlikely to be due to the extraction method as the same method was used in this study; it is more likely due to natural sample variations from different locations.

In Table 4, a comparison of SWE (at 150°C) with steam distillation and Soxhlet extraction is made by looking at percentage compositions of oil extracted from both samples using all the three methods. As is shown, the SWE (150°C) technique gave a slightly higher number of different analytes and a slightly better overall recovery of essential oil from both *O. onites* samples (Table 3). A range of lower molecular weight hydrocarbons were found to be absent in the Soxhlet extracts. It must be assumed that they were lost during the extraction or in the solvent reduction steps. Steam distillation and Soxhlet extraction are traditionally the most frequently used techniques in the extraction of organic materials from solid or liquid matrices. Steam distillation, especially, is popular in the extraction of essential oils from plant materials. Table 4 and recent publications [10–15] show that SWE is a very promising alternative to traditional techniques. Soxhlet extraction is time consuming (12 h) and uses a large amount of

Table 3 Yields of essential oils isolated from leaves of *O. onites* samples using various techniques

Condition	Essential oil yield (%)	
	Ortaca	Gözler
SWE, 100°C	2.35 (0.14)	3.12 (0.19)
SWE, 125°C	3.48 (0.24)	3.97 (0.31)
SWE, 150°C	3.76 (0.33)	4.11 (0.29)
SWE, 175°C	3.16 (0.16)	3.77 (0.34)
Steam distillation	3.58 (0.14)	4.03 (0.16)
Soxhlet extraction	3.62 (0.18)	3.91 (0.20)

Standard deviation in parentheses

Table 4 Percentage composition of the essential oils isolated from leaves of *O. onites* samples using SWE (150°C) and conventional techniques

Compound[a]	Ortaca (%)[b]			Gözler (%)[b]		
	SWE-150°C	Steam distillation	Soxhlet extraction	SWE-150°C	Steam distillation	Soxhlet extraction
Ethyl propionate	0.14	0.11	0.01	0.01	0.04	ND[c]
Tropilidene	0.08	0.15	ND	0.03	0.31	ND
2-Hexenal	0.04	0.04	ND	0.04	0.06	ND
Furfural	ND	ND	ND	0.02	ND	ND
3-Furaldehyde	0.02	ND	ND	0.01	ND	ND
α-Thujene	ND	ND	ND	ND	0.01	0.01
α-Pinene	0.08	0.05	0.02	0.07	0.12	0.05
Camphene	0.01	0.01	0.02	0.15	0.17	0.06
1-Octen-3-ol	0.34	0.24	0.19	0.49	0.49	0.29
Decane	0.01	0.01	0.02	0.02	0.01	0.01
3-Octanol	0.10	0.05	0.07	0.05	0.05	0.02
α-Phellandrene	0.12	0.01	0.05	0.01	ND	ND
o-Cymene	1.75	1.57	1.96	0.60	0.63	0.76
Eucalyptol	0.12	0.11	0.17	0.09	0.09	0.05
Limonene	0.01	0.01	0.02	0.01	ND	ND
Terpinolene	0.19	0.34	0.29	0.09	0.07	0.09
Camphenilone	ND	ND	0.01	ND	0.07	0.04
(*Z*)-α-Terpineol	2.96	2.79	2.98	2.51	2.59	2.69
γ-Terpinene	0.37	0.59	0.45	0.49	0.59	0.52
Undecane	0.18	0.03	0.09	0.02	0.02	0.01
Linalool	2.15	1.91	2.21	0.28	0.03	0.03
cis-Sabinenehydrate	0.04	0.05	0.05	0.09	0.37	0.33
Pinocarveol	ND	0.02	0.01	ND	ND	ND
Camphor	0.17	0.13	0.11	0.34	0.31	0.41
Borneol	5.12	5.46	5.95	3.48	3.31	3.61
Terpinen-4-ol	5.02	4.99	3.97	5.17	9.20	8.11
Carane	ND	0.08	0.05	ND	ND	ND
Dodecane	0.01	0.01	ND	0.02	0.02	0.01
α-Terpineol	0.57	0.36	0.43	0.72	0.71	0.65
cis-Piperitol	0.01	ND	ND	0.05	0.06	0.03
(*E*)-3-Caren-2-ol	0.03	ND	0.01	0.08	0.03	0.04
3-Thujen-2-one	ND	ND	ND	ND	0.01	0.02
1-Carvone	0.12	0.03	0.03	0.71	0.72	0.54
Linalyl anthranilate	ND	ND	ND	ND	0.01	ND
Thymol	1.86	1.40	1.70	2.46	2.17	2.50
Carvacrol	70.66	71.88	71.15	72.84	66.43	68.43
Caryophyllene	ND	ND	0.01	0.01	ND	ND
2-Caren-10-al	3.86	4.10	4.17	4.22	6.35	5.12
α-Bisabolene	0.02	0.07	0.15	0.19	0.27	0.35
Caryophyllene oxide	0.05	ND	ND	0.08	ND	ND
α-Cadinol	0.01	0.05	0.02	0.02	0.02	ND
Unknown	3.75	3.35	3.64	3.72	4.66	5.21

[a]As identified by GC×GC/TOF/MS software; names according to NIST mass spectral library

[b]Percentage of each component is calculated as peak area of analyte divided by peak area of total ion chromatogram

[c]*ND* not detected

environmentally unfriendly organic solvents. Due to the smaller quantity of oil being produced, it is necessary to use a solvent when performing SWE on a lab-scale. However, realistically, in a big extraction unit solvent will not be needed as the oil will float on the water extract making collection easy. Steam distillation can be performed in a shorter time but was found to produce slightly less essential oil than the even faster SWE technique at 150°C.

Conclusion

SWE was found to give recoveries comparable to those of steam distillation and Soxhlet extraction of essential oils from two *O. onites* samples. Soxhlet extraction is time consuming and labour intensive. Steam distillation is cheap but has no selectivity. In contrast, SWE is cheap, relatively fast and environmentally sound as solvents do not have to be used. It is also selective in that the operator is able to extract various polar and non-polar organic compounds (α-pinene, terpinen-4-ol, carvacrol, etc.) by choice by varying the temperature as long as the water is kept in a liquid state using minor adjustments in pressure. Comprehensive GC×GC/TOF/MS successfully achieved the separation and identification of compounds which cannot be separated by a conventional one-dimensional technique.

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