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Comparative Analysis of the Essential Oils of *Hypericum triquetrifolium* Turra. Extracted by Ultrasound, Hydrodistillation and Soxhlet/Dynamic Headspace

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ABSTRACT

The essential oil (EO) of *Hypericum triquetrifolium* Turra., obtained from the aerial parts by ultrasound extraction (USE), hydrodistillation (HD) and Soxhlet/dynamic headspace (SDH) were analyzed by GC-FID and GC-MS. The USE method gave a higher yield than HD and SDH. A total of 60 components were identified with *n*-octane, α-pinene, β-caryophyllene, 2-methyloctane, *n*-nonane, germacrene-D, α-selinene and β-cubebene being the main constituents. USE, when compared to HD and SDH, showed high efficiency concomitant to saving time, low energy cost and cleanliness.

Keywords: extraction procedure, GC-MS, *Hypericum triquetrifolium* Turra, volatile oil constituents

INTRODUCTION

Hypericum triquetrifolium Turra. belongs to the genus *Hypericum*, which contains approximately 460 species (Robson 2006). In Tunisia, this genus is represented by 8 species and *H. triquetrifolium* is the most abundant one with a wide range of ecological adaptations and morphological variations (Pottier-Alapetite 1979; Hosni *et al.* 2007).

Morphologically, *H. triquetrifolium* is a perennial herb with stem widely branched along most of length, usually forming pyramid. The stem is 2-lined with black glands in the lines and sometimes elsewhere. Leaves are sessile, triangular-lanceolate or rarely narrowly ovate to linear-oblong, concolorous, sometimes glaucous, chartaceous and margin crisped-ondulate. Lamina is characterised by the presence of numerous translucent glands which contains essential oil and resins. Leaves margins contain spaced black glands which considered as a typical trait of this species. Bright yellow flower are 8-12 mm in diameter and subequal to unequal five sepals oblong to ovate-oblong or lanceolate, acute to rounded-apiculate or rounded. Flowers contains from 15 to 40 stamens arranged in 3 fascicles with anther black glands. Capsules 3-5 × 2-3.5 mm are ovoid and contain 3 valves with longitudinal linear vittae and occasionally a few lateral vesicles. Capsule contains darkish brown seeds, subcylindric, not carinate or appendiculate (Robson 2002).

From a pharmacological standpoint, *H. triquetrifolium* extracts contain a complex mixture of bioactive substances, mainly flavonoids, naphtodianthrones (hypericin, pseudohypericin and their protoforms); phloroglucinols (hyperforine and adhyperforin), xanthones and tannins which possess a wide array of biological properties. Antioxydant, Antimicrobial, antifungal, antiviral, antinociceptive and cytotoxic activities have been reported (Couladis *et al.* 2002; Kizil *et al.* 2004; Cakir *et al.* 2005; Pistelli *et al.* 2005; Conforti *et al.* 2007). The contribution of the essential (EO) in a large part of those activities was also reported (Ozturk *et al.* 2002; Kizil *et al.* 2004; Pistelli *et al.* 2005; Toker *et al.* 2006). The latter components are usually extracted by hydrodistillation or steam distillation. These techniques present some shortcomings, namely losses of volatile compounds, low extraction efficiency, long extraction time, degradation of unsaturated or ester compounds through thermal or hydrolytic effects and toxic solvent residue (Lucchesi *et al.* 2004; Tam *et al.* 2007). These shortcomings associated with the expanding market of EO have led to the implantation of new techniques such as supercritical fluid extraction (SFE), pressurized fluid extraction (PFE), continuous supercritical water extraction (CSWE), accelerated solvent extraction (ASE), microwave assisted extraction (MAE) and ultrasound extraction (USE).

The USE has become a good alternative extraction method when compared to classical ones due to its high efficiency, low energy and water consumption (no reflux or refrigeration are needed). Besides, USE is a well established method in the processing of plant material, particularly to extract low molecular weight substances (Rodrigues and Pinto 2007). The mechanical effect of ultrasound is able to accelerate the extraction of active plant compounds, contained within the body of plants, due to disruption of the cell walls and enhanced mass transfer of cell contents (Toma *et al.* 2001). This method has been successfully used to extract phenolic compounds (Rodrigues and Pinto 2007), steroids and triterpenoids (Schinor *et al.* 2004), EO (Jerković *et al.* 2007), pigments, resins, alkaloids and flavonoids (Toma *et al.* 2001). In medicinal plants, particularly some species of the genus *Hypericum*, the use of ultrasound was limited to the extraction of bioactive principles notably hypericin, hyperforin and their derivatives (Smelcerovic *et al.* 2006; Hosni *et al.* 2010).

Investigations on the chemical composition of the EO of *H. triquetrifolium* are rather scarce and in the most of cases did not give homogeneous results though they are extracted mainly by hydrodistillation. Thus, the EO of *H. triquetrifolium* from the Hellenic peninsula showed an abundance of 2-methyl octane, α-pinene, *n*-nonane, β-caryophyllene and 3-methyl nonane (Petrakis *et al.* 2005). The SPME (solid phase microextraction) analysis showed higher yields of undecane and β-caryophyllene when compared with the corresponding hydrodistillation oil obtained from leaves and flowers of *H. triquetrifolium* (Bertoli *et al.* 2003).

Despite the substantial data on the extraction, identifica-

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tion and quantification of their "heavy" bioactive compounds (flavonoids, naphtodianthrone and phloroglucinols), species of the genus *Hypericum* were not essayed for their EO extraction by different methods. The main reasons corroborating this fact are the lower yield of their EO and the absence of a standard chemical composition.

Therefore, the aim of this study was to compare the chemical composition of the EO obtained by three extraction methods (hydrodistillation, soxhlet/dynamic headspace and ultrasound extraction) from the aerial parts of *H. triquetrifolium*. Until now, the use of soxhlet/dynamic headspace and ultrasound for the extraction of the EO from species of the genus *Hypericum* has not been reported.

MATERIALS AND METHODS

Plant material

The aerial parts of *H. triquetrifolium* Turra (top of 2/3 plants) were collected at the full flowering stage, during June 2005 in Seltene (North-eastern Tunisia; latitude 36°41' (N); longitude 10°24' (E); altitude 15 m; annual precipitation: 500 mm and mean temperature: 16.8°C). The sampling site was not grazed or mown during the period when the plants were gathered. The sampling was done by a randomised collection of 15 to 20 plants. To ovoid the sampling on the same plants, minimum distance of 10 m was required. The plant material was botanically characterized by Prof. Mohammed El Hedi El Ouni (Department of biology, Faculty of Sciences, Bizerte, Tunisia) and according to the morphological description presented in Tunisian flora (Pottier-Alapetite 1979). The harvested material was air-dried at room temperature (20 ± 2°C) for one week, and subsequently essayed for its EO composition.

Chemicals

Hexane and *n*-pentane of analytical grade were purchased from LabScan (Dublin, Ireland). Anhydrous Na_2SO_4 and activated charcoal were purchased from Fluka (Buchs, Switzerland). Linalool, α-pinene, β-pinene, 1,8-cineol, 6-Methyl-5-hepten-2-one, limonene, terpinen-4-ol, geraniol, β-caryophyllene, β-selinene, α-humulene and germacrene-D were purchased from Fluka (Steinheim, Germany). The 1-hexanol used as internal standard, *n*-octane and β-ionone were purchased from Sigma-Aldrich (Buchs, Switzerland).

Isolation procedures

1. Hydrodistillation (HD)

The EO was isolated from the air-dried material (100 g) by conventional HD for 3 h. The HD was performed by a simple laboratory Quikfit apparatus which consisted of a 2000 ml distillation flask, a condenser and a receiving vessel. The obtained distillate was extracted twice with *n*-pentane and dried over anhydrous Na_2SO_4. Choice of the solvent was based on its ability to extract the major constituents of the EO without loss of the high volatile components during the concentration step (Teixeira *et al.* 2007; Hosni *et al.* 2008).

The organic layer was then concentrated, at 35°C using a Vigreux column under atmospheric pressure. For the determination of the procedure yield, the solvent was removed by gentle nitrogen blowdown stream at low temperature to prevent the evaporation of the volatile constituents and the remaining oil was weighed on an analytical scale. After weighing, the whole sample was re-diluted in 1 mL of the extraction solvent and 1 µL was subsequently analyzed.

2. Soxhlet/Dynamic Headspace (SDH)

Twenty grams of dried plant material was mixed with 5 g of anhydrous Na_2SO_4 powder, loaded into 22 mm × 80 mm cellulose cartridge and extracted with 100 mL hexane for 6 h at boiling temperature of solvent (70°C), in a Soxhlet apparatus (2-3 cycle/h). The obtained solvent extract was concentrated to approximately 40 mL on a rotary-evaporator. Thirty millilitres of the concentrated solvent extract were subjected to a modified dynamic headspace technique. Thus, the solvent extract sample was introduced into Pyrex tube (25 mm × 400 mm), heated at 40°C in a water bath, stripped for 1h:30' with purified N_2 (1.2 dm^3/min) and trapped on 50 mg of activated charcoal (20-35 mesh) used as adsorbent agent. Yield of this procedure was calculated as the difference between the mass of saturated charcoal and the neutral one. The desorption step was achieved by adding 1 mL of *n*-pentane to the aforementioned adsorbant and an aliquot of 1µL was immediately analyzed.

3. Ultrasound extraction (USE)

Five grams of dried plant material were mixed with 1 g of anhydrous Na_2SO_4 and extracted with 50 mL of *n*-pentane for 30 min in an ultrasonic cleaning bath (Transsonic Type 310/H, Germany) working at a frequency of 35 KHz. The temperature of the water bath was regulated at 30°C. After decantation, the solvent extract was separated and the residual sample was recharged with 20 mL of fresh *n*-pentane and sonicated for a further 15 min. Joined organic extracts were concentrated and analyzed as described above (*cf* . paragraph 2.3.1).

Each type of extraction was performed in triplicate and the obtained EO was analyzed with three runs.

Chromatographic analysis

1. Gas chromatography (GC-FID)

Analytical gas chromatography was carried out on a Hewlett-Packard 6890 gas chromatograph series II (Agilent Technologies, Palo Alto, Ca, USA) equipped with HP-Innowax and HP-5 (60 m × 0.25 mm, 0.25 µm film thickness) capillary columns. Samples (1 µL) were injected with a split ratio of 1:60 and a continuous flow rate of 1.6 mL/min of chromatographic grade nitrogen was used. The oven temperature was initially held for 10 min at 35°C, ramped at 3°C/min up to 205°C and held isothermal for 10 min. Injector and FID detector temperature were held at 250 and 300°C, respectively.

2. Gas chromatography-Mass spectrometry (GC-MS)

The GC-MS analyses were performed on a gas chromatograph HP 6890 (II) interfaced with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, Ca, USA) with electron impact ionization (70 eV). A HP-5MS capillary column (60 m × 0.25 mm, 0.25 µm film thickness) was used. The column temperature was programmed to rise from 40 to 280°C at a rate of 5°C/min. The carrier gas was helium with a flow rate of 1.2 ml/min. Scan time and mass range were 1 s and 50-550 *m/z*, respectively.

3. Components identification

The volatile compounds were identified by comparison of their retention indices relative to (C_7-C_{40}) *n*-alkanes with those of literature and/or with those of authentic compounds available in our laboratory, and by matching their mass spectral fragmentation patterns with corresponding data (Wiley 275.L library) and other published mass spectra (Adams 2001) as well as by comparison of their retention indices with data from the Mass Spectral Library "Terpenoids and Related Constituents of Essential oils" (Dr. Detlev Hochmuth, Scientific consulting, Hamburg, Germany) using the MassFinder 3 software (www.massfinder.com). Quantitative data (%) were obtained from the electronic integration of the FID peak areas without the use of the correction factors.

Data (EO yields, percentage components and compounds chemical classes) were analyzed by Statistica v. 5 (Statsoft 1998) using ANOVA with the least significant difference (LSD) at the 0.05 probability level.

RESULTS AND DISCUSSION

Extraction yield and time

The use of HD, USE and SDH gave an EO with an average yield of 0.1, 0.36 and 0.07% (w/w), respectively (**Table 1**).

As is expected, the mean yield of EO obtained by USE

Table 1 Relative peak area (%) of the identified components of the essential oil extracted from the aerial parts of *H. triquetrifolium* by three different methods.

Compounds	RI[a]	HD[b]	USE[c]	SDH[d]
Yield (w/w)		0.1[b]	0.36[a]	0.07[b]
n-Octane	800 (800)	16.3[a]	17.6[a]	9.2[b]
trans-2-Hexenal	853 (1231)	0.1[b]	0.1[b]	1.3[a]
2-Methyl octane	882	8.3[a]	5.4[b]	3.4[c]
n-Nonane	900 (902)	3.5[ab]	5.1[a]	2.6[b]
α-Pinene	940 (1032)	11.3[b]	14.3[a]	8.8[c]
Camphene	954 (1076)	-	-	0.3[a]
Benzaldehyde	962 (1522)	0.1[a]	-	0.1[a]
Sabinene	979 (1132)	0.3[a]	0.1[b]	0.4[a]
3-Methyl nonane	980 (965)	3[a]	3.2[a]	0.1[b]
2-Pentylfuran	981 (1244)	0.1[b]	-	0.3[a]
β-Pinene	983 (1118)	0.6[a]	0.8[a]	0.1[b]
6-Methyl-5-hepten-2-one	988	0.1[a]	-	-
p-Cymene	1026 (1280)	-	-	0.4[a]
Limonene	1030 (1203)	0.7[c]	1.1[b]	1.7[a]
1-8-Cineol	1033 (1213)	0.2[a]	0.2[a]	0.1[b]
γ-Terpinene	1059 (1255)	-	-	1.2[a]
2-Methyl decane	1064 (1065)	0.6[b]	0.4[b]	0.8[a]
cis-Linalool oxide (furanoid)	1070 (1478)	0.3[a]	-	0.1[b]
trans- Linalool oxide (furanoid)	1087 (1450)	0.2[a]	-	0.1[ab]
Terpinolene	1088 (1290)	0.6[b]	0.3[c]	1.4[a]
Undecane	1098 (1100)	1.3[c]	1.9[b]	2.4[a]
Linalool	1101 (1553)	1.9[b]	2.4[a]	1.2[c]
cis-p-menth-2-en-1-ol	1130 (1638)	0.1[b]	0.3[a]	0.3[a]
Comphor	1144 (1532)	-	-	0.4[a]
Decanal	1180 (1722)	-	-	0.8[a]
Terpinene-4-ol	1187 (1611)	0.1[ab]	0.2[a]	-
α-Terpineol	1189 (1706)	1.1[b]	3.1[a]	1.5[b]
Geraniol	1255 (1857)	2[a]	0.2[b]	1.9[a]
Tridecane	1297 (1300)	0.1[c]	0.3[b]	0.8[a]
α-Cubebene	1348 (1468)	-	-	0.9[a]
α-Longipinene	1353	-	0.4[a]	-
Eugenol	1355 (2192)	0.7[c]	0.9[b]	1.6[a]
α-Copaene	1374 (1497)	0.4[a]	0.3[ab]	0.2[c]
Gearnyl acetate	1383 (1765)	0.1[a]	-	0.1[a]
Tetradecane	1398 (1400)	0.3[b]	0.4[b]	0.6[a]
β-Elemene	1406 (1596)	1[a]	0.8[a]	0.4[b]
β-Cubebene	1417 (1549)	3.5[a]	2.6[b]	1.9[c]
β-Caryophyllene	1420 (1612)	9.4[b]	11.8[a]	6.4[c]
trans-Cinnamyl acetate	1424	0.1[a]	0.1[a]	-
α-Humulene	1452 (1687)	1.5[ab]	1.9[a]	1.4[b]
Allo-Aromadendrene	1458 (1661)	2.2[a]	2.5[a]	1.6[b]
α- Amorphene	1474	3.8[a]	1.3[c]	2.5[b]
γ-Muurolene	1477 (1690)	0.1[b]	0.1[b]	0.3[a]
Germacrene-D	1480	4.9[a]	3.5[b]	3.4[b]
β-Ionone	1482 (1958)	0.1[a]	-	-
α-Selinene	1485 (1745)	3.1[b]	2.3[c]	3.4[a]
γ-Patchoulene	1503	2[a]	0.9[b]	0.5[c]
γ-Cadinene	1511 (1776)	1.9[b]	0.3[c]	2.5[a]
δ-Cadinene	1525 (1773)	3[a]	1.4[c]	2.3[b]
Lauric acid	1566 (2503)	-	0.1[b]	1.3[a]
Caryophyllene oxide	1569 (2008)	1.1[c]	2.4[a]	1.6[b]
Hexadecane	1598 (1600)	0.1[b]	0.2[b]	0.8[a]
Spathulenol	1576 (2153)	1.7[a]	0.5[b]	2[a]
T-Cadinol	1633 (2187)	1.6[a]	0.9[c]	1.2[b]
α-Muurolol	1642	0.1[b]	0.1[b]	0.3[a]
α-Cadinol	1652 (2255)	0.7[ab]	1[a]	0.4[b]
Octadecane	1800 (1800)	0.4[b]	0.5[b]	0.8[a]
Myristic acid	1808 (2713)	0.2[c]	0.4[b]	1.1[a]
Nonadecane	1897 (1900)	0.1[c]	0.2[b]	0.6[a]
Eicosane	1996 (2000)	0.3[c]	0.6[b]	1.3[a]
Total identified		97.71	95.79	83.15

[a] Retention Indices (RI) according to (C_7-C_{20}) on the HP-5MS and HP-Innowax column in parenthesis. -: Not detected.
[b] Hydrodistillation (HD)
[c] Ultrasound extraction (USE)
[d] Soxhlet/dynamic headspace
Mean values followed by different letters within raw are different at $P<0.05$ according to the LSD test.

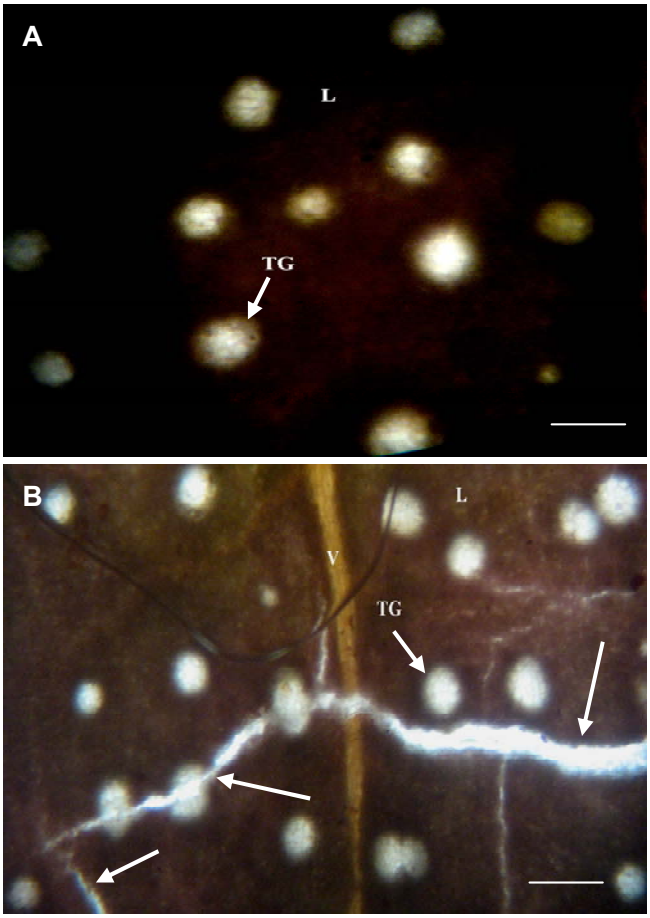

Fig. 1 *H. triquetrifolium* leaves before treatment with ultrasound (**A**) and after ultrasound treatment (**B**). V: Vein; L: Lamina; TG: Translucent glands. White bar (1 mm). White arrows show macrofracture induced by Ultrasound treatment.

was significantly higher than those obtained by HD and SDH. The yields of the latter extractives methods looked nearly identically. Comparing the expenditure of time, SDH does not represent a real alternative, while a significant reduction of the extraction time concomitant to an improvement of extraction efficiency were obtained by USE. The mechanical effects of ultrasound which induce a greater penetration of solvent into cellular materials and improve mass transfer were considered as the two major factors leading to the enhancement of extraction efficiency (Wang and Weller 2006).

In order to probe the impact of ultrasound vibrations on plant material, microscopic investigation was carried out. As shown in **Fig. 1**, ultrasound treatment induces significant degradation of leave tissues which is in good agreement with the results of Toma *et al.* (2001), who showed similar effects on mint leaves sonicated at 20 KHz.

Moreover, a clear macrofractures in close vicinity to translucent glands (TG) which contains EO could be easily distinguished but surprisingly, numerous TG stay intact. Unfortunately, because of the lack of equipment, it is not possible to perform electron microscopy of the treated samples. Nevertheless, it appears that the release of the EO from the TG could be enhanced by the ultrasound induced microfractures in the cell wall which becomes more malleable. This phenomenon allowed better diffusion of the extracting solvent and washing out the cell content as reported previously (Vinatoru 2001; Li *et al.* 2004).

Composition of the EO

The list of detected compounds with their retention indices and relative percentages are given in **Table 1** in order of their elution in the HP-5MS column. Altogether, 60 compo-

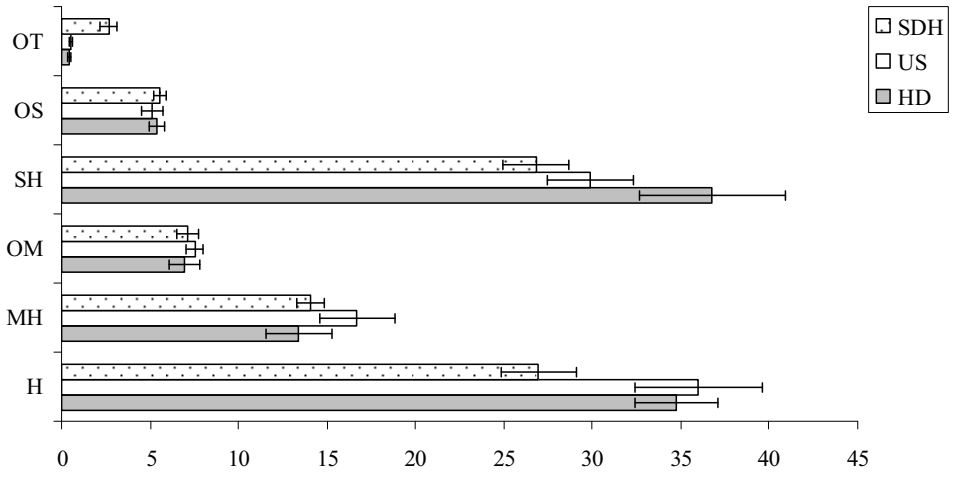

Fig. 2 Main chemical classes (%) of the essential oil composition obtained by three different methods. H: Hydrocarbons; MO: Monoterpene hydrocarbons; OM: Oxygenated monoterpenes; SH: Sesquiterpene hydrocarbons; OS: Oxygenated sesquiterpenes; OT: Other. HD: hydrodistillation ; USE : Ultrasound extraction; SDH : Soxhlet/dynamic headspace. Values represent mean ± Standard Error (SE). * Mean values followed by different letters are different at $P<0.05$ according to LSD test.

nents have been identified, accounting from 97.71, 95.76 and 83.15% for HD, USE and SDH, respectively of the whole oils. Forty two components of them are common to the three extractives methods. Hydrocarbons (26.94-36%) and sesquiterpene hydrocarbons (26.82-36.79%) predominated in all EO samples (**Fig. 2**).

The major components of the EO obtained by the aforementioned methods were *n*-octane (17.6-9.2%), α-pinene (14.3-8.8%), β-caryophyllene (11.8-6.4%), 2-methyloctane (8.3-3.4%), *n*-nonane (5.1-2.6%), germacrene D (4.9-3.4%), α-selinene (3.4-2.3%) and β-cubebene (3.5-1.9%). The monoterpene hydrocarbons fraction was present with higher amount in the EO obtained by USE when compared with those obtained by HD and SDH. This fraction was clearly dominated by α-pinene with a relatively higher content (14.3%) when extracted by USE. The lower content of this component in the EO obtained by HD (11.3%) and SDH (8.8%), respectively, was probably due to its transformation under the drastic condition notably higher temperature associated to acidic conditions during the extraction process.

The oxygenated fraction (mono- and sesquiterpenes) advocated as the most valuable components because of their high odoriferous characters (Roldán-Gutiérrez *et al.* 2008) were extracted with approximately the same content by the three methods. The content of this fraction was lower and do not exceed (7.5%) for oxygenated monoterpenes while the content of oxygenated sesquiterpenes was ranged from 5.1 to 5.6%. Geraniol, linalool and α-terpineol were the major oxygenated monoterpenes detected in all EO. Moreover, geraniol was preferably extracted by HD (2%) and SDH (1.9%), while linalool (2.4%) and α-terpineol (3.1%) were better extracted by USE. The major oxygenated sesquiterpenes; caryophyllene oxide and α-cadinol present the same trend of the oxygenated monoterpenes linalool and α-terpineol. In contrast, spathulenol and T-cadinol were preferably extracted by HD and SDH. Obviously, other components mainly myristic and lauric acids were predominantly presents in the EO obtained by SDH since this method was considered as a reference one for the extraction of fat and lipids.

Two minor components of the EO (6-methyl-5-hepten-2-one and β-ionone) were obtained by HD only, while camphene, camphor, *p*-cymene, γ-terpinene, terpinolene and decanal were only extracted by SHD. Terpinen-4-ol and *trans*-cinnamyl acetate were extracted by HD and USE.

Other minor components as benzaldehyde, amylfuran, linalool oxide (*cis* and *trans*) and geranyl acetate were found in the EO extracted by HD and SDH. These components namely benzaldehyde and linalool oxide were probably formed in the course of extraction. They could be produced by thermal degradation of carbohydrates via the Maillard and/or Strecker degradation reactions (Jerković *et al.* 2007; Adamiec *et al.* 2008). The component amylfuran seem to be a product of lipid peroxidation or thermal degradation of carbohydrate. Both formation pathways were accelerated by prolonged heating as reported by Ho *et al.* (2007) and Jerković *et al.* (2007). Hence, the occurrence of these compounds mainly amylfuran, benzaldehyde and linalool oxide in the EO obtained by HD and SDH could be considered as thermal artefacts since they are not detected in the EO obtained by USE.

Surprisingly, a significantly higher content of limonene, considered as a key taxonomic marker by Mathis and Ourisson (1964) for the infrageneric classification of the genus *Hypericum* was observed in the EO obtained by SDH (1.7%) and USE (1.1%). Enhancement of limonene extraction by USE was previously reported (Vinatoru 2001). Recently, the efficiency of USE for the extraction of limonene from laurel (*Laurus nobilis*) and oregano (*Oreganum majorana*) was evidenced by Roldán-Gutiérrez *et al.* (2007).

The insolubility of this component could justify its lower amount in the distillate contrarily to USE extraction where the ultrasonic vibration improved the solubility of limonene in the extracting solvent. In contrast, according to Chemat *et al.* (2004), the higher amount of limonene in the USE and SDH extract could be attributable to lipid oxidation.

Overall, obtained results differed greatly with those previously reported for the same species, but using HD as indicated above. Nevertheless, the abundance of hydrocarbons and sesquiterpene hydrocarbons considered as the characteristic components of *H. triquetrifolium* confirms the findings of Bertoli *et al.* (2003) and Petrakis *et al.* (2005).

CONCLUSIONS

The comparative analysis of the EO of *H. triquetrifolium* obtained by three extractive methods (USE, HD and SDH) has shown significant qualitative and quantitative differences. Due to its higher efficiency, saving time, low energy cost (the energy cost required for water or solvent evaporation in HD and SDH, respectively, surpassed that required in USE), reduction of thermal degradation and its capability to extract some valuable components notably α-pinene, linalool and β-caryophyllene, the use of USE seem to be a promising alternative in the possessing of EO. However, in the specific case of *H. triquetrifolium* and other related species from the same genus, it appears that the use of USE was limited since there is no standard chemical composition and our still incomplete knowledge of its detailed chemical

composition. The SDH methodology, since it presents the lower yield and thermal degradation was not recommended for the extraction of the EO.

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