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ORIGINAL ARTICLE

Chemical composition and bioactivity of essential oils of *Hypericum helianthemoides*, *Hypericum perforatum* and *Hypericum scabrum*

Abdollah Ghasemi Pirbalouti[1,2], Maryam Fatahi-Vanani[1], Lyle Craker[2], and Hamzeali Shirmardi[3]

[1]*Department of Medicinal Plants, Shahrekord Branch, Researches Center of Medicinal Plants & Ethno-veterinary, Islamic Azad University, Shahrekord, Iran,* [2]*Medicinal Plants Program, College of Natural Science, University of Massachusetts, Amherst, MA, USA, and* [3]*Herbarium of Research Center of Agriculture and Natural Resources, Chaharmahal va Bakhtiari, Iran*

Abstract

Context: A number *Hypericum* species are well known for their therapeutic efficacy and use in traditional medicine. The various species of *Hypericum* have been traditionally used for the treatment of wounds, eczema, burns, trauma, rheumatism, neuralgia, gastroenteritis, ulcers, hysteria, bedwetting and depression.
Objective: This study evaluated the *in vitro* antioxidant, antibacterial and phytochemical properties of essential oils of *Hypericum helianthemoides* (Spach) Boiss., *Hypericum perforatum* L. and *Hypericum scabrum* L. (Hypericaceae) collected from alpine region of Southwest Iran.
Materials and methods: The essential oils obtained from dried flowering aerial parts of three *Hypericum* species were analyzed by gas chromatography and gas chromatography/mass spectrometry to determine chemical compositions. The antibacterial activity of essential oils within concentration ranges from 16 to 500 μg/mL was individually evaluated against *Bacillus cereus, Listeria monocytogenes*, *Proteus vulgaris* and *Salmonella typhimurium*. The 1,1-diphenyl-2-picrilhydrazyl (DPPH) radical scavenging activity of essential oils was determined using DPPH assay.
Results: Essential oil yield of *H. helianthemoides*, *H. scabrum* and *H. perforatum* were 0.12, 0.20 and 0.21 mL/100 g dried material, respectively. The major constituents of the essential oils were α-pinene (12.52–49.96%), β-pinene (6.34–9.70%), (*E*)-β-ocimene (4.44–12.54%), β-caryophyllene (1.19–5.67%), and germacrene-D (2.34–6.92%). The essential oils of three *Hypericum* species indicated moderate-to-good inhibitory activities against four bacteria, especially against *L. monocytogenes*.
Discussion and conclusion: The essential oils of the three studied *Hypericum* species sourced in alpine region of West Iran were rich in monoterpene and sesquiterpenes hydrocarbons. Among the three tested species, the essential oil of *H. scabrum* showed the highest antibacterial and antioxidant activities.

Keywords

α-Pinene, antibacterial activity, antioxidant activity, high altitude

History

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Introduction

Hypericum L., an important genus in the family Hypericaceae, includes 484 species of herbs, shrubs and trees (Crockett & Robson, 2011). This genus, which grows in temperate regions, includes many species with traditional value as medicinal plants for treating wounds, eczema and burns (Yazaki & Okada, 1994). Many *Hypericum* species are used as treatment of trauma, rheumatism, neuralgia, gastroenteritis, ulcers, hysteria, bedwetting and depression (Miller, 1998). *Hypericum* species have also been used for sedative, anti-inflammatory and antiseptic effects (Baytop, 1984; Mukherjee & Suresh, 2000; Ozturk et al., 2002).

Primary constituents of *Hypericum* species include hypericin, tannins, flavonoids, phenolic acids, quercitrin, hyperoside, isoquercitrin, chlorogenic acid and rutin (Barnes et al., 2001; Dall'Agnol et al., 2003). Bioactivity properties of the plants include antimicrobial (Crockett, 2010; Dall'Agnol et al., 2003; Jayasuriya et al., 1989), anticancer (Agostinis et al., 2002), antidepressant (Butterweck et al., 2002), antiviral (Meruelo et al., 1988), antioxidant (Cakir et al., 2003), cytotoxic (Jayasuriya et al., 1989) and antifungal activities (Cakir et al., 2005; Fenner et al., 2005).

The essential oils of *Hypericum* species grown in different regions of the world have been extensively examined by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). For example, *Hypericum scabrum* L. and *Hypericum perforatum* L. in Turkey (Cakir et al., 1997; Erken et al., 2001), *Hypericum olympicum* L. and *H. perforatum* in Serbia (Gudzic et al. 2001) and *H. scabrum* and *H. perforatum* in Uzbekistan (Baser et al., 2002). Typical essential oil constituents for *Hypericum* species include the

Correspondence: Abdollah Ghasemi Pirbalouti, Department of Medicinal Plants, Shahrekord Branch, Researches Center of Medicinal Plants & Ethno-veterinary, Islamic Azad University, Rahmatieh 88146, Shahrekord, Iran. Tel: +1 (413) 545-2570. Fax: +1 (413) 545-3958. E-mail: ghasemi@iaushk.ac.ir; aghasemipir@psis.umass.edu.

monoterpenes α- and β-pinene, limonene and myrcene; the sesquiterpenes β-caryophyllene and caryophyllene oxide; and hydrocarbons such as *n*-decane, C_{16}- and C_{29} alkanes and C_{24}, C_{26} and C_{28} alkanols (Crockett, 2010; Nahrstedt & Butterweck, 1997). A study by Javidnia et al. (2008) indicated that the major constituent of *H. scabrum* collected from Fars (South Iran) was α-pinene, while the main constituents of *Hypericum helianthemoides* (Spach) Boiss., collected also from Fars (South Iran), were β-caryophyllene (23.3%) and spathulenol (17.4%). Motavalizadehkakhky (2012) reported the main constituents of essential oils of *H. scabrum, H. hyssopifolium* Chaix, *H. helianthemoides* and *H. perforatum* flowers collected from North-East Iran (Khorasan) were α-pinene and β-caryophyllene.

Species of this genus growing wild in western and northern parts of Iran include *H. helianthemoides*, *H. perforatum* and *H. scabrum*, which are perennial herbs, widely distributed at high altitude regions in Iran (Rechinger, 1963–1998). This study was designed to elucidate the chemical compositions, antibacterial and antioxidant activities of the essential oils of *H. helianthemoides*, *H. scabrum* and *H. perforatum* growing wild in Bakhtiari Zagros Mountains in Iran.

Materials and methods

Plant materials

Inflorescences of *H. helianthemoides*, *H. scabrum* and *H. perforatum* were collected during the mid-flowering stage from five plants of each species in three replications in July, 2012, the Bakhtiari Zagros Mountains (latitude 31° 20′ to 31° 50′; longitude 50° 50′ to 51° 05′; 2700 to 3000 m above sea level), Chaharmahal va Bakhtiari province, Iran. Plant identities were confirmed by Prof. V. Mozaffarian, and voucher specimens have been placed in the Herbarium of Research Center of Agriculture and Natural Resources, Chaharmahal va Bakhtiari, Iran (*H. helianthemoides* 1334; *H. perforatum* 1335; and *H. scabrum.* 1336).

The fresh samples of *H. helianthemoides*, *H. scabrum* and *H. perforatum* were dried inside for five days at room temperature (25 ± 5 °C), and ground to fine a powder using Moulinex food processor (Paris, France) and passed through a 20 mesh sieve to remove large pieces of debris. The ground samples were subsequently dried to a constant weight over a desiccant (Na_2SO_4) at room temperature (30 °C). The essential oil was extracted from 30 g of sample of tissue in 300 mL of water contained in a 500 mL flask and heated by heating jacket at 100 °C for 3 h in a Clevenger-type apparatus according to the British Pharmacopoeia (British Pharmacopoeia Commission, 1988). The collected essential oil was dried over anhydrous sodium sulfate and stored at 4 °C until analyzed.

Identification of the oil constituents

Composition of the essential oils were determined by GC and GC/MS. GC analysis was done on an Agilent Technologies 7890 GC equipped with flame ionization detector and a HP-5MS 5% capillary column (30.00 m × 0.25 mm, 0.25 μm film thicknesses). The carrier gas was helium at a flow of 0.8 mL/min. Initial column temperature was 60 °C and programmed to increase at 4 °C/min to 280 °C. The split ratio was 40:1. The injector temperature was set at 300 °C. The purity of helium gas was 99.999%, and 0.1 μL samples were injected manually in the split mode.

GC/MS analysis was done on the mentioned Agilent Technologies 5975 mass system. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 50–550. Constituents were identified by comparison of their Kovats index (KI) relative to C5–C24 *n*-alkanes obtained on a nonpolar DB-5MS column by comparison of the KI, provided in the literature, by comparison of the mass spectra with those recorded by the NIST 08 (National Institute of Standards and Technology, Gaithersburg, MD) and ChemStation Data System (WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany). The individual constituents were identified by retention indices and compared with constituents known from the literature (Adams, 2007; McLafferty, 2009). The percentage composition was computed from the GC peak areas without using any correction factors.

Antibacterial test

The four bacteria used as test organisms were as follows: *Bacillus cereus*, *Listeria monocytogenes*, *Proteus vulgaris* and *Salmonella typhimurium*. The bacteria strains were obtained from Food Microbiology Laboratory, Veterinary Medicine Faculty (I.A.U.), Iran. Bacterial strains were identified using polymerase chain reaction-restriction fragment length polymorphism and conventional morphological and biochemical tests. The density of bacteria culture required for the test was adjusted to 1.0 McFarland standards (1.0×10^7 colony-forming units (CFU)/mL) and measured using a spectrophotometer (Eppendorf AG, Hamburg, Germany). The minimum inhibitory concentration (MIC) values were evaluated using the broth serial dilution method according to standard methods (CLSI, 2012). Bacterial strains were cultured overnight at 37 °C in Muller Hinton broth (MHB). The essential oils and antimicrobial standards (ampicillin, ciprofloxacin and flumequine) dissolved in 5% dimethyl sulfoxide were first diluted to the highest concentration (500 μg/mL) to be tested, and then series of two-fold dilutions were made in a concentration range from 16 to 500 μg/mL in 10 mL sterile test tubes containing MHB. After incubation at 37 °C for 24 h, absorbance at 630 nm was used as a measurement of bacterial growth using a spectrophotometer (Zampini et al., 2005). The minimum bactericidal concentration (MBC) of essential oil were determined according to the MIC values, i.e., 5 μL from MIC tubes were transferred to agar plates and incubated at 37 °C for 24 h. The MBC was referred to the minimum concentration of essential oil with no viable bacteria. Experiments were performed at three different times.

Antioxidant test

The 1,1-diphenyl-2-picrilhydrazyl (DPPH) radical scavenging activity of essential oils was determined using the method proposed by Hung et al. (2005). The essential oils at different concentrations (16–500 μg/mL) were mixed with the same volume of 0.2 mM methanol solution of DPPH. The disappearance of DPPH by essential oils after 30 min of incubation at room temperature was determined

spectrophotometrically at 515 nm. Methanol was used to zero the spectrophotometer. The absorbance of the DPPH radical without antioxidant, i.e., the control, was measured daily. The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC_{50}) was calculated graphically. The percentage inhibition was calculated according to the equation:

$$\% \text{ inhibition} = \left[\frac{A_{C(0)} - A_{A(t)}}{A_{C(0)}}\right] \times 100$$

where $A_{C(0)}$ is the absorbance of the control at $t = 0$ min; and $A_{A(t)}$ is the absorbance of the antioxidant at $t = 30$ min. The food preservative butylated hydroxyanisole was used as positive control.

Statistical analyses

The data were statistically analyzed by SPSS (19.0) software (SPSS Inc., Chicago, IL) using a completely randomized design. Means of the traits were separated by Duncan's multiple range test at $p \leq 0.05$ level.

Results and discussion

Oil yield

All essential oils extracted from the flowering aerial parts of *H. helianthemoides*, *H. scabrum* and *H. perforatum* produced a clear, yellow liquid. An analysis of variance indicated no significant differences in oil yields obtained from the three *Hypericum* species. The essential oil yields were 0.12, 0.20 and 0.21 mL/100 g dry matter for *H. helianthemoides*, *H. scabrum* and *H. perforatum*, respectively (Table 1). *Hypericum* species are generally classed as essential oil-poor plants (oil yields generally <1%, w/w) (Roth, 1990). Gudzic et al. (2001) reported the oil yield of *H. perforatum* collected from Serbia was 0.32% (w/w). The yield of the oils extracted from *Hypericum* species was: 0.97% for leaves and 1.30% (w/w) for flowers of *H. androsaemum* L. from Iran (Morteza-Semnani & Saeedi, 2005), 0.10% (w/w) for *H. perfoliatum* and 0.13% for *H. tomentosum* L. (Hosni et al., 2008) and 0.2% for *H. scabrum* and 0.1% (w/w) for *H. perforatum* from Uzbekistan (Baser et al., 2002). Javidnia et al. (2008) reported the oil yields of *H. scabrum*, *Hypericum dogonbadanicum* Assadi, *H. helianthemoides* and *H. hirtellum* (Spach) Boiss., collected from south Iran (Fars province), were 0.05, 0.10, 0.06 and 0.07%.

Chemical compositions

In total, 33, 31 and 48 volatile constituents were identified representing 95, 96 and 89% of total volatiles in the essential oils of *H. helianthemoides*, *H. scabrum* and *H. perforatum*, respectively (Table 1). The main constituents in the volatile oil of *H. helianthemoides* were α-pinene (31.9 ± 1.9%), (*E*)-β-ocimene (12.5 ± 1.0%), β-phellandrene (8.4 ± 1.1%), β-pinene (6.3 ± 0.4%), β-caryophyllene (5.7 ± 0.4%) and germacrene-D (4.3 ± 0.7%). Javidnia et al. (2008) identified 71 constituents in the volatile oil of *H. helianthemoides* from south Iran. The major constituents were β-caryophyllene (23.3%), spathulenol (17.4%), 14-hydroxy-9-epi-(*E*)-caryophyllene (15.6%) and α-pinene (6.7%) (Javidnia et al., 2008). A report by Ferretti et al. (2005) indicated the main constituent in the oil of *H. richeri* Vill. from Italy was (*E*)-β-ocimene (19.5%). The major constituents in the volatile oil of *H. scabrum* were α-pinene (50.0 ± 7.6%), β-pinene (9.7 ± 3.7%), limonene (6.6 ± 2.9%), (*E*)-β-ocimene (5.6 ± 1.8%) and carvacrol (5.8 ± 3.1%). Results of a study by Javidnia et al. (2008) indicated that the main constituent in *H. scabrum* oil was α-pinene (59.3%); our results are in agreement with this report. Results from other studies (Javidnia et al., 2008; Sajjadi et al., 2001) indicated the major constituents of *H. dogonbadanicum* oils were α-pinene, β-pinene, limonene and camphene. The main constituents in the volatile oil of *H. perforatum* were α-pinene (12.5 ± 1.0%), β-pinene (8.3 ± 1.7%), undecane (7.0 ± 0.5) and germacrene-D (6.9 ± 0.1%). Many studies on the essential oil content of *H. perforatum* indicate the enormous variability inherent in the volatile chemistry of this species (Crockett, 2010).

Our results showed significant differences among the three studied *Hypericum* species for percentages of α-pinene, β-myrcene, limonene, α-terpinene, (*E*)-β-ocimene and germacrene-D, while no significant differences among the three studied *Hypericum* species for percentages of β-pinene and carvacrol were apparent (Table 1). The highest percentage of α-pinene was observed in *H. scabrum*, while the lowest percentage was obtained from *H. perforatum* (Table 1). Similarly, α-pinene is the major constituents in other *Hypericum* species (Baser et al., 2002; Couladis et al., 2001; Crockett et al., 2007; Javidnia et al., 2008; Nogueira et al., 2008; Yuce & Bagci, 2012). Akhbari et al. (2012) reported that the content of α-pinene differs greatly between the essential oil extracted from flowers (70.2%) and fruit (25.4%) of both *H. perforatum* and *H. scabrum* growing wild in Iran. An examination of *H. androsaemum* by Giuliani et al. (2010) indicated that the marginal glands of leaves contained β-caryophyllene and germacrene-B as the dominant volatile constituents, while the laminar glands contained mainly β-pinene and limonene.

Generally, monoterpene hydrocarbons (31–81%) and sesquiterpenes hydrocarbons (6.2–32.3%) were the main chemical groups in the volatile oils from the three *Hypericum* species tested in this study (Figure 1). Guedes et al. (2012) reported the main essential oil constituents identified in 40 species of *Hypericum* were monoterpenoids (α-pinene and β-pinene) and sesquiterpenoids (*E*-caryophyllene, germacrene-D, caryophyllene oxide, spathulenol and globulol) constituents. Characterization of essential oil from species of the *Hypericum* genus revealed the presence of monoterpenoid and sesquiterpenoid constituents, as well as alkanes and aldehydes, as the main constituents in the most of the plants (Mathis & Ourisson, 1964a,b,c). Our results are in agreement with those of others reporting monoterpenes as the main constituents.

A comparison of our results with the previous reports on the chemical composition of *Hypericum* species suggests differences in the volatile composition of the plant material could be attributed to genetic (genus, species, sub species and ecotype), chemotype, distinct environmental and climatic conditions, seasonal sampling periods, geographic origins, plant populations, vegetative plant phases and extraction and

Table 1. Identified constituents of the essential oils from the three studied *Hypericum* species.

			H. helianthemoides		*H. scabrum*		*H. perforatum*	
Components	RI[a]	ANOVA	Mean (%)[b]	±SD	Mean (%)	±SD	Mean (%)	±SD
Undecane, 5-methyl-	878						4.17	0.25
Nonane	903						1.39	0.39
α-Thujan	934		0.40	0.04	1.44	0.76	0.13	0.01
α-Pinene	941	$p \leq 0.05$	31.95ab[c]	1.85	49.96a	7.59	12.52b	1.00
α-Fenchene	953		0.23	0.04	0.07	0.08		
Camphene	954		0.70	0.11	0.24	0.08		
Nonane, 3-methyl-	969						2.39	0.39
β-Pinene	981	$p > 0.05$	6.34	0.39	9.70	3.67	8.26	1.74
β-Myrcene	994	$p \leq 0.05$	3.21a	0.15	3.64a	0.47	1.51b	0.71
Decane	1000						0.12	0.05
α-Phellandrene	1008		1.75	0.13	0.23	0.09	0.53	0.28
α-Terpinene	1019		1.84	0.10	0.72	0.40	0.11	0.01
p-Cymene	1027		1.15	0.45	0.34	0.06	0.18	0.06
Limonene	1028	$p \leq 0.05$	0.00b	0.00	6.66a	2.91	0.68b	0.21
β-Phellandrene	1029		8.37	1.07				
1,8-Cineole	1030						0.41	0.25
(*Z*) β-Ocimene	1035		1.13	0.11	0.38	0.20	1.71	0.14
(*E*) β-Ocimene	1046	$p \leq 0.01$	12.54a	1.02	5.64b	1.81	4.44b	0.16
γ-Terpinene	1057	$p \leq 0.01$	3.91a	0.23	1.69b	0.93	0.36b	0.12
Decane, 2-methyl-	1062						4.00	0.80
Terpinolene	1086		1.35	0.12	0.46	0.18	0.25	0.18
Undecane	1100						6.96	0.48
n-Nonanal	1104						0.22	0.12
1-Octanol, 2-methyl-	1104						1.05	0.07
Fenchol, endo	1111		0.17	0.03				
α-Campholene aldehyde	1125				0.10	0.09		
Borneol	1161		0.25	0.02	0.14	0.15		
Terpin 4 ol	1174		0.19	0.02	0.29	0.11		
Pulegone	1234						0.86	0.08
Thymol	1286		0.55	0.78	1.38	1.26		
Tridecane	1294						0.71	0.33
Carvacrol	1298	$p > 0.05$	2.13	2.79	5.84	3.12	0.00	0.00
α-Cubebene	1343		0.07	0.09	0.24	0.19	1.14	0.19
Ylangene	1365						0.49	0.03
α-Copaene	1369		0.49	0.04	0.12	0.11	1.57	0.63
β-Bourbonene	1378		0.28	0.03			0.22	0.12
β-Cubebene	1384						0.36	0.04
β-Elemene	1386		0.24	0.03	0.22	0.20		
α-Cedrene	1405						0.88	0.09
β-Caryophyllene	1412	$p \leq 0.01$	5.67a	0.41	1.19b	1.10	2.85b	0.37
Aromadendrene	1431		0.21	0.01			3.83	0.62
α-Humulene	1446		0.26	0.03	0.10	0.08	0.60	0.24
β-Farnesene <Z>	1452						0.87	0.06
Bicyclosesquiphellandrene < *Epi*->	1456						0.20	0.08
1-Dodecanol	1470						3.07	0.06
α-Amorphene	1471						1.77	0.14
Germacrene-D	1474	$p \leq 0.05$	4.32b	0.65	2.34b	1.14	6.92a	0.11
Bicyclogermacrene	1474		1.76	0.23	1.30	0.77		
β-Selinene	1480						2.39	0.46
α-Selinene	1489						4.18	0.24
α-Farnesene	1503						0.75	0.13
γ-Cadinene	1506	–	0.35	0.04	0.25	0.22	1.21	0.13
Δ-Cadinene	1516	–	0.91	0.10	0.41	0.30	2.07	0.08
1,4-Cadinadiene	1525						0.26	0.04
Spathulenol	1569	–	0.58	0.08	0.46	0.21	0.37	0.10
Caryophyllene oxide	1574						0.18	0.09
Germacrene B	1598						0.15	0.07
Hinesol	1629	–	0.25	0.03				
α-Cadinol < *Epi*->	1632				0.23	0.20		
β-Eudesmol	1640	–	0.58	0.06				
α-Eudesmol	1643	–	0.43	0.60				
Phytol	1648	–			0.23	0.20	1.03	0.10
Oil yield (v/w)		$p > 0.05$	0.12	0.05	0.22	0.08	0.20	0.07

[a]Retention indices (RI) relative to C5-C24 *n*-alkanes on HP-5MS capillary column.
[b]% GC peak.
[c]Means with different letters in a row are statistically significant at the 5% level probability. Values of major compounds are given as means ± SD.

quantification methods (Ghasemi Pirbalouti et al., 2013; Guedes et al., 2004; Petrakis et al., 2005; Radusiene et al., 2005; Southwell & Bourke, 2001; Teixeira et al., 2013).

Antibacterial and antioxidants activities

An antibacterial test of the essential oils from the three *Hypericum* species indicated relatively high inhibitory activities against four pathogenic bacteria tested (Table 2). The MICs of the essential oils were within concentration ranges from 62 to 250 µg/mL and the respective MBCs were from 250 to 500 µg/mL. The essential oil from *H. scabrum* had higher inhibitory activity against bacteria than the essential oil from the other two *Hypericum* species. The antibacterial activity of *H. scabrum* oil could be attributed to the relatively high level of α-pinene, a constituent with known antimicrobial properties (Stojkovic et al., 2008). Lipophilic constituents, including terpenoid derivatives, have been shown to disrupt cellular membranes in bacteria and fungi, thus inhibiting cellular respiration and ionic transport (Hayouni et al., 2008). The mechanisms by which essential oil can inhibit microorganisms vary. In some cases, it may be due to the hydrophobicity of the chemical (oil), which penetrates into the lipid bilayer of the cell membrane and makes the cells more permeable, leading to leakage of vital cell contents (Burt, 2004). The essential oil constituents move into the membrane, causing swelling and reducing membrane function that leads to cell death (Holly & Patel, 2005). Antimicrobial activity of essential oils may be due to the presence of synergy between the major constituents and other constituents of the oils leading to various degrees of antimicrobial activity.

Free radicals cause auto-oxidation of unsaturated lipids in food (Kaur & Perkins, 1991), and the antioxidant activity of essential oils could be attributed to their hydrogen donating ability. Antioxidant properties are very important in counteracting the deleterious role of free radicals in foods or biological systems. The antioxidant activity of essential oils of the three studied *Hypericum* species are expressed as IC_{50}. A low IC_{50} value indicates an active ability of the oil to act as a DPPH scavenger (Figure 2). The highest antioxidant activity of *H. scabrum* oil could be attributed to the relatively high level of α-pinene.

In conclusion, a comparison of our results with other reports on essential oil constituents and biological activities of *H. helianthemoides*, *H. perforatum* and *H. scabrum*

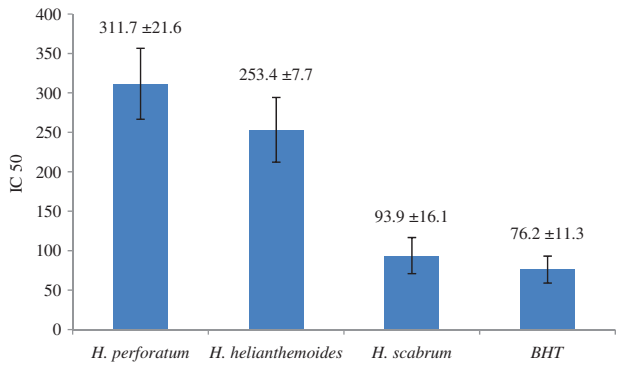

Figure 2. Antioxidant activity of the essential oils of the three studies *Hypericum* species using DPPH assay.

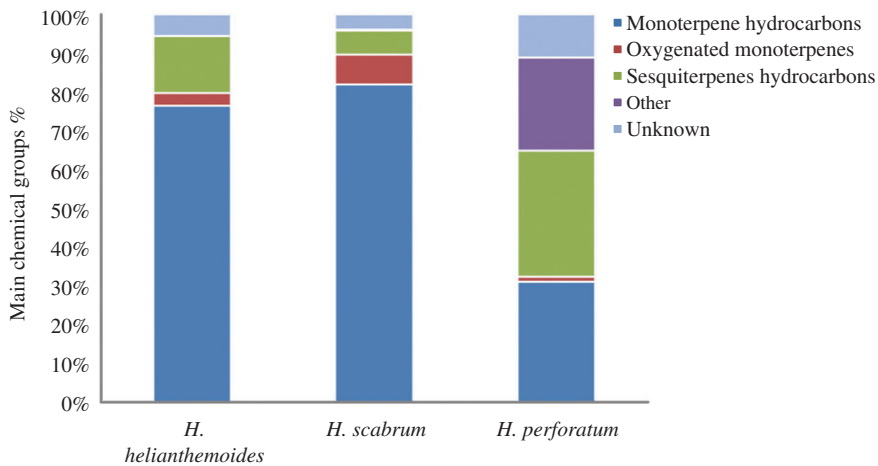

Figure 1. Comparison of main chemical groups (%) of the essential oils of the three studies *Hypericum* species.

Table 2. MICs and MBCs (µg/mL) of the essential oils of the three studied *Hypericum* species against four food-borne pathogens.

	H. helianthemoides		*H. scabrum*		*H. perforatum*		Fl[a]	Ci[a]	Am[a]
Pathogens	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MIC	MIC
Bacillus cereus	250	500	125	250	250	500	125	125	62
Listeria monocytogene	125	250	62	125	250	500	125	62	62
Pseudomonas aeruginosa	250	500	125	250	250	500	125	125	62
Salmonella typhimurium	125	500	125	250	500	500	62	125	125

[a]Fl: Flumequine; Ci: Ciprofloxacin; and Am: Ampicillin.

demonstrated these species have considerable variation in essential oil compositions and biological activities.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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