INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17F–107/2018/20–1–203–208
DOI: 10.17957/IJAB/15.0520
http://www.fspublishers.org

Full Length Article

Chemical Composition and Antioxidant Activity of the Essential Oil of *Hypericum patulum* (Family: Clusiaceae)

Jingyu Duan, Yaotang Zhang, Weihong Wu, Huankai Yao, Yan Li and Chunping Zhang*
Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, School of Pharmacy, Xuzhou Medical University, Xuzhou 221004, Jiangsu, China
*For correspondance: chunpingzhang520@163.com

Abstract

In this paper, the chemical constituents and antioxidant activity of the essential oils of aerial parts of *Hypericum patulum* Thumb. have been described. GC-MS was used for chemical analysis; whereas, DPPH and $ABTS^{\bullet+}$ radicals scavenging assays were performed to assess the antioxidant activities. Among 109 chemical constituents detected from essential oils before and after drying, the relative contents of nonane were highest being 32.57 and 17.12%, respectively. Monoterpenoids, sesquiterpenoids and alkanes were the main components of the volatile oils. Besides, azulenoids and other compounds were also detected. The antioxidant capacity assays showed that essential oils had weak antioxidant activity, but ethanol and methanol extracts exhibited significant scavenging activities against both DPPH and $ABTS^{\bullet+}$ radicals. Results suggested further constituent based activity studies on *H. patulum*. © 2018 Friends Science Publishers

Keywords: *Hypericum patulum* Thumb.; Essential oil; Antioxidant activity; GC-MS; Methanolic extract

Introduction

Hypericum patulum Thumb. (Family: Clusiaceae) is a half evergreen or handsome evergreen shrub and mainly distributed in the southwest, central and Taiwan area of China. Because of the beautiful green leaves and yellow flowers, *H. patulum* has been usually used as ornamental plants in the courtyard greening. It has also been used for the treatment of numerous disorders such as hepatitis, influenza, diarrhea, gonorrhea, traumatic injury, etc. (Jiangsu New Medical College, 2006). The genus Hypericum is represented by 350 species distributed throughout the world (Chinese Flora Editorial Board in Chinese Academy of Sciences, 1990; Wu *et al*., 2014). Up to now, numerous compounds with documented biological activities have been isolated from the plants of this genus, e.g., phloroglucinol, xanthones, flavonoids, volatile oil etc. Besides, Hypericum shows various bioactivities including antidepressive, antiviral, antibacterial and antitumor effects (Zhang *et al*., 1999; Lv *et al*., 2002; Xiao and Mu, 2007). Because of their various important biological activities, the pharmaceutical, chemical and biological scholars are stimulated to explore the chemical composition and bioactivities of this genus.

Free radicals are extensively involved in oxidative damages and can lead to various diseases including aging, carcinogenesis, inflammation, etc. (Bokhari *et al*., 2013). Antioxidants can interfere with oxidation process, chelate catalytic metals and act as oxygen scavengers to effectively eliminate these free radicals (Jayaprakash *et al*., 2001). The current synthetic antioxidants have been suspected to have side effects; therefore, an increasing attention has been paid to antioxidants from natural products (Guedes *et al*., 2013). Many herbal and medicinal plants are potential sources of natural antioxidants. The biologically active constituents including flavonoids, quinones, triterpenoids, steroids, etc. are known to possess potential antioxidant activity (Yagi *et al*., 2013). Chemical composition of the extracts of cell suspension cultures of *H. patulum* has been reported previously (Ishiguro *et al*., 1993, 1995a, 1995b, 1996, 1997, 1998, 1999). It was reported that the total extraction of this plant had cytotoxic (Vijayan *et al*., 2003) and antibacterial effects (Mukherjee *et al*., 2002). But there were no related reports about chemical composition of volatile oils and antioxidant activity of both volatile oils and the extract of aerial part of *H. patulum*. In this paper, essential oils from *H. patulum* aerial parts before and after drying were analysed for chemical composition and antioxidant activity.

Materials and Methods

Plant Material Identification

Aerial parts of *Hypericum patulum* were collected from Suqian, Jiangsu, China in July 2013, identified by an expert in the School of Pharmacy, Xuzhou Medical University. A voucher specimen has been kept for future references (HPT-01).

To cite this paper: Duan, J., Y. Zhang, W. Wu, H. Yao, Y. Li and C. Zhang, 2018. Chemical composition and antioxidant activity of the essential oil of *Hypericum patulum* (Family: Clusiaceae). *Int. J. Agric. Biol*. 20: 203–208

Chemicals

2, 2'-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan). The diammonium salt of 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS), and 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Steinheim, Germany). Vitamin C (VC) was provided by the Beijing Chemical Ltd Co., China. All organic solvents were analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). These solvents were distilled with rotary evaporator before use.

Isolation of the Essential Oils

The aerial parts were dried in shade, cut into 1 cm of small section and stored in a well closed vessel for future use. Fresh and dried aerial parts of plant materials (100 g) were respectively subjected to hydro-distillation for about 8 h according to the Chinese Pharmacopeia. The oils were dried with anhydrous sodium sulfate and stored in dark at + 4ºC until tested.

Organic Solvents Extracts Preparation

Ten grams of plant material (dried aerial parts of *H. patulum*) was finely powdered and introduced into five 100 mL covered conical flasks containing 100 mL each chloroform, ethyl acetate, acetone, ethanol and methanol as solvents. The flasks were placed at room temperature for 2 h. Then, the ultrasound-assisted extraction was performed in an ultrasonic bath (SK3300H, Shanghai Kedao Ultrasound Co. Ltd., China) for 30 min to obtain extracts 1 to 5 (E_1 to E_5). The extracts were filtrated by a polytetrafluoroethylene membrane (average pore size was 0.8 μm) and the filtrate was distillated and then further freeze dried. These extracts were stored in vacuo dryer for further antioxidant assays.

GC-MS Analysis and Identification of Compounds

The chemical constituents analysis of essential oils was carried out by a Hewlett Packard 6890/5973 gas chromatography/mass spectrometer (GC-MS, Hewlett-Packard, USA). The GC-MS spectrometer was equipped with a HP-5MS capillary column (crosslink 5% PH ME siloxane, 0.25 m film thickness 60 m × 0.25 mm i.d.,) and a quadrupole analyzer. Helium was used as the carrier gas. The temperature of injector and detector was 250 and 150ºC, respectively. The initial temperature was 60 ºC (kept for 2 min) and then gradually increased to 150ºC (held for 2 min) at a rate of 5ºC /min, then raised from 150 to 200ºC (held for 2 min) at a rate of 2ºC /min, finally, it raised from 200 to 280ºC (and held for 3 min) at a rate of 10ºC /min. The carrier gas linear velocity was 1.0 mL/min and the split ratio was 20:1. EI was used as the ion source and its temperature was 230ºC. The electron impact potential was 70 eV. The mass range scanned was from 33 to 500 amu.

In order to identify the structures of constituents of oils, the recorded mass spectra must be matched with the standard mass spectra from the National Institute of Standards and Technology (NIST05.LIB) libraries data offered by GC-MS system, literature data and standards of the main constituents.

DPPH Radical Scavenging Activity

This assay was carried out following Sunil *et al*. (2013) and modified for use by measuring absorbance at 517 nm. Basically, 0.1 mM ethanol solution of DPPH was prepared daily and stored in dark. Each sample was dissolved with ethanol in a volumetric flask to obtain a certain concentration ethanol solution. The DPPH radical scavenging capability was calculated with the following equation:

$$\text{DPPH Scavenging effect (\%)} = (1 - A_1/A_0) \times 100 \quad (1)$$

A_0 was the absorbance of the mixture of 1.9 mL DPPH solution and 100 μL ethanol. A_1 was the absorbance of the 2 mL mixture reaction system, including 1.9 mL DPPH solution, a certain volume sample solution and complemented ethanol. The analysis was carried out in triplicate.

The DPPH radical scavenging percentage was plotted versus the concentration of samples and the concentrations of samples required to obtain 50% inhibition (IC_{50}) were derived from the graph. Lower IC_{50} value corresponds to greater radical scavenging activity and antioxidant activity.

$ABTS^{\bullet+}$ Radical Scavenging Assay

The assay was conducted following Re *et al.* (1999) and modified. Briefly, 5 mL of fresh 7 mM ABTS solution in H_2O and 88 μL of 140 mM $K_2S_2O_8$ solution in H_2O were reacted at room temperature in dark place for about 16 h to produce the $ABTS^{\bullet+}$ cation radical. Then the $ABTS^{\bullet+}$ solution must be diluted to get an absorbance of 0.700 ± 0.025 at 734 nm with ethanol before usage, The $ABTS^{\bullet+}$ radical scavenge capability was calculated by the following equation:

$$ABTS^{\bullet+}\text{ Scavenging effect (\%)} = (1 - A_1/A_0) \times 100 \quad (2)$$

A_0 was the absorbance of the mixture of 1.9 mL $ABTS^{\bullet+}$ solution and 100 μL ethanol. A_1 was the absorbance of the reaction system of 1.9 mL $ABTS^{\bullet+}$ solution, a certain volume sample solution and complemented ethanol. All experiments were performed at least three times, and in triplicate. Slopes were calculated for the linear portion of graphs of absorbance versus concentration.

Trolox was a standard and the $ABTS^{\bullet+}$ radical scavenging capacity was expressed as the Trolox concentration (the Trolox equivalent antioxidant capacity, TEAC) which equals to the same scavenging capacity of the $ABTS^{\bullet+}$ radical as 1 mg/mL concentration at 734 nm.

Results

The Volatile Components of *H. patulum* before and after Drying

The hydro-distillation of the aerial parts of *H. patulum* before and after drying yielded 0.3 and 0.1% (v/w), respectively of pale yellowish oils. The chemical constituents of the essential oils are summarized in Table 1. In total, 109 compounds were detected from essential oils of *H. patulum*. Of these, 92 and 94 compounds were identified in essential oils of respective undried and dried aerial parts of the plant indicating two additional compounds in dried plant material. The most abundant component was nonane in essential oils before and after drying with contents of 32.57 and 17.12%, respectively. Monoterpenoids, sesquiterpenoids and alkanes were the main components of volatile oils of *H. patulum.* Besides, azulenoids and other compounds were also tested. All the constituents determined from the oils of this plant were the first reported so far.

Of all the 109 compounds, 32 kinds of monoterpenoids identified included five kinds acyclic monoterpenoids (No. 20, 26, 27, 34, 35), 12 kinds monocyclic monoterpenoids (No. 17, 22, 23, 24, 25, 28, 29, 30, 36, 40, 41 and 44) and 15 bicyclic monoterpenoids (No. 9, 10, 12, 13, 14, 15, 18, 34, 37, 38, 39, 42, 47, 49 and 51). The other monocyclic monoterpenoids except No. 29, 36 and 44 were all methane monocyclic monoterpenoids. Except No. 12, the other bicyclic monoterpenoids belonged to pinane and fenchane bicyclic monoterpenoids. In all monoterpenoids detected, the relative content of (1*R*)-2, 6, 6-Trimethylbicyclo [3.1.1] hept-2-ene (No.13) with 13.24 and 12.99% was highest in oils of *H. patulum* before and after drying, respectively. *D*-Limonene (No. 25) with the relative content of more than 5.0% may be the part of the fragrance of oils from *H. patulum*.

It also detected 42 kinds of sesquiterpenoids from the oils of *H. patulum* before and after drying. The majority was bicyclic sesquiterpenoids, including eight kinds of valerine sesquiterpenoids (No. 74, 87, 88, 91, 92, 98, 103 and 104), 13 kinds of cadinane sesquiterpenoids (No. 67, 69, 71, 72, 78, 79, 80, 81, 82, 85, 99, 100 and 102) and two kinds of copane sesquiterpenoids (No. 57 and 70). The relative contents of caryophyllene (No.62), (*E*)-β-famesene (No.65), humulene (No.68) and α-farnesene (No.76) were respectively more than 3.5% and significantly higher than that of other components (Table 1). These constituents may contribute to the key fragrance source of essential oils of *H. patulum* as well as *D*-Limonene.

It was worth mentioning that seven kinds of azulenoids (No. 60, 64, 66, 83, 89, 95 and 96) were detected from the essential oils of *H. patulum* before and after drying. Their structures have been shown in Fig. 1.

The substituent groups of azulene ring could be replaced and azulenoids had many good biological activities; therefore, azulenoids have become one of research focuses in pharmaceutical and pesticide field. At present, a large number of azulenoids have been synthesized with heterocyclic ring containing N and S atoms (Shoji *et al*., 2010, 2012).

DPPH Radical Scavenging Activity

The DPPH radical scavenging capacity results of all samples as well as vitamin C have been presented in Table 2. The essential oils of *H. patulum* before and after drying had little antioxidant activity to scavenge DPPH radical and the IC_{50} values of which were more than 20 mg of this plant per mL and could be neglected. E_5 showed the highest scavenging activity to DPPH radical with an IC_{50} value of 0.073 mg/mL. In addition, as a positive control, IC_{50} of vitamin C was 4.18 μg/mL.

$ABTS^{\bullet+}$ Radical Scavenging Activity

The reaction kinetics of samples and vitamin C showed that the absorbance value remained stable 40 min later, so all samples were detected after 40 min. Results (Table 2) revealed that all samples and vitamin C displayed the proton-donating ability. E_4 and E_5 gave the highest TEAC value of 22.75 μmol/L and showed the highest $ABTS^{\bullet+}$ radical scavenging capacity which was in accordance with DPPH radical scavenging capacity. Meanwhile, the essential oils of *H. patulum* before and after drying revealed weak $ABTS^{\bullet+}$ free radical scavenging capacity with low TEAC values. In addition, E_1, E_2 and E_3 exhibited more stronger antioxidant activity with higher TEAC values than vitamin C.

Discussion

Earlier studies have reported isolation and structural identification of prenylated xanthones, epicatechin, patulone, (-)-epicatechin, phloroglucinol derivatives, and imethylchromene derivatives from the extracts of cell suspension cultures of *H. patulum* (Ishiguro *et al*., 1993, 1995a, 1995b, 1996, 1997, 1998 and 1999). As far as ascertained, however, chemical composition and antioxidant activity of volatile oils/extracts of aerial part of *H. patulum* have not been investigated. This paper, therefore is the first report on chemical composition and antioxidant activity of the essential oils of undried and undried aerial parts of *H. patulum*. The present results revealed that monoterpenoids, sesquiterpenoids and alkanes were the main components of volatile oil detected in this study. Besides, azulenoids and other compounds were also detected. *D*-Limonene, caryophyllene, (*E*)-β-famesene, humulene and α-farnesene may contribute to the key fragrance source of essential oils of *H. patulum.* The relative content of monoterpenoids in the volatile oil of *H. patulum* before drying was higher than that of after drying. On the contrary, after drying, the relative content of sesquiterpenoids in the volatile oil was higher than that before drying.

Table 1: Chemical constituents of volatile oils from *H. patulum* Thumb. before and after drying

No.	t/min	Name of Components	matching degree	dried before φ/%	dried after φ/%
Monoterpenoids					
9	10.11	1,7,7-Trimethylbicyclo[2.2.1]hept-2-ene	96	—	0.009
10	10.13	2,7,7-Trimethylbicyclo[2.2.1]hept-2-ene	91	0.007	—
12	10.72	1-Isopropyl-4-methylbicyclo[3.1.0]hex-2-ene	94	0.136	0.034
13	10.98	(1*R*)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	96	13.238	12.986
14	11.38	7,7-Dimethyl-2-methylenebicyclo[2.2.1]heptane	95	0.055	0.105
15	11.44	Camphene	96	0.145	0.348
17	12.12	β-Phellandrene	91	0.022	0.026
18	12.28	(1*S*)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane	97	10.871	7.67
20	12.50	α-Myrcene	90	2.096	1.096
22	13.04	α-Phellandrene	91	0.027	0.024
23	13.40	1-Isopropyl-4-methylcyclohexa-1,3-diene	97	0.093	0.051
24	13.64	*p*-Cymene	97	0.063	0.221
25	13.79	*D*-Limonene	99	5.339	5.397
26	13.89	trans-β-Ocimene	96	0.358	0.115
27	14.23	(Z)-3,7-Dimethylocta-1,3,6-triene	97	0.586	0.183
28	14.67	gamma.-Terpinene	95	0.226	0.243
29	15.08	*cis*-Linaloloxide	90	—	0.074
30	15.59	1-Methyl-4-(propan-2-ylidene)cyclohex-1-ene	96	0.275	0.511
32	15.81	3,7-Dimethylocta-1,6-dien-3-ol	96	0.038	0.121
34	16.44	1,3,3-Trimethylbicyclo[2.2.1]heptan-2-ol	98	0.035	0.098
35	16.71	(4*E*,6*E*)-2,6-Dimethylocta-2,4,6-triene	94	0.019	—
36	16.78	2-(2,2,3-Trimethylcyclopent-3-en-1-yl)acetaldehyde	90	—	0.056
37	17.26	(1*S*,3*R*,5*S*)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptan-3-ol	96	—	0.187
38	17.96	Pinocarvone	90	—	0.093
39	18.06	endo-Borneol	91	0.023	0.065
40	18.35	(*R*)-1-Isopropyl-4-methylcyclohex-3-enol	93	0.069	0.065
41	18.71	α-Terpineol	86	0.759	0.867
42	18.94	(-)-Myrtenol	94	0.089	0.483
44	19.64	2,6,6-Trimethylcyclohex-1-enecarbaldehyde	94	0.012	0.033
47	21.52	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-yl acetate	96	0.023	0.115
49	21.94	6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptan-3-yl acetate	90	0.018	0.06
51	22.77	Myrtenyl acetate	91	0.116	0.246
Sesquiterpenoids					
54	23.72	α-Cubebene	95	0.053	0.096
56	24.61	Ylangene	99	0.059	0.112
57	24.78	Copaene	99	0.177	0.468
58	25.17	(-)-β-Bourbonene	97	0.169	0.433
59	25.27	(1*R*,2*S*,4*R*)-1-Methyl-2,4-di(prop-1-en-2-yl)-1-vinylcyclohexane	90	0.055	0.049
61	26.09	(2*S*)-1,1,5,5-Tetramethyl-2,3,4,5,6,7-hexahydro-1*H*-2,4a-methanonaphthalene	93	0.026	0.036
62	26.52	Caryophyllene	99	2.796	4.409
63	26.82	γ-Elemene	97	0.935	0.638
65	27.40	(*E*)-β-Famesene	95	3.793	4.261
67	27.67	*cis*-Muurola-3,5-diene	93	0.013	
68	27.87	Humulene	97	4.893	8.216
69	28.05	4-Isopropyl-1,6-dimethyl-1,2,3,7,8,8a-hexahydronaphthalene	91	0.026	0.051
70	28.37	β-Copaene	93	0.024	—
71	28.62	γ-Muurolene	99	—	3.33
72	28.77	α-Muurolene	97	0.063	0.114
73	28.92	2,6-Dimethyl-6-(4-methylpent-3-en-1-yl)bicyclo[3.1.1]hept-2-ene	93	1.565	1.442
74	29.20	(4a*R*,7*R*,8a*S*)-4a-Methyl-1-methylene-7-(prop-1-en-2-yl)decahydronaphthalene	99	0.216	0.558
75	29.38	(*S*,1*E*,6*E*)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	90	0.203	0.642
76	29.51	α-Farnesene	93	5.887	5.567
77	30.11	(*R*)-3,5,5,9-Tetramethyl-2,4a,5,6,7,8-hexahydro-1*H*-benzo[7]annulene	91	0.501	—
78	30.22	(1*S*,4a*R*,8a*R*)-1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene	95	0.864	—
79	30.23	1-Isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene	97	—	2.552
80	30.51	(1*S*,4a*R*,8a*S*)-1-Isopropyl-4,7-dimethyl-1,2,4a,5,8,8a-hexahydronaphthalene	94	1.759	—
81	30.53	(1*S*,8a*R*)-1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	94	—	2.735
82	30.60	(1*S*,4*S*)-4-Isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalene	96	—	0.186
84	30.79	(-)-Aristolene	92	0.029	—
85	30.97	4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7-hexahydronaphthalene	99	0.14	0.163
86	31.19	5,5,9-Trimethyl-3-methylene-2,3,5,6,7,8,9,9a-octahydro-1H-benzo[7]annulene	95	0.953	—
87	31.21	(3*R*,4a*R*,5*S*)-4a,5-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	93	—	2.033

Table 1: Continued

Table 1: Continued

No.	t/min	Name of Components	matching degree	dried before	dried after
				φ/%	φ/%
88	31.50	4a,8-Dimethyl-2-(propan-2-ylidene)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	99	0.515	1.428
90	32.20	1-Methyl-2-(prop-1-en-2-yl)-4-(propan-2-ylidene)-1-vinylcyclohexane	91	0.713	0.259
91	32.34	(4a*R*,8a*S*)-4a-Methyl-1-methylene-7-(propan-2-ylidene)decahydronaphthalene	90	0.047	0.129
92	33.07	(-)-Spathulenol	94	0.067	0.358
93	33.38	1,4-Dimethyl-3-(2-methylprop-1-en-1-yl)-4-vinylcyclohept-1-ene	91	0.249	—
94	33.39	Caryophyllene oxide	91	—	1.091
97	34.53	(1*R*,3*E*,7*E*,11*R*)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	87	0.139	1.043
98	35.01	2-((2*R*,4a*R*)-4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalen-2-yl)propan-2-ol	96	0.078	0.094
99	35.75	1-Isopropyl-4-methyl-7-methylene-1,2,3,4,4a,5,6,7-octahydronaphthalene	90	0.384	0.579
100	36.41	α-Cadinol	91	0.466	0.632
102	37.37	4-Isopropyl-1,6-dimethylnaphthalene	99	—	0.107
103	38.42	(1*R*,4a*R*,8a*R*)-1,4a-Dimethyl-7-(propan-2-ylidene)decahydronaphthalen-1-ol	92	0.235	—
104	38.43	(2*R*,4a*R*,8a*R*)-4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	90	—	0.204
Azulenoids					
60	25.53	(3*S*,3a*S*,5*R*)-3,8-Dimethyl-5-(prop-1-en-2-yl)-1,2,3,3a,4,5,6,7-octahydroazulene	91	0.054	0.116
64	27.29	1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulene	99	0.278	1.173
66	27.55	(1*S*,4*R*,7*R*)-1,4,9,9-Tetramethyl-1,2,3,4,5,6,7,8-octahydro-4,7-methanoazulene	90	0.017	—
83	30.67	Isoledene	95	0.036	—
89	31.71	(1a*R*,4*R*,4a*R*,7b*S*)-1,1,4,7-Tetramethyl-1a,2,3,4,4a,5,6,7b-octahydro-1H-cyclopropa[e] azulene	94	0.116	0.072
95	33.76	(1a*R*,4*S*,4a*S*,7*R*,7a*S*,7b*S*)-1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol	94	0.107	0.108
96	34.27	Ledol	93	0.058	0.094
Alkanes					
1	6.18	Ethylcyclopentane	94	0.029	0.023
3	7.33	n-Octane	91	0.277	0.052
6	8.86	2-Methyloctane	94	0.147	0.086
8	9.83	n-Nonane	94	32.567	17.117
11	10.23	1,1-Dimethyl-2-(2-methylprop-1-en-1-yl)cyclopropane	91	0.014	0.016
16	11.86	3-Methylnonane	91	0.639	0.856
21	12.71	Decane	95	0.047	0.047
31	15.75	Undecane	95	2.275	3.212
43	19.54	(7*S*,7a*S*)-1-Methyloctahydro-1H-4,7-methanoindene	91	—	0.042
48	21.65	n-Tridecane	93	0.009	0.058
109	56.37	n-Heptacosane	95	0.067	—
Others					
2	6.8	Toluene	93	0.039	0.035
4	8.44	5-(tert-butyl)Cyclopenta-1,3-diene	91	0.005	0.005
5	8.66	(*E*)-Hex-2-enal	98	0.049	—
7	9.68	3,3-Dimethyl-6-methylenecyclohex-1-ene	95	0.002	0.008
19	12.36	6-Methylhept-5-en-2-one	90	—	0.025
33	15.93	Nonanal	91	—	0.089
45	19.78	(*Z*)-Hex-3-en-1-yl pentanoate	90	0.018	—
46	20.73	2,4,5,5,8a-Pentamethyl-3,5,6,8a-tetrahydro-2H-chromene	92	0.012	0.023
50	22.49	(2*S*,8a*R*)-2,5,5,8a-Tetramethyl-3,5,6,8a-tetrahydro-2H-chromene	92	0.031	0.075
52	23.45	3,7-Dimethyloct-6-en-1-yl acetate	91	0.009	0.033
53	23.52	2,7-Dimethyl-5-(prop-1-en-2-yl)nona-1,8-diene	94	0.015	0.008
55	23.96	1,1,6-Trimethyl-1,2-dihydronaphthalene	91	—	0.084
101	37.04	2,2,8a-Trimethyl-2,3,6,7,8,8a-hexahydro-1,3-methanonaphthalen-4(1H)-one	90	0.069	0.059
105	44.69	6,10,14-Trimethylpentadecan-2-one	83	0.018	0.351
106	45.93	Diisobutyl phthalate	90	0.039	0.279
107	49.57	Isophytol	91	0.013	0.027
108	54.37	Phytol	91	0.114	0.229

The reason was that monoterpenoids was more volatile than sesquiterpenoids, so it would reduce greatly if the plant was placed for a longer period of time.

Conclusion

The essential oils of *H. patulum* before and after drying were comparatively explored from the aspects of chemical composition for the first time. It was found that ethanol and methanol extracts of *H. patulum* possess prominent antioxidant capacities, especially the $ABTS^{\bullet+}$ radical scavenging activity was much stronger than that of vitamin C. On the contrary, the essential oil was found ineffective in antioxidant assays. *H. patulum* could be considered as promising antioxidant, and thus can be used in raw material in food and/or pharmaceutical fields because of its strong antioxidant capacities. However, further studies are in progress on the chemical constituents and the safety of this plant for humans and to improve the potency and stability for practical use.

Table 2: Antioxidant activity of the essential oils and E_1 to E_5

Samples	DPPH-scavenging	ABTS-scavenging
	IC_{50}	TEAC value (μmol/L)
Before drying	>20	<1
After drying	>20	<1
E_1	6.46±0.15	3.73±0.07
E_2	3.42±0.08	8.41±0.06
E_3	0.633±0.04	17.1±0.18
E_4	0.091±0.0013	22.75±0.34
E_5	0.073±0.0051	22.75±0.38
vitamin C[a]	4.18±0.4[b]	2.66±0.4[c]

IC_{50}values represent the mean ± S.E.M. (n = 3)
Before drying, After drying, E_1-E_5, IC_{50} (mg plant /mL)
[a] Standard substance, [b] IC_{50} (μg/mL), [c] TEAC value, the concentration of Trolox giving the same scavenging capacity of the radical cation as 1 μg/mL vitamin C

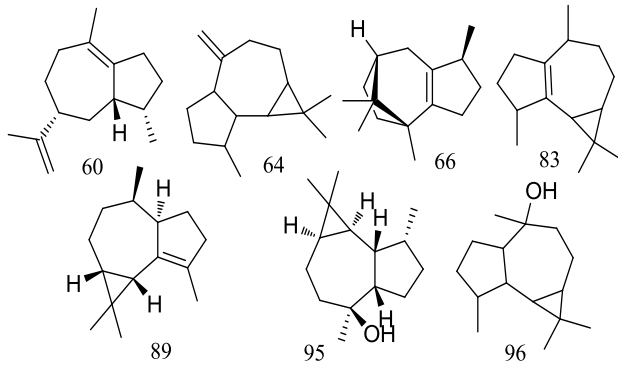

Fig. 1: Structures of azulenoids detected from the essential oils of *H. patulum* Thumb. before and after drying

Acknowledgments

This research was supported by Natural Science Foundation of Jiangsu Province (No. BK20130214), National Natural Science Foundation of China (No. 31300222), and Director Fund of Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy (No. ZR-XY201401).

References

Bokhari, J., M.R. Khan, M. Shabbir, U. Rashid, S. Jan and J.A. Zai, 2013. Evaluation of diverse antioxidant activities of *Galium aparine*. *Spectrochim. Acta Part A: Mol. Biomol. Spectr*., 102: 24–29

Chinese Flora Editorial Board in Chinese Academy of Sciences, 1990. *The Flora of China*, (Fiftieth volume, Second fascicle). Science Press: Beijing, China

Guedes, A.C., H.M. Amaro, M.S. Gião and F.X. Malcata, 2013. Optimization of ABTS radical cation assay specifically for determination of antioxidant capacity of intracellular extracts of microalgae and cyanobacteria. *Food Chem*., 138: 638–643

Ishiguro, K., H. Fukumoto, A. Suitani, M. Nakajima and K. Isoi, 1996. Prenylated xanthones from cell suspension cultures of *Hypericum patulum*. *Phytochemistry*, 42: 435–437

Ishiguro, K., H. Fukumoto, M. Nakajima and K. Isoi, 1993, Xanthones in cell suspension cultures of *Hypericum Patulum*. *Phytochemistry*, 33: 839–840

Ishiguro, K., M. Nakajima, H. Fukumoto and K. Isoi, 1995a. Co-occurrence of prenylated xanthones and their cyclization products in cell suspension cultures of *Hypericum patulum*. *Phytochemistry*, 38: 867–869

Ishiguro, K., M. Nakajima, H. Fukumoto and K. Isoi, 1995b. A xanthone substituted with an irregular monoterpene in cell suspension of *Hypericum patulum*. *Phytochemistry*, 39: 903–905

Ishiguro, K., N. Nagareya and H. Fukumoto, 1998. A phloroglucinol derivative from cell suspension cultures of *Hypericum patulum*. *Phytochemistry*, 47: 1041–1043

Ishiguro, K., N. Nagareya, A. Suitani and H. Fukumoto, 1997. A prenylated xanthone from cell suspension cultures of *Hypericum patulum*. *Phytochemistry*, 44: 1065–1066

Ishiguro, K., R. Yamamoto and H. Oku, 1999. Patulosides A and B, novel xanthone glycosides from cell suspension cultures of *Hypericum patulum*. *J. Nat. Prod*., 62: 906–908

Jayaprakash, G.K., R.P. Singh and K.K. Sakariah, 2001. Antioxidant activity of grape seed (*Vitis vinefera*) extracts on peroxidation models *in vitro*. *Food Chem*., 73: 285–290

Jiangsu New Medical College, 2006. *Dictionary of Traditional Chinese Medicine*, 1st volume, Shanghai Publishing House of Science and Technology: Shanghai, China

Lv, H.F., Q.G. Chu and Z.H. Hu, 2002. Advances in study on chemical constituents in *Hypericum* Linn. *Chin. Trad. Herb Drugs*, 33: 1135–1138

Mukherjee, P.K., G.S. Saritha and B. Suresh, 2002. Antimicrobial potential of two different Hypericum species available in India. *Phytother. Res*., 16: 692–695

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad. Biol. Med*., 26: 1231–1237

Shoji, T., K. Okada, S. Ito, K. Toyota and N. Morita, 2010. Synthesis of 1-(pyridyl, quinolyl, and isoquinolyl) azulenes by Reissert–Henze type reaction. *Tetrahedron Lett*., 51: 5127–5130

Shoji, T., Y. Inoue and S. Ito, 2012. First synthesis of 1-(indol-2-yl) azulenes by the Vilsmeier–Haack type arylation with triflic anhydride as an activating reagent. *Tetrahedron Lett*., 53: 1493–1496

Sunil, C., V. Duraipandiyan, S. Ignacimuthu and N.A. Al-Dhabi, 2013. Antioxidant, free radical scavenging and liver protective effects of friedelin isolated from *Azima tetracantha* Lam. Leaves. *Food Chem.*, 139: 860–865

Vijayan, P., K.S. Vinod, S.A. Dhanaraj, P.K. Mukherjee and B. Suresh, 2003. In Vitro cytotoxicity and antitumour properities of *Hypericum mysorense* and *Hypericum patulum*. *Phytother. Res*., 17: 952–956

Wu, L., D. Yang, F. Wang and H. Zhang, 2014. New taxa of *Hypericum* (Clusiaceae) from China. *Acta Phytotaxon. Sin*., 42: 73–78

Xiao, Z.Y. and Q. Mu, 2007. Advances on chemical investigation of *Hypericum*. *Nat. Prod. Res. Dev*., 19: 344–355

Yagi, S., N. Drouart, F. Bourgaud, M. Henry, Y. Chapleur and D. Laurain-Mattar, 2013. Antioxidant and antiglycation properties of *Hydnora johannis* roots. *S. Afr. J. Bot*., 84: 124–127

Zhang, L., Z. Hu and G. Zhao, 1999. An overview and some suggestions on studies of medical plants in genus *Hypericum*. *Chin. Trad. Pat. Med*., 21: 647–650

(Received 29 August 2017; Accepted 27 September 2017)