Chemical composition of *Toona sinensis* essential oil and DPPH scavenging activity of different fractions in ethanol extract of *Toona sinensis* from China

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Abstract
Chemical components of essential oil of Toona sinensis (A.Juss.) Roem from China, separated by simultaneous distillation solvent extraction (SDE), were analyzed by GC-FIRD and GC-MS. The DPPH scavenging activity of the essential oil and different fractions of the associated ethanol extract was also evaluated. Seventy-seven compounds were identified in the essential oil of the leaves and eighty-one compounds were identified in the essential oil of the common petioles, the sum of relative mass fraction accounting for 98.86% and 98.64% respectively. The main components in the essential oil from leaves were β-caryophyllene (19.51%), humulen-(v1) (16.04%), himachala-2, 4-diene (5.71%), seychellene (4.82%), longifolene-(V4) - (4.42%), caryophyllene oxide (4.03%), aristolene (3.18%).

The main components in the essential oil from common petioles were longifolene- (V4) - (16.4%), himachala - 2, 4-diene (14.64%), β - caryophyllene (6.6%), δ-cadinene (4.41%), humulen-(v1) (4.38%), α-guaiene (3.89%), tau - cadinol (3.85%), α-copaene (3.18%). The essential oil of Toona sinensis can be used as food additives with a special flavor. The weak DPPH scavenging activity of Toona sinensis essential oil indicated weak antioxidant capacity. The ethyl acetate fraction and ethanol extracts from Toona sinensis leaves and common petioles exhibited high DPPH scavenging activity, with that of the common petiole material exceeding that of the leave materials. The results indicated that Toona sinensis may be a useful new source of natural antioxidants.

Keywords: *Toona sinensis* essential oil, GC-MS, extracts, DPPH scavenging activity, antioxidants.

Introduction

Toona sinensis (A. Juss) Roem, a perennial hardwood called "Xiangchun" in Chinese, is a common plant in China. It is uniquely aromatic and its young leaves and shoots can be used as a kind of vegetable called "Xiangchun Ya", known as a "tree vegetable". The leaves of *Toona sinensis* are also used in Chinese traditional medicine for the treatment of diarrhea, chronic dysentery, bloody stools, seminal emissions, leucorrhoea and metrorrhagia[1]. The extracts from the plant can alter lipid metabolism[2,3], alleviate hyperglycemia[4], alleviate liver fibrosis[5], induce apoptosis of cancer cells[6], inhibit tumor growth[7,8], increase dynamic activity of human sperm[9], protect MDCK cells from DNA damage by hydrogen peroxide-induced oxidative stress[10] and exhibit anti-oxidative activity *in vitro*[11,12] as well as antibacterial activity[13,14].

Previous studies on *Toona sinensis* have led to the isolation of flavones, triterpenes, phenolic compounds, alkaloids, anthraquinones and tannins.[1,15,16.] Many volatile compounds[17, 20] with bioactivity are also present such as β-caryophyllene is used as a fragrance chemical and has the effects of local anesthetic, anti-inflammatory, repelling insects and treating general anxiety neurosis and depression[21-24]. Caryophyllene oxide has the effects of analgesic and anti-inflammatory, antifungal activities, cytotoxicity[25-27]. Understanding the chemical composition of volatile compounds in *Toona sinensis* is an essential step in scientifically assessing its possible commercial use.

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical which has an unpaired valence electron at one atom of nitrogen bridge. Scavenging of DPPH radical is the basis of the popular DPPH antioxidant assay[28]. DPPH assay has been used to predict the oxidative stability of edible oils and essential oils[29-32] and antioxidant activity of solvent extracts of the plant[11, 31-35]. However, there have been few published reports on the chemical composition[17-20] and the DPPH scavenging activity of the essential oil and different fractions of ethanol extracts of *Toona sinensis* leaves and common petioles.

The aim of the present work is to determine volatile components and to conduct a comparative study of the DPPH scavenging activity of the essential oil and different fractions of ethanol extracts of *Toona sinensis* leaves and common petioles. The study is intended to provide a firm foundation for investigating the use of essential oil as additives and these extracts as natural antioxidants and to guide further study into the isolation and identification of the active components.

Material and Methods

Plant material and chemicals: Samples of *Toona sinensis*

(A Juss.) Roem, grown in Zhuzhou, Hunan Province in China were collected in November 2007. The plants were identified and authenticated by Professor Kewang Liu of the Central South University of Forestry and Technology. The leaves and common petioles of *Toona sinensis* were collected and air-dried about a week. The common petioles were cut into small pieces about 5mm.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (USA). Rutin, quercetin, gallic acid and other biochemical reagents were purchased from Shanghai Sinopharm Chemical Reagent Co. S-8 macroporous resin and NKA-9 macroporous resin were made in China. All other chemicals, including ascorbic acid, ethanol, petroleum ether (the boiling point is 60-90 ^{o}C), ethyl acetate, n-butanol, sodium chloride and sodium sulfate made in China were of analytical grade.

Essential oil extraction and GC-MS analysis: The essential oil samples were isolated from the leaves and common petioles of *Toona sinensis* by SDE (simultaneous-distillation and solvent-extraction) for 4 h in Likens–Nickerson apparatus[36] using ethyl ether (50 ml) as the extracting solvent. The extractions were carried out at atmospheric pressure. The sample was kept at 120±2 ^{o}C, the solvent at 50±2 ^{o}C and the condensation system at 5.0±0.1 ^{o}C. The extracts were dried with anhydrous sodium sulfate and concentrated at room temperature by rotatory evaporator under vacuum until the solvent evaporated. The collected essential oil was stored at 4^{o}C in the sealed brown vials until analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis[37] was performed using a TRACE GC-POLARISQ MS (made by Thermo Finnigan) using a DB-1 silica capillary column (i.d.= 0.25mm, length=30mand film thickness=0.25μm) and equipped with an flame ionization detector (FID). Helium was used as a carrier gas, flowing at 1ml/min, not split. The oven temperature was maintained at 80 ^{o}C for 2 min, then raised at the rate of 5 ^{o}C/ min to 240 ^{o}C and finally kept at that temperature for 20 min. Injector and detector temperatures were set at 250 ^{o}C and 290 ^{o}C respectively. Diluted samples (1/1000 in acetone, v/v) of 10 μl were injected, not split.

The MS detector was used in the electron impact ionization (EI) mode with an ionization voltage of 70 eV, electron multiplier voltage of 1.2 kV and scan range of 40~400 amu. Identification of the components was based on the comparison of the mass spectrum of each compound with that of known compounds found in the available library data of the GC/MS system (NIST 98 data software) and correlation literature[17-20]. Quantification was expressed as the percentage contribution of each compound to the total amount of material detected after peak area normalization.

Preparation of *Toona sinensis* extracts/fractions: The air-dried leaves and common petioles were extracted with ethanol (φ=90%). The extracted solution was filtered and concentrated by rotary evaporation, yielding ethanol (φ=90%) extracts. Then most of the ethanol (φ=90%) extracts was suspended in water and partitioned successively by petroleum ether (60-90 ^{o}C), ethyl acetate and n-butanol. All extracted solutions were concentrated by rotary evaporation, yielding samples referred to as petroleum ether (60-90 ^{o}C) fraction ethyl acetate fraction and n-butanol fraction. The residual aqueous phase fraction was divided into two parts, one of which was passed through S-8 macroporous resin and the other passed through NKA-9 macroporous resin. The saturated macroporous resins were eluted with ethanol and the resulting eluants concentrated by rotary evaporation, yielding the S-8 fraction and NKA-9 fraction.

Determination of DPPH scavenging activity of the essential oil and the extracts/fractions: The DPPH scavenging activities of extracts and fractions were determined by methods described in the literature[11]. A series of extracts/fractions solutions with varying concentrations was prepared by dissolving different mass of the extracts/fractions in 25 ml of ethanol. 1 ml of these different solutions were added to 4.0 ml of 51.54 mg·L^{-1} DPPH solution. After 50 min of incubating at room temperature, the optical absorbance was measured at 517 nm. The DPPH scavenging activity was calculated by eq. (1):

$$Y = \frac{1-(A_s - A_r)}{A_0} \times 100 \quad (1)$$

where Y is DPPH Scavenging Effect (%), A_0 is the absorbance of DPPH ethanol solution (mixture of 4.0 ml of 51.54 mg·L^{-1}DPPH ethanol solution and 1.0 ml ethanol), A_r is the absorbance of the sample in the presence of the ethanol solution (mixture of 4.0 ml of ethanol and 1.0 ml sample ethanol solution) and A_s is the absorbance of the solution after incubation (4.0 ml of 51.54 mg·L^{-1} DPPH ethanol solution and 1.0 ml sample ethanol solution). IC_{50} is sample ethanol solution concentration when its DPPH scavenging effect is 50%. IC_{50} values also calculated according to regression analysis equation were used to determine the relationship between the concentration (C) of essential oil, ethanol extracts or different polarity fractions and the DPPH scavenging effect (Y). IM_{50} is the removed DPPH mass when antioxidant sample generated a 50% DPPH scavenging effect, IM_{50} values were calculated as per eq. (2):

$$IM_{50} = \frac{M_{DPPH}}{M_{sample}} = \frac{0.5 \times 4 \times 10^{-3} \times 51.54}{1.0 \times 10^{-3} \times IC_{50}} \quad (2)$$

i.e. $$IM_{50} = \frac{103.08}{IC_{50}}$$

M_{DPPH} is 50% DPPH mass added when antioxidant sample generated a 50% DPPH scavenging effect, M_{sample} is sample

mass added when antioxidant sample generated a 50% DPPH scavenging effect. The IM_{50} value stands for removed DPPH mass when antioxidant sample generated a 50% DPPH scavenging effect. The higher the IM_{50} value is, the stronger the DPPH scavenging ability of the sample is.

Results and Discussion

Chemical composition of *Toona sinensis* essential oil: 4.0kg of *Toona sinensis* leaves and 2.5 kg of common petioles were subjected to distillation and solvent extraction (SDE). 5.0 ml essential oil (No.3) (which was a yellow, oily-liquid with a spicy fragrance) was obtained from the leaves and 3.0 ml of essential oil (No.4) (which was a greyish yellow, oily liquid with a woody odor) was obtained from the common petioles i.e. respective yields of 1.25 ml·kg^{-1} and 1.20 ml·kg^{-1}.

Total ion chromatograms of these essential oils are shown in fig.1 and 2. The components identified are listed in table 1 in order of their retention times and retention indices. It can be seen that there was a little difference between the components or composition content (relative mass fractions) of the essential oils derived from leaves and common petioles. More specifically, 77 and 81 compounds were identified in the essential oils extracted from the leaves (No.3) and the common petioles (No.4), accounting for relative mass fractions of 98.86% and 98.64% respectively.

Among the 77 compounds in the leaf oil were 5 kinds of monoterpene oxygenated compounds, 25 kinds of sesquiterpenes, 21 kinds of sesquiterpene oxygenated compounds and 26 kinds of non-terpenoid compounds, but no monoterpene. Among the 81 compounds in the common petioles oil were 5 kinds of monoterpene oxygenated compounds 26 sesquiterpenes, 24 sesquiterpene oxygenated compounds and 26 kinds of non-terpenoid compounds, but no monoterpenes. The relative mass fraction of each component is shown in table 1. The chemical structure of the main components is shown in table 2.

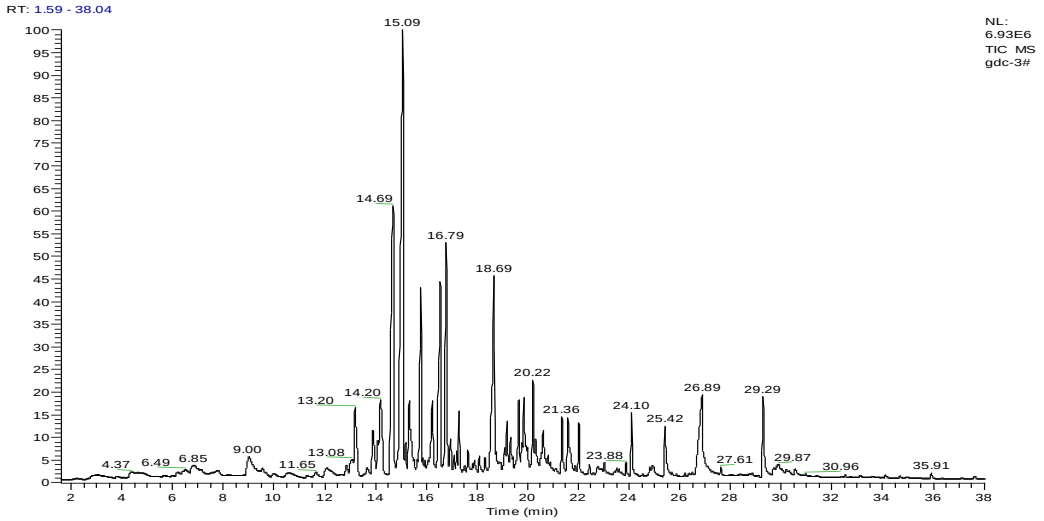


Fig.1: Total ion chromatogram of chemical components in essential oil (3#) from *Toona sinensis* leaves grown in Hunan Zhuzhou in China collected in November 2007

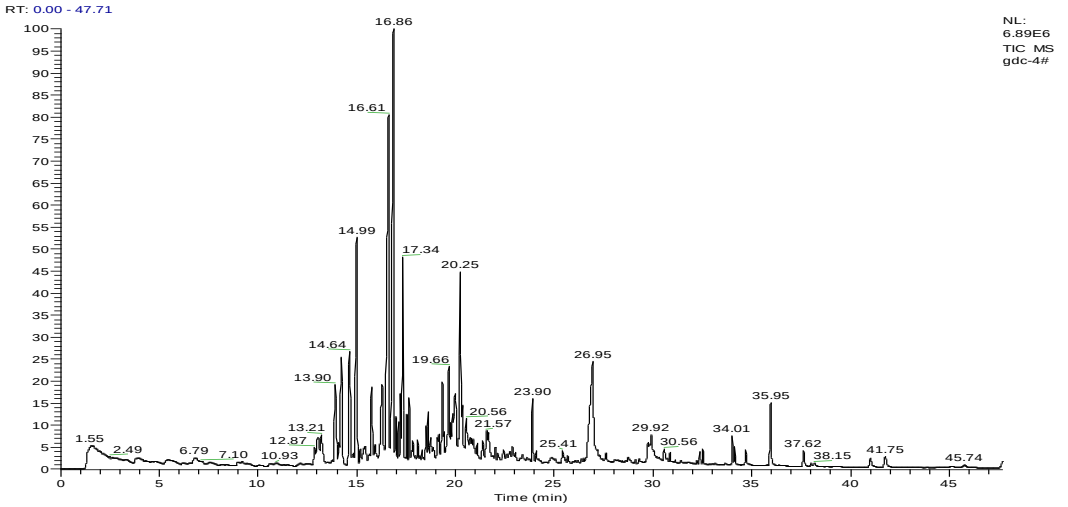


Fig. 2: Total ion chromatogram of chemical components in essential oil (4#) from *Toona sinensis* common petioles from Hunan Zhuzhou in China, collected in November, 2007

Table 1
Chemical components and relative mass fraction in the essential oil of *Toona sinensis* leaves and common petioles from Hunan Zhuzhou in China

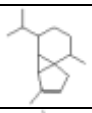
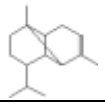
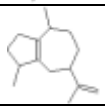
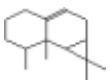
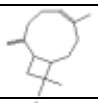
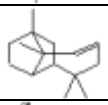
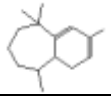
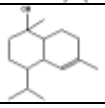
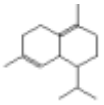
S.N.	Compound name	M.F.	R.T. (min)		R.M.F. (%)	
			No.3	No.4	No.3	No.4
1	Methylthiirane	C_3H_6S	—	1.55	—	0.49
2	Thiophene, 2,4-dimethyl-	C_6H_8S	—	3.35	—	0.11
3	5-Thiatricyclo[4.1.0.0(2,4)]heptane	C_6H_8S	3.8	3.81	0.05	0.1
4	Cyclopropanemethanol,2-isopropylidene-α-methyl-	$C_8H_{14}O$	4.35	—	0.22	—
5	Isogeraniol	$C_{10}H_{18}O$	—	4.77	—	0.05
6	(-)-cis-Sabinol	$C_{10}H_{16}O$	—	5.39	—	0.12
7	α-Limonene diepoxide	$C_{10}H_{16}O_2$	6.78	6.78	0.80	0.39
8	Isopinocarveol	$C_{10}H_{16}O$	—	7.56	—	0.08
9	2-Isopropylidene-3-methylhexa-3,5-dienal	$C_{10}H_{16}O$	8.99	8.96	0.70	0.08
10	β-Cyclocitral	$C_{10}H_{16}O$	9.54	—	0.11	—
11	Z,Z,Z-4,6,9-Nonadecatriene	$C_{19}H_{34}$	10.54	10.59	0.27	0.06
12	Cyclohexene,1-(2-nitro-2-propenyl)-	$C_9H_{13}NO_2$	—	10.89	—	0.11
13	Ionone	$C_{13}H_{20}O$	11.27	—	0.06	—
14	Cyclopentanone,2-(2-nitro-2-heptenyl)-	$C_{12}H_{19}NO_3$	11.65	—	0.10	—
15	3,5-Heptadienal,2-ethylidene-6-methyl-	$C_{10}H_{14}O$	12.06	—	0.33	—
16	τ-Elemene	$C_{15}H_{24}$	12.85	12.85	0.25	0.30
17	p-Eugenol	$C_{10}H_{12}O_2$	13.02	13.02	0.43	0.84
18	α-Cubebene	$C_{15}H_{24}$	13.20	13.21	2.70	0.88
19	6,6-Dimethyl-1,2,3b,6,7,8-hexahydrocyclopenta[2,3]cyclopropa[1,2-a]cyclohepten-3(3ah)-one	$C_{13}H_{18}O$	13.67	—	0.31	—
20	α-Copaene	$C_{15}H_{24}$	13.89	13.91	2.12	3.18
21	β-Bourbonene	$C_{15}H_{24}$	14.08	14.09	0.39	0.21
22	α-Guaiene	$C_{15}H_{24}$	14.2	14.22	2.85	3.89
23	Humulen-(v1)	$C_{15}H_{24}$	14.70	14.63	16.04	4.38
24	β-Caryophyllene	$C_{15}H_{24}$	15.08	14.99	19.51	6.60
25	Isoledene	$C_{15}H_{24}$	15.19	15.19	0.51	0.37
26	Aristolene	$C_{15}H_{24}$	15.33	15.42	3.18	0.84
27	β-Guaiene	$C_{15}H_{24}$	15.64	15.63	0.10	0.09
28	Seychellene	$C_{15}H_{24}$	15.79	15.76	4.82	2.12
29	(+)-Aromadendrene	$C_{15}H_{24}$	15.94	15.93	0.20	0.28
30	Curcumene	$C_{15}H_{22}$	16.24	—	1.86	—
31	(±)-Cadinene	$C_{15}H_{24}$	—	16.29	—	2.41
32	Longifolene-(V4)-	$C_{15}H_{24}$	16.56	16.61	4.42	16.40
33	Himachala-2,4-diene	$C_{15}H_{24}$	16.79	16.85	5.71	14.64
34	α-Himachalene	$C_{15}H_{24}$	16.97	16.98	0.90	1.09
35	τ-Muurolene	$C_{15}H_{24}$	17.10	17.12	0.31	0.80
36	Cadina-1,3,5-triene	$C_{15}H_{22}$	17.20	17.22	0.34	1.08
37	δ-Cadinene	$C_{15}H_{24}$	17.3	17.33	1.28	4.41
38	Cadina-1,4-diene	$C_{15}H_{24}$	17.52	17.54	0.23	1.04
39	Cadala-1(10),3,8-triene	$C_{15}H_{22}$	17.65	17.65	0.52	1.34
40	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	17.91	17.84	0.28	0.70
41	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane	$C_{15}H_{22}O$	18.09	18.08	0.80	0.70
42	(-)-Spathulenol	$C_{15}H_{24}O$	18.57	18.51	0.14	0.76
43	Caryophyllene oxide	$C_{15}H_{24}O$	18.69	18.62	4.03	0.92
44	τ-Gurjunenepoxide-(2)	$C_{15}H_{24}O$	18.75	18.74	0.08	0.43
45	Cedren-13-ol, 8-	$C_{15}H_{24}O$	18.93	18.88	0.20	0.20
46	Aromadendrene oxide-(1)	$C_{15}H_{24}O$	19.19	19.16	1.12	1.00
47	1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo[5.3.1.0	$C_{15}H_{24}$	19.33	19.34	0.69	1.56

	(4,11)]undec-8-ene					
48	α-Copaen-11-ol	$C_{15}H_{24}O$	19.65	19.66	1.64	1.85
49	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	$C_{15}H_{24}O$	19.77	19.77	0.21	0.29
50	Cedr-8(15)-en -ol	$C_{15}H_{24}O$	19.86	19.85	1.10	0.25
51	Cubenol	$C_{15}H_{26}O$	20.00	20.01	0.25	1.85
52	tau -Cadinol	$C_{15}H_{26}O$	20.22	20.25	2.01	3.85
53	2-(4α-,8-Dimethyl-1,2,3,4,4α,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	$C_{15}H_{24}O$	20.31	20.33	0.47	0.49
54	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	$C_{15}H_{24}O$	20.61	20.56	0.67	1.18
55	Eudesm-4(14)-en-11-ol	$C_{15}H_{26}O$	21.10	21.10	0.11	0.27
56	Ledene oxide-(II)	$C_{15}H_{24}O$	21.36	21.37	1.26	0.47
57	cis-Z-α-Bisabolene epoxide	$C_{15}H_{24}O$	21.58	—	1.22	—
58	6-Isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	—	21.57	—	0.78
59	δ-Cadinol	$C_{15}H_{26}O$	22.02	22.01	1.32	0.25
60	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one	$C_{15}H_{22}O$	—	22.12	—	0.21
61	Murolan-3,9(11)-diene-10-peroxy	$C_{15}H_{24}O_2$	22.43	22.43	0.49	0.51
62	2,2,6-Trimethyl-1-[(1E)-3-methyl-1,3-butadienyl]-5-methylene-7-oxabicyclo[4.1.0]heptane	$C_{15}H_{22}O$	22.90	22.88	0.30	0.38
63	Cycloisolongifolene, 8,9-dehydro-	$C_{15}H_{22}$	—	23.25	—	0.05
64	2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	$C_{15}H_{22}O$	—	23.39	—	0.14
65	Androst-2,16-diene	$C_{19}H_{28}$	23.52	—	0.10	—
66	Bicyclo[4.4.0]dec-5-ene,1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-	$C_{15}H_{24}O_2$	23.60	23.61	0.07	0.08
67	1R,3Z,9S-2,6,10,10-Tetramethylbicyclo[7.2.0]undeca-2,6-diene	$C_{15}H_{24}$	23.88	23.90	0.32	1.35
68	Menthol, 1'-(butyn-3-one-1-yl)-,(1S,2S,5R)-	$C_{14}H_{22}O_2$	24.10	24.09	1.42	0.23
69	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	$C_{21}H_{32}O_2$	24.92	24.84	0.49	0.23
70	Santalol, cis, α-	$C_{15}H_{24}O$	25.42	25.41	1.10	0.27
71	Androst-5,7-dien-3-ol-17-one	$C_{19}H_{26}O_2$	25.68	25.68	0.05	0.13
72	Strophanthidol	$C_{23}H_{34}O_6$	26.36	—	0.05	—
73	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	26.88	26.93	1.46	1.99
74	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	—	29.11	—	0.07
75	Cedrane-8,13-diol	$C_{15}H_{26}O_2$	27.61	27.62	0.19	0.21
76	Phytol	$C_{20}H_{40}O$	29.29	—	2.02	—
77	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	29.69	29.74	0.11	0.27
78	2-Hydroxy-1-(hydroxymethyl)ethyl (9E,12E,15E)-9,12,15-octadecatrienoate	$C_{21}H_{36}O_4$	29.87	29.93	0.30	0.41
79	6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	30.55	30.56	0.29	0.39
80	1,1-Dimethyltetradecyl hydrosulfide	$C_{16}H_{34}S$	—	30.85	—	0.19
81	2-(7-Heptadecynyloxy)tetrahydro-2H-pyran	$C_{22}H_{40}O_2$	—	32.21	—	0.08
82	Pregna-4,6-diene-3,20-dione,17-hydroxy-6,16α-dimethyl-	$C_{23}H_{32}O_3$	—	32.37	—	0.32
83	Heptadecane, 9-octyl-	$C_{25}H_{52}$	32.51	32.52	0.06	0.33
84	Corynan-17-ol,18,19-didehydro-10-methoxy-, acetate ester	$C_{22}H_{28}N_2O_3$	33.09	—	0.05	—
85	Pregnan-20-one, ,6-epoxy-3,17-dihydroxy-16-methyl-, (3a,5a,6a,16a)-	$C_{22}H_{34}O_4$	33.61	—	0.06	—
86	1,7-Dicyclopentyl-4-n-octylheptane	$C_{25}H_{48}$	—	34.01	—	0.63
87	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	34.11	34.13	0.07	0.36

88	1,1-Cyclopropanedicarboxylic acid, 2-[2-cyano-1,1-bis(methoxycarbonyl)propyl]-, dimethyl ester	$C_{15}H_{19}NO_8$	34.68	34.69	0.05	0.38
89	Tetracosane, 11-decyl-	$C_{34}H_{70}$	35.91	35.95	0.15	1.54
90	5α-Pregn-16-en-20-one, 3β,12α-dihydroxy-, diacetate	$C_{25}H_{36}O_5$	37.61	37.62	0.09	0.43
91	17-Pentatriacontene	$C_{35}H_{70}$	—	37.99	—	0.08
92	3-Ethyl-5-(2'-ethylbutyl)octadecane	$C_{26}H_{54}$	40.99	41.00	0.13	0.35
93	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	$C_{27}H_{42}O_4$	41.74	41.74	0.12	0.36
94	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	—	45.74	—	0.09
95	17-(Acetyloxy)-4,4-dimethyl-3-oxoandrost-5-en-19-yl acetate	$C_{25}H_{36}O_5$	47.72	—	0.10	—

No.3 is the essential oil derived from *Toona sinensis* leaves from Hunan Zhuzhou in China, collected in November, 2007; No.4 is the essential oil derived from *Toona sinensis* common petioles of the same source. R.T. is retention time, R.M.F. is relative mass fraction. The same is in table 2.

Table 2
The chemical structure of the main components in the essential oil of *Toona sinensis* leaves and common petioles from Hunan Zhuzhou in China

Compound name	Chemical structure	M.F.	R.M.F. (%)		Compound name	Chemical structure	M.F.	R.M.F. (%)	
			No.3	No.4				No.3	No.4
α-Cubebene		$C_{15}H_{24}$	2.7	0.88	α-Copaene		$C_{15}H_{24}$	2.12	3.18
α-Guaiene		$C_{15}H_{24}$	2.85	3.89	Humulen-(v1)		$C_{15}H_{24}$	16.04	4.38
Aristolene		$C_{15}H_{24}$	3.18	0.84	β-Caryophyllene		$C_{15}H_{24}$	19.51	6.60
Seychellene		$C_{15}H_{24}$	4.82	2.12	Longifolene-(V4) -		$C_{15}H_{24}$	4.42	16.40
Himachala-2,4-diene		$C_{15}H_{24}$	5.71	14.64	tau -Cadinol		$C_{15}H_{26}O$	2.01	3.85
δ-Cadinene		$C_{15}H_{24}$	1.28	4.41	Caryophyllene oxide		$C_{15}H_{24}O$	4.03	0.92

DPPH scavenge activity of essential oil and ethanol extracts or different polarity fractions of *Toona sinensis* ethanol extracts: The IC_{50} and IM_{50} of DPPH scavenging for essential oil and ethanol extracts or different polarity fractions of *Toona sinensis* ethanol extracts calculated according to eq. (1) and (2) are shown in table 3. Regression analysis was used to determine the relationship between the concentration (*C*) of essential oil and ethanol extracts or different polarity fractions and the DPPH scavenging effect (*Y*) shown in table 3.

The IM_{50} of DPPH scavenging of rutin, quercetin, ascorbic acid and gallic acid were 3.300, 6.734, 4.730 and 13.506 g DPPH/g respectively[11]. Among the DPPH scavenging capacity of the ethanol extract of *Toona isnensis* leaves and common petioles and different polarity fractions, ethyl acetate fractions were the highest, exceeding that of rutin, quercetin and ascorbic acid. The highest DPPH scavenging activity in *Toona sinensis* leaves and common petioles is primarily in the ethyl acetate fraction, reflecting the presence of antioxidant chemicals whose specific identities must await further study.

In general, the IM_{50} of DPPH scavenging for *Toona sinensis* essential oil was low i.e. less than 0.3 g DPPH/g essential oil which may be related to the elevated temperature used in the simultaneous distillation and extraction. This low value reflects the oil's weak antioxidant capacity.

Table 3
Relationship between DPPH scavenging effect and the concentration of *Toona sinensis* essential oil and different polarity fractions of ethanol extracts

Extracts	Relationship	R^2	Concentration range ($mg \cdot L^{-1}$)	IC_{50} ($mg \cdot L^{-1}$)	IM_{50} ($g \cdot g^{-1}$)
Leaves ethanol extract	$Y = 0.9249C + 9.7733$	0.9955	9.55~76.40	43.28	2.3816
Leaves petroleum ether fraction	$Y = 0.0615C + 6.8414$	0.9961	7.74~1239.10	701.77	0.1469
Leaves ethyl acetate fraction	$Y = 5.7702C - 2.8192$	0.9725	4.61~13.84	9.15	11.2609
Leaves n-butanol fraction	$Y = 0.8147C + 8.8828$	0.9662	5.35~85.65	50.47	2.0424
Leaves residual aqueous phase fraction	$Y = 0.2021C + 8.6255$	0.9963	38.38~307.05	204.74	0.5035
Leaves S-8 fraction	$Y = 1.5601C + 6.1606$	0.9896	0.84~33.50	28.10	3.6682
Common petioles ethanol extract	$Y = 9.1417C + 8.6583$	0.9917	0.84~6.70	5.79	17.807
Common petioles petroleum ether fraction	$Y = 0.7141C + 9.0553$	0.9997	6.40~64.00	57.34	1.7977
Common petioles ethyl acetate fraction	$Y = 10.2730C + 6.1466$	0.9966	0.65~5.16	4.27	24.1473
Common petioles n-butanol fraction	$Y = 0.7638C + 8.7275$	0.9960	2.21~84.50	54.047	1.9076
Common petioles residual aqueous phase fraction	$Y = 0.1292C + 3.3406$	0.9870	78.00~390.00	361.14	0.2854
Leaves essential oil	$Y = 0.0262C + 5.8321$	0.9910	15.4~1540.0	1685.8	0.06115
Common petioles essential oil	$Y = 0.0073C + 17.028$	0.9754	31.5~6300.0	4516.7	0.02282

Conclusion

Chemical components of essential oil of *Toona sinensis* (A. Juss.) Roem from China, separated by simultaneous distillation solvent extraction (SDE), were analyzed by GC-MS. The DPPH scavenging activity of the essential oil and different fractions of the associated ethanol extract was also evaluated. Seventy-seven compounds were identified in the essential oil of the leaves and eighty-one in the common petioles, accounting for 98.86% and 98.64% mass fraction respectively. The main components in the essential oil from leaves were β-caryophyllene (19.51%), humulen-(v1) (16.04%), himachala-2,4-diene (5.71%), seychellene (4.82%), longifolene-(V4)- (4.42%), caryophyllene oxide (4.03%), aristolene (3.18%), α-guaiene (2.85%), α-cubebene (2.70%), α-copaene (2.12%), phytol (2.02%), tau - cadinol (2.01%), curcumene (1.86%), α-copaen-11-ol (1.64%).

The main components in the essential oil from common petioles were longifolene-(V4)- (16.4%), himachala -2, 4-diene (14.64%), β-caryophyllene (6.6%), δ-cadinene (4.41%), humulen-(v1) (4.38%), α-guaiene (3.89%), tau-cadinol (3.85%), α-copaene (3.18%), (±)-cadinene (2.41%), seychellene (2.12%), l-(+)-ascorbic acid 2,6-dihexadecanoate (1.99%), α-copaen-11-ol (1.85%), cubenol (1.85%), 1R,4S,7S,11R-2,2,4,8- tetramethyl tricycle[5.3.1.0(4,11)]undec-8-ene (1.56%), tetraco-sane,11-decyl- (1.54%). The essential oil of *Toona sinensis* can be used as additives with a special flavor. The DPPH scavenging activity of *Toona sinensis* essential oil was weak, reflecting its weak antioxidant capacity.

The highest DPPH scavenging capacity is found in the ethyl acetate fractions, with the fraction from the petioles being greater than that from the leaves, In fact, it appears that the ethyl acetate fraction and the ethanol extract of *Toona sinensis* leaves and commom petioles possess significant antioxidant activities that may be a potential source of natural antioxidants.

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References

1. Luo X. D., Wu S. H., Ma Y. B. and Wu. D. G., Studies on chemical constituents of Toona sinensis , *Chinese Traditional and Herbal Drugs*, **32**, 390–391 **(2001)**

2. Zhang J. F., Zhang Q., Lu G. and Zhou. L., Effects of Toona Sinensis Leaves Extract on Lipid Metabolism and Antioxidant Activity of Mice with Hyperlipidemia, *Journal of Chinese Institute of Food Science and Technology*, **7,** 3–7 **(2007)**

3. Hsu H. K., Yang Y. C., Hwang J. H. and Hong. S. J., Effects of Toona sinensis leaf extract on lipolysis in differentiated 3T3-L1 adiposities, *Kaohsiung Journal of Medicine Science*, **19**, 385–390 **(2003)**

4. Wang P. H., Tsai M. J., Hsu C. Y., Wang C. Y., Hsu H. K. and Weng. C. F., Toona sinensis Roem (Meliaceae) leaf extract alleviates hyperglycemia via altering adipose glucose transporter, *Food and Chemical Toxicology*, **46**, 2554–2560 **(2008)**

5. Fan S., Chen H. N., Wang C. J., Tseng W. C., Hsu H. K. and Weng C. F., Toona sinensis Roem (Meliaceae) leaf extract alleviates liver fibrosis via reducing TGFβ1 and collagen, *Food and Chemical Toxicology*, **45,** 2228–2236 **(2007)**

6. Yang H. L., Chang W. H., Chia Y. C., Huang C. J., Lu F. J., Hsu H. K. and Hseu Y. C., Toona sinensis extracts induces apoptosis via reactive oxygen species in human premyelocytic leukemia cells, *Food and Chemical Toxicology*, **44**, 1978–1988 **(2006)**

7. Chang H. C., Hung W. C., Huang M. S. and Hsu H. K., Extract from the leaves of Toona sinensis roemor exerts potent antiproliferative effect on human lung cancer cells, *The American Journal of Chinese Medicine*, **30**, 307–314 **(2002)**

8. Chang H. L., Hsu H. K., Su J. H., Wang P. H., Chung Y. F., Chia Y. C., Tsai L. Y., Wu Y. C. and Yuan S. S. F., The fractionated Toona sinensis leaf extract induces apoptosis of human ovarian cancer cells and inhibits tumor growth in a murine xenograft model, *Gynecologic Oncology*, **102**, 309–314 **(2002)**

9. Wang M. F., Xu W. M. and Xu S. G., The effect of Toona sinensis on the aging male mice sperm quality, *Taiwan Agriculture Chemistry and Food science*, **43(2)**, 38–45 **(2005)**

10. Hsieh T. J., Liu T. Z., Chia Y. C., Chern C. L., Lu F. J., Chuang M. C., Maud S. Y., Chend S. H., Syud Y. H. and Chend C. H., Protective effect of methyl gallate from Toona sinensis (Meliaceae) against hydrogen peroxide-induced oxidative stress and DNA damage in MDCK cells, *Food and Chemical Toxicology*, **42**, 843–850 **(2004)**

11. Chen C. J., Huang K. Y., Li D. L., Wang X. Q. and Yuan S. S., Determining Method of DPPH Free Radical Scavenging Activity of Toona sinensis (A. Juss.) Roem. Leaves Extracts, *Chemistry and Industry of Forest Products*, **26**, 69–72 **(2006)**

12. Hseu Y. C., Chang W. H., Chen C. S., Liao J. W., Huang C. J., Lu F. J., Chia Y. C., Hsu H. K., Wu J. J. and Yang H. L., Antioxidant activities of Toona Sinensis leaves extracts using different antioxidant models, *Food and Chemical Toxicology*, **46**, 105–114 **(2008)**

13. Tian D. Y. and Yang R. H., The study on antibacterial activitives of Toona sinensis, *Food Industry Science and Technology*, **23**, 21–22 **(2002)**

14. Ouyang J., Wu Y. W. and Lu X. R., Comparison of Anti-bacterial Activities of the Extracts from Young and Old Leaves of Toona sinensis, *Natural Products Research and Development*, **20**, 427–430 **(2008)**

15. Chen T. S., Luo Z. P., Cui H. A., Zhen X. Q. and Liu Z. Z., Preliminary study on the chemical constituents of Toona sinensis, *Shanxi Forest Science and Technology*, **20**, 1–2, **(2000)**

16. Wang K. J., Yang C. R. and Zhang Y. J., Phenolic antioxidants from Chinese toon (fresh young leaves and shoots of Toona sinensis, *Food Chemistry*, **101**, 265–371 **(2007)**

17. Mu R. M., Wang X. R., Liu S. X., Yuan X. L., Wang S. B. and Fan Z. Q., Rapid Determination of Volatile Compounds in Toona sinensis (A. Juss.) Roem.by MAE-HS-SPME followed by GC–MS, *Chromatographia*, **65**, 463–467 **(2007)**

18. Chen C. J., Huang K. Y., Li J. J. and Zeng D., Comparative Research on Volatile Oil from Toona sinensis (A.Juss.) Roem Buds Extracted by Different Methods, *Chinese Journal Analysis Laboratory*, **28**, 30–35 **(2009)**

19. Liu X. P., Zhang C., Yu A. N., Tan Z. W. and Liu Y. X., Study of Chemical Components in Volatile Oil from Toona sinensis Roem, *Fine Chemicals*, **25**, 41–44 **(2008)**

20. Chen C. J., Yang G. E., Yuan L. J. and Huang K. Y., Analysis of Volatile Components from Toona sinensis (A. Juss.) Roem Buds and Leaves by Headspace-Solid Phase Micro-extraction Gas Chromatography- Mass Spectrometry, *Fine Chemicals*, **26**,1080–1084 **(2009)**

21. Skold M., Karlberg A. T., Matura M. and Borje A., The fragrance chemical β-caryophyllene—air oxidation and skin sensitization, *Food and Chemical Toxicology*, **44**, 538–545 **(2006)**

22. Ghelardini C. et al, Local anaesthetic activity of β-caryophyllene, *Il Farmaco***, 56**, 387–389 **(2001)**

23. Cho J. Y., Chang H. J., Lee S. K., Kim H. J., Hwang J. K. and Chun H. S., Amelioration of dextran sulfate sodium-induced colitis in mice by oral administration of β-caryophyllene, a sesquiterpene, *Life Science***, 80**, 932–939 **(2007)**

24. Liu X. Y., Chen X. B. and Chen G. Y., Research progress in bioactivity and synthesis of β-caryophyllene and its derivatives, *Chemistry and Industry of Forest Products*, **32**, 104–110 **(2012)**

25. Chavan M. J., Wakte P. S. and Shinde D. B., Analgesic and anti-inflammatory activity of caryophyllene oxide from Annona squamosa L. bark, *Phytomedicine*, **17**, 149–151 **(2010)**

26. Yang D., Michel L., Chaumont J. P. and Millet-Clerc J., Use of caryophyllene oxide as an antifungal agent in an in vitro experimental model of onycho-mycosis, *Mycopathologia*, **148**, 79–82 **(1999)**

27. Monzote L., Stamberg W., Staniek K. and Gille L., Toxic effects of carvacrol, caryophyllene oxideand ascaridole from essential oil of Chenopodium ambrosioides on mitochondria, *Toxicology and Applied Pharmacology*, **240**, 337–347 **(2009)**

28. Sharma O. P. and Bhat T. K., DPPH antioxidant assay revisited, *Food Chemistry*, **113**, 1202–1205 **(2009)**

29. Lee J. M., Chung H., Chang P. S. and Lee J. H., Development of a method predicting the oxidative stability of edible oils using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), *Food Chemistry*, **103**, 662–669 **(2007)**

30. Scherer R. and Godoy H. T., Antioxidant activity index (AAI) by the 2, 2-diphenyl-1- picrylhydrazyl method, *Food Chemistry* **112**, 654–658 **(2007)**

31. Gholiv M. B., Rahimi-Nasrabadi M., Batooli H. and Ebrahimabadi A. H., Chemical composition and antioxidant activities of the essential oil and methanol extracts of Psammogeton canescens, *Food and Chemical Toxicology*, **48**, 24–28 **(2010)**

32. Ahmadi F., Sadeghi S., Modarresi M., Abiri R. and Mikaeli A., Chemical composition, in vitro anti-microbial, antifungal and antioxidant activities of the essential oil and methanolic extract of Hymenocrater longiflorus Benth. of Iran, *Food and Chemical Toxicology*, **48**, 1137–1144 **(2010)**

33. EI Ouariachi E. M., Tomi P., Bouyanzer A., Hammouti B., Desjobert J. M., Costa J. and Paolini J., Chemical composition and antioxidant activity of essential oils and solvent extracts of Ptychotis verticillata from Morocco, *Food and Chemical Toxicology***, 49**, 533–536 **(2011)**

34. Serbetci T., Ozsoy N., Demirci B., Canb A., Kultur S. and Can Base K. H., Chemical composition of the essential oil and antioxidant activity of methanolic extracts from fruits and flowers of Hypericum lydium Boiss, *Industrial Crops and Products*, **36(1)**, 599–606 **(2012)**

35. Siatka T. and Kasparova M., Seasonal Variation in Total Phenolic and Flavonoid Contents and DPPH Scavenging Activity of Bellis perennis L. Flowers, *Molecules*, **15**, 9450-9461 **(2010)**

36. Valette L., Fernandez X., Poulain S., Loiseau A. M., Lizzani-Cuvelier L., Levieil R. and Restier L., Volatile constituents from Romanesco cauliflower, *Food Chemstry*, **80**, 353–358 **(2003)**

37. Xu R. S., Liu Y. H. and Yuan K., HS-SPME and SD Extraction and Analysis of Volatile Oil in Tridaxprocumbens. L by GC/MS, *Research Journal of Chemistry and Environment*, **12(4)**, 116-127 **(2008)**.

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