Chemical Composition and Antimicrobial and DPPH Scavenging Activity of Essential Oil of *Toona sinensis* (A. Juss.) Roem from China

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The chemical components of essential oil of *Toona sinensis* leaf blades and their petioles from China were extracted by simultaneous distillation solvent extraction (SDE) and were analyzed by GC-MS. The antimicrobial and DPPH scavenging activity of the essential oil were evaluated. The results showed that there were differences in chemical compositions and content among essential oils extracted from *T. sinensis* in different parts and different geographical areas in China, but the main components of essential oils were sesquiterpene and sesquiterpene oxygenated compounds, accounting for 90.1% (No. 1), 92.6% (No. 2), and 80.9% (No. 3) of the relative mass fraction, respectively. *T. sinensis* essential oil exhibited noticeable growth inhibitory activity against the tested microorganisms. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of different essential oils against microorganisms were different. For all essential oil samples, MIC and MBC against *Escherichia coli* and *Bacillus subtilis* were less than 25 μg·mL^{-1}, MIC and MBC against *Penicillium citrinum* were 200 and 400 μg·mL^{-1}, respectively, and MIC and MBC against *Colletotrichum gloeosporioides* were 50 and 200 μg·mL^{-1}, respectively. The IM_{50} of DPPH scavenging for *T. sinensis* essential oil was less than 0.3 g DPPH per g essential oil. The results indicated that *T. sinensis* essential oil may be a useful natural antiseptic source from forest products.

Keywords: Toona sinensis essential oil; Chemical components; GC-MS; Antimicrobial activity; DPPH scavenging activity

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INTRODUCTION

Toona sinensis (A. Juss) Roem, a species of *Toona*, is widely distributed in North Korea south through most of eastern, central, and southwestern China to Nepal, northeastern India, Myanmar, *etc*. It is a perennial hardwood also called "Xiangchun" in Chinese and 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 *T. sinensis* are also used in Chinese traditional and herbal drugs for the treatment of diarrhea, chronic dysentery, bloody stools, seminal emissions, leucorrhoea, and metrorrhagia (Luo *et al*. 2001). Extracts from *T. sinensis* leaves can alter lipid metabolism (Hsu *et al*. 2003; Zhang *et al*. 2007), alleviate hyperglycemia (Wang *et al.* 2008), alleviate liver fibrosis (Fan *et al.* 2007), induce apoptosis of cancer cells (Yang *et al.* 2006), inhibit tumor growth (Chang *et al.* 2002; Chang *et al.* 2006), increase

locomotor activity of human sperm (Wang *et al.* 2005), prevent H_2O_2-induced oxidative stress and DNA damage in cultured Madin-Darby canine kidney cells (Hsieh *et al.* 2004), and exhibit anti-oxidative activity *in vitro* (Chen *et al.* 2006; Hseu *et al.* 2008); they also exhibit antibacterial activity (Tian and Yang 2002; Ouyang *et al.* 2008).

Previous studies on *T. sinensis* have led to the isolation of flavones, triterpene, phenolic compounds, alkaloids, anthraquinone, and tannins (Chen *et al.* 2000; Wang *et al.* 2007). Many volatile compounds (Mu *et al.* 2007; Liu *et al.* 2008; Chen *et al.* 2009a, 2009b) with bioactivity are also present, such as β-caryophyllene, the main volatile compound of *T. sinensis* leaves (Chen *et al.* 2009b) is used as a fragrance chemical and has the effects of a local anesthetic, anti-inflammatory substance, insect repellant, and treatment for general anxiety neurosis and depression (Cho *et al.* 2007; Ghelardini *et al.* 2001; Liu *et al.* 2012; Skold *et al.* 2006). Caryophyllene oxide is an analgesic and has anti-inflammatory, antifungal, and cytotoxicity activities (Chavan *et al.* 2010; Monzote *et al.* 2009; Yang *et al.* 1999). Spathulenol, one of the main volatile compounds of *T. sinensis* essential oil, has shown the capacity to inhibit proliferation in lymphocytes and to induce apoptosis in these cells, possibly through a caspase-3 independent pathway (Ziaei *et al.* 2011). The essential oil compounds from plants grown in different geographical areas or different parts of the plants may be different either quantitatively or qualitatively (Maggi *et al.* 2009; Ouni *et al.* 2011; Saei-Dehkordi *et al.* 2010; Youssef *et al.* 2011). 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical that has an unpaired valence electron at one atom of the nitrogen bridge. Scavenging of DPPH radicals is the basis of the popular DPPH antioxidant assay (Sharma and Bhat 2009). The DPPH assay has been used to predict the oxidative stability of edible oils (Lee *et al.* 2007; Scherer and Godoy 2009). However, there have been few published reports on the chemical composition, antimicrobial, and DPPH scavenging activity of the essential oil (Mu *et al.* 2007; Liu *et al.* 2008; Chen *et al.* 2009a, 2009b) especially from different parts of *T. sinensis*, in different geographical areas in China.

The effect of the geographical origin in which plants grow and the parts of the plants on the yield, composition, and bioactivities of *T. sinensis* essential oils is of importance. The aim of the present work is to determine any volatile components and to carry out a comparative evaluation of yield, components, antimicrobial, and DPPH scavenging activity of the essential oil of *T. sinensis* leaf blades and petioles from different geographical areas in China.

EXPERIMENTAL

Materials

Plant material and chemicals

Samples of *T. sinensis*, grown in Nandan and Dongnan county, Guangxi Province, China, were collected in August, 2007. The plants were identified and authenticated by Professor He Taiping of the Forestry College, Guangxi University. The leaf blades and their petioles from *T. sinensis* were collected and air-dried. The petioles were cut into small pieces of about 5 mm. 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. All other chemicals, including ascorbic acid, ethanol, ethyl ether, sodium sulfate, sodium chloride, dextrose,

agar, peptone, beef extract, and potato extract, were made in China and were of analytical grade.

Microorganisms and media

The essential oil was tested against four microorganisms: bacteria (*Escherichia coli*, *Bacillus subtilis*) and fungi (*Penicillium citrinum*, *Colletotrichum gloeosporioides*). All microorganisms were provided by the microbiology laboratory of Central South University of Forestry and Technology.

Each microorganism was inoculated in test-tube slant medium. Bacteria were cultured for 24 h at 37 ºC, and fungi were cultured for 72 h at 28 ºC in an electro-thermal incubator. On a sterile workbench, inocula were suspended in sterile saline and the suspension was adjusted to contain approximately 10^5 to 10^6 colony forming units (CFU) *per* mL bacteria suspension or 10^5 to 10^6 spores *per* mL of fungi suspension using a blood cell counting chamber.

Beef extract peptone agar medium for bacteria consisted of 5 $g \cdot L^{-1}$ beef extract, 10 $g \cdot L^{-1}$ peptone, 20 $g \cdot L^{-1}$ agar, and 5 $g \cdot L^{-1}$ sodium chloride, with a pH value of 7.2 to 7.4. Potato dextrose agar medium for fungi consisted of 6 $g \cdot L^{-1}$ potato extract, 20 $g \cdot L^{-1}$ dextrose, and 20 $g \cdot L^{-1}$ agar, with a pH value of 5.6.

Methods

Essential oil extraction and GC-MS analysis

The essential oil samples were isolated from the leaf blades and their petioles of *T. sinensis* by simultaneous-distillation and solvent-extraction (SDE) for 4 h in a Likens–Nickerson apparatus (Ansorena *et al.* 2001; Chung *et al.* 2002; Valette *et al.* 2003) using ethyl ether (50 mL) as the extracting solvent. The extraction was carried out at atmospheric pressure. The sample was kept at 105 ± 2 ºC, and the solvent was kept at 50 ± 2 ºC. The extracts were dried with anhydrous sodium sulfate and concentrated under vacuum at room temperature by a rotatory evaporator, until the solvent was evaporated. The collected oil was stored at 4 ºC in the sealed brown vials until analysis.

GC-MS analysis was performed on a TRACE GC-POLARISQ MS (manufactured by Thermo Finnigan, America) using a DB-1 silica capillary column (i.d. = 0.25 mm, length = 30 m, and film thickness = 0.25 μm) and equipped with a flame ionization detector (FID). Helium was used as a carrier gas with a flow rate of 1 $mL \cdot min^{-1}$. The oven temperature was maintained at 80 ºC for 2 min, raised to 240 ºC at a rate of 5 $^oC \cdot min^{-1}$, and finally kept for 20 min at 240 ºC. Injector and detector temperatures were set at 250 ºC and 290 ºC, 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 to 400 amu. An FID was used for quantification of the constituents in the essential oil.

Identification of the components was based on the comparison of the mass spectrum of each compound with that of known compounds searched in the NIST mass spectral library data, and analysis after consulting correlation literature (Chen *et al.* 2009a, 2009b; Liu *et al.* 2008; Mu *et al.* 2007). Quantification was expressed as the percentage contribution of each compound to the total amount of material detected after peak area normalization.

Determination of the growth inhibition zone diameter of essential oil

The growth inhibition zone diameter of different essential oil samples against bacteria and fungi experiment was evaluated using cylinder plate assay methods (Jiao *et al.* 2007; Liu *et al.* 2008a; Zhang *et al.* 2012; Zulfiya 2008). Ten milliliters of agar medium was poured into each test tube. After autoclaving, the agar solution in each tube was cooled to about 45 °C, then aseptically poured into a sterile Petri dish (90 × 15 mm) and allowed to solidify. After solidification, 0.1 mL of microbial suspension containing approximately 10^5 to 10^6 colony forming units (CFU) *per* mL bacteria or 10^5 to 10^6 spores *per* mL of fungi was swabbed on the top of the solidified medium and dried. Oxford cups (bore diameter, 6.0 ± 0.1 mm; outer diameter, 7.8 ± 0.1 mm; height, 10 ± 0.1 mm) were placed on each plate. Then, 100 μL of essential oils was dispensed into individual oxford cups and left for 30 min at room temperature to allow for diffusion. The plates were incubated for 24 h at 37 ºC for bacteria and 72 h at 28 ºC for fungi in an electro-thermal incubator. When essential oils in the oxford cups diffused completely, the cups were removed from the agar plate. The growth inhibition zone diameters were measured horizontally and vertically. The experiment was repeated twice, and the values were averaged.

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of essential oil

A serial agar macrodilution method (Arias *et al.* 2004; Buwa and van Staden 2006; Duraipandiyan and Ignacimuthu 2007) was used to assess the activity of different essential oil samples against bacteria and fungi. The initial test concentration of the various essential oil samples (0.8 $mg \cdot mL^{-1}$) was serially diluted two-fold with ethanol. Then, 1.0 mL serial two-fold dilution of each essential oil sample (400 $\mu g \cdot mL^{-1}$, 200 $\mu g \cdot mL^{-1}$, 100 $\mu g \cdot mL^{-1}$, 50 $\mu g \cdot mL^{-1}$, 25 $\mu g \cdot mL^{-1}$) was mixed with 9.0 mL of 50 to 60 ºC sterile beef extract peptone agar for bacteria and sterile potato dextrose agar for fungi and put into Petri dishes (90 × 15 mm) as soon as possible. After the medium was solidified and cooled, each Petri plate was inoculated with spots of 10 μL of suspension containing 10^5 to 10^6 $cfu \cdot mL^{-1}$ of bacteria or 10^5 to 10^6 $spore \cdot mL^{-1}$ of fungi and incubated for 24 h at 37 ºC for bacteria and 72 h at 28 ºC for fungi (Ait-Ouazzou *et al.* 2012; Lv *et al.* 2011). Negative control experiments were conducted using ethanol and distilled water at the same time. Experiments were repeated twice at each concentration.

MIC for microorganisms was determined as the lowest concentration of the essential oil sample at which no colony was observed after the bacteria were incubated for 24 h at 37 ºC (fungi for 72 h at 28 ºC). MBC for microorganisms was defined as the lowest concentration of the essential oil sample at which no colony was observed after the bacteria were incubated for 72 h at 37 ºC (fungi for 120 h at 28 ºC).

Determination of DPPH scavenging activity of the essential oil

The DPPH scavenging activity of the essential oil was determined by methods described in the literature (Chen *et al.* 2006). A series of essential oil ethanol solutions with varying concentrations was prepared by dissolving various masses of essential oil in 25 mL of ethanol. Then, 1 mL of essential oil ethanol solution was added to 4.0 mL of 51.54 $mg \cdot L^{-1}$ DPPH ethanol solution. After 50 min of incubation at room temperature, the optical absorbance was measured at 517 nm. The DPPH scavenging activity of essential oil was calculated by Eq. 1,

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

where Y is the 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 essential oil in the presence of the ethanol solution (mixture of 4.0 mL of ethanol and 1.0 mL essential oil ethanol solution), and A_s is the absorbance of essential oil ethanol solution after incubation (4.0 mL of 51.54 mg·L^{-1} DPPH ethanol solution and 1.0 mL essential oil ethanol solution). IC_{50} (the essential oil ethanol solution concentration that generated a 50% scavenging effect) values were also calculated according to Eq. 1, and IM_{50} (the removed DPPH mass when essential oil generated a 50% DPPH scavenging effect) values were calculated as follows:

$$IM_{50} = \frac{M_{DPPH}}{M_{sample}} = \frac{0.5 \times 4 \times 10^{-3} \times 51.54}{1.0 \times 10^{-3} \times IC_{50}} \qquad IM_{50} = \frac{103.08}{IC_{50}} \quad (2)$$

M_{DPPH} is 50% DPPH mass added when antioxidant sample generated a 50% DPPH scavenging effect, and M_{sample} is sample mass added when antioxidant sample generated a 50% DPPH scavenging effect. The IM_{50} value is the removed DPPH mass when essential oil generated a 50 % DPPH scavenging effect. The higher the IM_{50} value is, the stronger the DPPH scavenging ability of the essential oil is. Rutin, quercetin, gallic acid, and ascorbic acid were used as positive controls.

RESULTS AND DISCUSSION

Yield and Character of the Essential Oils from Different *T. sinensis* Samples

The yield and characteristics of the essential oil from different *T. sinensis* samples are shown in Table 1.

Table 1. Yield and Character of the Essential Oils from Different *T. sinensis* Samples

Geographical origin	Parts	No.	Appearance character	Yield (mL·kg^{-1})
Nandan county	Leaf blades	1	Brownish-orange oily liquid, fragrant odor	7.33
Dongnan county	Leaf blades	2	Gray-green oily liquid, fragrant odor	4.80
Dongnan county	common Petioles	3	Light gray-green oily liquid, light Fragrance	1.33

All samples were from Guangxi Province, China, and collected in August, 2008

As can be seen in Table 1, the yield and appearance of the essential oils from different parts of *T. sinensis* in different geographical areas were different, and the yield of the essential oil from Guangxi Nandan county in China in August were the highest (7.33 mL·kg^{-1}).

Chemical Composition of Essential Oils

The total ion chromatograms of essential oils from *T. sinensis* leaf blades and common petioles extracted by simultaneous distillation solvent extraction (SDE) are shown in Figs. 1 to 3. The components identified in these essential oils of *T. sinensis* are listed in Table 2 in order of their experimental retention time and retention indices. There were differences among the components and contents (relative mass fraction) of essential oils from *T. sinensis* in different geographical areas. A total of 63 compounds were identified in the essential oil of leaf blades grown in Nandan county in Guangxi province, China (No. 1), and 63 compounds in the essential oil of leaf blades (No. 2) and 72 compounds in the essential oil of common petioles (No. 3) grown in Dongnan county in Guangxi province, China, were also identified, accounting for 99.35%, 99.25%, and 96.78% (relative mass fraction), respectively. The relative mass fraction of each component is shown in Table 2.

Among the 63 kinds of compounds identified in essential oil (No. 1) were 25 kinds of sesquiterpenes (accounting for 44.30%), 20 kinds of sesquiterpene oxygenated compounds (accounting for 45.80%), and 16 kinds of non-terpenoid compounds (sulfur-containing compounds 0.2%, guaiazulene 1.5% among them). The main compounds in essential oil (No. 1) were (-)-spathulenol (13.24%), β-himachalenoxide (12.79%), α-copaene (7.35%), (±)-cadinene (5.07%), 1R,4S,7S,11R-2,2,4,8-tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene (5.07%), himachala-2,4-diene (3.59%), δ-cadinene (3.42%), cubenol (3.12%), 6-isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydro-naphthalen-2-ol (3.08%), tau-cadinol (2.86%), cadala-1(10),3,8-triene (2.52%), longifolene-(V4)– (2.48%), 7-tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol,4,4,11,11-tetramethyl- (2.24%), aristolene (2.01%), (+)-aromadendrene (1.88%), tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl- (1.81%), isolongifolene,4,5,9,10-dehydro- (1.51%), guaiazulene (1.50%), in which guaiazulene was found in *T. sinensis* the first time. The mass spectrogram and structural formula of guaiazulene are shown in Fig. 4.

Among the 63 kinds of compounds identified in essential oil (No. 2) were one kind of monoterpene oxygenated compound (0.12%), 25 kinds of sesquiterpenes (accounting for 61.67%), 19 kinds of sesquiterpene oxygenated compounds (accounting for 30.97%), and 17 kinds of non-terpenoid compounds (sulfur-containing compounds 0.34%, guaiazulene 0.75% among them). The main compounds in essential oil (No. 2) were α-copaene (10.96%), himachala-2,4-diene (9.09%), α-himachalene (6.90%), α-guaiene (6.63%), 6-isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydro-naphthalen-2-ol (6.29%), tau-cadinol (5.41%), (±)-cadinene (5.07%), (-)-spathulenol (4.56%), β-himachalene (4.17%), δ-cadinene (3.69%), 1R,4S,7S,11R-2,2,4,8-tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene (3.68%), cadala-1(10),3,8-triene (2.73%), aromadendrene oxide-(2.02%), tricyclo[5.2.2.0(1,6)]undecan-3-ol,2-methylene-6,8,8-trimethyl- (1.91%), cubenol (1.72%), β-caryophyllene (1.48%).

Among the 72 kinds of compounds identified in essential oil (No. 3) were 3 kinds of monoterpenes oxygenated compounds (accounting for 0.37%), 26 kinds of sesquiterpenes (accounting for 53.23%), 18 kinds of sesquiterpene oxygenated compounds (accounting for 27.64%), and 22 kinds of non-terpenoid compounds (sulfur-containing compounds 7.96%, guaiazulene 0.29% among them). The main compounds in essential oil (No. 3) were α-copaene (11.32%), methylthiirane (7.29%), α-copaen-11-ol (6.47%), δ-cadinene (5.66%), cubenol (4.85%), himachala-2,4-diene (4.2%), β-caryophyllene (4.11%), tau-cadinol (4.09%), (-)- spathulenol (4.00%), longifolene-(V4)–

(3.8%), α-cubebene (3.74%), cadala-1(10),3,8-triene (2.99%), l-(+)-ascorbic acid 2,6-dihexadecanoate (2.74%), aromadendrene oxide-(1) (1.86%), humulen-(v1) (1.72%), (±)-cadinene (1.62%), aromadendrene, dehydro- (1.52%).

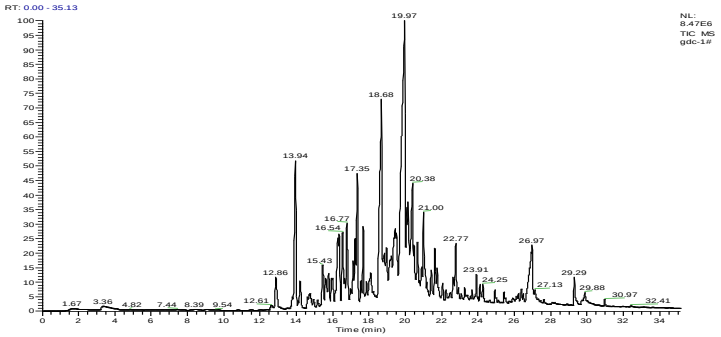

Fig. 1. Total ions chromatogram of chemical components in essential oil (No. 1) from *T. sinensis* leaves

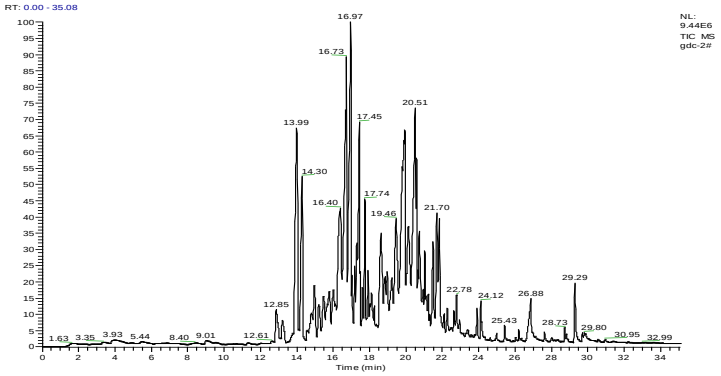

Fig. 2. Total ion chromatogram of chemical components in essential oil (No. 2) from *T. sinensis* leaves

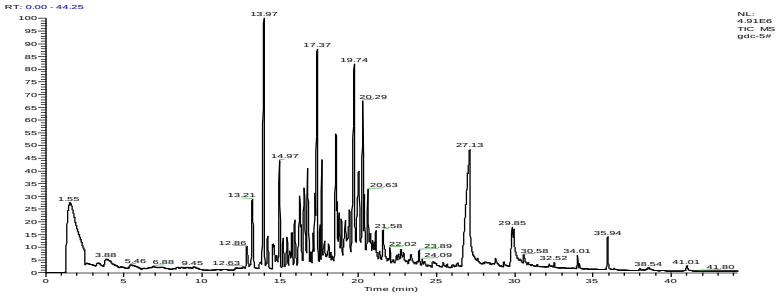

Fig. 3. Total ion chromatogram of chemical components in essential oil (No. 3) from *T. sinensis* common petioles

There were 57 shared chemical components in essential oils No. 1 and 2, including one kind of the same sulfur compounds: thiophene, 4-dimethyl-, one kind of the same azulenoids: guaiazulene, 24 kinds of the same sesquiterpene compounds, and 21 kinds of the same oxygenated sesquiterpene compound; however, the relative mass fraction of each components was different.

There were 54 shared chemical components in essential oils No. 2 and 3, including 3 kinds of the same sulfur compounds: methylthiirane, thiophene,2,4-dimethyl-

, 5-thiatricyclo[4.1.0.0(2,4)]heptanes, one kind of the same azulenoids: guaiazulene, 24 kinds of the same sesquiterpene compounds, and 19 kinds of the same oxygenated sesquiterpene compound; however, the relative mass fraction of each component was different.

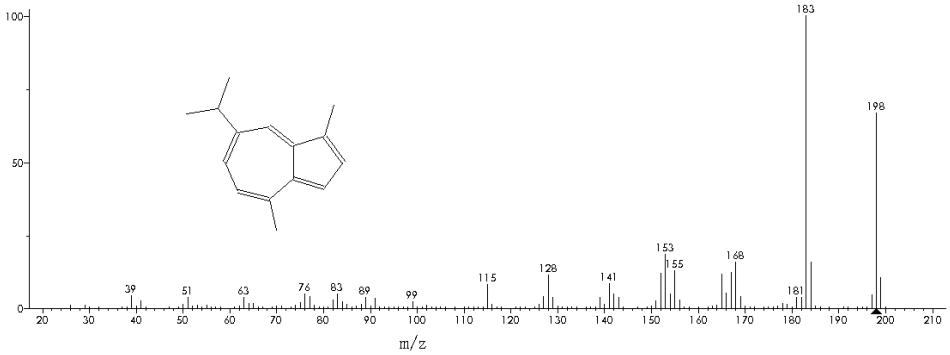

Fig. 4. Mass spectrogram of guaiazulene

Table 2. Chemical Components and Relative Mass Fraction in the Essential Oil from *T. sinensis*

No	Compounds	Formula	M.W.	R.M.F. (%)		
				No. 1	No. 2	No. 3
1	Methylthiirane	C_3H_6S	74	—	0.07	7.29
2	2-Furanmethanethiol, 5-methyl-	C_6H_8OS	128	—	—	0.06
3	Thiophene, 2,4-dimethyl-	C_6H_8S	112	0.2	0.1	0.25
4	5-Thiatricyclo[4.1.0.0(2,4)]heptane	C_6H_8S	112	—	0.17	0.2
5	Isogeraniol	$C_{10}H_{18}O$	154	—	—	0.10
6	Carveol	$C_{10}H_{16}O$	152	—	—	0.14
7	3,3,6,6-Tetramethyl-4,5-didehydro-2,3,6,7-tetrahydrothiepine 1-oxide	$C_{10}H_{16}OS$	184	—	—	0.16
8	2-Isopropylidene-3-methylhexa-3,5-dienal	$C_{10}H_{14}O$	150	—	0.12	—
9	3,5-Heptadienal, 2-ethylidene-6-methyl-	$C_{10}H_{14}O$	150	—	—	0.13
10	3,6-Dimethoxy-1a,2,2a,3,6,6a,7,7a-octahydro-1-oxacyclopropa[b]naphthalene	$C_{12}H_{18}O_3$	210	0.16	0.06	—
11	β-Elemene	$C_{15}H_{24}$	204	1.19	0.81	1.14
12	α-Cubebene	$C_{15}H_{24}$	204	—	1.08	3.74
13	(+)-Cyclosativene	$C_{15}H_{24}$	204	0.15	0.07	0.25
14	α-Copaene	$C_{15}H_{24}$	204	7.35	10.96	11.32
15	α-Guaiene	$C_{15}H_{24}$	204	1.23	6.63	1.20
16	Humulen-(v1)	$C_{15}H_{24}$	204	0.22	0.29	1.72
17	β-Iraldeine	$C_{14}H_{22}O$	206	0.64	0.62	—
18	β-Caryophyllene	$C_{15}H_{24}$	204	0.29	1.48	4.11
19	Isoledene	$C_{15}H_{24}$	204	0.27	1.12	1.04
20	Aristolene	$C_{15}H_{24}$	204	2.01	1.14	1.04
21	β-Guaiene	$C_{15}H_{24}$	204	0.92	0.18	0.42
22	Seychellene	$C_{15}H_{24}$	204	1.04	0.78	1.09
23	(+)-Aromadendrene	$C_{15}H_{24}$	204	1.88	1.23	1.33
24	(±)-Cadinene	$C_{15}H_{24}$	204	5.07	5.07	1.62
25	Longifolene-(V4) -	$C_{15}H_{24}$	204	2.48	—	3.80
26	Himachala-2,4-diene	$C_{15}H_{24}$	204	3.59	9.09	4.20
27	α-Himachalene	$C_{15}H_{24}$	204	0.31	6.90	0.32
28	τ-Muurolene	$C_{15}H_{24}$	204	0.82	0.89	0.72
29	Cadina-1,3,5-triene	$C_{15}H_{22}$	202	1.18	0.81	1.17
30	δ-Cadinene	$C_{15}H_{24}$	204	3.42	3.69	5.66

31	Cadina-1,4-diene	$C_{15}H_{24}$	204	0.58	0.76	0.99
32	Cadala-1(10),3,8-triene	$C_{15}H_{22}$	202	2.52	2.73	2.99
33	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	204	0.38	0.88	0.72
34	Isolongifolene, 4,5,9,10-dehydro-	$C_{15}H_{20}$	200	1.51	0.52	0.41
35	β-Himachalene	$C_{15}H_{24}$	204	0.08	0.30	0.36
36	(-)-Spathulenol	$C_{15}H_{24}O$	220	13.24	4.56	4.00
37	τ-Gurjunenepoxide-(2)	$C_{15}H_{24}O$	220	0.50	0.80	1.08
38	Cedren-13-ol, 8-	$C_{15}H_{24}O$	220	0.94	—	—
39	Aromadendrene oxide-(1)	$C_{15}H_{24}O$	204	1.34	2.02	1.86
40	1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene	$C_{15}H_{24}$	204	5.07	3.68	1.51
41	α-Copaen-11-ol	$C_{15}H_{24}O$	220	—	—	6.47
42	β- Himachalenoxide	$C_{15}H_{24}O$	220	12.79	4.17	—
43	Cubenol	$C_{15}H_{26}O$	222	3.12	1.72	4.85
44	tau.-Cadinol	$C_{15}H_{26}O$	222	2.86	5.41	4.09
45	2-(4α-,8-Dimethyl-1,2,3,4,4α,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	$C_{15}H_{24}O$	220	0.48	0.08	1.18
46	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	$C_{15}H_{24}O$	220	1.81	1.91	0.44
47	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	$C_{15}H_{24}O$	220	2.24	1.29	0.47
48	Eudesm-4(14)-en-11-ol	$C_{15}H_{26}O$	222	0.16	0.37	0.51
49	Ledene oxide-(II)	$C_{15}H_{24}O$	220	0.06	0.12	0.33
50	6-Isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	220	3.08	6.29	0.92
51	δ-Cadinol	$C_{15}H_{26}O$	222	0.59	0.46	0.41
52	Murolan-3,9(11)-diene-10-peroxy	$C_{15}H_{24}O_2$	236	0.50	0.75	0.43
53	Isolongifolene, 7,8-dehydro-8α-hydroxy-	$C_{15}H_{24}O$	220	0.59	0.44	0.21
54	Guaiazulene	$C_{15}H_{18}$	198	1.50	0.75	0.29
55	2,2,6-Trimethyl-1-[(1E)-3-methyl-1,3-butadienyl]-5-methylene-7-oxabicyclo[4.1.0]heptane	$C_{15}H_{22}O$	218	0.42	0.35	0.27
56	2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	$C_{15}H_{22}O$	218	0.49	0.17	0.33
57	Bicyclo[4.4.0]dec-5-ene,1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-	$C_{15}H_{24}O_2$	236	0.25	0.04	0.05
58	1R,3Z,9S-2,6,10,10-Tetramethylbicyclo[7.2.0]undeca-2,6-diene	$C_{15}H_{24}$	204	0.74	0.58	0.36
59	Menthol, 1'-(butyn-3-one-1-yl)-,(1S,2S,5R)-	$C_{14}H_{22}O_2$	222	0.49	0.89	0.14
60	6,9-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	0.52	—	0.18
61	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)	$C_{15}H_{24}O_2$	236	0.60	0.05	0.23
62	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	$C_{21}H_{32}O_2$	316	0.08	—	—
63	(6E,9E)-18,18-Dimethoxy-6,9-octadecadiene	$C_{20}H_{38}O_2$	310	—	0.21	—
64	Santalol, cis, α-	$C_{15}H_{24}O$	220	0.55	0.33	0.11
65	Androst-5,7-dien-3-ol-17-one	$C_{19}H_{26}O_2$	286	0.09	—	0.08
66	5-(7α-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-penta-2,4-dien-1-ol	$C_{20}H_{32}O$	288	0.21	0.09	0.12
67	2-Methylenecholestan-3-ol	$C_{28}H_{48}O$	400	0.29	0.20	—

68	Strophanthidol	$C_{23}H_{34}O_6$	406	0.79	—	0.13
69	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	1.95	0.87	2.74
70	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	256	0.05	0.26	0.19
71	Cedrane-8,13-diol	$C_{15}H_{26}O_2$	238	0.10	0.16	—
72	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.91	1.29	—
73	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	0.11	0.14	—
74	α-Glyceryl linolenate	$C_{21}H_{36}O_4$	352	0.35	0.19	0.63
75	6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	346	—	0.06	0.42
76	2-(7-Heptadecynyloxy)tetrahydro-2H-pyran	$C_{22}H_{40}O_2$	336	—	—	0.08
77	Heptadecane, 9-octyl-	$C_{25}H_{52}$	352	—	—	0.13
78	1,7-Dicyclopentyl-4-n-octylheptane	$C_{25}H_{48}$	348	—	—	0.36
79	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	324	—	—	0.10
80	Tetracosane, 11-decyl-	$C_{34}H_{70}$	478	—	—	1.02
81	17-Pentatriacontene	$C_{35}H_{70}$	490	—	—	0.07
82	21-Acetoxypregnenolone acetate	$C_{25}H_{36}O_5$	416	—	—	0.11
83	3-Ethyl-5-(2'-ethylbutyl)octadecane	$C_{26}H_{54}$	366	—	—	0.19

No. 1 is the essential oil derived from *T. sinensis* leaves grown in Guangxi, Nandan county in China, collected in August, 2008; No. 2 is the essential oil derived from *T. sinensis* leaves grown in Guangxi, Dongnan county in China, collected in August, 2008; and No. 3 is the essential oil derived from *T. sinensis* common petioles grown in Guangxi, Dongnan county in China, collected in August, 2008. M.W. is molecular weight, R.T. is retention time, and R.M.F. is relative mass fraction.

Antimicrobial Activity

Growth inhibition zone diameters of different essential oil samples against microorganisms

All essential oil samples of *T. sinensis* exhibited significant growth inhibitory activity against the tested microorganisms (Table 3). The growth inhibition zone diameters of oil samples against the tested bacteria and fungi were 26 ± 0.1 mm ~ 60 ± 0.1 mm and 11 ± 0.1 mm ~ 50 ± 0.1 mm, respectively. *T. sinensis* essential oil was found to exhibit growth inhibitory activity against *C. gloeosporioides* for the first time.

Table 3. Growth Inhibition Zone Diameters of the Essential Oils against Microorganisms (mm)

Microorganisms	Essential oil samples		
	No.1	No.2	No.3
Escherichia coli	60 ± 0.1	50 ± 0.1	26 ± 0.1
Bacillus subtilis	36 ± 0.1	35 ± 0.1	36 ± 0.1
Penicillium citrinum	16 ± 0.1	11 ± 0.1	14 ± 0.1
Colletotrichum gloeosporioides	31 ± 0.1	50 ± 0.1	50 ± 0.1

There were differences among the growth inhibitory activities of different essential oil samples against different microorganisms. The order of inhibitory activity of essential oil samples from strong to weak was, for essential oil No. 1: *E. coli* > *B. subtilis* > *C. gloeosporioides* > *P. citrinum*; for essential oil No. 2: *E. coli* = *C. gloeosporioides* > *B. subtilis* > *P. citrinum*; and for essential oil No. 3: *C. gloeosporioides* > *B. subtilis* > *E. coli* > *P. citrinum*.

Different microorganisms had different sensitivities to the same essential oil samples. *E. coli* exhibited the strongest sensitivity to essential oil No. 1, with a growth

inhibition zone diameter of 60 ± 0.1 mm, and the weakest sensitivity to essential oil No. 3, with a growth inhibition zone diameter of 26 ± 0.1 mm. *B. subtilis* exhibited similar sensitivity to the three essential oils, with a growth inhibition zone diameter 35 ± 0.1 mm ~ 36 ± 0.1 mm. *P. citrinum* exhibited the strongest sensitivity to essential oil No. 1, with a growth inhibition zone diameter of 16 ± 0.1 mm, and the weakest sensitivity to essential oil No. 2, with a growth inhibition zone diameter of 11 ± 0.1 mm. *C. gloeosporioides* exhibited the same sensitivity to essential oils Nos. 2 and 3, with growth inhibition zone diameters of 50 ± 0.1 mm, greater than its sensitivity to essential oil No. 1, with a growth inhibition zone diameter of 31 ± 0.1 mm. The three essential oils exhibited noticeable growth inhibitory activity against *C. gloeosporioides.*

MIC and MBC of essential oils against microorganisms

MIC and MBC of different essential oils against microorganisms are shown in Table 4.

Table 4. MIC and MBC of Essential Oils against Microorganism (μg·mL^{-1})

Microorganism	No. 1 essential oil		No. 2 essential oil		No. 3 essential oil	
	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	< 25	< 25	< 25	< 25	< 25	< 25
Bacillus subtilis	< 25	< 25	< 25	< 25	< 25	< 25
Penicillium citrinum	200	400	200	400	200	400
Colletotrichum gloeosporioides	50	200	50	200	50	200

All essential oils of *T. sinensis* exhibited noticeable growth inhibitory activity against the tested microorganisms. The MIC and MBC of different essential oils against microorganisms were different. The MIC and MBC against *E. coli* and *B. subtilis* of all essential oil samples were less than 25 μg·mL^{-1}. The MIC against *P. citrinum* of all essential oil samples was 200 μg·mL^{-1}, and the MBC against *P. citrinum* of all essential oil samples was 400 μg·mL^{-1}. The MIC against *C. gloeosporioides* of all essential oils was 50 μg·mL^{-1}, and the MBC against *C. gloeosporioides* of all essential oils was 200 μg·mL^{-1}.

T. sinensis essential oils were a complex mixture of components, and the difference in antimicrobial activity among the different samples resulted from different chemical compositions. There were differences in chemical compositions and contents among essential oils (No. 1, No. 2, and No. 3) extracted from *T. sinensis* from different parts and in different geographical areas in China, but the main components of essential oils were sesquiterpene and sesquiterpene oxygenated compounds, accounting for 90.10%, 92.64%, and 80.87% of the relative mass fraction, respectively. The primary compounds in essential oils are shown in Table 5.

The antimicrobial effect of the essential oils is the result of their chemical composition synergy. The major antimicrobial components and the antimicrobial chemical compositions' antimicrobial action mechanisms should be subject to further study.

Table 5. Primary Compounds in Essential Oils Extracted from *T. sinensis*

No	Compounds	Formula	M.W.	R.M.F. (%)		
				No. 1	No. 2	No. 3
1	Methylthiirane	C_3H_6S	74	—	0.07	7.29
11	β-Elemene	$C_{15}H_{24}$	204	1.19	0.81	1.14
12	α-Cubebene	$C_{15}H_{24}$	204	—	1.08	3.74
14	α-Copaene	$C_{15}H_{24}$	204	7.35	10.96	11.32
15	α-Guaiene	$C_{15}H_{24}$	204	1.23	6.63	1.20
16	Humulen-(v1)	$C_{15}H_{24}$	204	0.22	0.29	1.72
18	β-Caryophyllene	$C_{15}H_{24}$	204	0.29	1.48	4.11
19	Isoledene	$C_{15}H_{24}$	204	0.27	1.12	1.04
20	Aristolene	$C_{15}H_{24}$	204	2.01	1.14	1.04
22	Seychellene	$C_{15}H_{24}$	204	1.04	0.78	1.09
23	(+)-Aromadendrene	$C_{15}H_{24}$	204	1.88	1.23	1.33
24	(±)-Cadinene	$C_{15}H_{24}$	204	5.07	5.07	1.62
25	Longifolene-(V4) -	$C_{15}H_{24}$	204	2.48	—	3.80
26	Himachala-2,4-diene	$C_{15}H_{24}$	204	3.59	9.09	4.20
27	α-Himachalene	$C_{15}H_{24}$	204	0.31	6.90	0.32
29	Cadina-1,3,5-triene	$C_{15}H_{22}$	202	1.18	0.81	1.17
30	δ-Cadinene	$C_{15}H_{24}$	204	3.42	3.69	5.66
32	Cadala-1(10),3,8-triene	$C_{15}H_{22}$	202	2.52	2.73	2.99
34	Isolongifolene, 4,5,9,10-dehydro-	$C_{15}H_{20}$	200	1.51	0.52	0.41
36	(-)-Spathulenol	$C_{15}H_{24}O$	220	13.24	4.56	4.00
37	τ-Gurjunenepoxide-(2)	$C_{15}H_{24}O$	220	0.50	0.80	1.08
39	Aromadendrene oxide-(1)	$C_{15}H_{24}O$	204	1.34	2.02	1.86
40	1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene	$C_{15}H_{24}$	204	5.07	3.68	1.51
41	α-Copaen-11-ol	$C_{15}H_{24}O$	220	—	—	6.47
42	β- Himachalenoxide	$C_{15}H_{24}O$	220	12.79	4.17	—
43	Cubenol	$C_{15}H_{26}O$	222	3.12	1.72	4.85
44	tau.-Cadinol	$C_{15}H_{26}O$	222	2.86	5.41	4.09
45	2-(4α-,8-Dimethyl-1,2,3,4,4α,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	$C_{15}H_{24}O$	220	0.48	0.08	1.18
46	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	$C_{15}H_{24}O$	220	1.81	1.91	0.44
47	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	$C_{15}H_{24}O$	220	2.24	1.29	0.47
50	6-Isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	220	3.08	6.29	0.92
54	Guaiazulene	$C_{15}H_{18}$	198	1.50	0.75	0.29

DPPH Scavenging Activity of Essential Oil

The IC_{50} and IM_{50} values of essential oils scavenging DPPH calculated according to Eqs. 1 and 2 are shown in Table 6. Relationships between DPPH scavenging effects and the concentration of essential oil determined by regression analysis methods are also shown in Table 6. The IM_{50} values of scavenging DPPH of rutin, quercetin, gallic acid, and ascorbic acid were 3.300, 6.734, 4.730, and 13.506 g DPPH per g sample, respectively (Chen *et al.* 2006). It can be seen from Table 7 that the IM_{50} of scavenging DPPH of *T. sinensis* essential oil was low, less than 0.3 g DPPH per g essential oil, which may be related to the elevated temperature used in the simultaneous distillation and extraction. The weak DPPH scavenging activity of *T. sinensis* essential oil indicated its weak antioxidant capacity.

Table 6. Relationship between DPPH Scavenging Effect and the Concentration of *T. sinensis* Essential Oil

Essential oil samples	Relationship	R^2	Concentration range ($mg \cdot l^{-1}$)	IC_{50} ($mg \cdot l^{-1}$)	IM_{50} ($g \cdot g^{-1}$)
No. 1	Y = 0.0514 C + 6.7243	0.9690	38.0~860.0	841.94	0.1224
No. 2	Y = 0.0157 C + 12.645	0.9638	48.8~2440.0	2379.30	0.04332
No. 3	Y = 0.09 C + 6.7813	0.9905	11.6~464.0	480.21	0.2147

R is the correlation coefficient, C is the concentration of the essential oil ($mg \cdot L^{-1}$), and Y is the DPPH scavenging effect (%).

CONCLUSIONS

1. A total of 63 compounds were identified in the essential oil of leaf blades grown in Nandan county in Guangxi province, China (No. 1), 63 in the essential oil of leaf blades (No. 2), and 72 in the essential oil of common petioles (No. 3) grown in Dongnan county in Guangxi province, China in August, accounting for 99.35%, 99.25% and 96.78% of the relative mass fraction, respectively. There were differences in chemical compositions and content among the essential oils extracted from different parts of *Toona sinensis* in different geographical areas in China, but the primary components of essential oils were sesquiterpene and sesquiterpene oxygenated compounds, accounting for 90.10%, 92.64%, and 80.87% of the relative mass fraction, respectively.
2. All essential oils of *T. sinensis* exhibited noticeable growth inhibitory activity against the tested microorganisms. MIC and MBC of different essential oil samples against each microorganism were different. MIC and MBC against *Escherichia coli* and *Bacillus subtilis* of all essential oil samples were less than 25 $\mu g \cdot mL^{-1}$. MIC and MBC against *Penicillium citrinum* of all essential oil samples were 200 $\mu g \cdot mL^{-1}$ and 400 $\mu g \cdot mL^{-1}$, respectively. MIC and MBC against *Colletotrichum gloeosporioides* of all essential oil samples were 50 $\mu g \cdot mL^{-1}$ and 200 $\mu g \cdot mL^{-1}$, respectively. *T. sinensis* essential oil samples were a complex mixture of components, and the difference in antimicrobial activity among the different essential oil samples resulted from different chemical compositions. The major antimicrobial components and the antimicrobial chemical compositions' antimicrobial action mechanisms are subjects for further study.
3. The IM_{50} of scavenging DPPH of *T. sinensis* essential oil was low, less than 0.3 g DPPH *per* g essential oil. The weak DPPH scavenging activity of *T. sinensis* essential oil indicated its weak antioxidant capacity.
4. The results indicated that *T. sinensis* essential oil may be a useful new source of natural antiseptic from forest products.

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