Asian Journal of Chemistry; Vol. 26, No. 9 (2014), 2557-2560

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.15727

Chemical and Antimicrobial Analyses of Essential Oil of *Toona sinensis* from China

CHUN-LIN WANG[1,*], JUN-XIU SHI[2] and YUN WU[2]

[1]Longdong Key Laboratory of Protection and Utilization of Bio-resource, Qingyang 745000, Gansu Province, P.R. China
[2]College of Chemistry and Chemical Engineering, Longdong University, Qingyang 745000, P.R. China

*Corresponding author: Tel: +86 934 8658905; E-mail: ldxywangchunlin@126.com

Received: 4 May 2013; *Accepted*: 18 March 2014; *Published online*: 28 April 2014; AJC-15068

Toona sinensis Roem, belonging to the *Toona* family, was used as one of the traditional Chinese medicines in China. The chemical composition of essential oil obtained by hydrodistillation from the dried leaves of *T. sinensis* was analyzed by gas chromatography-mass spectrometry (GC-MS). Sixty-two compounds, constituting about 92.46 % of the total oil, were identified. The main constituents were *trans*-phytol (9.76 %), β-caryophyllene (8.79 %), palmitic acid (5.76 %), *endo*-borneol (5.03 %), α-cubebene (4.98 %), α-farnesene (4.12 %), camphor (3.85 %), borneyl acetate (3.34 %) and β-cubebene (3.02 %). The antimicrobial activity of the essential oil was evaluated against 6 microorganisms using agar disc diffusion and broth microdilution methods. The essential oil was found to show a broad spectrum of antimicrobial activity against all the tested bacterial strains. The essential oil had more sensitivity to gram-positive (*Staphylococcus aureus* and *Streptococcus pneumoniae*) than gram-negative (*Escherichia coli, Pseudomonas aeruginosa*, *Shigella flexneri* and *Salmonella typhi*) bacteria.

Keywords: *Toona sinensis* Roem, Essential oil, GC-MS, Antimicrobial activity.

INTRODUCTION

Toona sinensis Roem, also known as Chinese *Toona*, belonging to the family *Meliaceae*, is widely distributed in North China and Southeast China, especially in Gansu, Hebei and Shandong Provinces. It is a rapidly growing, perennial deciduous tree vegetable and its fresh, young leaves and shoots are edible and nutritious. More importantly, almost all parts of *T. sinensis* including seeds, bark, root bark, petioles and leaves have medicinal effects to human health[1-3]. Its leaves has been used for the treatments of enteritis, dysentery, carbuncles, boils, dermatitis, scabies, tinea blanca, heliosis and for the health improvement as a traditional Chinese medicine[4]. The bark is used as an astringent and as a depurative substance, the powdered root is used as a corrective and the fruits are used as an astringent and for the treatment of eye infection[5]. As far as our literature survey could ascertain, the antimicrobial activity of essential oil has not been published previously, although there are some reports on the essential oil composition isolated from this species[6,7]. Therefore, we focused our study on the chemical composition and antimicrobial properties of the essential oil of the leaves of *T. sinensis* from China.

EXPERIMENTAL

The leaves of *T. sinensis* Roem were collected during germination stage in May 2011 from Qingyang in Gansu province, China.

Extraction: Dried leaves of *T. sinensis* (200 g) were distilled for 2.5 h using a Clevenger type apparatus. The essential oil obtained was dried over anhydrous Na_2SO_4 and stored at -4 °C until tested and analyzed.

Analysis and identification: The analysis of the essential oil was performed in a Hewlett-Packard 6890 gas chromatograph equipped with a HP-5 MS fused silica capillary column (30 m × 0.25 mm, film thinkness 0.25 μm) and a Hewlett-Packard 5973 MSD (280 °C). The mass spectra were obtained by electron ioniazation at 70 eV. The column temperature was programmed from 50 to 80 °C at 5 °C/min, isothermal at 80 °C for 4 min, then increased to 150 °C at 5 °C/min, from 150 to 170 °C at 2 °C/min and from 170 to 280 °C at 5 °C/min, respectively and isothermal at 280 °C for 15 min. The temperature at the interface was 260 °C. The carrier gas was helium (99.999 %) with a column head pressure of 58.5 kPa and a flow-rate of 1 mL/min and fractional ratio of 40:1. The volume of the sample injection was 0.2 μL. The mass scanning range was 35-450 amu. By means of GC-MS technique, the gas chromatogram and mass spectrogram of the constituents of the essential oils were achieved (Fig. 1). The constituents were tentatively analyzed and identified by comparing the experimental retention indices (RI) and MS fragmentation pattern with corresponding reference data (Table-1)[8,9]. A standard solution of *n*-alkanes (C_6-C_{30}) was used to obtain the retention indices.

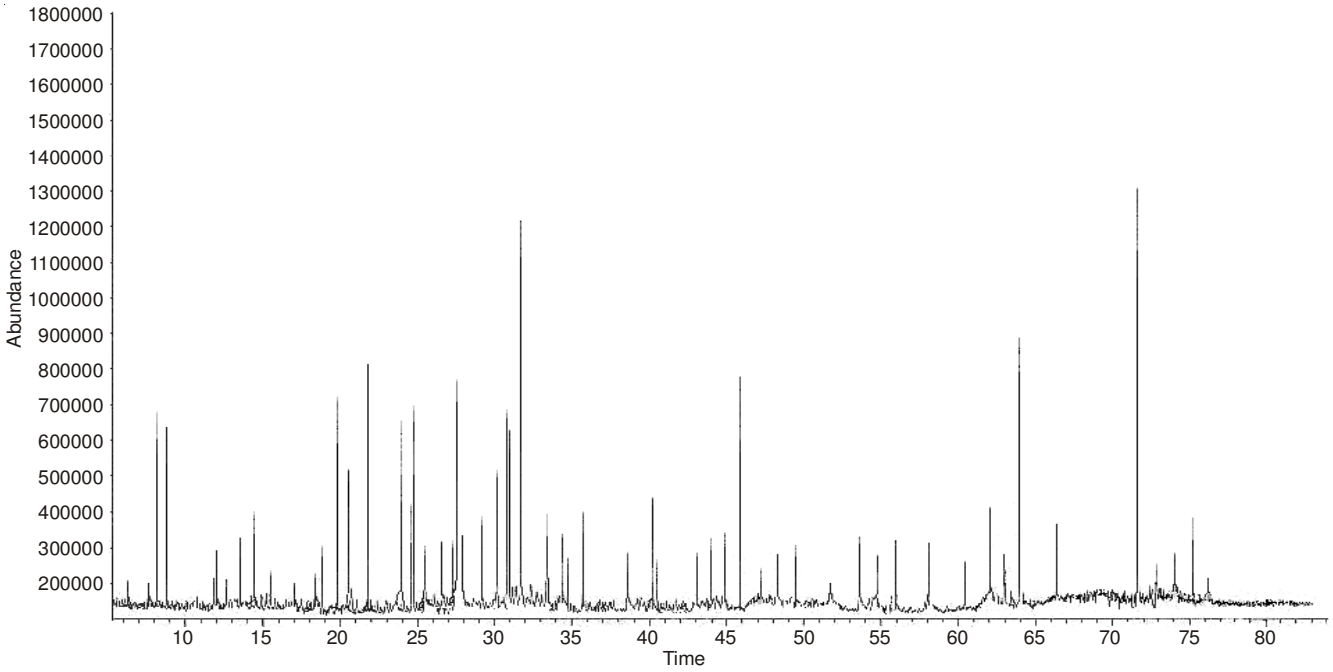

Fig. 1. Total ion chromatogram of the essential oil from *Toona sinensis* Roem

TABLE-1
CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF *T. sinensis*[*]

Compounds	RI[a]	m.f.	Contents (%)	Compounds	RI[a]	m.f.	Contents (%)
Hexanal	801	$C_6H_{12}O$	0.12	α-Humulene	1453	$C_{15}H_{24}$	0.47
Ethyl acetate	803	$C_4H_8O_2$	Tr.	(E)-β-Farnesene	1459	$C_{15}H_{24}$	1.43
(E)-2-Hexenal	853	$C_6H_{10}O$	2.95	γ-Curcumene	1475	$C_{15}H_{22}$	0.35
cis-3-Hexenol	858	$C_6H_{12}O$	2.34	(E)-β-Ionone	1481	$C_{13}H_{20}O$	0.61
Heptanal	903	$C_7H_{14}O$	Tr.	β-Selinene	1485	$C_{15}H_{24}$	0.87
α-Pinene	937	$C_{10}H_{16}$	0.19	β-Guaiene	1501	$C_{15}H_{24}$	0.94
Camphene	951	$C_{10}H_{16}$	0.46	α-Farnesene	1509	$C_{15}H_{24}$	4.12
Benzaldehyde	962	C_7H_6O	0.18	β-Bisabolene	1510	$C_{15}H_{24}$	0.24
(E)-2-Heptenal	979	$C_7H_{12}O$	0.96	β-Cadinene	1518	$C_{15}H_{24}$	0.45
(E,E)-2,4-Heptadienal	1010	$C_7H_{10}O$	1.32	δ-Cadinene	1539	$C_{15}H_{24}$	0.71
Phenylacetaldehyde	1045	C_8H_8O	0.31	α-Cadinene	1541	$C_{15}H_{24}$	Tr.
Linalool	1099	$C_{10}H_{18}O$	0.11	Geraniene-B	1556	$C_{15}H_{24}$	0.85
n-Nonanal	1103	$C_9H_{18}O$	0.28	*trans*-Nerolidol	1564	$C_{15}H_{26}O$	0.69
endo-Fenchol	1114	$C_{10}H_{18}O$	0.53	Spathulenol	1574	$C_{15}H_{24}O$	Tr.
Camphor	1145	$C_{10}H_{16}O$	3.85	Caryophyllene oxide	1581	$C_{15}H_{24}O$	0.82
Isoborneol	1149	$C_{10}H_{18}O$	2.12	α-Cedrol	1598	$C_{15}H_{26}O$	0.83
endo-Borneol	1167	$C_{10}H_{18}O$	5.03	Torreyol	1643	$C_{15}H_{26}O$	0.36
cis-3-Hexenyl butyrate	1186	$C_{10}H_{16}O_2$	2.86	(E,E)-Farnesol	1723	$C_{15}H_{26}O$	1.81
Geraniol	1256	$C_{10}H_{18}O$	1.51	(E,Z)-Farnesol	1742	$C_{15}H_{26}O$	0.75
Borneyl acetate	1286	$C_{12}H_{20}O_2$	3.34	Hexadecanal	1820	$C_{16}H_{32}O$	Tr.
Isobornyl acetate	1288	$C_{12}H_{20}O_2$	0.68	(E,E)-Farnesyl acetate	1843	$C_{17}H_{28}O_2$	Tr.
(E,E)-2,4-Decadienal	1304	$C_{10}H_{16}O$	0.87	Palmitic acid	1961	$C_{16}H_{32}O_2$	5.76
δ-Elemene	1341	$C_{15}H_{24}$	0.76	*cis*-Phytol	2114	$C_{20}H_{40}O$	0.43
α-Cubebene	1354	$C_{15}H_{24}$	4.98	*trans*-Phytol	2135	$C_{20}H_{40}O$	9.76
Eugenol	1356	$C_{10}H_{12}O_2$	0.66	Linoleic acid	2153	$C_{18}H_{32}O_2$	0.42
α-Copaene	1375	$C_{15}H_{24}$	1.15	Tetracosane	2400	$C_{24}H_{50}$	0.67
β-Bourbonene	1383	$C_{15}H_{24}$	2.10	Pentacosane	2500	$C_{25}H_{52}$	1.12
β-Cubebene	1392	$C_{15}H_{24}$	3.02	Hexacosane	2600	$C_{26}H_{54}$	0.19
β-Elemene	1395	$C_{15}H_{24}$	2.32	Total identified	92.46		
β-Caryophyllene	1418	$C_{15}H_{24}$	8.79	Monoterpene hydrocarbons	0.65		
trans-α-Bergamotene	1435	$C_{15}H_{24}$	1.23	Oxygenated monoterpenes	17.17		
α-Guaiene	1440	$C_{15}H_{24}$	0.94	Sesquiterpene hydrocarbons	37.57		
Aromadendrene	1443	$C_{15}H_{24}$	0.59	Oxygenated sesquiterpenes	5.26		
(Z)-β-Farnesene	1444	$C_{15}H_{24}$	1.26	Others	31.81		

[*]Method of identification: GC-MS, RI; [a]RI: Retention indices on HP-5MS relative to C_6-C_{30} *n*-alkanes. Tr.: Trace (<0.1%).

The relative proportions of the constituents were percentages obtained by FID peak area normalization without any correction factors.

Antimicrobial activity

Test microorganisms: The *in vitro* antimicrobial activity of the essential oil was evaluated against *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 49619, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Shigella flexneri* ATCC 12022 and *Salmonella typhi* ATCC 50013. Microorganisms were provided by the College of Life-Science at Northwest University, Xi'an (China). Bacterial strains were cultured overnight at 37 °C in Mueller Hinton agar (MHA).

Agar disc diffusion method: The antimicrobial activity of the essential oil was investigated by agar disc diffusion method. Briefly, a suspension of the tested micro-organism (2×10^8 CFU/mL) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were individually soaked with 15 µL of the diluted oil and placed on the inoculated plates, after remaining at + 4 °C for 2 h, were incubated at 37 °C for 24 h. The diameters of inhibition zones (DIZ) were measured and expressed in millimeters (Table-2). Each test was applied in triplicate. Levofloxacin served as positive control.

TABLE-2
ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL FROM *T. sinensis*

Test organism	Essential oil			Levofloxacin		
	DIZ[a]	MIC[b]	MBC[t]	DD[c]	MIC[d]	MBC[c]
(+)*Staphylococcus aureus* ATCC 25923	31	1.57	1.57	29	0.30	0.30
(+)*Streptococcus pneumoniae* ATCC 49619	26	1.57	3.13	23	0.61	1.22
(-)*Escherichia coli* ATCC 25922	24	3.13	6.25	34	0.61	0.61
(-)*Pseudomonas aeruginosa* ATCC 27853	25	3.13	3.13	28	0.30	0.61
(-)*Shigella flexneri* ATCC 12022	14	12.50	50.00	23	2.44	2.44
(-)*Salmonella typhi* ATCC 50013	21	6.25	12.50	29	1.22	1.22

[a]DIZ: Diameter of inhibition zones (mm) including disc diameter of 6 mm (as 3 mg/disc); [b]Values given as as mg/mL; [c]Tested at a concentration of 5. µg/disc; [d]Values given as µg/mL; (-), Inactive; (7-14), moderately active; (> 14), highly active

Broth microdilution method: A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)[10]. All tests were performed in Mueller Hinton broth (MHB) supplemented with Tween 80 at a final concentration of 0.5 % (v/v). Test strains were suspended in MHB to give a final density of 4×10^4 CFU/mL and these were confirmed by viable counts. Geometric dilutions ranging from 0.05 to 200 mg/mL of the essential oil, were prepared in a 96-well micro-titre plate, including one growth control (MHB + Tween 80) and one sterility control (MHB + Tween 80 + test oil). Plates were incubated under normal atmospheric conditions at 37 °C for 24 h. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The microorganism growth was indicated by the turbidity (by measuring optical density at 600 nm). To determine MBC, 10 µL broth was taken from each well and inoculated in MHB for 24 h at 37 °C. The MBC is defined as the lowest concentration of the essential oil at which inoculated microorganism was completely killed (99.99 %). The number of surviving organism was determined by viable count. The MIC and MBC of Levofloxacin was also determined in parallel experiments in order to control the sensitivity of the test microorganisms (Table-2). East test was performed in triplicate.

RESULTS AND DISCUSSION

By hydrodistillation the dried leaves of *T. sinensis* yielded 0.16 % (v/w) of essential oil. By using a chromatographic procedures 62 compounds, representing 92.46 % of the oil were identified. The chemical constituents, relative percentages and retention indices are listed according to their elution orders on HP-5MS column in Table-1. The essential oil consisted mainly of sesquiterpenes (42.83 %) and monoterpenes (17.82 %). β-Caryophyllene (8.79 %), α-cubebene (4.98 %), α-farnesene (4.12 %) and β-cubebene (3.02 %) were the main sesquiterpenes, while *endo*-borneol (5.03 %), camphor (3.85 %) and borneyl acetate (3.34 %) were the main monoterpenes. Furthermore, *trans*-phytol (9.76 %) and palmitic acid (5.76 %) were also significant.

In a previous paper, the leaves oil of *T. sinensis* from Tianjin of China were reported to contain caryophyllene (39.19 %), aromadendrene (6.91 %), nonadecane (5.32 %) and phytol (5.32 %) as the major constituents[6], while the oil from Taian of China mainly included *trans*-caryophyllene (21.42 %), 2-hexenal (14.68 %), 5-ethyl thiazole (11.81 %), 2-ethyl thiazole (7.55 %), 2,5-dimethyl thiophene (4.23 %), limonene (3.55 %) *etc*.[11]. These differences might have been derived from several factors, such as genotypic variation, geographical, geological, climatic, seasonal and experimental conditions[12].

Results obtained from disc diffusion method, followed by measurements of MIC and MBC (Table-2), indicate that the essential oil of *T. sinensis* displayed a broad antimicrobial spectrum and exerted a much stronger antimicrobial effect against Gram-positive bacteria than Gram-negative bacteria. However, the oil was not as active as Levofloxacin. According to the results given in Table-2, S. *aureus* was the most sensitive microorganism tested with the largest inhibition zone (31 mm) and *S. flexneri* exhibited the smallest inhibition zone (14 mm). The results of MIC indicated *S. aureus* and *S. pneumoniae* had the lowest MIC (1.57 mg/mL), the higest MIC was 12.50 mg/mL for *S. flexneri*. The lowest MBC was 1.57 mg/mL for *S. aureus*; *S. flexneri* had the highest MBC of 50 mg/mL. The antibacterial activity of the oils can be attributed, to a considerable degree, to the existence of constituents, such as linalool, camphor, α-pinene, caryophyllene oxide, *endo*-borneol, *etc*.[13].

ACKNOWLEDGEMENTS

The work was supported by the Natural Science Foundation of Qingyang City, China (Grant No. ZJ201303).

REFERENCES

1. J.M. Edmonds and M. Staniforth, *Curtis's Bot. Mag*., **15**, 186 (1998).
2. X.D. Luo, S.H. Wu, Y.B. Ma and D.G. Wu, *Fitoterapia*, **71**, 492 (2000).
3. E.J. Cho, T. Yokozawa, D.Y. Rhyu, S.C. Kim, N. Shibahara and J.C. Park, *Phytomedicine*, **10**, 544 (2003).
4. H.L. Chang, H.K. Hsu, J.H. Su, P.H. Wang, Y.F. Chung, Y.C. Chia, L.Y. Tsai, Y.C. Wu and S.F. Yuan, *Gynecol. Oncol*., **102**, 309 (2006).
5. S. Fan, H.-N. Chen, C.-J. Wang, W.-C. Tseng, H.-K. Hsu and C.-F. Weng, *Food Chem. Toxicol*., **45**, 2228 (2007).
6. M.H. Chen, C.L. Wang, L. Li, Y.R. Wang and S.H. Jiang, *Int. J. Food Eng*., **6**, 1 (2010).
7. Y.L. Chen, Z.P. Ruan, L.S. Lin and C.L. Li, *J. Changzhi Med. Coll*., **22**, 315 (2008).
8. R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy, Allured Publishing Corp. (2007).
9. ACIS (Aroma Chemical Information Service), ESO-97 Database of Essential Oils (1997).
10. I.H.N. Bassole, A.S. Quattara, R. Nebie, C.A.T. Quattara, Z.I. Kabore and S.A. Traore, *Phytochemistry*, **62**, 209 (2003).
11. R.M. Mu, X.R. Wang, S.X. Liu, X.L. Yuan, S.B. Wang and Z.Q. Fan, *Chromatographia*, **65**, 463 (2007).
12. N.B. Perry, R.E. Anderson, N.J. Brennan, M.H. Douglas, A.J. Heaney, J.A. McGimpsey and B.M. Smallfield, *J. Agric. Food Chem*., **47**, 2048 (1999).
13. X. Xianfei, C. Xiaoqiang, Z. Shunying and Z. Guolin, *Food Chem*., **100**, 1312 (2007).