Chemical components of the volatile oil from leaves of *Cananga odorata* and its anti-oxidant activity

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Abstract: *Cananga odorata* (Lamk.) Hook. f. et Thoms., belonging to Annonaceae, is an evergreen tree. The oils extracted from its flower are a famous perfume and used in daily chemical and food industry. Although this plant has been widely cultivated in tropical regions of the world, the yield of oils from its flower is very limited. In order to develop the other parts of this plant, the chemical constituents of the volatile oils from the leaves of *C. odorata* was analyzed by gas chromatography/flame ionization detector (GC-FID) and GC/mass spectrometry (GC-MS). And the volatiles showed nitric oxide (NO) inhibitory activity with an IC_{50} value of 37.61μg/mL and anti-oxidant activity with an IC_{50} value of 3.84mg/mL, respectively.

Keywords: *Cananga odorata*, volatile oil, chemical components, anti-inflammation, anti-oxidant activity.

INTRODUCTION

Cananga odorata (Lamk.) Hook. f. et Thoms., belonging to the family Annonaceae, is an evergreen tree (Manner and Elevitch, 2006; Chen *et al*, 1982). Its flower is regarded as a famously aromatic resource and used to extract oils, named "Ylang-Ylang oil", which is widely used in the daily chemical industry, such as shampoos, creams, and lotions, and food industry, such as ice creams, candies and baked goods flavors. Approximate 100 ton of this oil is imported into the United States every year (Gaydou *et al*, 1986). Notably, some aromatherapists claimed that Ylang-Ylang oil is useful for depression, distressed breathing, high blood pressure, anxiety, as well as anorgasmia ((Manner and Elevitch, 2006). Although this plant has been widely cultivated in tropical regions of the world, the yield of oils from its flower is very limited, and the other parts of this plant have not been utilized effectively.

Previous investigations have showed the volatile oil of the flowers from *C. odorata* has some anti-microbial (Fiona and Kevin, 1999**)**, acaricidal (In-Sook *et al*, 2006), anti-oxidative (Gaydou, *et al*, 1986; Tapanee *et al*, 2006; Baratta *et al*, 1998; Gianni *et al*, 2005; Miguel *et al*, 2002), relaxing (Miguel *et al*, 2002) and harmonizing (Hongratanaworakit *et al*, 2002) activities. And some species in genus *Cananga* contains many kinds of chemical constituents, including alkaloids (Rao *et al*, 1986; Rahman *et al*, 2005; Yang and Huang, 1988), unusual lactones (Caloprisco *et al*, 2002), linear acetogenins (Nikhom *et al*, 2011), sesquiterpenes (Hsieh *et al*, 2001), monoterpenes and monoterpene glucosides (Jiro *et al*, 2010; Matsunami *et al*, 2010). In order to further study and develop other parts from of *C. Odorata*, the volatiles was extracted from its leaves by steam distillation and analyzed by gas chromatography/flame ionization detector (GC-FID) and GC/mass spectrometry (GC-MS) for the first time. Meantime, potentially anti-inflammatory and anti-oxidant activities of this volatiles were evaluated by NO production in lipopolysaccharide (LPS)-induced RAW264.7 cells and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging bioassays, respectively.

MATERIALS AND METHODS

Material and extraction of volatiles

The leaves of *C. odorata* were collected on October, 2012 from Yunnan, China and identified by associate Prof. Mi Zhang (Faculty of Life Science and Technology, Kunming University of Science and Technology). The vouchers were stored in the laboratory of Faculty of Life Science and Technology, Kunming University of Science and Technology. The leaves (50g) were soaked in water for 1.5 hour, and extracted by steam distillation for 3 hour (Gong *et al*, 2012). Then, anhydrous sodium sulfate was added to the extract for removing the residual water. The yield of volatiles was represented as volume/weight (v/w).

Chemicals

All chemicals and reagents used in our experiments were of AR grade, and obtained from Shengshi Chemical Co., Ltd. (Wuhan, China). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and a series of n-alkanes were purchased from Sigma-Aldrich Chemie (Steinheim, Germany).

GC-FID and GC-MS analyses

The volatile oil was analyzed by using the Agilent Technologies 7820A gas chromatograph (flame ionization detector, FID). Separation was carried out on HP-5 capillary column (30m × 0.10mm × 0.10μm) and nitrogen was used as carrier gas at a flow rate of 1.0mL/min. The temperatures of detector and injector were set to 280 and

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250°C, respectively. The volume of inject was set to 1μL (2μL volatile oil in 1mL of ethanol) on split injection mode (20:1). Program temperatures were set to 50°C to 250°C at the rate of 2°C/min and held for 5min finally.

GC/mass spectrometry (GC-MS) was determined on an Agilent GC–MS, equipped with a HP-5 capillary column (cross-linked 5% phenymethyl polysiloxane, 30m × 0.10 mm × 0.10μm). For GC/MS detections, helium was used as the carrier gas at a rate of 0.5mL/min. The temperatures of detector, injector and ion source were set to 280, 250 and 250°C, respectively. Ionization energy was 70eV and electron ionization was used over a scan range of 50-550 atomic mass units. MS quad was 150 °C. The temperature of column was kept at 50°C for 5 min initially, then increased to 250°C at the rate of 2°C/min, and held for 5min finally. 1μL of the volatile oil was injected using split mode (20:1).

The oil was determined based on retention index (RI) relative to (C_8-C_{40}) n-alkanes using the same experimental conditions according to previous reports (Bektas *et al*, 2005; Gullucea *et al*, 2007; Daíse *et al*, 2008; Lioliosa *et al*, 2009). The compositions in oil were identified by matching their mass spectra with those stored in the mass spectral library of the GC/MS date system and other reports (Liu *et al*, 2014; Wang *et al*, 2009; Zhao *et al*, 2013; Mohamed *et al*, 2013). The percentage compositions were calculated by peak areas without using correction factors. And the identified compositions in volatile oil were listed in table 1.

Nitric Oxide (NO) production bioassay

RAW264.7 cells were seeded in 96-well cell culture plates (1×10^5 cells/well), supplemented with DMEM and 10% FBS. Cells were incubated with different concentrations (1, 10, 100 and 500μg/mL) of the sample, followed by stimulation with LPS (2μg/mL) for 24h, and the control group was treated with LPS only. NO production in the supernatant was determined by Griess reagents. The absorbance at 540 nm was measured with a micro plate reader.

DPPH radical scavenging bioassay

The anti-oxidative activity of the volatile oil was evaluated by DPPH radical scavenging test according to the reported method (Zhao *et al*, 2014). Each test was done in triplicate.

RESULTS

In this work, the yield (v/w) of volatile oil was 0.6-1.3%. The identified compounds in the volatile oil were listed in table 1. 63 chemical components were detected by GC-FID and GC-MS analysis, accounting for 91.9% of the sample. The mainly chemical compositions were spathulenol (31.6%), humulene expoxide-(II) (7.1%), α-cadinol (4.5%), 2-methylene-6,8,8-trimethyl-tricyclo [5.2.2.0 (1,6)] undecan-3-ol (3.1%), tridecanoic acid (2.9 %) and α-curcumene (2.6%).

In bioassays, the volatiles from leaves of *C. odorata* exhibited significant NO inhibition with an IC_{50} value of 37.61μg/mL on NO production in lipopolysaccharide-induced RAW264.7 cells, while it showed some anti-oxidant effect with an IC_{50} value of 3.84mg/mL by DPPH radical scavenging method (table 2).

DISCUSSION

Compared the constituents extracted from leaves of *C. odorata* with those from flowers, the main chemical substances and ration's differences were displayed between them (Gaydou *et al*, 1986), although there are some chemical substances' s similarity in the volatiles extracted from flowers and leaves, respectively. The main compositions from flower volatiles were characterized by β-caryophyllene (10.7%), α-gurjunene (4.6%), linalool (2.9%), epi-α-muurolol (1.7%) and γ-muurolene (1.5%) (Gaydou *et al*, 1986). However, the number and variety of chemical substances from leave volatiles of this plant was far beyond those from flower. In *vitro* tests, the volatiles from leaves of *C. odorata* produced some effects on NO inhibition and radical scavenging, suggesting it has potentially anti-oxidant activity and may be used for treating some diseases associated with oxidative stress.

So far, the development and utilization of the volatiles from flowers of *C. odorata* has been full relatively scarce study of the other parts of *C. odorata*. Meantime, the demand for Ylang-Ylang volatiles is on the increase every year, but the yield of that volatiles and biomass of flowers are limited. Thus, using this plant resource fully, assessing the whole plant value scientifically and developing Ylang-Ylang volatiles substitutes gradually, are effective ways to avoid wasting resources. Our study can provide some research foundation for development of the leaves of *C. odorata*.

CONCLUSION

In our current study, the chemical constituents of volatiles from leaves of *C. odorata* were analyzed by GC-FID and GC-MS and its potentially anti-oxidant activities were tested for the first time. This research displayed that the volatiles from leaves of *C. odorata* is abundant in sesquiterpenoids, which showed anti-oxidant activities, and can be used as an addition agent in medicine and food.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Fundation of China (No. 21466018) and Personnel Training Project of Yunnan, China (KKSY201326042).

Table 1: Constituents of the volatiles extracted from the leaves of *C. Odorata*

No. of compound[a]	RIE[b]	RM[c]	Leave volatiles (%)	Flower volatiles (%)
1. 3,3,6-trimethyl-1,4-Heptadien-6-ol	751	919	0.39	
2. Eucalyptol	897	950	0.14	
3. *β*-Pinene	981	944	0.47	
4. Linalool	1095	932	0.13	2.9 (Fiona and Kevin 1999)
5. 5,5-dimethyl-Hexanal	1162	817	0.17	
6. 1,3,4-trimethyl,3-Cyclohexene-1-carboxaldehyde	1173	901	0.17	
7.1-(3-methylphenyl)-2,2,4-trimethyl, 3-Cyclohexene-1-methanol,(S)-Ethanone	1185	918	0.14	
8. Nonanoic acid	1277	839	0.14	
9. *α*-Copaene	1322	910	0.16	0.7
10. *β*-Elemene	1332	913	0.35	0.3 (Fiona and Kevin 1999)
11. *β*-Caryophyllene	1387	939	0.19	10.7 (Gaydou *et al*, 1986)
12. Patchoulene	1396	898	0.13	
13. Thujopsene	1400	839	0.11	0.9 (Yang and Huang, 1988)
14. Z-a-trans-Bergamotol	1405	770	0.15	
15. *α*-Curcumene	1416	967	2.56	
16. Benzyl alcohol	1450	778	0.37	0.1 (Gaydou *et al*, 1986)
17. *β*-Cubebene	1479	841	0.28	0.2 (Gaydou *et al*, 1986)
18. *γ*-Muurolene	1491	881	0.22	1.5 (Gaydou *et al*, 1986)
19. Calamenene	1498	907	0.32	0.3 (Gaydou *et al*, 1986)
20. 3,8-triene, Cadala-1(10)	1518	890	0.49	
21. *β*-Caryophyllene oxide	1549	914	1.70	
22. Globulol	1570	896	0.78	
23. Ledol	1572	859	0.48	
24. Spathulenol	1585	880	31.6	
25. Humulane-1,6-dien-3-ol	1592	868	2.01	
26. Isoaromadendrene epoxide	1597	859	0.37	
27. Cedrol	1600	759	0.96	0.85 (Yang and Huang, 1988)
28. Humulene expoxide-(II)	1609	901	7.09	
29. *β*-Himachalene	1625	797	1.17	
30. Ledene oxide-(II)	1630	837	0.70	
31. 4,4-dimethyl-Tetracyclo[6.3.2.0(2,5). 0(1,8)]tridecan-9-ol	1635	885	0.83	
32. epi-*α*-Muurolol	1639	921	1.13	1.7 (Gaydou *et al*, 1986)
33.*γ*-Gurjunene epoxide-(2)	1645	907	0.71	
34. *α*-Cadinol	1653	919	4.54	0.6 (Gaydou *et al*, 1986)
35. 2-methylene-6,8,8-trimethyl-Tricyclo[5.2.2.0(1,6)]undecan-3-ol	1670	828	3.07	
36. 4-Isopropenyl-4,7-dimethyl-1-oxaspiro[2.5]octane	1679	814	1.08	
37. trans-Longipinocarveol	1682	807	0.83	
38. (-)-Isolongifolol, methyl ether	1687	739	0.95	
39. Alloaromadendrene oxide-(2)	1695	811	1.32	
40. Calarene epoxide	1705	844	0.81	
41. *γ*-Bisabolene epoxide	1716	805	1.53	
42. [e]azulene-4,5,6-triol 1,1,4,6-tetramethyl Perhydrocyclopropa	1722	834	0.55	
43. Aromadendrene oxide-(1)	1735	806	0.72	
44. Acetic acid	1741	843	1.75	
45. 8*S*,13-Cedran-diol	1749	781	0.79	
46. 1-Heptatriacotanol	1757	784	0.78	
47. Methyl (Z)-5,11,14,17-eicosatetraenoate	1762	826	1.67	
48. 5,5-dimethyl-4-(3-methyl-1,3-butadienyl)-1-Oxaspiro[2.5]octane	1768	789	2.52	
49. Diepicedrene-1-oxide	1777	844	0.73	
50. Murolan-3,9(11)-diene-10-peroxy	1780	860	0.72	

51.4,5,6-triol,1,1,4,6-tetramethyl Perhydrocyclopropa [e]azulene	1788	808	0.38	
52. Santalol	1792	814	0.39	
53. Ledene oxide-(I)	1797	819	0.39	
54. (-)-Globulol	1915	786	1.32	
55. 2-methylene-Cholestan-3-ol	2063	836	0.63	
56. Vitamin A aldehyde	2071	877	0.60	
57. Thunbergol	2085	785	0.75	
58. 1,2-Longidione	2110	753	0.40	
59. Ambrosin	2117	812	0.43	
60. Verrucarol	2145	852	0.63	
61. Tridecanoic acid	2155	855	2.88	
62. Oleic acid	2302	848	0.19	
63. Formic acid	2385	730	0.14	
Monoterpenes oxygenated			1.44	
Sesquit. hydrocarbon			5.45	
Sesquit. oxygenated			62.85	
Acides			4.96	
Others			17.16	
Total			91.86	

Notes: Compounds[a] are showed in order of their elution from an HP-5 column. RIE[b] – retention indices as tested on HP-5 column using the homologous series of C8-40 n-alkanes. RM[c] – relative match according to the mass spectral library.

Table 2: Anti-oxidant activities of the volatiles extracted from the leaves of *C. Odorata*.

Experiment	IC_{50}
Nitric Oxide (NO) Production bioassay	37.61 μg/mL
DPPH radical scavenging bioassay	3.84 mg/mL

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