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Research Article

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Chemical composition and antioxidant activity of the essential oil from the flowers of *Artemisia austro-yunnanensis*

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ABSTRACT

Artemisia austro-yunnanensis, belonging to the family Asterales, is distributed mainly in south Asia regions. So far, there is no chemical and biologically active researches about this plant. In current study, for the first time, essential oil from the the flowers of Artemisia austro-yunnanensis was extracted by hydrodistillation and analyzed by gas chromatography (GC) and GC/mass spectrometry (GC-MS). The main compositions were Humulane-1,6-dien-3-ol (16.52 %), 3,3,6-Trimethyl-1,5-heptadien -4-ol (7.36 %), Agarospirol (6.10 %), Caryophyllene oxide (6.05 %), Borneol (3.34 %) and β-Bisabolo (3.00 %). And the antioxidant activity of the essential oil from that plant was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

Key words: *Artemisia austro-yunnanensis*; essential oil; chemical composition; antioxidant activity

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INTRODUCTION

Artemisia austro-yunnanensis is a semi-herbaceous shrubs in the Compositae family, mainly distributed in southwestern China, India, Myanmar, Thailand and other south Asia regions [1]. As one of species belonging in the genus *Artemisia* possessing variety of bioactivities [2-7], *A. austro-yunnanensis* has not been studied about its chemical composition and biological activity so far. In order to further study about bioactive compositions from *Artemisia* plants, essential oil from the flowers of *A. austro-yunnanensis* was extracted by hydrodistillation and analyzed by gas chromatography/flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC-MS) for the first time. The main compositions were Humulane-1,6- dien-3-ol (16.52 %), 3,3,6-Trimethyl-1,5-heptadien-4-ol (7.36 %), Agarospirol (6.10 %), Caryophyllene oxide (6.05 %), Borneol (3.34 %) and *β*-Bisabolo (3.00 %). And the antioxidant activity of essential oil of that plant was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

EXPERIMENTAL SECTION

Plant material and isolation of essential oil

Flowers of *A. austro-yunnanensis* were collected on October 2012 from Yunnan province in China. Specimen was identified by associate Prof. Mi Zhang (Faculty of Life Science and Technology, Kunming University of Science and Technology) and vouchers were stored in our lab.

Chemicals

Anhydrous sodium sulfate, ethanol and all other chemicals and reagents with AR grade used in this study were purchased from Shengshi Chemical Co. (Wuhan, China). 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the series of n-alkanes were purchased from Sigma-Aldrich Chemie (Steinheim, Germany).

Extraction of essential oil

Dried flowers (50 g) of *A. austro-yunnanensis* were soaked for 1.5 hours in water, and then extracted by

hydrodistillation for 3 hours. Anhydrous sodium sulfate was added to the sample to remove the remaining water. Oil yields are expressed as volume/weight (v/w).

GC-FID and GC-MS analysis

The oil was analyzed on the Agilent Technologies 7820A gas chromatograph (flame ionization detector, FID). Analytical conditions: HP-5 capillary column (30 m ×0.10 mm× 0.10 μm), nitrogen was as carrier gas at a flow rate of 1.0mL/minute, detector and injector temperatures were 280℃ and 250℃, respectively, inject volume was 1 μL (2 μL oil in 1mL of ethanol) with split injection mode (20:1). Program temperatures were 50℃ to 250℃ (2℃/min) and finally held for 5 minutes.

GC/mass spectrometry (GC-MS) was carried out on a Agilent Technologies GC–MS, equipped with a HP5-MS (cross-linked 5 % phenymethylpolysiloxane) capillary column (30 m ×0.10 mm× 0.10 μm). Regarding GC/MS detections, helium was the carrier gas at a flow rate of 0.5 mL/min. Detector and injector temperature were 280℃ and 250℃, respectively. The temperature of the ion source was 250℃. Electron ionization with ionization energy of 70 eV was used over a scan range of 50-550 atomic mass units. Column temperature was initially kept at 50℃ for 5minutes, then increased to 250℃ at the rate of 2℃/min and finally held for 5minutes. 1 μL of the oil was injected in split mode (20:1).

The determination of the mixture was done on the basis of retention index (RI) relative to (C_8-C_{40}) *n*-alkanes under same experimental conditions with those of literature [8-13]. Further determination was assigned by matching their mass spectra with those stored in the mass spectral library of the GC/MS date system and other published literatures [14-25]. Identification of the percentage constitution was computed by peak areas without using correction factors. The GC-MS chromatogram of oil sample is showed in Figure 1.

Antioxidant activity

The DPPH radical scavenging method was used to evaluate the antioxidant activity. The sample was dissolved in ethanol. 150 *u*L essential oil (1 mg/mL, 2 mg/mL, 5 mg/mL, 10 mg/mL) was added to aliquots (50 *u*L) of alcohol solution of DPPH (50 mg/mL). Absorbance measurements were recorded at 490 nm every 10 minutes in 1 h in the dark at room temperature. All experiments were performed in triplicate. Absorption of a blank sample containing the same amount of alcohol and DPPH solution was used as the negative control. The percentage inhibition of the DPPH radical by the oil was computed according to the formula:

$$\% \text{ Inhibition}=[(A_B-A_A/A_B)]\times 100$$

While A_B is the absorption of the blank sample (t = 0 min) and A_A represent the absorption of the tested oil or substance solution in 1h.

RESULTS AND DISCUSSION

Compositions of essential oil from the flowers of *A. austro-yunnanensis*

In this work, oil yield (v/w) was 0.8-1.2 %. Table **1** showed the identified compositions from the oil. In total, 81 components were determined accounting for 95.49 % of the tested sample. The main compositions were Humulene-1,6-dien-3-ol (16.52 %), 3,3,6-Trimethyl-1,5-heptadien-4-ol (7.36 %), Agarospirol (6.10 %), Caryophyllene oxide (6.05 %), Borneol (3.34 %) and *β*-Bisabolo (3.00 %). And the determined compounds were dominated by oxygenated monoterpenes (19.28 %) and oxygenated sesquiterpenes (57.61 %). According to the reports, Agarospirol considered to be an neuroleptic [20], and Caryophyllene oxide showed significant central, as well as peripheral, analgesic, along with anti-inflammatory activity [21]. Meantime, borneol can specifically inhibit the nAChR-mediated effects in a noncompetitive way [22]. Based on the above, it is necessary to find bioactive constituents from the essential oil of *A. austro-yunnanensis*.

Antioxidant activity

DPPH radical scavenging method has been widely applied to determinate the antioxidant activity of some essential oil [18-20]. Fig. **2** showed the result of DPPH tests. The DPPH inhibition (%) of the sample was positively correlated with the time and concentration of sample. It indicated that DPPH radical scavenging power is may not related to the concentration of the sample, but also has a relationship with the reaction time. The chemical composition of the essential oil may has an impact on its antioxidant due to the presence of some chemical compositions.

Table 1. Constituents of the essential oil received from the flowers of *A. austro-yunnanensis*

NO. of compound[a]	RI[b]	FID[c]	RM[d]
1. 2,4,6-Trimethyl, 1,3,6-heptatriene	927	0.39%	914
2. 1-Octen-3-ol	976	0.14%	931
3. 3,3,6-Trimethyl, 1,4-heptadien-6-ol	997	2.70%	918
4. Eucalyptol	1056	0.47%	959
5. **3,3,6-Trimethyl, 1,5-heptadien-4-ol**	1081	**7.36%**	934
6. Terpinolene	1093	0.23%	927
7. trans, 1-methyl-4-(1-methylethyl), 2-Cyclohexen-1-ol	1116	0.20%	837
8. Isopinocarveol	1132	0.13%	900
9. 4,6,6-trimethyl, Bicyclo[3.1.1]hept-3-en-2-ol	1141	0.27%	907
10. trans, 4,5-epoxy-Carane	1153	0.13%	843
11. **Borneol**	1162	**3.34%**	960
12. 4-methyl-1-(1-methylethyl)-3-Cyclohexen-1-ol	1173	1.15%	877
13. Thymol	1181	0.31%	855
14. αα4-trimethyl, 3-Cyclohexene-1-methanol	1186	1.28%	948
15. 6,6-dimethyl, Bicyclo[3.1.1]hept-2-ene-2-methanol	1191	0.52%	953
16. cis, 3-methyl-6-(1-methylethyl), 2-Cyclohexen-1-ol	1201	0.16%	914
17. 3-methyl, 2-Hexanone	1206	0.23%	787
18. 3-(1-methylethyl), Phenol	1223	0.22%	921
19. Bornyl acetate	1281	0.25%	927
20. 4-(1-methylethyl), Benzenemethanol	1285	0.36%	941
21. 4-(1-methylethenyl), 1-Cyclohexene-1-methanol	1293	0.15%	898
22. Ascaridole epoxide	1296	0.15%	856
23. αα4-trimethyl, 3-Cyclohexene-1-methanol	1345	0.11%	926
24. 3-Allyl-6-methoxyphenol	1348	0.18%	805
25. 3,3,6-trimethyl, 1,5-Heptadien-4-ol	1355	0.14%	798
26. Isobornyl propionate	1372	0.82%	907
27. 8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	1387	0.12%	868
28. 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester, Acetic acid	1409	0.80%	863
29. β-Caryophyllene	1453	0.18%	862
30. decahydro-4,8,8-trimethyl-9-methylene, 1,4-Methanoazulene	1482	1.43%	885
31. 8-Cedren-13-ol	1497	0.21%	812
32. exo, 3-methyl-, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, Butanoic acid	1501	2.47%	935
33. Cubenol	1508	0.29%	833
34. 2-methyl, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, Butanoic acid	1511	0.29%	899
35. endo, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, Pentanoic acid	1518	0.77%	805
36. (2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl) benzene	1531	0.47%	867
37. Diepi-πcedrene epoxide	1540	0.40%	839
38. cis-Z-πBisabolene epoxide	1549	0.37%	819
39. trans, Longipinocarveol	1555	0.25%	848
40. Globulol	1565	0.58%	847
41. **Caryophyllene oxide**	1579	**6.05%**	903
42. Ledene oxide-(II)	1584	0.39%	831
43. Octahydro-1,5,5,8a-tetramethyl, 1,4-Methanoazulen-7(1H)-one	1589	1.95%	831
44. Longifolenaldehyde	1595	0.95%	772
45. Epiglobulol	1599	1.31%	835
46. Calarene epoxide	1604	2.57%	824
47. Alloaromadendrene oxide-(2)	1607	1.33%	824
48. Humulane-1,6-dien-3-ol	1609	0.70%	843
49. Aromadendrene oxide-(2)	1619	0.39%	865
50. **Agarospirol**	1625	**6.10%**	940
51. αGuaiene	1632	2.39%	800
52. tau-Cadinol	1643	1.37%	911
53. **Humulane-1,6-dien-3-ol**	1651	**16.5%**	872
54. 2-methylene-6,8,8-trimethyl-Tricyclo[5.2.2.0(1,6)]undecan-3-ol	1662	1.15%	855
55. trans, Z-αBisabolene epoxide	1666	1.68%	842
56. 8,14-Cedranoxide	1674	1.56%	826
57. **β-Bisabolo**	1678	**3.00%**	946
58. 3,7,11-trimethyl, 2,6,10-Dodecatrien-1-ol	1682	1.69%	771
59. cis, Lanceol	1697	2.02%	831
60. (+)-octahydro-4,8,8,9-tetramethyl-, 1,4-Methanoazulen-7(1H)-one	1798	0.70%	817
61. endo, 8-hydroxy, Cycloisolongifolene	1805	0.86%	831
62. Ledene alcohol	1809	0.67%	833
63.1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester, 3-hydroxy-6-isopropenyl-4,8a-dimethyl, Acetic acid	1815	1.03%	830
64. Isoaromadendrene epoxide	1833	1.26%	827
65. αGurjunenepoxide-(2)	1841	0.54%	850
66.1,8-dimethyl-8,9-epoxy-4-isopropyl, Spiro[4.5]decan-7-one	1844	0.62%	803
67. 1-Heptatriacotanol	1849	0.86%	732
68. Methyl hinokiate	1865	0.47%	779
69. αGurjunenepoxide-(1)	2083	0.24%	838
71.1-methyl-4-isopropyl-7,8-dihydroxy-,(8S), Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane]	2123	0.23%	824
72. 6,10,14-trimethyl, 2-Pentadecanone	2130	1.28%	956
73. isobutyl octadecyl ester, Phthalic acid	2157	0.20%	859

74. Sclareoloxide	2163	0.44%	904
75.1,1,4,6-tetramethyl, Perhydrocyclopropa[e]azulene-4,5,6-triol	2206	0.13%	830
76. Sclareoloxide	2247	0.29%	857
77. n-Hexadecanoic acid	2277	1.79%	914
78. 1-Heptatriacotanol	2285	0.27%	732
79. Hexadecanoic acid, ethyl ester	2307	0.22%	836
80. 2-methylene, Cholestan-3-ol	2327	0.12%	818
81. 1,8-dimethyl-8,9-epoxy-4-isopropyl, Spiro[4.5]decan-7-one	2349	0.13%	813
Identified compounds		95.49%	
Monoterpene hydrocarbons		0.39%	
Oxygenated monoterpenes		19.28%	
Sesquiterpene hydrocarbons		3.12%	
Oxygenated sesquiterpenes		57.61%	
Lipids		5.58%	
others		9.51%	

Notes: Compounds[a] are showed in order of their elution from an HP-5 column. RI[b] – retention indices as tested on HP-5 column using the homologous series of C_{8-40} n-alkanes. RM[d] – Relative Match according to the mass spectral library.

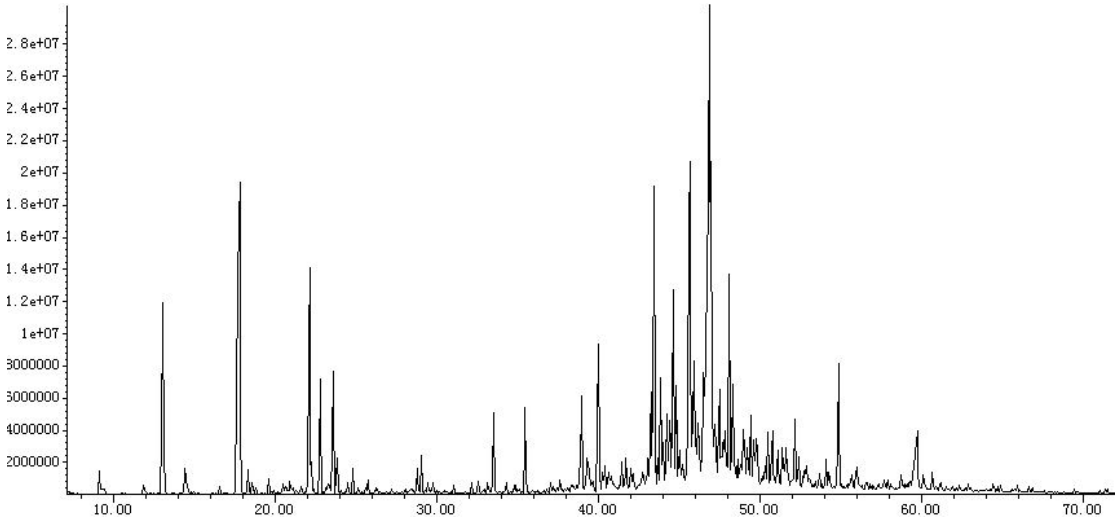

Fig.1. Total ion chromatograms of chemical compositions identified in essential oil of the flower of *A. austro-yunnanensis*

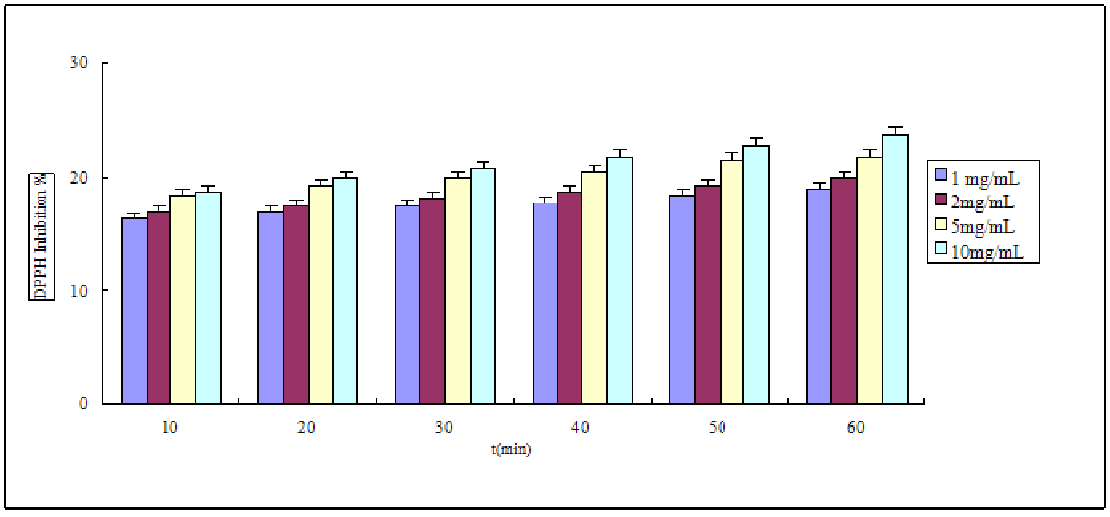

Fig. 2. Antioxidant activity determined by DPPH tests

CONCLUSION

In current stdy, the essential oil from the flowers of *A. austro-yunnanensis* was extracted by hydrodistillation and analyzed by GC-FID and GC-MS for the first time. And it showed weak antioxidant activity by DPPH radical scavenging assay. It will provide a basis for the further study on the bioactive constituents from *A. austro-yunnanensis.*

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