Molecules **2006**, *11*, 549-555

molecules

ISSN 1420-3049
http://www.mdpi.org

Full Paper

Chemical Composition and Antimicrobial Activity of the Essential Oil from *Ambrosia trifida* L.

Peng Wang [1,2], Chui Hua Kong [1,*] and Chao Xian Zhang [3]

[1] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, P.R. China
[2] Graduate School, Chinese Academy of Sciences, Beijing 100039, P.R. China
[3] Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, P.R. China
E-mails: wangpeng@iae.ac.cn (Peng Wang); cxzhang@wssc.org.cn (Chao Xian Zhang)

* Author to whom correspondence should be addressed; e-mail: kongch@iae.ac.cn; Fax: (+86)-24-83970300

Received: 1 June 2006; in revised form: 19 July 2006 / Accepted: 20 July 2006 / Published: 25 July 2006

Abstract: The essential oil obtained by steam distillation of dried aerial parts of *Ambrosia trifida* L. from Northeast China was analyzed by GC and GC–MS. The essential oil yield based on dried plant material was 0.12% and thirty-five compounds (corresponding to 86.7% of the total weight) were identified. The main components were: bornyl acetate (15.5%), borneol (8.5%), caryophyllene oxide (8.3%), α-pinene (8.0%), germacrene D (6.3%), β-caryophyllene (4.6%), *trans*-carveol (2.9%), β-myrcene (2.6%), camphor (2.4%) and limonene (3.2%). *A. trifida* essential oil demonstrated bactericidal and fungicidal activity against six bacterial strains and two fungal strains, using the agar diffusion method.

Keywords: *Ambrosia trifida* L.; antimicrobial activity; chemical composition; essential oil.

Introduction

The genus *Ambrosia* (Asteraceae) is classified as part of the tribe Heliantheae. It comprises some 35-40 species, mostly found in the Americas. *A. trifida* L. (great or giant ragweed) and *A.*

artemisiifolia L. (common ragweed) are two common species found in China, where *A. trifida* occurs mainly in Northeast China. *A. trifida* is American in origin and in ecological terms it is spreading worldwide as a pioneer species [1]. It invades cultivated fields and reduces crop productivity [2, 3]. This plant is wild and sometimes cultivated by the North American Indians for food and medicine. It is used as a tea in the treatment of pneumonia, fevers, nausea, intestinal cramps, diarrhoea and mucous discharges and menstrual disorders [4]. *A. trifida* leaves are very astringent, emetic and febrifugal and are applied to insect bites and various skin complaints. The pollen is harvested commercially and manufactured into pharmaceutical preparations for the treatment of allergies to the plant, but ingesting or touching the pollen of *A. trifida* can cause allergic reactions and hay fever in some people [5].

Many kinds of metabolites including sesquiterpene lactones, phenolics, ambrosin, isabelin, and psilostachyin have been isolated and identified from *A. artemisiifolia* [6, 7] and more recently, the chemical composition and antimicrobial activity of essential oil from *A. artemisiifolia* have been reported [8]. However, there are only a few investigations on phytochemistry of *A. trifida*. Several sesquiterpenes and thiarubrines were isolated and identified from *A. trifida* tissues [6, 9]. The volatile chemicals from *A. trifida* leaves and their allelopathic potential on other plant species were investigated in our previous paper [10]. This study concerns chemical composition and antimicrobial activity of the essential oil from *A. trifida*.

Results and Discussion

The essential oil obtained by steam distillation of aerial parts of *A. trifida* collected in Northeast China was isolated with a yield of 0.12% (based on dried plant material). The isolated oil was a yellowish liquid with a strong aromatic fragrance. Table 1 shows its chemical composition. Thirty-five compounds were identified by comparison of their retention indexes and the mass spectra of each GC component with those of standards and with reported data. Terpenes and their derivatives predominated, with the most abundant one being bornyl acetate (15.5%), followed by borneol (8.5%), caryophyllene oxide (8.3%), *α*-pinene (8.0%), germacrene D (6.3%), *β*-caryophyllene (4.6%), *trans*-carveol (2.9%), *β*-myrcene (2.6%), camphor (2.4%) and limonene (2.2%), respectively These main components comprised more than 86 % of the essential oil. Although most of these compounds are well documented as essential oil components in various plant species [11], to our knowledge this is the first report of their occurrence in the essential oil of *A. trifida*.

Interestingly, there were significant differences between the main components of the essential oil of *A. trifida* L. and those previously determined in *A. artemisiifolia* L. [8], which belongs to the same genus. Thus, terpene alcohols such as spathulenol, longipinanol, isospathuienol, *α*-eudesmol and *γ-epi*-eudesmol are quantitatively abundant in *A. artemisiifolia* oil, whilst they were only present in much smaller quantities in *A. trifida* oil (Table 2).

Table 1. Chemical composition of the essential oil from *A. trifida* from Northeast China

Peak Number	Compound	Retention index	Relative amount (%)
1	*α*-pinene	935	8.0
2	*β*-myrcene	992	2.6
3	limonene	1031	2.2
4	*α*-terpinolene	1087	1.8
5	camphor	1143	2.4
6	cis-*β*-terpineol	1145	0.6
7	borneol	1170	8.5
8	trans-carveol	1217	2.9
9	bornyl acetate	1275	15.5
10	*α*-cubebene	1350	1.5
11	*α*-terpinyl acetate	1354	0.6
12	isoledene	1376	1.1
13	*β*-caryophyllene	1420	4.6
14	*β*-farnesene	1450	1.8
15	germacrene D	1480	6.3
16	(E)-methylisoeugenol	1500	1.4
17	*γ*-cadinene	1520	0.8
18	*δ*-cadinene	1530	0.7
19	longipinanol	1565	1.1
20	caryophyllene oxide	1581	8.3
21	spathulenol	1583	0.6
22	globulol	1586	0.4
23	carotol	1594	0.6
24	cubenol	1630	0.4
25	isospathulenol	1638	0.8
26	*β*-cedren-9-*α*-ol	1645	1.9
27	*α*-eudesmol	1654	0.7
28	*γ*-eudesmol acetate	1780	1.0
29	hexahydrofarnesyl acetone	1844	1.5
30	2-methyl-nonadecane	1890	0.9
31	isophytol	1945	0.5
32	manoyl oxide	2000	1.1
33	heneicosane	2100	0.6
34	docosane	2200	0.5
35	abieta-8,11,13-trien-7-one	2312	0.5
-	total isolate	-	86.7
-	unknown	-	13.3

Compounds were listed in order of elution. Retention indices were calculated from retention times relative to those of *n*-alkanes (C5 to C26) on the non-polar HP-5 column.

Table 2. Main composition of the essential oils from *A. trifida* and *A. artemisiifolia*

A. trifida **(Relative amount, %)**	***A. artemisiifolia*** **[8] (Relative amount, %)**
bornyl acetate (15.5%)	germacrene D (24.1%),
borneol (8.5%)	limonene (16.8%)
caryophyllene oxide (8.3%)	*α*-pinene (8.0%)
α-pinene (8.0%)	*β*-myrcene (7.4%)
germacrene D (6.3%)	borneol (2.9%)
β-caryophyllene (4.6%)	spathulenol (1.6%)
trans-carveol (2.9%)	longipinanol (1.6%)
β-myrcene (2.6%)	isospathulenol (1.5%)
camphor (2.4%)	*α*-eudesmol(1.4%)
limonene (2.2%)	*γ-epi*-eudesmol (1.3%)

Results of the antimicrobial activity tests of the *A. trifida* essential oil against bacteria and fungi are given in Table 3. Dilute solutions (2% or 4%) of the oil demonstrated bactericidal and fungicidal activity against all microorganisms tested. Particularly significant were the inhibition zone diameters observed for 4% essential oil solution against *Staphylococcus aureus* and *Candida albicans*, and for 2% essential oil solution against *Klebsiella pneumoniae*, while *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Asperigillus niger* were less sensitive to the oil.

Table 3. Antimicrobial activity of the essential oil from *A. trifida* from Northeast China

Microorganisms	Diameters of inhibition zones (mm)		
	2% oil solution	4% oil solution	Control
Gram (+) bacteria			
Bacillus subtilis	14.0	12.5	9.0
Staphylococcus aureus	22.5	25.5	10.0
Enterococcus faecalis	20.0	18.5	9.5
Gram (-) bacteria			
Escherichia coli	20.0	17.0	10.5
Pseudomonas aeruginosa	14.5	16.5	9.0
Klebsiella pneumoniae	23.5	20.5	11.0
Fungi			
Asperigillus niger	16.0	14.5	11.0
Candida albicans	19.0	23.5	10.5

Data are average diameters of inhibition zones from two independent determinations.

Conclusions

Our GC and GC-MS study of the essential oil from *A. trifida* from Northeast China led to the identification of 35 compounds, representing 86.7% of the total mass. The major components were terpenes and their derivatives, and the most prominent one was bornyl acetate (15.5%). The antimicrobial activity results presented here demonstrate that this plant essential oil has a commercial potential.

Experimental

Plant Material and Isolation of the Essential Oil

Flowering aerial parts of *A. trifida* were collected from the Shenyang Experimental Station of Ecology, Chinese Academy of Sciences (Northeast China, N 41º31´, E 123º24´) in August 2005. Harvested plant material was air-dried in a shaded area at ambient temperature. A voucher specimen was deposited in Institute of Applied Ecology, Chinese Academy of Sciences, China. The essential oil was obtained by steam distillation in a Clevenger-type apparatus, according to the literature [12]. Isolated oil was dried over a layer of anhydrous sodium sulphate and submitted to chemical and microbiological analysis.

Essential Oil Analysis

The oil was analyzed by capillary GC and GC-MS. Oil (25 µL) was diluted in dichloromethane (2 mL) before injection and 1 µL of this solution was directly used for analysis. GC analysis of the oil was performed on a Hewlett-Packard 5890A gas chromatograph equipped with a split/splitless injector (250 ºC, split ratio 1:30) and a FID operated at 250 ºC. A HP-5 fused silica capillary column (25 m × 0.32 mm i.d., 0.52 µm film thickness) was used. The operating conditions were as follows: 5 min at 50°C initial hold, then from 50-280°C at 2.5 °C/min.; injector temperature, 250 °C; detector temperature, 280 °C; carrier gas, H_2 at 1 mL/min. Retention indices were determined with C5 to C26 alkane standards as reference. Relative amounts of individual components are based on peak areas obtained without FID response factor correction. Identification of the components was assigned by comparison of their retention indices and confirmed by GC-MS [13, 14]. GC–MS analyses were performed on a Hewlett-Packard 5890/5970A system, equipped with a HP-5 MS capillary column (30 m ×0.25 mm i.d., film thickness 0.25 µm). Helium was used as carrier gas, the inlet pressure was 200 kPa, the linear velocity 1 mL/min (70 ºC), split flow 10mL/min. Temperature programme: 40–260 °C at a rate of 4 °C/min; injector temperature, 250 °C; detector temperature, 260 °C. The electron energy was 70 eV. Mass spectra were obtained by automatic scanning of the mass range m/z 45 to 629 amu. at 2 scan/s. Chromatographic peaks were checked for homogeneity with the aid of the mass chromatograms of the characteristic fragment ions reported in the NIST 98 and WILEY 138 databases.

Antimicrobial Activity

The essential oil diluted in absolute ethanol was each tested for antimicrobial activity by the agar diffusion method [15] at concentrations of 4% and 2% (v/v). Two fungal strains (*Candida albicans* and *Asperigillus niger*) and six bacterial strains (*Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 29665) and *Escherichia coli* (ATCC 25922)) were used. All innocula were subcultures from stock cultures into nutrient broth (10 mL) and incubated at 37 °C. After 24 h the broth culture was subcultured into fresh broth (10 mL) and incubated for a further 18 h under the same conditions. The resulting broth culture was then used as the inoculum for the agar diffusion test. The tests were carried out by pouring agar into Petri dishes to form 4 mm thick layers and adding dense inocula of the tested microorganisms (about 106 microorganisms/mL) in order to obtain semiconfluent growth. One drop of 4% and one of 2% essential oil solution in absolute ethanol were poured onto the agar, prepared as required. Reference samples were produced according to WHO standards [16]. Sensitivity of the bacteria was tested on Mueller-Hinton agar and Saburaud dextrose agar was used to test oil activity against fungi. Incubation lasted 18 h at 37 °C. Reading of results was carried out by measuring the diameters of the zones of inhibition and clear growth (in mm) and comparing to absolute ethanol as control.

Acknowledgements

This work was financially supported by Hundreds-Talent Program, Chinese Academy of Sciences and the Tenth Five-year Plan of Science & Tech Program of China (2004BA509B07).

References

1. Abul-Fatih, H. A.; Bazzaz, F. A. The biology of *Ambrosia trifida* L. I. Influence of species removal on the organization of the plant community. *New Phytol.* **1979**, *83*, 813-816.
2. Weaver, S. E. Correlations among relative crop and weed growth stages. *Weed Sci.* **2003**, *51*, 163-170.
3. Harrison, S. K.; Regnier, E. E. Postdispersal predation of giant ragweed (*Ambrosia trifida*) seed in no-tillage corn. *Weed Sci.* **2003**, *51*, 955-964.
4. Foster, S.; Duke, J. A. *A Field Guide to Medicinal Plants. Eastern and Central N. America*; Houghton Mifflin Co.: Boston, MA, USA, **1990**; pp. 135-143.
5. Facciola, S. *Cornucopia - A Source Book of Edible Plants*; Kampong Publications: Vista, CA, USA, **1990**; pp. 212-217.
6. Bloszyk, E.; Rychlewska, U.; Szczepanska, B.; Budesinsky, M.; Drozdz, B.; Holub, M. Sesquiterpene lactones of *Ambrosia artemisiifolia* L. and *Ambrosia trifida* L. species. *Collect. Czech. Chem. Comm.* **1992**, *57*, 1092-1102.
7. Tamura, Y.; Hattori, M.; Konno, K.; Kono, Y.; Honda, H.; Ono, H.; Yoshida, M. Triterpenoid and caffeic acid derivatives in the leaves of ragweed, *Ambrosia artemisiifolia* L. (Asterales:

Asteraceae), as feeding stimulants of *Ophraella communa* LeSage (Coleoptera: Chrysomelidae). *Chemoecology* **2004**, *14*, 113-120.

8. Chalchat, J. C.; Maksimovic, Z. A.; Petrovic, S. D.; Gorunovic, M. S. Chemical composition and antimicrobial activity of *Ambrosia artemisiifolia* L. Essential Oil. *J. Essential Oil Res.* **2004**, *16*, 270-273.
9. Lu, T. S.; Parodi, F. J.; Vargas, D.; Quuano, L.; Mertooetomo, E. R.; Hjortso, M. A.; Fischer, N.H. Sesquiterpenes and thiarubrines from *Ambrosia trifida* and its transformed roots. *Phytochemistry* **1993**, *33*, 113-116.
10. Wang, P.; Liang, W. J.; Kong, C. H.; Jiang, Y. Allelopathic potentials of volatile allelochemicals of *Ambrosia trifida* L. on other plants. *Allelopathy J.* **2005**, *15*, 131-136.
11. Zhu, L. F.; Li, Y. H.; Li, B. L.; Lu, B.Y.; Zhang, W. L. *Aromatic plants and essential constituents (supplement 1).* South China Institute of Botany, Chinese Academy of Sciences (Ed); Hai-Feng Publishing Co.: Hong Kong, **1995.**
12. Yáñez, X. Pinzón, M. L. Solano, F.. Sánchez, L. R Chemical Composition of the Essential Oil of *Psidium caudatum* McVaugh. *Molecules* **2002**, *7*, 712-716.
13. Jennings, W.; Shibamoto, T. *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic Press: New York, **1980**.
14. Adams RP. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*. Allured: Carol Stream, IL, USA, **2001**.
15. Benson, H. J. *Microbiological Applications*, 5th ed; William C. Brown Pub: Dubuque, IA, USA, **1990**.
16. *WHO Expert Committee on Biological Standardization, Thirty-second Report. World Health Organization Technical Reports Series 673*; World Health Organization: Geneva, Switzerland, **1982**; http://whqlibdoc.who.int/trs/WHO_TRS_673.pdf

Sample Availability: Samples of the essential oil are available from the authors.