Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 103 (2007) 355–358
Food Chemistry
www.elsevier.com/locate/foodchem

Chemical composition, cytotoxic and antioxidant activity of the leaf essential oil of *Photinia serrulata*

Hou Jie, Sun Tao, Hu Jun, Chen Shuangyang, Cai Xiaoqiang, Zou Guolin *

State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan 430072, People's Republic of China

Received 6 June 2006; received in revised form 9 July 2006; accepted 13 July 2006

Abstract

The leaf essential oil of *Photinia serrulata* was obtained by hydrodistillation and analyzed by GC and GC-MS. Seventy-one components were identified in the essential oil and the main components of the oil were 10-epi-γ-eudesmol (12.72%), pinene (6.85%), sabinene (5.93%), α-humulene (5.87%) and α-thujene (5.47%). The *in vitro* cytotoxicity of the oil on human cancer cell lines Hela, A-549 and Bel-7402 was examined. The oil was found to be very active against all the three human tumor cell lines tested with low IC_{50} of 0.0427 μl/ml (Hela), 0.0219 μl/ml (A-549) and 0.0301 μl/ml (Bel-7402). The oil was also found to possess antioxidant activity as demonstrated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical method.

Keywords: *Photinia serrulata*; Essential oil; Cytotoxicity; Antioxidant

1. Introduction

Photinia serrulata Lindl, a plant named Shi-Nan in Chinese traditional Medicine, is a well-known herb which has a long history in China as an efficient folk medicine for nephropathy, rheumatism and spermatorrhea. The plant is native in southern china, and people there always pick leaves off from *P. serrulata* and dip the leaves into the wine for a couple of months before drinking, which can develop the wine's distinct flavour and medical effect.

The herb is known to contain hydrocyanic acid, prunasin, ursolic acid and saponins (Jiangsu New Medicine College, 1977). To our knowledge, there are no published reports on the chemical composition, cytotoxic and antioxidant activity of the leaf essential oil of *P. serrulata*. The chemical composition of this oil was analyzed and its cytotoxicity on a series of human cancer lines Hela (cervical), Bel-7402 (liver) and A-549 (lung) investigated. The antioxidant activity of the oil was also evaluated.

* Corresponding author. Tel.: +86 27 87645674; fax: +86 27 68752560.
E-mail addresses: jadyhou@hotmail.com (J. Hou), zouguolin@whu.edu.cn (G. Zou).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.07.060

2. Experimental

2.1. Plant material

The leaves of *P. serrulata* were collected in Jiangxi Province of China in Spring of 2004. Voucher specimen was deposited at the herbarium at the College of Life Sciences, Wuhan University, China.

2.2. Essential oil extraction and GC-MS analysis

The leaves of *P. serrulata* were air dried and then distilled for 3 h using a Clevenger type apparatus. The essential oil obtained was dried with anhydrous sodium sulphate and stored at −20 °C until used.

The oil was analyzed by GC-MS with two different fused silica capillary columns (30 m × 0.25 mm i.d.; film thickness 0.25 μm) of different polarities [DB-5 and HP-innowax from Agilent company (Palo Alto, California, USA)]. The oven temperature was programmed from 50 to 250 °C at 3 °C/min rate and finally held isothermal for 10 min. Injector and interface temperatures were 220 and 250 °C. Carrier gas was helium at 1.0 ml/min. Diluted

samples (1.0 μl, 1/10 in ether) were injected manually and the split ratio was adjusted to 40:1. GC-MS analyses were performed with a Thermo Finnigan TRACE GC coupled with a TRACE MS plus (EI 70ev) of the same company. The components were identified by comparison of their mass spectra with those of NIST98 library data of the GC-MS system and Adams libraries spectra (Adams, 2001). The results were further confirmed by comparison with their retention indices with literature data (Davies, 1990; Pedro et al., 2001). Retention indices of the components were determined relative to the retention times of a series of *n*-alkanes with linear interpolation.

2.3. Cell culture

The human cervical carcinoma Hela, human lung carcinoma A-549 and liver carcinoma cell line Bel-7402 were obtained from China Centre for Typical Culture Collection, Wuhan University. The cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, 1% (w/v) glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were cultured in a humidified atmosphere at 37 °C in 5% CO_2.

2.4. Cytotoxicity assay

Cytotoxicity was measured using a modified MTT assay (Plumb, Milroy, & Kaye, 1989). Briefly, the cells (5×10^4) were seeded in each well containing 100μl of the RPMI medium supplemented with 10% FBS in a 96-well plate. After 24 h for adhesion, a serial of doubling dilution of the essential oil was added to triplicate wells over the range of 1.0000–0.0078 μl/ml. The final concentration of ethanol in the culture medium was maintained at 0.5% (volume/volume) to avoid toxicity of the solvent (Sylvestre, Legault, Dufour, & Pichette, 2005). After 2 days, 10 μl of MTT (5 mg/ml stock solution) were added and the plates were incubated for an additional 4 h. The medium was discarded and the formazan blue, which formed in the cells, were dissolved with 100 μl dimethyl sulphoxide (DMSO). The optical density was measured at 490 nm. The cell survival curves were calculated from cells incubated in the presence of 0.5% ethanol. Cytotoxicity is expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50}). All tests and analyses were run in triplicate and mean values recorded.

2.5. Antioxidant activity

The antioxidant activity was measured by a modification of the DPPH radical-scavenging method of Ramos et al. (2003). Two hundred microlitres of distilled water were mixed with 160 μl DPPH (0.5 mM in ethanol), then 40 μl samples of the oil in ethanol (ranging from 0.250 to 0.875 μl/ml) were added. The mixture was shaken and left to stand at room temperature. The absorbance was measured 30 min later at 517 nm. Antioxidant activity was expressed as the concentrations of drug scavenge DPPH radical by 50% (IC_{50}). All tests and analyses were run in triplicate and mean values recorded.

3. Results and discussion

Hydrodistillation of dried leaves of *P. serrulata* yielded 1.4% (v/w) of a transparent essential oil. Seventy-one compounds, representing 97.61% of the oil, were identified using two chromatographic producers (see Section 2l). The chemical composition of the leaf essential oil is listed in Table 1.

The essential oil consisted mainly of monoterpene hydrocarbons (25.02%), sesquiterpene hydrocarbons (36.48%) and oxygenated sesquiterpenes (24.85%), whereas oxygenated monoterpenes were weakly represented (2.27%). A portion (2.39%) of the total composition was not identified. The major components of the oil were 10-epi-γ-eudesmol (12.72%), pinene (6.85%), sabinene (5.93%), α-humulene (5.87%) and α-thujene (5.47%).

To investigate the cytotoxic activity of *P. serrulata* essential oil, we evaluated its effect on a selection of human cell lines Hela (human cervical carcinoma), A-549 (human lung carcinoma) and Bel-7402 (human liver carcinoma) by MTT assay. All cell lines were submitted to growing concentrations of *P. serrulata* leaf essential oil for 48 h. As shown in Fig. 1, the *P. serrulata* essential oil was active against all three human cancer cell lines tested. It induced a concentration-dependent inhibitory effect on all cell lines tested in the dilutions ranging from 0.01 to 0.125 μl/ml. The IC_{50} values of the oil were 0.0427, 0.0219 and 0.0301 μl/ml for the Hela, A-549 and Bel-7402 cell lines, respectively (Table 2).

The cytotoxic activity of the *P. serrulata* leaf essential oil may be attributed to specific components of the oil. A few of the compounds found in *P. serrulata* leaf essential oil have been tested for cytotoxic properties. It has been reported that α-humulene (5.87%) shows activity against MCF-7, PC3, A-549, DLD-1, M4BEU and CT-26 cell lines (Legault, Dahl, Debition, Pichette, & Maldemont, 2003). There is still controversy over whether caryophyllene oxide (0.47%) is cytotoxic or not. Kubo et al. (1996) and Sibanda et al. (2004) reported that it exhibited a modest cytotoxic activity, while some reports also showed that it was inactive against tumor cell lines (Kanada et al., 1992; Legault et al., 2003; Tambe, Tsujiuchi, Honda, Ikeshiro, & Tanaka, 1996). γ-Elemene (0.39%) and δ-elemene (0.06%) have also been reported to be cytotoxic (Duh, Wang, Weng, Chiang, & Dai, 1999.). Altogether, the compounds with cytotoxic activity of the oil are mainly sesquiterpenes. In fact, it has been reported that sesquiterpenes are responsible for the cytotoxic activity of *Myrica gale L.* essential oil (Sylvestre et al., 2005). However, the low concentration of α-humulene (5.87%), caryophyllene oxide (0.47%), γ-elemene (0.39%) and δ-elemene (0.06%) cannot fully explain the high cytotoxic activity of *P. serrulata* leaf essential oil, which means some other compounds, probably sesquiter-

Table 1
Chemical composition of the leaf essential oil of *P. serrulata*

Coumpoud	RI (this work)	RI (Adams, 2001)	Area (%)
Monoterpene hydrocarbon			**25.02**
Artemisia triene	924	930	0.43
α-Thujene	932	930	5.47
Camphene	947	954	0.09
Sabinene	972	975	5.93
Pinene	977	979	6.85
Myrcene	988	991	0.83
para-Mentha-1(7),8-diene	1007	1004	0.46
α-Terpinene	1016	1017	0.56
para-Cymene	1023	1025	0.04
δ-3-Carene	1030	1031	3.01
(E)-β-Ocimene	1045	1050	0.29
γ-Terpinene	1057	1060	0.86
para-Mentha-2,4(8)-diene	1084	1088	0.20
Oxygenated monoterpenes			**2.27**
cis-Sabinene hydrate	1069	1070	0.04
Linalool		1097	0.65
cis-para-Menth-2-en-1-ol	1122	1122	0.06
trans-para-Menth-2-en-1-ol	1140	1141	0.04
Terpinen-4-ol	1179	1177	1.32
Dihydro carveol	1194	1194	0.13
trans-Piperitol	1208	1208	0.03
Sesquiterpene hydrocarbons			**36.48**
δ-Elemene	1334	1338	0.06
α-Cubebene	1346	1351	0.22
Cyclosativene	1367	1371	0.57
α-Ylangene	1373	1375	0.51
β-Bourbonene	1381	1388	0.59
iso-Italicene	1401	1402	0.85
α-Bergamotene	1411	1413	0.59
(E)-Caryophyllene	1417	1419	3.88
β-Copaene	1426	1432	0.14
γ-Elemene	1432	1437	0.39
Acromadendrene	1441	1441	0.10
cis-Muurola-3,5-dine	1446	1450	0.44
α-Humulene	1453	1455	5.87
Aromadendrene (l)	1456	1460	2.29
γ-Curcumene	1476	1483	2.47
ar-Curcumene	1479	1481	1.34
cis-Eudesma-6,11-diene	1486	1490	1.74
β-Selinene	1486	1490	1.74
α-Selinene	1493	1498	2.97
Epizonarene	1505	1502	0.97
β-Bisabolene	1508	1506	0.37
β-Curcumene	1513	1516	3.06
Zonarene	1520	1530	0.69
trans-Calamenene	1522	1529	0.59
β-Sesquiphellandrene	1526	1523	1.27
trans-Candina-1(2),4-diene	1533	1535	0.58
Selina-3,7(11)-dinene	1536	1547	0.16
α-Calacorene	1541	1546	0.29
Germacrene B	1556	1561	1.74
Oxygenated sesquiterpenes			**25.72**
Elemol	1549	1550	0.8
Spathulenol	1575	1578	0.87
Globulol	1583	1585	0.11
Carotol	1595	1595	0.74
Humulene epoxide II	1605	1608	0.49
10-epi-γ-Eudesmol	1622	1624	12.72
α-Acorenol	1630	1633	1.22
cis-Cadin-4-en-7-ol	1639	1637	0.14
α-Muurolol	1644	1646	0.76
Eudesmol	1652	1654	4.30
epi-β-Bisabolol	1669	1672	3.00
α-Bisabolol	1685	1686	0.57
Other			**8.12**
2E-Hexenal	850	855	0.10
Heptanal	902	902	0.01
Heptanal	902	902	0.01
2-acetyl-5-methyl-Furan	1035	1037	0.19
n-Nonanal	1103	1101	0.01
Isobornyl acetate	1284	1286	0.07
2-Undecanone	1294	1294	0.18
3-Dodecanone	1388	1391	1.34
Geranyl butanoate	1564	1564	5.74
Caryophyllene oxide	1579	1583	0.47
Total			**97.61**

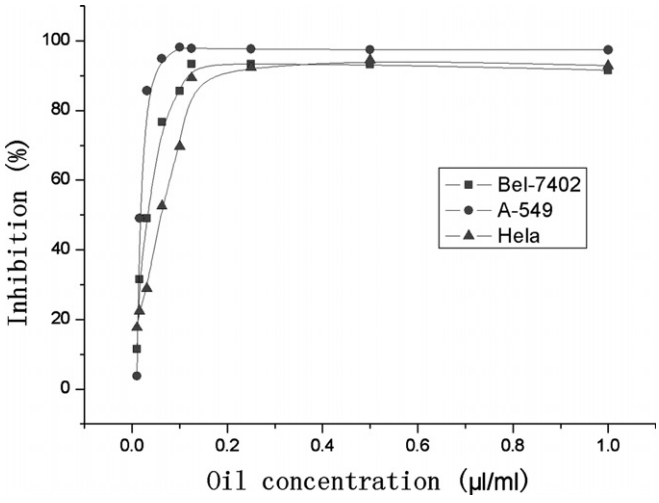

Fig. 1. Cytotoxic activity of *P. serrulata* leaf essential oil on cancer lines. Cell viability was assessed by MTT.

Table 2
Cytotoxic activity of *P. serrulata* leaf essential oil

Cell line	IC_{50} (μl/ml)
Hela	0.0427
A-549	0.0219
Bel-7402	0.0301

penes, are active in the essential oil. In addition, minor components could also contribute to cytotoxic activity of the oil. It is also possible that the minor components may be involved in some type of synergism with the other active compounds (Lattaoui & Tantaoui-Elaraki, 1994; Yu, Lei, Yu, Cai, & Zou, 2004).

The antioxidant activity of the leaf essential oil of *P. serrulata* is also tested by DPPH radical-scavenging method. The scavenging curve is presented in Fig. 2; the

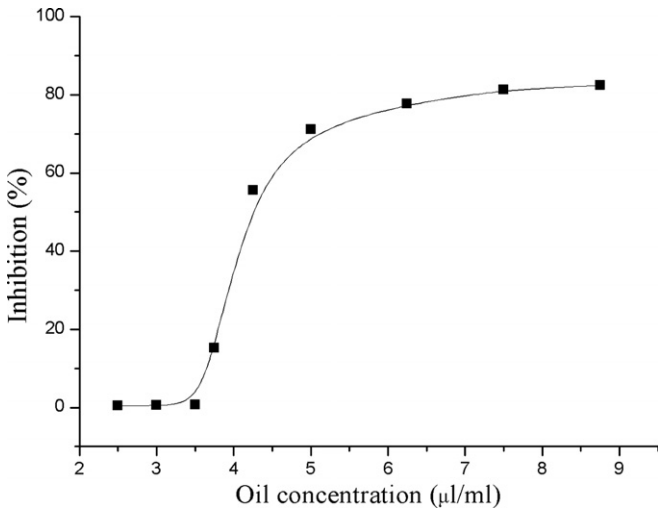

Fig. 2. Antioxidant activity of *P. serrulata* leaf essential oil. Antioxidant activity was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical reduction.

IC_{50} was 5.45 μl/ml. Many antioxidants have been demonstrated to be cytotoxic, such as *Panax ginseng* (Keum et al., 2000), *Xanthones*(Lee et al., 2005) and methanol extract of *Betula platyphylla* var.*japonica* (Ju, Lee, Hwang, & Kim, 2004). Some reports support the relationship of cytotoxicity with antioxidant activity (Dwivedi, Muller, Goetz-Parten, Kasperson, & Mistry, 2003; Owen et al., 2000). So the antioxidant activity of *P. serrulata* leaf essential oil might contribute to its cytotoxic activity.

4. Conclusions

Most components of *P. serrulata* essential oil were identified and their cytotoxic and antioxidant activity investigated. The results clearly showed that the oil presented strong cytotoxic activity against three human tumor cell lines tested. The antioxidant activity of the oil was moderate. The cytotoxic activity could be explained partly by the presence of α-humulene, caryophyllene oxide, γ-elemene and δ-elemene. However, as their concentration was low, a further study is needed to find new active components and unravel the mechanism of their cytotoxic activity. The results of this work also demonstrate the potential of *P. serrulata* essential oil as a new cytotoxic agent.

Acknowledgments

We thank our Chinese traditional doctor Mr. Hou Lizhen, highly appreciate for his assistance to collect and identify the plant. We also gratefully acknowledge the National Science Foundation of China (30370366) and the Research Fund for the Doctoral Program of Higher Education for financial support.

References

Adams, R. P. (2001). *Identification of essential oils components by gas chromatography/mass spectroscopy*. IL, USA: Allured Publishing Corporation.

Davies, N. W. (1990). Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbouwax 20 M phases. *Journal of Chromatography A, 503*, 1–24.

Dwivedi, C., Muller, L. A., Goetz-Parten, D. E., Kasperson, K., & Mistry, V. V. (2003). Chemopreventive effects of dietary mustard oil on colon tumor development. *Cancer Letter, 196*, 29–34.

Duh, C. Y., Wang, S. K., Weng, Y. L., Chiang, M. Y., & Dai, C. F. (1999). Cytotoxic terpenoids from the Formosan soft coral *Nephthea brassica*. *Journal of Natural Products, 62*, 1518–1521.

Jiangsu New Medicine College. (1977). *Dictionary of Chinese Material* Shanghai: Medical Science and Technology Press of Shanghai.

Ju, E. M., Lee, S. E., Hwang, H. J., & Kim, J. H. (2004). Antioxidant and anticancer activity of extract from *Betula platyphylla* var. *japonica*. *Life Sciences, 74*, 1013–1026.

Kanada, N., Pezzuto, J. M., Kinghorn, A. D., Earnsworth, N. R., Santisuk, T., Tuchinda, P., et al. (1992). Plant anticancer agents, cytotoxic triterpenes from *Sandoricum Koetjape* stems. *Journal of Natural Products, 55*, 654–659.

Keum, Y. S., Park, K. K., Lee, J. M., Chun, K. S., Park, J. H., Lee, S. K., et al. (2000). Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. *Cancer Letter, 150*, 41–48.

Kubo, I., Chaudhuri, S. K., Kubo, Y., Sanchez, Y., Ogura, T., Saito, T., et al. (1996). Cytotoxic and antioxidative sesquiterpenoids from *Heterotheca inuloides*. *Planta Medica, 62*, 427–430.

Lattaoui, N., & Tantaoui-Elaraki, A. (1994). Individual and combined oils. *Rivista Italoana EPPOS, 13*, 13–19.

Lee, B. W., Lee, J. H., Lee, S. T., Lee, H. S., Lee, W. S., Jeong, T. S., et al. (2005). Antioxidant and cytotoxic activities of xanthones from *Cudrania tricuspidata*. *Bioorganic and Medicinal Chemistry Letters, 15*, 5548–5552.

Legault, J., Dahl, W., Debition, E., Pichette, A., & Maldemont, J. C. (2003). Antitumor activity of balsam fir oil: production of reactive oxygen species induced by α-Humulene as possible mechanism of action. *Planta Medica, 69*, 402–407.

Owen, R. W., Giacosa, A., Hull, W. E., Haubner, R., Wurtele, G., Spiegelhalder, B., et al. (2000). Olive-oil consumption and health: the possible role of antioxidants. *The Lancet Oncology, 1*, 107–112.

Pedro, L. G., Santos, P. A., Silva, J. A., Figueiredo, A. C., Barrso, J. G., Deans, S. G., et al. (2001). Essential oils from *Azorean Laurus azorica*. *Phytochemistry, 57*, 245–250.

Plumb, J. A., Milroy, R., & Kaye, S. B. (1989). Effects of the pH dependence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. *Cancer Research, 49*, 4435–4440.

Ramos, A., Visozo, A., Piloto, J., Garcia, A., Rodriguez, C. A., & Rivero, R. (2003). Screening of antimutagenicity via antioxidant activity in Cuban medicinal plants. *Journal of Ethnopharmacology, 87*, 241–246.

Sibanda, S., Chigwada, G., Poole, M., Gwebu, E. T., Noletto, J. A., Schmidt, J. M., et al. (2004). Composition and bioactivity of the leaf essential oil of *Heteropyxis dehniae* from Zimbabwe. *Journal of Ethnopharmacology, 92*, 107–111.

Sylvestre, M., Legault, J., Dufour, D., & Pichette, A. (2005). Chemical composition and anticancer activity of leaf essential oil of *Myrica gale* L. *Phytomedicine, 12*, 299–304.

Tambe, Y., Tsujiuchi, H., Honda, G., Ikeshiro, Y., & Tanaka, S. (1996). Gastric cytoprotection of the non-steroidal anti-flammatory sesquiterpenes, beta-caryophyllene. *Planta Medica, 62*, 469–470.

Yu, J. Q., Lei, J. Q., Yu, H. D., Cai, X., & Zou, G. L. (2004). Chemical composition and antimicrobial activity of essential oil of *Scutellaria barbata*. *Phytochemistry, 65*, 881–884.