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Chemical Composition, Antioxidant and Antimicrobial Activity of the Essential Oil of *Phellodendron amurense* (Rupr.) from China

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The essential oil of *Phellodendron amurense* has been isolated from fruits collected in their native habitat China. Hydrodistillation of *P. amurense* afforded a pale yellow oil in 1.4 % (w/w) yield. The chemical composition of this essential oil was analyzed by GC-MS. Twenty-one compounds were identified, accounting for 97 % of the total oil. There were five most abundant constituents (greater than 5 %) were myrcene (51.7 %), 2-methyl-6-methyleneocta-3,7-dien-2-ol (7.4 %), 1,2-benzenedicarboxylic acid-*bis*(2-methylpropyl)-ester (7.2 %), 2-methyl-6-methyleneocta-1,7-dien-3-ol (7.1 %) and α-phellandrene (5.2 %).The essential oil of *P. amurense* showed antioxidant activity (IC_{50}, 2.32 μg/mL) and broad spectrum fumigant and contact antimicrobial activity against all microbial strains tested (MIC or IC_{50}, 0.12-1.36 μg/mL). These findings suggest that the essential oil may be used as an antioxidant or antimicrobial agent.

Keywords: *Phellodendron amurense*, Essential oil, Antimicrobial activity, Rutaceae.

INTRODUCTION

Phellodendron amurense (Rupr.), commonly called the Amur cork tree, is a species of tree in the family Rutaceae, native to northeast Asia[1]. *P. amurense* is found primarily in the northeastern regions of China. The bark is the principal part of *P. amurense* used medicinally because it produces berberine, a compound with antibacterial and antifungal properties[2]. Plants in the family Rutaceae, such as *Citrus* spp.[3-5], *Zanthoxylum bungeanum*[6] and *P. chinense*[7], are known as aromatic plants. Their fruits are rich in essential oils that exhibit potent antimicrobial activity. Essential oils from these plants are considered to be good sources of antimicrobial agents[8-10]. Few studies have been published to date on the chemical constituents in the essential oil of *P. amurense* collected from its native habitat. Lis *et al.*[11] reported the chemical composition of the essential oil of *P. amurense* from Poland, but those authors did not investigate its biological activity. Wang *et al.*[12] reported the antimicrobial activity of bark extracts from *P. amurense* and noted that berberine is one of the major active compounds[13]. However, little is known about the biological activity of the fruits of *P. amurense*, especially with respect to the essential oil from these fruits. To our best of knowledge, this is the first report on the antioxidant and antimicrobial activity of essential oil from Chinese *P. amurense* fruits. The activity of this oil against plant pathogens is described and its chemical composition is compared with that reported previously.

EXPERIMENTAL

Five kilograms (fresh weight) of ripe *P. amurense* fruits (moisture content 50.2 %) was collected in November 2011 from the Wuying Forestry Bureau in the Wuying region (altitude 320 meters, coordinates 48°04' North, 129°15' East) of the Lesser Khingan Range forest in northeast China. The *P. amurense* has been identified and authenticated by Prof. Qinggui Wang (Heilongjiang University) and Prof. Wei Cao (The Herbarium of Northeast China). The voucher specimens were deposited in the College of Agricultural Resource and Environment, Heilongjiang University and the Herbarium of Northeast China (the voucher specimen number is IFP0215142). All microorganisms were obtained from Heilongjiang University and Northeast Agricultural University. Two Gram-positive bacteria (*Bacillus megaterium* pv. cerealis HLJA109 and *Clavibacter michiganensis* subsp. *michiganensis* HLJA126) and two Gram-negative bacteria (*Pseudomonas syringae* pv. *glycinea* HLJA112 and *Xanthomonas oryzae* pv. *oryzae* HLJA107) were used for broth microdilution. Two oomycetes (*Pythium aphanidermatum* HLJA011 and *Phytophthora sojae* HLJA002) and seven other filamentous

fungi (*Alternaria solani* ATCC 26670, *Bipolaris maydis* HLJA011, *Fusarium graminearum* HLJA026, *F. oxysporum* HLJA012, *Magnaporth grisea* HLJA032, *Penicillium italicum* ATCC 48114 and *Rhizoctonia solani* HLJA018) were used for agar diffusion and vapor diffusion.

Extraction of essential oil: The ripe fruits of *P. amurense* were ground and subjected to steam distillation for 2 h using a clevenger apparatus to yield the essential oils. Essential oils were dehydrated by the addition of anhydrous sodium sulfate and stored at 4 °C[14].

GC and GC-MS analysis: The essential oil was analyzed using a SHIMADZU GC-2010, equipped with a flame ionization detector (FID) and an SE-54 silica capillary column (30 m × 0.32 mm × 0.50 μm). The column temperature was increased from 50 °C (1 min) to 150 °C (2 min) at 3 °C/min, then increased from 150 to 250 °C at 10 °C/min. Injector and detector temperatures were set at 250 °C. The percentage composition of the essential oil was obtained from the normalization of the GC peak areas, without using correction factors. GC-MS analyses were carried out using a SHIMADZU GCMS-QP2010 system operating in the EI mode at 70 eV. The operating conditions were same as those reported for the GC analyses. Helium (99.99 %) was used as the carrier gas at a flow rate of 1 mL/min. Injector and transfer line temperatures were 250 °C. The ion source temperature was 280 °C. Split injection was used with a split ratio of 10:1. Diluted samples (1 μL, 1/100 in acetone) were injected manually. Retention indices (RI) of the components were determined relative to the retention times of a series of *n*-alkanes C8-C40 (Sigma) on the SE-54 column using the linear interpolation method described by Van den Dool and Kratz[15]. The constituents of the essential oil were identified by RI and GC-MS and through comparison of their RI and mass spectra with those reported in the NIST05.LIB library, LRI web library and existing literature.

Antioxidant activity: Antioxidant activity was investigated by measuring the radical scavenging capacity (RSC) of essential oils against the DPPH radical[16]. Two-fold serial dilutions of essential oil were prepared and tested (final concentrations: 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 μg/mL). Absorption values were recorded at 515 nm. Trolox was used as a positive control. Three replicates were investigated for each sample. The percentage radical scavenging capacity (RSC) was determined from the equation: RSC (%) = $[(A_{blank}-A_{sample})/A_{blank}] \times 100$, where A is the absorption value at 515 nm. The IC_{50} values, the concentrations of essential oil required to achieve 50 % growth inhibition, were determined from linear relationships between the concentration logarithms and radical scavenging capacity value probabilities[7].

Antimicrobial activity: Modified broth dilution in microtiter plates was used to determine minimum inhibitory concentrations (MIC)[17,18]. The MIC is defined as the lowest concentration at which no microbial growth is observed. Microbial growth was indicated using the chromogenic reagent 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). All assays were performed in LB broth for bacteria. Serial dilutions of the oils were prepared with 10 % dimethyl sulfoxide (DMSO). Dilutions of the oils were added into a 96-well microtiter plate containing the broth and assay strain at a final concentration of 1×10^5 colony forming units/mL (CFU/mL). The final concentrations of oil in the wells ranged from 0.06 to 2 μg/mL and the final DMSO concentration in each well was 1 % (v/v). Plates were incubated at 37 °C for 24 h for bacteria. MTT stock solution (10 μL, 0.5 mg/mL) was added to each well (total volume 100 μL) as a growth indicator and incubated for an additional 1 h. Control wells contained 1 % DMSO. Each assay was performed in triplicate. Antifungal activity was determined using an agar diffusion method to determine fungal growth rates[19]. Potato dextrose agar (PDA, 30 mL) was inoculated with different concentrations of essential oil in 1 % aqueous DMSO in a 9 cm Petri dish with a 5 mm agar disc. PDA without oil was inoculated with the fungal disc as a control. The diameters of assay colonies were measured when the control colony reached a diameter of 8 cm. Inhibition (%) was calculated from the following equation: Inhibition (%) = $[(D_{blank}-D_{sample})/D_{blank}] \times 100$, where D is the colony diameter. IC_{50} values were determined from linear relationships between the concentration logarithms of the essential oil and fungal inhibition probabilities[7]. Antifungal activity was determined using the vapor diffusion method[6,7]. Two uncovered 6 cm petri dishes were placed in a 15 cm petri dish with a sealed cover. One 6 cm petri dish contained water agar (15 g agar per 1 L water) with different concentrations of the essential oil. The other 6 cm dish containing PDA was inoculated with a 5 mm agar disc of assay fungus. The IC_{50} values of volatile compounds from essential oil were calculated as described above. Water agar without oil was used as control. Each antifungal experiment was performed in triplicate. Streptomycin Sulfate, Amphotericin B and Azoxystrobin were used as positive controls in the antimicrobial assays.

RESULTS AND DISCUSSION

Hydrodistillation of ripe *P. amurense* fruits from China afforded a pale yellow oil in 1.4 % yield (w/w) based on the original mass of the fruit. The yield of our essential oil was higher than that (1.1 %) recovered by Lis *et al.*[11] from ripe fruits from Poland. In both cases, however, the yield in dry weight was 2.8 %. This apparent discrepancy is due to different moisture contents in the ripe fruits resulting from different collection times. Twenty-one compounds were identified and each constituent represented more than 0.3 % of the total oil. These compounds collectively accounted for 97 % of the total oil (Table-1). The chemical composition of the essential oil in this study was distinct from that derived from fruits from Poland. Differences in the growth environments of *P. amurense* (a forest in northeastern China compared to the Medicinal Plant Garden of the University of Medicine in Wroclaw, Poland), time of fruit collection (November compared to October), moisture content (50.2 % compared to 60.3 %) and isolation methods account for the difference in chemical composition between these essential oils. Lis *et al.*[11] reported 17 main constituents (each representing more than 0.3 % of total oil) and trace amounts of 48 other constituents (each less than 0.3 %) in their oil, accounting for 94 % of the total oil. Our oil contained 15 constituents (compounds **1-5**, **7-12**, **14** and **16-18** in Table-1) that were also detected by Lis *et al.*[11] These 15 constituents represented 76.7 % of our total oil. The other 6v

TABLE-1
CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM *P. amurense* fruits

No.	Compound	RI[a]	Area (%) ± SD[b]
1	α-Pinene	935	1.9 ± 0.09
2	Sabinene	976	0.8 ± 0.05
3	Myrcene	994	51.7 ± 3.50
4	α-Phellandrene	1026	5.2 ± 0.35
5	δ-3-Carene	1042	0.6 ± 0.05
6	2-Methyl-6-methyleneocta-3,7-dien-2-ol	1115	7.4 ± 0.15
7	2-Methyl-6-methyleneocta-1,7-dien-3-one	1162	1.2 ± 0.05
8	2-Methyl-6-methyleneocta-1,7-dien-3-ol	1181	7.1 ± 0.20
9	Bornyl acetate	1298	0.5 ± 0.01
10	Undecan-2-one	1302	0.5 ± 0.03
11	Undecan-2-ol	1316	0.4 ± 0.02
12	Neryl acetate	1432	0.6 ± 0.05
13	Isoledene	1444	0.6 ± 0.02
14	(E)-Caryophyllene	1476	2.5 ± 0.01
15	1,4,7,11-1,3,4,7- Tetramethyltricyclo [5,3,1,0(4,11)]undec-2-en-8-one	1533	0.6 ± 0.01
16	Germacrene D	1539	2.4 ± 0.03
17	δ-Cadinene	1575	0.7 ± 0.02
18	Caryophyllene oxide	1618	0.6 ± 0.02
19	1,2-Benzenedicarboxylic acid-bis(2-methylpropyl)ester	1746	7.2 ± 0.28
20	9,10-Dehydro-cycloisolongifolene	1775	3.9 ± 0.25
21	1-Methyl-4-(5-methyl-1-methylene-4-hexenyl)-,(s)-cyclohexene	1789	0.6 ± 0.03

[a]RI was calculated against C_8-C_{40} *n*-alkanes on the column
[b]Area (%) is the average of four samples; SD, standard deviation

constituents (compounds **6**, **13**, **15** and **19-21** in Table-1), representing 20.3 % of our total oil, have not been reported previously in *P. amurense* oil. Five compounds (compounds **3**, **4**, **6**, **8** and **19** in Table-1) in our oil accounted for more than 5 % of the total: myrcene (51.7 %), 2-methyl-6-methyleneocta-3,7-dien-2-ol (7.4 %), 1,2-benzenedicarboxylic acid-*bis*(2-methylpropyl)-ester (7.2 %), 2-methyl-6-methyleneocta-1,7-dien-3-ol (7.1 %) and α-phellandrene (5.2 %). These five constituents collectively represent 78.6 % of the total oil. Myrcene (70.3 %) and β-caryophyllene (6.8 %) were the most abundant constituents (greater than 5 % of total oil) reported by Lis *et al.*[11], accounting for 77.1 % of their oil. A single compound, monoterpene hydrocarbon myrcene, accounts for the majority of the mass in both samples of essential oils. Myrcene occurs frequently as a constituent of essential oils from other plants, including *Aniba roseaodora*, *Citrus aurantium* and *Pelargonium roseum*[20]. Two of the constituents abundant in our oil were not found in the oil reported by Lis *et al.*[11]: 2-methyl-6-methyleneocta-3,7-dien-2-ol (7.4 %) and 1,2-benzenedicarboxylic acid-*bis*(2-methylpropyl)-ester (7.2 %). Another constituent abundant in our oil, α-phellandrene (5.2 %), was found in the oil described by Lis *et al.*[11]; however, it represented only 0.1 % of their total oil. It is possible that the compositions of essential oils from the fruits of *P. amurense* differ due to their different growth environments[21,22]. Although 2-methyl-6-methyleneocta-3,7-dien-2-ol was not observed by Lis *et al.*[11], 2-methyl-6-methyleneocta-1,7-dien-3-ol (7.1 %) was found in their ripe (3 %) and unripe fruit oil (less than 0.1 %). It may be that the concentration of 2-methyl-6-methyleneocta-1,7-dien-3-ol increases over time. Our fruit was collected later in the year compared with that collected by Lis *et al.*[11] and 2-methyl-6-methyleneocta-1,7-dien-3-ol is an abundant constituent in our oil. In addition, β-caryophyllene was abundant in the oils reported by Lis *et al.*[11] (6.8 % in ripe fruit oil and 10.5 % in unripe fruit oil). It seems possible that the concentration of β-caryophyllene decreases over time. The content of β-caryophyllene in our ripe fruit oil was only 2 % of the total. It is possible that the collection time accounts for this difference. All of the identified constituents in our oil have been reported in other plants, such as *Adiantum flabellulatum* and *Alpinia* conchigera[23,24].

The antioxidant activity of the essential oils from *P. amurense* fruits was investigated by measuring the radical scavenging capacity in a DPPH radical assay. As shown in Fig. 1, the essential oils from the fruit of *P. amurense* showed more potent scavenging efficacy than the positive control Trolox, a common antioxidant. The IC_{50} value for the essential oils was 2.32 μg/mL (y = 2.4306x + 4.1135, R^2 = 0.9932), while the IC_{50} of Trolox was 3.58 μg/mL (y = 3.2272x + 3.2139, R^2 = 0.9892). Wang *et al.*[12] reported the antioxidant activity of the aqueous (IC_{50} 6.73 mg/mL) and enthanolic extract (IC_{50} 4.26 mg/mL) of dried *P. amurense* bark in the DPPH radical assay. It appears that our oil extracted from the fruits of *P. amurense* has more potent antioxidant activity than the bark extracts. This finding suggests that the essential oil can be used as a natural antioxidant.

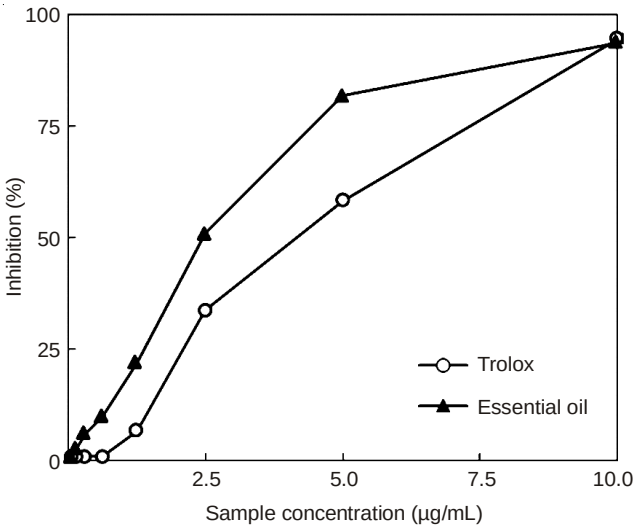

Fig. 1. Antioxidant activity of essential oil from *P. amurense* fruit

All of the 13 microorganisms used for antimicrobial assays were plant pathogens. *in vitro* antibacterial experiments showed that growth was inhibited in all of these bacteria by the essential oil of *P. amurense*. MIC values in these assays ranged from 0.19 to 0.50 μg/mL (Table-2). Vapor assays and direct contact assays were used to determine the antifungal activity of the essential oil. Comparisons between antifungal activities measured by vapor and direct contact assays are shown in Table-2 (all R^2 values were greater than 0.90). Antifungal activities observed in direct contact assays (IC_{50} 0.12 to 0.46 μg/mL) were generally stronger than those observed in vapor assays (IC_{50} 0.16 to 1.36 μg/mL). There was a direct correlation in relative antimicrobial activities between the contact and

TABLE-2
ANTIBACTERIAL MIC (μg/mL) AND ANTIFUNGAL IC_{50} (μg/mL) OF ESSENTIAL OIL FROM *P. amurense* FRUIT

Bacterium		MIC	Control[a] MIC
B. megaterium pv. *cerealis*		0.19	0.06
C. michiganensis subsp. *michiganensis*		0.38	0.13
P. syringae pv. *glycinea*		0.50	0.25
X. oryzae pv. *oryzae*		0.38	0.13
Fungus	Contact IC_{50}	Vapor IC_{50}	Control[a] IC_{50}
A. solani	0.23	0.33	0.10
B. maydis	0.16	0.46	0.09
F. graminearum	0.15	0.39	0.11
F. oxysporum	0.12	0.16	0.15
M. grisea	0.18	0.42	0.09
P. italicum	0.46	1.36	0.16
R. solani	0.18	0.57	0.13
Oomycete	Contact IC_{50}	Vapor IC_{50}	Control[a] IC_{50}
P. aphanidermatum	0.21	0.95	0.11
P. sojae	0.29	0.84	0.09

[a]Positive controls of bacterium, fungus and oomycete were streptomycin sulfate, Amphotericin B and Azoxystrobin, respectively, in the contact assay

vapor assays in most cases. The results of the vapor antifungal assays may be more significant due to the small size and ubiquity of most fungi, which can spread through air. As shown in Table-2, the essential oil derived from the fruit of *P. amurense* showed similar antimicrobial activity against bacteria, fungi and oomycetes (MIC or IC_{50}, 0.12-1.36 μg/mL). Our oil was more potent in antibacterial assays compared with the essential oil from *P. chinense* (IC_{50} 0.24 to 1.46 mg/mL). This finding is striking, given that both plants belong to the same genus and have 53.2 % chemical similarity, as described above[7]. However, these values are close to those reported for the essential oil from the fruits of *C. sinensis* (MIC 2.5 to 3 μg/mL)[6,7], a plant that also belongs to the family Rutaceae. Our essential oil was also more potent in antimicrobial assays than those reported for the bark extracts from *P. amurense* (MIC 3.68 to 50 mg/mL)[12]. Myrcene was the most abundant constituent in our oil. Previous reports have described the antimicrobial activity of myrcene[5,25,26]. The antimicrobial activity of other constituents in our oil, including carene and pinene, has also been reported[5,27]. Although some constituents of our oil have been reported to possess antimicrobial activity, a more detailed explanation of antimicrobial activity will be reported after each purified compound has been tested for antimicrobial activity.

Conclusion

The essential oil of *P. amurense* fruits from China was characterized and was found to have a unique chemical composition. This oil exhibited antioxidant and antimicrobial activity against various plant pathogens, including bacteria, fungi and oomycetes and might therefore be used as a natural antioxidant and antimicrobial.

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REFERENCES

1. K. Kishi, K. Yoshikawa and S. Arihara, *Phytochemistry*, **31**, 1335 (1992).
2. Y. Ida, Y. Satoh, M. Ohtsuka, M. Nagasao and J. Shoji, *Phytochemistry*, **35**, 209 (1993).
3. D.R.L. Caccioni, M. Guizzardi, D.M. Biondi, A. Renda and G. Ruberto, *Int. J. Food Microbiol*., **43**, 73 (1998).
4. N. Sharma and A. Tripathi, *Microbiol. Res*., **163**, 337 (2008).
5. S. Droby, A. Eick, D. Macarisin, L. Cohen, G. Rafael, R. Stange, G. McColum, N. Dudai, A. Nasser, M. Wisniewski and R. Shapira, *Postharvest Biol. Technol*., **49**, 386 (2008).
6. Y. Gong, Y. Huang, L. Zhou, X. Shi, Z. Guo, M. Wang and W. Jiang, *J. Essent. Oil Res*., **21**, 174 (2009).
7. Y. Gong, L. Zhou, X. Shi, Z. Ma, Z. Guo, M. Wang, J. Wang and X. Li, *J. Essent. Oil Res*, **23**, 108 (2011).
8. K.A. Hammer, C.F. Carson and T.V. Riley, *J. Appl. Microbiol*., **86**, 985 (1999).
9. M.B. Isman, *Crop Prot*., **19**, 603 (2000).
10. S. Burt, *Int. J. Food Microbiol*., **94**, 223 (2004).
11. A. Lis, E. Boczek and J. Gora, *Flavour Fragrance J*., **19**, 549 (2004).
12. W. Wang, Y.G. Zu, Y.J. Fu, J. Reichling, U. Suschke, S. Nokemper and Y. Zhang, *Am. J. Chin. Med*., **37**, 195 (2009).
13. H. Yu, K. Kim, J. Cha, H. Kim, Y. Lee, N.-Y. Choi and Y.-O. You, *J. Med. Food*, **8**, 454 (2005).
14. M. Tan, L. Zhou, M. Qin, D. Li, W. Jiang, Y. Wang and X. Hao, *J. Essent. Oil Res*., **19**, 197 (2007).
15. H. Van den Dool and P.D. Kratz, *J. Chromatogr. A*, **11**, 463 (1963).
16. N. Mimica-Dukic, B. Bozin, M. Sokovic and N. Simin, *J. Agric. Food Chem*., **52**, 2485 (2004).
17. NCCLS, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard, Wayne, USA, edn 5 (2001).
18. L. Xu, L. Zhou, J. Zhao, J. Li, X. Li and J. Wang, *J. Appl. Microbiol*., **46**, 68 (2008).
19. J. Wang, H. Liu, J. Zhao, H. Gao, L. Zhou, Z. Liu, Y. Chen and P. Sui, *Molecules*, **15**, 5807 (2010).
20. C.L. Wilson, J.M. Solar, A. El Ghaouth and M.E. Wisniewski, *Plant Dis*., **81**, 204 (1997).
21. P. Sandra and C. Bicchi, Capillary Gas Chromatography in Essential Oil Analysis, Alfred Huethig Verlag, Heidelberg, p. 3 (1987).
22. K.H.C. Baser and G. Buchbauer, Handbook of Essential Oils: Science, Technology and Applications, Taylor & Francis, Boca Raton, pp. 1-32 (2010).
23. Z.W.Y. Kang, Q. Ji and J.M. Wang, *Chem. Nat. Comp*., **45**, 575 (2009).
24. Q.Z. Faridah, A.H.A. Abdelmageed, A.N. Nor Hazirah and M. Yaacob, *J. Med. Plants Res*., **4**, 2700 (2010).
25. Y. Inoue, A. Shiraishi, T. Hada, H. Hamashima and J. Shimada, *FEMS Microbiol. Lett.,* **237**, 325 (2004).
26. L. Jirovetz, S. Bail, G. Buchbauer, Z. Denkova, A. Slavchev, A. Stoyanova, E. Schmidt and M. Geissler, *Sci. Pharm*., **74**, 189 (2006).
27. H.J.D. Dorman and S.G. Deans, *J. Appl. Microbiol*., **88**, 308 (2000).