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Comparative Analysis of Essential Oil Components and Antioxidant Activity of Extracts of *Nelumbo nucifera* from Various Areas of China

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This study was designed to examine the composition of extracts and essential oil components from *Nelumbo nucifera* leaves from the principal habitats in China. The amounts of phenolics, flavonoids, and proanthocyanidins in the lotus leaf extracts varied widely, ranging from 354 to 487 mg/g gallic acid equivalents, from 172 to 236 mg/g rutin equivalents, and from 124 to 179 mg/g catechin equivalents, respectively. All of the extracts had strong antioxidant activity in comparison to the standard compounds butylated hydroxytoluene and vitamin C. Wild lotus samples from Baiyangdian Lake and Weishan Lake exhibited a stronger free radical scavenging effect and greater reducing power than the cultural samples, but no such differences were observed in the inhibition of lipid oxidation. Chemical variation in the essential oils from the various samples was analyzed by GC-MS. The main constituents were l-(+)-ascorbic acid 2,6-dihexadecanoate (0−33.5%), *trans*-phytol (5.1−24.1%), hexahydrofarnesyl acetone (5.6−15.3%), pentadecyl acrylate (2.2−12.4%), geranyl acetone (1.9−8.0%), and β-ionone (0−8.0%). The rhizome lotus and seed lotus samples were clustered into separate groups by hierarchical cluster analysis according to the composition of the corresponding essential oils. No significant relationship was found between essential oil composition and geographical distribution of the 11 populations. However, the results indicated that region of origin and growing conditions could significantly affect both the bioactivities of the lotus leaf and the content of bioactive compounds in the leaves. Thus, the existence of chemical polymorphism in the *N. nucifera* leaf in China was demonstrated.

KEYWORDS: *Nelumbo nucifera*; antioxidant activity; essential oil; chemical variation; China

INTRODUCTION

Lotus (*Nelumbo nucifera* Gaertn.) is widely used not only as an ornamental plant but also as a dietary staple in Eastern Asia, particularly in China. In China, the cultured edible lotus is found as two varieties, the rhizome lotus and the seed lotus. Several studies have shown that the rhizome extract possesses antioxidant, antidiabetic, and anti-inflammatory activities (*1*−*3*), and it also can improve learning and memory (*4*). Seed extracts showed hepatoprotective and free radical scavenging effects (*5*), and stamen extracts showed an antioxidant effect (*6*).

Since ancient times, the leaf of *N. nucifera*, a health food "He Ye", has been thought to possess useful medicinal properties. This aromatic, blue-green herb is traditionally used to disperse body heat during summer and, according to folk medicine, is believed to increase essential body energies. Our previous study of the leaf showed a strong antioxidant activity (*7*). It also showed antiobesity, antimalarial, and antifungal effects (*8*, *9*) and as well as an antidiabetic effect (*10*).

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In recent years, lotus leaf has become popular as an ingredient of antioxidant beverages and tea bags in China. However, lotus leaf as the main raw material of these products is extensively cultivated in several principal habitats in China, and the annual production of lotus leaf now exceeds 800,000 tons. Therefore, the study of the variation in the antioxidant activity of lotus leaf extracts was directly related to the quality of the products. In addition, few studies (*11*) have examined the essential oils of the lotus leaf. The comparative study of the chemical composition of the essential oil of lotus leaf from principal habitats in China could lay the foundation for the further chemical and bioactivity studies of the essential oil of lotus leaf, which may help to broaden its applications, resulting in greater use of this abundant resource, providing income for lotus producers and the regional economy. In the present study, we collected samples during August 2007 from 11 *N. nucifera* populations that included rhizome lotus (RL), seed lotus (SL), and wild lotus (WL) from 9 Chinese provinces. Our goal was to study the chemical composition of the essential oils and lotus leaf extracts and to examine variations between the principal habitats in China.

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MATERIALS AND METHODS

Plant Materials and Chemicals. Leaves of *N. nucifera* were collected during August 2007 from 11 locations in China (**Figure 1**) and identified at the College of Pharmacy, Wuhan University. The plant material from each location was obtained from three populations, and each population contained 10 individual plants. Each interval between populations was not less than 1000 m. As the single individual lotus may occupy an area of 2−4 m^2, and the main rhizome may reach 3−6 m, an interval of 10 m was used in sampling the individual plants. Plants were dried at room temperature. No obvious morphological variation was found among the specimens. Voucher specimens, no. 532 (Wuhan), 533 (Xiaogan), 534 (Baoying), 535 (Guangzhou), 536 (Xiangtan), 537 (Honghu), 538 (Jiande), 539 (Jianning), 540 (Guangchang), 541 (Weishan Lake), and 542 (Baiyangdian Lake), have been deposited in the Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy of Wuhan University.

Ascorbic acid (vitamin C), gallic acid, catechin, rutin, and butylated hydroxytoluene (BHT) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). α,α-Diphenyl-β-picrylhydrazyl (DPPH) and 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used for analysis were of AnalaR grade, obtained from China Medicine (Group) Shanghai Chemical Reagent Corp. (Shanghai, China).

Extraction of the Essential Oils. A sample of lotus leaves (500 g) was subjected to hydrodistillation for 3 h using a Clevenger-type apparatus (*12*). The oils were dried over anhydrous sodium sulfate and preserved in a sealed vial at 4 °C until further analysis.

Preparation of Plant Extract. Dried *N. nucifera* leaf powder (50 g) was decocted with 60% ethanol (1.5 L) for 2 h at 80 °C. The extracts were filtered, and solvent was evaporated by rotary evaporation at 35 °C. The residues were evaporated to dryness in vacuo, and the resulting dry powder was stored at 4 °C.

Analysis of the Oils. Samples diluted by acetone (1 μL, 1%) were injected into GC-MS (QP-2010, Shimadzu Co., Kyoto, Japan) and separated on an Rtx-5MS column (length = 30 m, i.d. = 0.25 mm, thickness = 0.25 μm) using helium as carrier gas. The GC oven was operated at an initial temperature of 60 °C for 2 min with a programmed rate of increase of 10 °C/min to a final temperature of 250 °C and a 10 min isotherm. The injector and detector temperatures were set at 240 and 280 °C, respectively. The mass spectrometer was operated in the 70 eV EI mode with scanning from 30 to 500 amu at 0.5 s, and the ion source was set at 200 °C. The essential oil components were quantitated by relative percent peak area of TIC from the MS signal and identified by comparing their mass fragmentation pattern with those stored in the spectrometer database using NIST05.LIB and NIST05s.LIB (National Institute of Standards and Technology).

Determinations of Total Phenolics, Flavonoid, and Proanthocyanidin Contents. Total phenolic content of the extract was determined using the Folin−Ciocalteu assay according to the previously described method (*13*). The amount of total phenolic compounds was calculated as milligrams of gallic acid equivalents from the calibration curve derived from a gallic acid standard solution. Results were expressed as gallic acid equivalents, in milligrams per gram of dry extract.

Total flavonoid content of the extract was determined by colorimetric assay (*14*). A calibration curve was generated using rutin as reference compound, and the results were expressed as milligrams of rutin equivalents per gram of dry extract.

Proanthocyanidin determination was based on the previously described procedure (*15*). Extract solution (0.5 mL) was mixed with 3 mL of a 4% vanillin−methanol solution and 1.5 mL of hydrochloric acid, and the mixture was allowed to stand for 15 min. Absorbance was measured at 500 nm and used to calculate the final result expressed as milligrams of catechin equivalents per gram dry extract.

DPPH and ABTS Radical Scavenging Assay. The DPPH free radical scavenging activity was measured according to a previous procedure (*16*) with minor modifications. Extract solution at a range of concentrations (0.3 mL) was mixed with a solution of 0.2 mM DPPH in methanol (2.7 mL). The mixture was shaken vigorously and allowed to stand for 1 h before the absorbance was measured at 517 nm. DPPH radical scavenging activity was calculated as a percentage according to the equation $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$ (where A_{DPPH} = absorbance without sample and A_S = absorbance with sample). BHT and vitamin C at the same concentration as the samples were used as references.

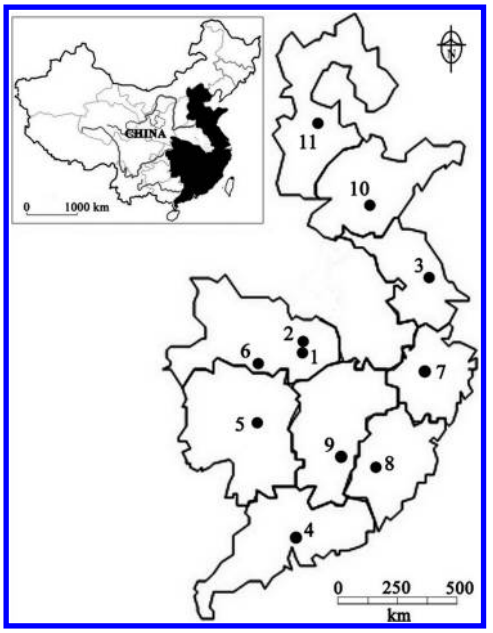

Figure 1. Eleven sampling locations of *Nelumbo nucifera* in China: 1, Wuhan; 2, Xiaogan; 3, Baoying; 4, Guangzhou; 5, Xiangtan; 6, Honghu; 7, Jiande; 8, Jianning; 9, Guangchang; 10, Weishan Lake; 11, Baiyangdian Lake.

The ability of the extract to scavenge the ABTS radical cation was determined by the method given in ref *17* with slight modifications. A solution of ABTS radical cation ($ABTS^+$) was prepared by the reaction of 7 mM ABTS and 2.45 mM potassium persulfate at room temperature in the dark for 16 h. The $ABTS^+$ solution was then diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. Extract solution at a range of concentrations (0.3 mL) was mixed with $ABTS^+$ solution (2.7 mL), the reaction mixture was allowed to stand at 30 °C for exactly 30 min, and absorbance at 734 nm was recorded. ABTS radical scavenging activity was calculated as a percentage according to the equation $[(A_{ABTS} - A_S)/A_{ABTS}] \times 100$ (where A_{ABTS} = absorbance without sample and A_S = absorbance with sample). BHT and vitamin C at the same concentration as the samples were used as references.

Reducing Power Assay. Reducing power was determined according to the method of Oyaizu (*18*). Extracts (50−200 μg) were diluted to a range of concentrations in 1.0 mL of distilled water, and the diluted extracts were mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). After incubation at 50 °C for 20 min, trichloroacetic acid (2.5 mL, 10%) was added, and each mixture was centrifuged at 3000 rpm for 10 min. The upper layer (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. BHT and vitamin C were used as references for comparison.

Antioxidant Activity in the Linoleic Acid System with Ferrothiocyanate (FTC) and Thiobarbituric Acid (TBA). The FTC method was adapted from that of Osawa and Namiki (*19*). Extracts (400 μg) in ethanol (4 mL) were mixed with linoleic acid in ethanol (4 mL, 2.5%), phosphate buffer (8 mL, 0.05 M, pH 7.0), and distilled water (4 mL) and incubated at 40 °C in screw-cap tubes in the dark. Aliquots (0.1 mL) were withdrawn and mixed with ethanol (9.7 mL, 75%) and ammonium thiocyanate (0.1 mL, 30%). Ferrous chloride (0.1 mL, 20 mM in 3.5% hydrochloric acid) was added to each aliquot, and precisely 3 min later the absorbance at 500 nm was measured. Aliquots were withdrawn and assayed similarly at 24 h intervals until a constant maximum value was reached. Controls without extract and standards containing 400 μg of BHT or vitamin C in place of leaf extracts were subjected to the same procedure.

Table 1. Essential Oil and Extract Yields and Contents of Total Phenolics, Flavonoids, and Proanthocyanidins in Extracts of *Nelumbo nucifera* Leaf Collected in Different Locations in China[a]

sample	place of collection (locality/province)	plant material	type	essential oil yield (%)	extract yield (%)	total phenolics (mg/g of extract)	flavonoids (mg/g of extract)	proanthocyanidins (mg/g of extract)
1	Wuhan/Hubei	cultured	rhizome lotus	0.03 ± 0.01	18.5 ± 1.9	374 ± 7.5	203 ± 7.0	145 ± 8.3
2	Xiaogan/Hubei	cultured	rhizome lotus	0.05 ± 0.01	20.1 ± 2.1	392 ± 9.5	183 ± 5.5	135 ± 6.5
3	Baoying/Jiangsu	cultured	rhizome lotus	0.02 ± 0.01	18.6 ± 0.5	372 ± 9.8	183 ± 4.2	144 ± 6.2
4	Guangzhou/Guangdong	cultured	rhizome lotus	0.03 ± 0.01	19.3 ± 1.6	365 ± 7.2	172 ± 9.1	124 ± 11.7
5	Xiangtan/Hunan	cultured	seed lotus	0.02 ± 0.01	17.5 ± 2.0	358 ± 12.1	195 ± 5.7	131 ± 7.5
6	Honghu/Hubei	cultured	seed lotus	0.02 ± 0.01	17.2 ± 1.6	370 ± 23.5	176 ± 6.1	145 ± 12.1
7	Jiande/Zhejiang	cultured	seed lotus	0.03 ± 0.01	17.5 ± 2.1	399 ± 13.5	197 ± 5.5	135 ± 7.8
8	Jianning/Fujian	cultured	seed lotus	0.04 ± 0.01	17.6 ± 1.9	368 ± 7.5	181 ± 8.6	151 ± 9.2
9	Guangchang/Jiangxi	cultured	seed lotus	0.02 ± 0.01	18.4 ± 1.9	397 ± 6.2	186 ± 6.1	130 ± 11.5
10	Weishan Lake/Shandong	wild	−	0.05 ± 0.01	18.7 ± 1.2	478 ± 16.5	232 ± 5.9	170 ± 2.3
11	Baiyangdian Lake/Hebei	wild	−	0.03 ± 0.01	18.6 ± 1.7	487 ± 12.1	236 ± 10.8	179 ± 9.0

[a] Each value is presented as the mean ± SD of three replicate determinations. (−), unknown.

The method of Kikuzaki and Nakatani (*20*) was used to determine the antioxidant activity of the extracts using TBA. Aqueous TBA (2 mL) and trichloroacetic acid (2 mL, 20%) were added to 1 mL of extract solution prepared as in the FTC method above. The mixture was placed in a boiling water bath for 10 min, cooled, and centrifuged at 3000 rpm for 20 min. Absorbance of the resulting supernatant was measured at 532 nm.

Statistical Analyses. All experiments were done in triplicate, and results were reported as mean ± SD. The data were analyzed by one-way ANOVA; statistically significant effects were further analyzed, and means were compared using Duncan's multiple-range test. Statistical significance was determined at $P < 0.05$. The data obtained from the studies of essential oils were analyzed by hierarchical cluster with SPSS version 13.0 to investigate relationships among the 11 populations.

RESULTS AND DISCUSSION

The yields of extract and essential oil from lotus leaves are shown in **Table 1**. No obvious variation in the yield of extracts was found among samples from the 11 regions; these ranged between 17.2 and 20.1%, w/w. These samples yielded essential oils as 0.02−0.05% (v/w) yellowish oils, similar to the yield obtained from *N. nucifera* in Egypt (*11*). Although the essential oil content is low, it may be sufficient to meet needs given the substantial annual production of lotus leaf and the readily available raw materials.

Table 2 shows the chemical constituents of the extracts identified by GC-MS. In total, 95 compounds were identified, accounting for 95.5−99.2% of the total oil composition. The essential oils in the samples from Wuhan, Xiaogan, Baoying, Guangzhou, Xiangtan, Honghu, Jiande, Jianning, Guangchang, Weishan Lake, and Baiyangdian Lake were composed of 49, 44, 50, 39, 45, 42, 54, 50, 41, 41, and 43 identified compounds, accounting for 98.4, 95.5, 97.8, 97.1, 97.8, 99.2, 98.5, 97.3, 96.9, 97.4, and 98.7% of the total oil, respectively. The main constituents in the essential oil in the extracts from the 11 regions are shown in **Figure 2**. Using hierarchical cluster analysis, these extracts were placed in three main groups according to the chemotypes represented in the essential oil component data (**Figure 3**). The extracts in group 1 (from Wuhan, Guangzhou, Baoying, Weishan Lake, and Xiaogan) contained a high level of L-(+)-ascorbic acid 2,6-dihexadecanoate (14.3−33.5%) and a relatively low level of *trans*-phytol (6.2−14.6%). In group 2 (Honghu and Guangchang), the relative abundance of these two components was reversed; L-(+)-ascorbic acid 2,6-dihexadecanoate was present at 6.1−13.2%, and *trans*-phytol at 21.4−24.1%. In group 3 (Jiande, Baiyangdian Lake, Xiangtan, and Jianning), the contents of both L-(+)-ascorbic acid 2,6-dihexadecanoate (0−8.5%) and *trans*-phytol (5.1−7.3%) were generally lower than in group 1 or group 2. Thus, the three principal chemotype groups may be characterized by the concentration of just two of the major components.

From the dendrogram shown in **Figure 3**, it is noteworthy that the RL samples were in one group and well separated from the SL samples, whereas the SL samples were split between two groups. The two WL samples, from Weishan Lake and Baiyangdian Lake, were clustered with RL and SL samples, respectively. Overall, no significant relationship was found between the essential oil composition and the geographical distribution of the 11 populations. The main constituents of the Chinese extracts were quite different from those found in lotus samples from Egypt (*11*), which comprised 10.95% myristic acid, 8.76% edulan, 5.16% phytol, and 31.89% hydrocarbons. The explanation for this may be variation in growing conditions and genetic and environmental factors between the two regions. Various studies with other plant species have also observed variation in essential oil composition between samples from different collection areas. In Lithuania, samples of *Thymus pulegioides* L. representing two varieties of subsp. *silvestris* and four varieties of subsp. *pulegioides* from 11 different localities were hierarchical clustered into 5 chemotypes according to the essential oils' component data. There was no clear relationship in essential oil composition between the studied taxa and the chemical types detected (*21*). The samples of *Ephedra sinica* Stapf. from six populations of Inner Mongolia in northeastern China were divided into two chemotypes based on the component data of the essential oil, one rich in α-terpineol and *p*-vinylanisole and the other rich in phytol, γ-eudesmol, and eudesm-7(11)-en-4-ol (*12*). In Iran, the essential oils of 19 accessions belonging to 6 different *Achillea* species were analyzed. There was a significant variation in oil composition among and within these species. According to the compositions of essential oils, four chemotypes were defined (*22*). These results suggest that variation in the compositions of essential oils could be due to various influencing factors, such as growing conditions, genetic difference of inter- and intraspecies, soil type, and climatic conditions.

Phenolic compounds have been reported to be responsible for the antioxidant activity of *N. nucifera* (*1*, *5*). In the present study, the amounts of total phenolics, flavonoids, and proanthocyanidins in lotus leaf extracts from 11 regions varied widely, ranging from 354 to 487 mg/g of extract, from 172 to 236 mg/g of extract, and from 124 to 179 mg/g of extract, respectively (**Table 1**). These results showed no obvious variations between RL and SL samples, whereas the WL values from Baiyangdian Lake and Weishan Lake were much higher than those of the RL and SL samples from any other cultural location. This suggests that variation in the levels of phenolics between extracts could be partly due to differences in growing conditions. It was reported that under field conditions, where the phenolic composition of shoots of tea varied under the effect of seasonal, genetic, and

Table 2. Composition of the Essential Oils from Different Locations in China

			sample										
peak	RI[a]	compound	1	2	3	4	5	6	7	8	9	10	11
1	814	leaf aldehyde	–	–	–	–	–	–	0.2	0.2	0.2	–	–
2	845	tyranton	0.3	–	–	–	–	0.7	–	–	–	–	–
3	931	(*S*)-3-ethyl-4-methylpentanol	–	–	1.3	–	0.1	–	–	0.3	–	–	–
4	938	6-methyl-5-hepten-2-one	–	–	–	–	0.4	–	tr	0.4	0.3	–	–
5	1040	2-pentylfuran	0.5	0.2	–	0.3	0.8	tr	1.0	1.0	0.5	0.1	1.1
6	1072	4-methoxystyrene	–	–	0.2	–	0.3	–	–	0.6	–	–	–
7	1082	β-linalool	1.3	tr	1.1	1.2	1.6	1.3	1.6	3.1	1.2	–	0.3
8	1088	2,3-octanedione	–	–	tr	–	0.2	–	0.1	0.5	–	–	–
9	1104	nonanal	0.3	tr	tr	0.1	0.7	–	2.2	0.4	0.1	–	0.2
10	1143	α-terpineol	–	–	0.1	0.8	0.4	0.1	–	0.6	0.2	–	–
11	1159	3-decen-5-one	–	–	–	–	0.8	–	–	–	–	–	–
12	1186	safranal	1.6	0.5	0.9	1.3	1.7	1.4	1.4	1.5	1.1	0.4	1.2
13	1196	dihydrocarveol	–	–	–	–	0.3	–	0.3	tr	tr	–	–
14	1204	β-cyclocitral	1.2	0.3	0.5	1.1	0.7	0.5	0.8	1.0	0.6	tr	0.8
15	1224	epoxylinalool oxide	–	–	–	–	–	0.5	–	–	0.4	–	0.1
16	1228	geraniol	0.8	–	0.4	–	0.4	0.2	0.3	0.8	0.2	–	0.3
17	1231	naphthalene	–	–	–	1.3	–	–	tr	–	–	–	tr
18	1272	nonanoic acid	0.4	tr	0.8	–	0.9	–	0.9	0.5	–	–	–
19	1277	bornyl acetate	1.0	0.2	1.0	1.4	0.4	1.1	2.6	1.2	0.3	0.9	2.2
20	1309	5,5,8a-trimethylhexa-hydro-2*H*-chromene	2.5	0.8	3.4	1.7	–	1.4	1.1	1.6	–	–	–
21	1321	hexahydropseudoionone	–	–	–	–	0.5	–	0.3	0.3	–	–	–
22	1333	α-terpineol acetate	tr	–	–	0.7	–	–	–	–	–	–	–
23	1393	2-butyloctyl alcohol	–	–	–	4.3	4.0	–	–	3.9	–	–	–
24	1393	dihydro-(−)-neoclovene	0.9	–	–	–	–	–	–	0.2	1.3	–	0.6
25	1396	1,1,6-trimethyl-1,2-dihydro-naphthalene	0.3	–	–	–	0.6	–	tr	0.1	0.4	0.2	0.3
26	1402	lauraldehyde	–	0.2	–	–	0.4	–	–	–	–	–	0.2
27	1413	tetradecane	1.0	0.4	0.2	0.9	0.6	0.3	0.8	1.8	0.6	0.4	0.7
28	1420	geranyl acetone	4.5	1.9	2.4	2.2	8.0	3.9	3.1	6.7	3.4	2.3	4.6
29	1426	tetrahydro-4,4,7a-tri-methyl-2(4*H*)-benzofuranone	–	–	0.2	–	0.1	–	0.4	0.4	–	–	–
30	1428	pseudoionone	0.9	–	–	–	3.0	0.1	0.2	1.7	–	0.6	0.3
31	1440	4-(2,4,4-trimethylcyclo-hexa-1,5-dienyl)but-3-en-2-one	0.5	–	0.2	tr	0.6	0.1	–	0.2	–	0.5	–
32	1454	β-ionone epoxide	–	–	–	–	4.2	–	–	–	–	–	–
33	1454	megastigmatrienone	tr	0.5	0.4	–	–	3.5	–	–	2.7	–	–
34	1456	2-pentanone, 4-(2,6,6-trimethyl-2-cyclohexenyl)	0.5	–	–	–	–	–	0.7	–	–	–	–
35	1457	dodecyl alcohol	1.1	1.1	–	–	–	1.6	1.9	–	–	1.3	1.8
36	1457	β-ionone	6.2	6.8	4.6	5.2	–	–	4.1	6.2	–	2.8	8.0
37	1465	2-(5-oxohexyl)cyclopentanone	–	–	–	–	1.5	–	0.7	–	0.6	2.6	–
38	1481	propionic acid decyl ester	3.8	2.8	5.4	–	–	3.0	6.4	–	–	5.0	6.2
39	1483	5,6-dipropyldecane	tr	–	0.2	–	0.1	–	0.4	–	–	0.1	–
40	1512	pentadecane	–	–	0.8	–	–	0.6	–	–	–	1.7	2.2
41	1522	α-elemol	0.2	–	0.4	–	–	–	0.2	–	–	–	–
42	1541	2,5,5,8a-tetramethylhexa-hydro-7*H*-chromen-7-one	–	0.9	0.6	–	0.3	11.8	–	–	10.9	–	–
43	1543	cedrol	–	–	1.2	tr	0.5	–	0.7	–	–	–	–
44	1555	2,4-di-*tert*-butylphenol	0.6	0.8	0.6	0.9	0.3	1.5	1.0	0.4		0.8	1.4
45	1556	tridecyl alcohol	–	–	1.5	–	1.4	–	–	–	–	–	–
46	1563	hexahydrofarnesol	–	–	–	–	0.6	–	–	–	–	tr	0.4
47	1568	asarone	–	1.7	–	–	–	0.6	–	1.2	–	6.5	3.2
48	1576	cyclohexyl ketone	1.4	1.1	3.4	1.4	–	1.1	1.5	1.8	0.4	–	0.6
49	1593	eudesm-4(14)-en-11-ol	–	–	0.8	0.1	1.0	–	0.5	0.4	–	–	1.8
50	1601	tetradecanal	–	–	–	–	0.1	1.7	–	–	–	–	–
51	1605	3(10)-caren-4-ol, acetoacetic acid ester	0.2	0.1	–	–	0.2	0.2	–	0.6	–	–	tr
52	1626	γ-eudesmol	1.6	0.9	0.5	–	1.1	0.4	–	–	1.1	–	5.0
53	1643	methyl-3-methylfuran-butyl-oxiran-ethanone	0.3	–	0.5	–	1.4	–	0.4	–	0.7	tr	1.1
54	1653	norphytan	0.1	tr	0.3	0.4	0.6	–	0.6	0.8	–	0.7	0.7
55	1701	pentadecanal	0.2	–	–	–	0.4	–	–	–	1.5	0.3	0.6
56	1710	farnesol	0.1	–	–	–	0.2	0.6	tr	–	1.2	0.2	–
57	1711	heptadecane	1.1	–	–	1.9	–	–	1.7	2.7	–	–	–
58	1753	phytane	–	tr	0.2	–	0.3	–	0.3	0.7	–	0.1	0.3

Table 2. Continued

peak	RI[a]	compound	sample 1	2	3	4	5	6	7	8	9	10	11
59	1754	hexahydrofarnesyl acetone	8.5	5.6	7.1	7.6	15.3	10.1	11.4	10.0	8.3	13.3	9.2
60	1769	tetradecanoic acid	1.5	1.3	2.7	–	0.3	–	–	–	–	1.5	–
61	1779	ethyl myristate	–	–	–	–	–	–	–	0.8	–	–	–
62	1782	anthracene	0.3	–	–	–	–	0.3	–	–	–	–	–
63	1790	2-hexyl-1-decanol	0.7	tr	0.5	–	2.9	0.5	1.2	0.6	0.3	1.7	1.2
64	1800	hexadecanal	–	0.7	–	–	0.6	–	–	–	–	–	–
65	1808	(*Z*)-7-hexadecenal	–	0.3	–	0.9	–	–	–	–	–	–	–
66	1810	octadecane	1.2	2.0	–	–	1.8	–	1.0	4.3	2.0	0.5	0.7
67	1831	1,1-dimethyltetradecyl hydrosulfide	–	0.4	–	–	–	–	0.8	–	–	–	–
68	1899	isophytol	1.1	0.7	tr	0.7	0.9	0.8	–	0.3	1.1	2.5	0.2
69	1902	farnesyl acetone	3.8	2.5	1.9	2.1	8.9	3.9	2.7	5.3	4.8	3.5	3.2
70	1908	diisobutyl phthalate	1.5	0.6	2.2	2.8	1.6	1.9	2.8	0.8	1.9	0.8	0.6
71	1968	pentadecyl acrylate	9.4	6.6	12.4	9.6	6.3	8.5	11.7	7.3	2.2	8.8	12.3
72	1968	palmitic acid	–	–	–	–	–	–	–	–	–	–	9.1
73	1978	ethyl palmitate	1.0	2.2	–	–	–	0.5	1.5	8.5	1.9	–	–
74	1983	dodecyl 3-mercaptopropionate	1.0	tr	1.4	2.0	–	–	0.8	–	–	2.4	0.7
75	1986	ethyl 9-hexadecenoate	–	–	–	–	–	–	–	1.0	–	–	–
76	2009	eicosane	1.0	–	–	1.3	–	–	0.2	–	0.6	0.1	–
77	2036	chlorooctadecane	–	–	tr	0.4	–	–	0.5	0.9	–	–	–
78	2037	butyl phthalate	–	–	–	–	–	4.4	5.1	–	–	–	4.3
79	2044	*cis*-1-chloro-9-octadecene	–	–	–	0.9	–	–	–	1.3	0.9	–	–
80	2045	*trans*-phytol	11.9	9.8	6.2	13.5	5.1	21.4	5.8	6.9	24.1	14.6	7.3
81	2101	14-bromopenta-decanoic acid	–	2.0	–	–	–	–	0.4	–	0.2	–	–
82	2109	heneicosane	3.3	3. 9	2.2	5.6	4.0	1.4	4.2	4.7	4.8	3.1	3.4
83	2119	acetic acid, 3,7,11,15-tetramethylhexadecyl ester	0.2	0.1	0.1	–	1.2	0.1	1.1	–	0.2	1.5	–
84	2167	stearic acid	–	1.3	–	–	–	–	–	–	–	0.7	–
85	2183	*cis*, *cis*-linoleic acid	–	0.5	–	–	–	–	–	–	–	–	–
86	2245	4-fluorobenzoic acid tridec-2-ynyl ester	–	–	–	–	–	0.6	–	–	–	–	–
87	2295	hexadecylsulfonyl chloride	–	–	0.1	–	–	–	0.4	–	–	0.2	–
88	2316	methyl eicosa-5,8,11,14,17-pentaenoate	–	–	0.3	–	0.4	tr	–	–	0.2	–	–
89	2362	retinol acetate	–	–	–	0.4	–	–	–	–	–	–	–
90	2407	ethyl arachidonate	–	–	0.7	1.3	–	–	–	–	–	0.2	–
91	>2500	isooctyl phthalate	–	–	1.0	0.4	–	–	–	–	–	–	–
92		andrographolide	–	–	–	–	–	0.5	–	0.6	0.3	–	–
93		oxalic acid, 2-ethylhexyl octadecyl ester	–	–	–	0.1	–	–	–	0.2	–	–	0.3
94		tetracontane	–	0.3	0.3	1.2	–	–	–	–	–	0.2	–
95		L-(+)-ascorbic acid 2,6-dihexadecanoate	16.6	33.5	24.6	19.1	6.8	6.1	8.5	–	13.2	14.3	–
	total identified		98.4	95.5	97.8	97.1	97.8	99.2	98.5	97.3	96.9	97.4	98.7
	aliphatics												
	alkanes, alkenes		9.7	8.5	4.5	14.2	9.1	3.2	11.1	20.3	11.1	13.9	13.2
	alcohols		15.7	12.8	10.1	19.4	15.3	26.5	10.7	12.4	25.5	20.9	12.3
	aldehydes		2.1	1.7	0.9	2.3	3.9	3.1	3.8	2.1	2.9	0.7	2.2
	ketones		10.7	8.1	11.6	9.0	19.9	23.0	14.8	12.7	21.2	15.9	10.9
	fatty acids and aliphatic esters		35.6	51.0	51.8	35.7	17.8	25.8	39.6	20.7	20.1	35.2	33.5
	terpenoids												
	oxygenated monoterpenes		18.9	10.0	14.1	14.3	20.1	12.7	14.4	23.4	9.0	7.1	16.6
	oxygenated sesquiterpenes		5.7	3.4	4.8	2.2	11.7	4.9	4.1	5.7	7.1	3.7	10.0

[a] RI, retention index; tr (trace), relative content <0.1%; (–) not detected. Samples: 1, Wuhan; 2, Xiaogan; 3, Baoying; 4, Guangzhou; 5, Xiangtan; 6, Honghu; 7, Jiande; 8, Jianning; 9, Guangchang; 10, Weishan Lake; 11, Baiyangdian Lake.

agronomic factors, the level of theaflavins produced by these shoots during fermentation was highly correlated to the concentration of (−)-epigallocatechin in the shoots (*23*); the aril samples from six pomegranate (*Punica granatum* L.) cultivars obtained from various sites from the Mediterranean region of Turkey were evaluated for their antioxidant properties. The results showed that only selected cultivars had high amounts of phenolics and probably represent only a portion of the native germplasm (*24*).

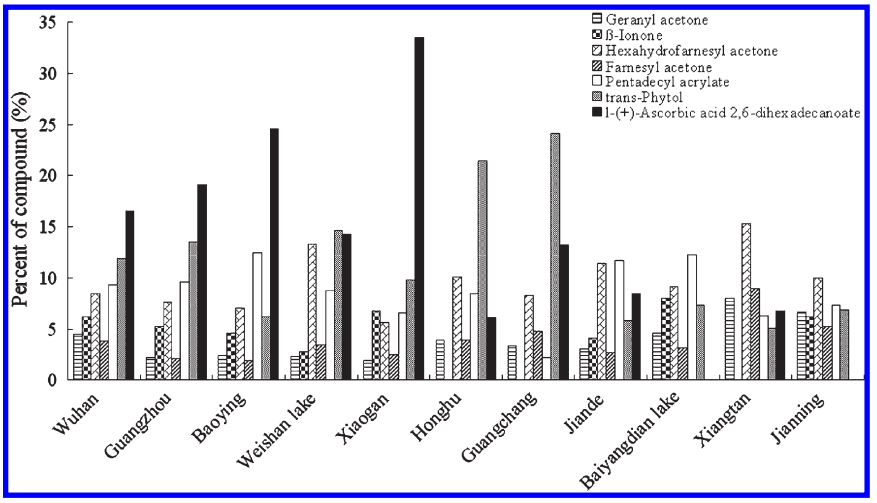

Figure 2. Comparison of major components of the essential oils of *Nelumbo nucifera* leaf from 11 regions in China.

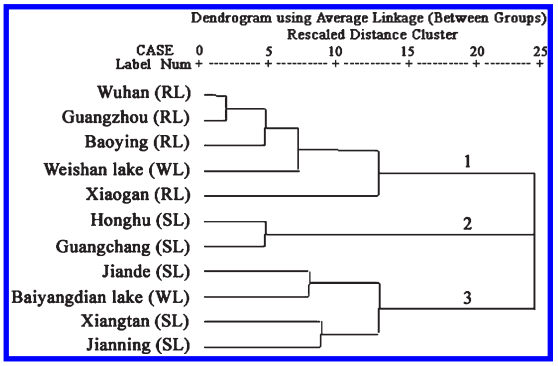

Figure 3. Dendogram of 11 populations of *Nelumbo nucifera* in China based on hierarchical cluster analysis: 1, group 1; 2, group 2; 3, group 3.

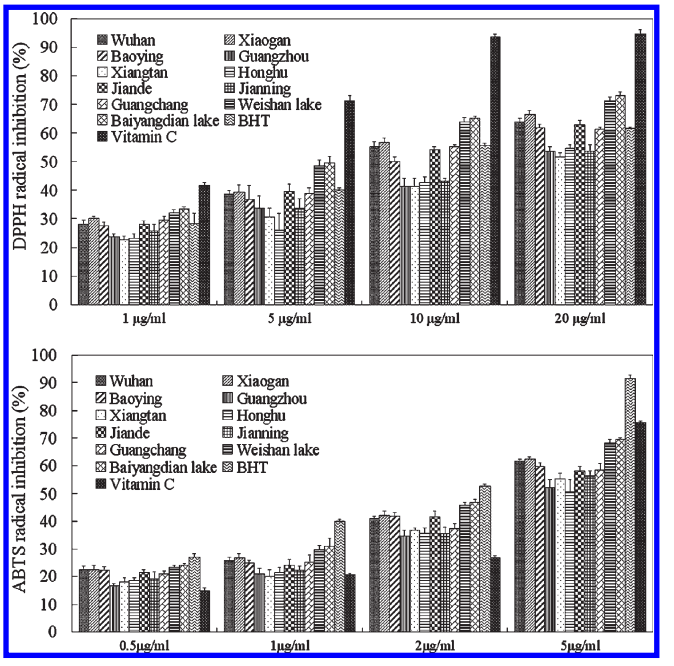

Figure 4. DPPH and ABTS radical scavenging activities of extract of *Nelumbo nucifera* leaf from 11 regions in China.

Phenolic content has also been reported to depend on growing conditions such as temperature and rainfall, the stage of maturation, and the region. The polyphenolic concentrations in

Table 3. IC_{50} Values of Extracts of *Nelumbo nucifera* Leaf Collected in Different Locations in China Using Two Free Radical Assays

	IC_{50} value[a] (μg/mL)	
sample	DPPH	ABTS
Wuhan	10.855 ± 0.579	3.710 ± 0.004
Xiaogan	9.971 ± 0.697	3.577 ± 0.007
Baoying	12.28 ± 1.022	3.770 ± 0.006
Guangzhou	16.749 ± 1.091	4.574 ± 0.010
Xiangtan	17.881 ± 0.954	4.508 ± 0.008
Honghu	16.608 ± 1.211	4.675 ± 0.012
Jiande	11.077 ± 0.662	3.861 ± 0.006
Jianning	16.456 ± 0.968	4.361 ± 0.010
Guangchang	11.254 ± 0.927	3.972 ± 0.009
Weishan Lake	6.977 ± 0.679	3.068 ± 0.005
Baiyangdian Lake	6.290 ± 0.461	2.913 ± 0.004
BHT	11.068 ± 0.552	1.786 ± 0.004
vitamin C	2.050 ± 0.594	3.299 ± 0.003

[a] IC_{50} value was determined to be the effective concentration at which DPPH and ABTS radicals were inhibited by 50%, respectively. Each value is presented as the mean ± SD of three replicate determinations.

10 different apple cultivars grown commercially in New Zealand, each sourced from three different geographic regions, were measured. There were significant differences in polyphenolic concentrations in fruit from different regions (*25*). The samples of emblica (*Phyllanthus emblica* L.) fruit from six regions in China were measured for their antioxidant activities, and the results indicated that these samples had various levels of phenolic contents (*26*).

To investigate the antioxidant activity of the lotus leaf extracts, we evaluated their ability to scavenge the two stable free radicals DPPH and ABTS, which have been widely used to test free radical scavenging activity (*5*). Results of this evaluation at various concentrations of extracts and the reference compounds BHT and vitamin C are shown in **Figure 4**, and the concentrations required to inhibit each radical by 50% (IC_{50}) are shown in **Table 3**.

Most of the lotus leaf extracts showed appreciable free radical scavenging activities in the DPPH assay. Notably, the WL samples from Baiyangdian Lake (IC_{50} = 6.290 μg/mL) and Weishan Lake (IC_{50} = 6.977 μg/mL) had the strongest activities, higher than that of BHT (IC_{50} = 11.068 μg/mL). The activities of extracts from Wuhan, Xiaogan, Baoying, Jiande, and Guangchang were also comparable with that of BHT, whereas the Guangzhou, Xiangtan, Honghu, and Jianning samples exhibited lower activity. Positive correlation was found between the DPPH

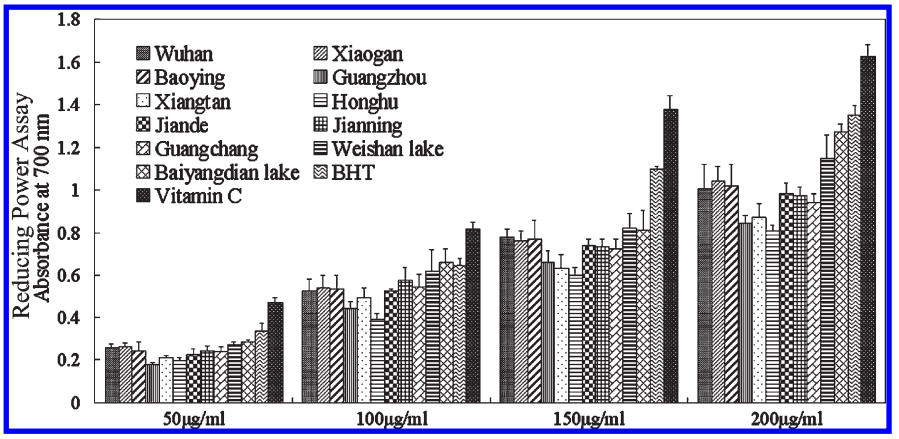

Figure 5. Reducing power activities of extract of *Nelumbo nucifera* leaf from 11 regions in China.

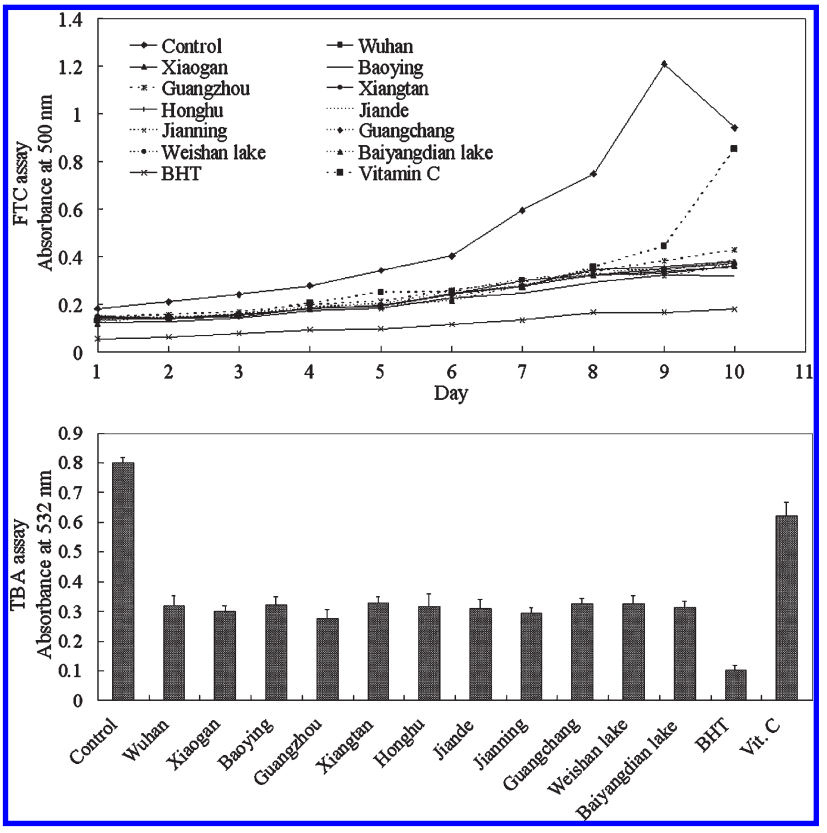

Figure 6. Capabilities of lipid peroxidation inhibition using FTC method and TBA method.

free radical scavenging activity, as measured by IC_{50}, and the total phenolic content shown in **Table 1** ($R^2 = 0.723$, $P < 0.01$).

Similar scavenging activity patterns were seen in the ABTS assay. The radical scavenging ability of the extracts from Baiyangdian Lake ($IC_{50} = 2.913$ μg/mL) and Weishan Lake ($IC_{50} = 3.086$ μg/mL) was stronger than that of the RL and SL extracts from other regions. Positive correlation was found between the ABTS free radical scavenging activity (IC_{50}) and total phenolic content ($R^2 = 0.739$, $P < 0.01$).

Reducing power has been used as an important measure of the antioxidant capability of medicinal herbs. Reductive ability was determined by monitoring the $Fe^{3+}-Fe^{2+}$ transformation in the presence of the extracts; increase in absorbance of the reaction mixture indicated reducing power. As shown in **Figure 5**, all of the extracts showed high, concentration-dependent reducing power, ranked in a similar order to DPPH and ABTS radical scavenging activity.

The FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. Peroxides formed during linoleic acid oxidation may be detected and quantified by their ability to oxidize Fe^{2+} to Fe^{3+}. Fe^{3+} ions form a thiocyanate complex with SCN^-, which has a maximum absorbance at 500 nm (*27*). Therefore, samples with high antioxidant activity tested by this method are revealed by low absorbance values. As shown in **Figure 6**, all of the extracts countered the linoleic acid oxidation with strong antioxidant activity, but there were no obvious differences between RL, SL, and WL samples ($P > 0.05$).

During storage of linoleic acid, peroxide is gradually decomposed to lower molecular weight malondialdehyde. The amount of malondialdehyde may be measured by the TBA assay, providing a means to evaluate the extent of lipid peroxidation. At low pH and high temperature (100 °C), malonaldehyde binds TBA to form a red complex that can be quantified by its absorbance at 532 nm. An

increase of the amount of red pigment formed correlates with the oxidative rancidity of the lipid. As shown in **Figure 6**, the TBA results were in agreement with those obtained by the FTC method; the antioxidant activity of the WL samples was similar to that of the RL and SL samples ($P > 0.05$). These lipid peroxidation results from both the FTC and TBA assays differed from those obtained in the free radical scavenging and reducing power assays in that no significant correlation was found between inhibitory ability on lipid oxidation and total phenolic content. The lack of correlation with phenolic content may be due to the presence of other phytochemicals in the extracts that also contribute to the lipid antioxidant activity. Similar observations have been reported for other plants, such as *P. emblica* L. (*26*), blackberry (*28*), and Chinese jujube (*29*). The flavonoids and phenolic compounds are the main antioxidants that occur in the lotus leaf, and many of them have been identified as hyperin, isoquercetin, astragalin, kaempferol, myricetin, catechin, etc. (*30*). However, details of the composition and structures of the phenolics in the lotus leaf extracts from different regions are still unknown, so further studies will be necessary to fully analyze these extracts.

In this work, we related the phenolic content of ethanolic extracts of *N. nucifera* leaves from 11 regions in China to their antioxidant activities. Variation in the chemical composition of essential oils from these extracts was also determined. Our results indicated that the region of origin and the growing conditions could significantly affect both the content of bioactive compounds and the bioactivity of the lotus leaf extracts. Furthermore, our results revealed chemical polymorphism in the essential oils of the *N. nucifera* leaf in China, suggesting that genetic and environmental factors should be taken into account to ensure consistent essential oil quality from the lotus leaf. The bioactivity of the essential oils remain undefined. Therefore, additional studies should be conducted to evaluate the bioactivity of the essential oils.

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