HPLC PROFILING OF PHENOLIC ACIDS AND FLAVONOIDS AND EVALUATION OF ANTI-LIPOXYGENASE AND ANTIOXIDANT ACTIVITIES OF AQUATIC VEGETABLE *LIMNOCHARIS FLAVA*

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Abstract: *Limnocharis flava* is an edible wetland plant, whose phenolic acid and flavonoid compositions as well as bioactivities were underexplored. This study analyzed the profiles of selected hydroxybenzoic acids, hydroxycinnamic acids and flavonoids in the aqueous extracts of *L. flava* leaf, rhizome and root by high performance liquid chromatography (HPLC). Anti-lipoxygenase and antioxidant (iron chelating, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and nitric oxide (NO) scavenging) activities of the extracts were also evaluated. Leaf extract had the highest phenolic contents, being most abundant in *p*-hydroxybenzoic acid (3861.2 nmol/g dry matter), ferulic acid (648.8 nmol/g dry matter), and rutin (4110.7 nmol/g dry matter). Leaf extract exhibited the strongest anti-lipoxygenase (EC_{50} 6.47 mg/mL), iron chelating (EC_{50} 6.65 mg/mL), DPPH scavenging (EC_{50} 15.82 mg/mL) and NO scavenging (EC_{50} 3.80 mg/mL) activities. Leaf extract also had the highest ferric reducing ability. This is the most extensive HPLC profiling of phenolic acids and flavonoids in *L. flava* to date. In conclusion, *L. flava* leaf is a source of health-promoting phenolics, anti-lipoxygenase agents and antioxidants.

Keywords: antioxidant, flavonoid, hydroxybenzoic acid, hydroxycinnamic acid, lipoxygenase inhibition, metal chelating

Worldwide, there is an increasing interest to search for natural antioxidants and other therapeutically relevant bioactive constituents from edible plants. A large number of plant food-derived bioactive compounds reported in the literature are members of the phenolic acid and flavonoid families (1, 2). Many phenolic acids and flavonoids exhibit chemopreventive or therapeutic properties (3, 4). For instance, protocatechuic acid, a hydroxybenzoic acid, exhibits growth inhibitory effects against numerous human cancer cell lines (5-7). Sinapic acid, a hydroxycinnamic acid, shows protective effect against cardiac hypertrophy and dyslipidemia in animal models (8). Rutin, a flavonoid, upregulates activities of antioxidative enzymes such as superoxide dismutase and catalase in cerebral ischemia injury in rats (9). Several studies also revealed that rutin is a potent anti-inflammatory phytochemical (10-12).

Lipoxygenase (LOX) pathway plays an important role in the inflammatory response in the human body (13). The main enzyme involved in the LOX pathway is 5-LOX. The increased activity of this enzyme has been correlated with certain diseases, including asthma (14) and inflammatory bowel diseases (15). Thus, 5-LOX is a potential therapeutic target for such diseases and there is strong interest among researchers to search for natural products with anti-LOX activity. On the other hand, there is also continuing interest among researchers to search for plant food-derived antioxidants, which are perceived to have fewer side effects than synthetic antioxidants commonly in use at present (16).

A number of aquatic plants are cultivated or harvested from the wild and consumed as vegetables (17). Nevertheless, literature substantiating the health-promoting and/or therapeutic potential of such edible aquatic plants is overall limited. Information obtained from investigations on the bioactive constituents and bioactivities of edible aquatic plants may help to promote their utilisation, hence boosting their economic values, as vegetables.

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Moreover, it may promote the application of edible aquatic plants in the development of nutraceuticals or functional food, in addition to their application as an alternative bioresource for the management of human diseases.

Limnocharis flava (L.) Buchenau (Alismataceae), commonly known as yellow velvetleaf, is an edible aquatic plant. *L. flava* inhabits shallow swamps, ditches and stagnant fresh water. The bud, flower, and leaves of the plant are consumed as salad or cooked vegetables in Vietnam, Indonesia, Bangladesh (18), Thailand (19) and Malaysia. At present, there is a gap of knowledge about the health-promoting phenolic constituents and bioactivities of *L. flava*. Thus, this study was undertaken to analyze the profiles of health-promoting phenolic acids and flavonoids in *L. flava*. In addition, the anti-LOX, iron chelating, radical scavenging, and ferric reducing antioxidant activities of this edible aquatic plant were also evaluated.

EXPERIMENTAL

Collection of plant samples and species identification

Healthy specimens of *L. flava* were collected from shallow streams in the town of Tronoh Mines, Perak State, Malaysia. The species of the plant was verified by Professor Hean-Chooi Ong, University of Malaya. Herbarium voucher was stored at the Faculty of Science, Universiti Tunku Abdul Rahman, for future reference.

Preparation of aqueous extracts

The plant samples were rinsed under running tap water to wash off sands and mud. The plant was separated into three parts, namely leaf, rhizome and root, and dried in an oven at 45°C for 48 h. The dried samples were then pulverized to powder by using a blender. Next, the plant powder was suspended in deionized water at a ratio of 1 : 20 (dry weight : volume) and incubated in a 95°C water bath for 2 h with continuous agitation (120 rpm). Subsequently, the suspension was vacuum-filtered using cheesecloth and Buchner funnel. The filtrates were centrifuged at 9000 rpm and 4°C for 10 min. The supernatant was aliquoted and stored at -20°C until further use. The concentration of the supernatant was taken as 50 mg dry matter (DM)/mL.

High performance liquid chromatography (HPLC) analysis

The HPLC system used in this analysis was comprised of Shimadzu LC-20D binary pumps, Shimadzu CTO-10AS column oven, and Shimadzu Prominence SPD-20A UV/Vis detector. Phenomenex-Gemini 5 µm (150 mm length × 4.6 mm internal diameter, 110 Å pore size, 5 µm particle size) was the C-18 reversed phase column used. The choice of solvent, solvent composition and elution program were adopted from (20, 21) with minor modifications. Gradient elution was executed in this analysis with a flow rate of 0.8 mL/min, at an oven temperature of 38°C and injection volume of 20 µL. The mobile phase were deionized water acidified with acetic acid (pH 2.8) as solvent A and acetonitrile as solvent B. Gradient elution was performed as follows: 0-5 min, 5-9% solvent B; 5-15 min, 9% solvent B; 15-22 min, 9-11% solvent B; 22-38 min, 11-18% solvent B; 38-43 min, 18-23% solvent B; 43-44 min 23-90% solvent B; 44-45 min, 90-80%, solvent B; 45-55 min, 80% solvent B; 55-60 min, and 80-5% solvent B. The column was flushed and equilibrated with 5% acetonitrile for 20 min at the completion of each gradient elution program. Phenolic compounds were identified by comparing their respective retention times with those of pure external standards. Hydroxybenzoic acid standards used were protocatechuic acid (PCCA), *p*-hydroxybenzoic acid (*p*-HBA), gallic acid (GA), and vanillic acid (VA). Hydroxycinnamic acid standards used were ferulic acid (FA), caffeic acid (CFA), *p*-coumaric acid (*p*-CA), sinapic acid (SNA), and chlorogenic acid (ChA). Flavonoid standards used were myricetin, rutin and quercetin. Detection wavelengths for hydroxybenzoic acids, hydroxycinnamic acids and flavonoids were 280, 320 and 370 nm, respectively. Chromatograms with positive detection were analyzed using the LabSolution software, compared with standards calibration curves.

Anti-lipoxygenase (LOX) assay

Anti-LOX activity was determined with the microplate-based method reported by (22). First, 20 µL of extract (0-50 mg/mL) was pipetted into a well containing 50 µL of 440 ng/mL LOX prepared in 50 mmol/L Tris-HCl (pH 7.4). The microplate was then incubated in darkness for 5 min at room temperature. Subsequently, 50 µL of 616 µmol/L linoleic acid was added to the mixture, followed by a further incubation of 20 min. Next, 100 µL of freshly prepared ferric oxidation of xylenol orange (FOX) reagent was added to the mixture. The mixture was incubated in the dark again for 30 min. The absorbance of the mixture was determined at 560 nm. FOX reagent was prepared by adding 15 µmol/L xylenol orange and 15 µmol/L $FeSO_4$ into a mixture of 15 mL of 300 mmol/L H_2SO_4 and 135

mL of methanol. Nordihydroguaiaretic acid was used as the positive control. The lipoxygenase inhibition activity was calculated by using the formula:

$$\text{Anti-LOX activity (\%)} = [(A_{control} - A_{sample})/ A_{control})] \times 100$$

where $A_{control}$ is the absorbance of the reaction mixture in which the extract was excluded; A_{sample} is the absorbance of the reaction mixture containing an extract. EC_{50} value is defined as the extract concentration required to achieve 50% inhibition of LOX activity.

Determination of iron chelating activity

The iron chelating assay described in (20) was modified into a microplate format. First, 80 µL of 0.1 mmol/L $FeSO_4$ was pipetted into a well followed by 80 µL of plant extract (0-50 mg/mL). The mixture was incubated for 5 min at room temperature. Next, 160 µL of 0.25 mmol/L ferrozine was added into the mixture, followed by an incubation for 10 min at room temperature. The absorbance of the reaction mixture was measured at 562 nm. Disodium ethylenediaminetetraacetic acid (disodium EDTA) was used as the positive control. Iron chelating activity was calculated by using the formula:

$$\text{Iron chelating activity (\%)} = [(A_{control} - A_{sample})/ A_{control})] \times 100$$

where $A_{control}$ is the absorbance of the reaction mixture in the absence of a plant extract; A_{sample} is the absorbance of the reaction mixture containing a plant extract. EC_{50} value is defined as the extract concentration required to achieve 50% iron chelating activity.

Antioxidant assays
Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The method described in (23) was modified into a microplate assay. The assay was started by pipetting 10 µL of extract (0-50 mg/mL) to a well, followed by 300 µL of freshly prepared 0.004% (weight/volume) methanolic DPPH solution. The mixture was then incubated in darkness for 30 min at room temperature. The absorbance of the mixture was determined at 517 nm. Ascorbic acid was the positive control used. DPPH scavenging activity was calculated by using the formula:

$$\text{DPPH scavenging activity (\%)} = [(A_{control} - A_{sample})/ A_{control})] \times 100$$

where $A_{control}$ is the absorbance of the reaction mixture from which plant extract was omitted; A_{sample} is the absorbance of reaction mixture containing a plant extract. EC_{50} value is defined as the extract concentration required to scavenge 50% of DPPH free radical in the reaction medium.

Determination of nitric oxide (NO) scavenging activity

NO scavenging activity was determined by using the method reported in (24). Firstly, 90 µL of extract (0-50 mg/mL) was pipetted into a well, followed by addition of 30 µL of 5 mmol/L sodium nitroprusside prepared in phosphate buffer saline (pH 7.4). The microplate was then incubated under light at room temperature for 150 min. Next, 90 µL of freshly prepared Griess reagent (1% sulfanilamide and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride in 5% phosphoric acid) was added into the well. The microplate was further incubated in the dark for 10 min after which the absorbance of the reaction mixture was measured at 560 nm. Ascorbic acid was used as the positive control. NO scavenging activity was calculated by using the formula:

$$\text{NO scavenging activity (\%)} = [(A_{control} - A_{sample})/ A_{control})] \times 100$$

where $A_{control}$ is the absorbance of the reaction mixture in which the plant extract was excluded; A_{sample} is the absorbance of reaction mixture containing a plant extract. EC_{50} value is defined as the extract concentration required to scavenge 50% of NO free radical in the reaction medium.

Determination of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was modified from (25) into a microplate assay. FRAP reagent was prepared freshly by mixing 300 mmol/L acetate buffer pH 3.6, 10 mmol/L 2,4,6-tripyridyl-s-triazine in 40 mmol/L HCl and 20 mmol/L $FeCl_3 \times 6H_2O$ at a ratio of 10 : 1 : 1. A standard calibration curve was constructed with $FeSO_4 \times 7H_2O$ (0.1 to 1.0 mmol/L). The assay was performed by pipetting 10 µL of extract into a well, followed by 300 µL of FRAP reagent. The microplate was then incubated for 5 min at room temperature. The absorbance of the reaction mixture was measured at 593 nm. FRAP values were expressed as µmol of Fe^{2+} equivalents per g of DM of plant sample. Butylated hydroxytoluene (BHT) was used as the positive control.

Data analysis

Experiments were performed in triplicates. Results were expressed as the mean ± standard errors. Statistical Analysis System (SAS) software version 9.2 was used for statistical analysis. Data collected were analyzed by using the one-way ANOVA test. Fisher's Least Significant Difference (LSD) test was used to separate means of significant differences at $\alpha = 0.05$. The determination of EC_{50} values for bioactivities was carried out by using lin-

ear regression analysis performed with Microsoft Office Excel 2007.

RESULTS

Hydroxybenzoic acid, hydroxycinnamic acid and flavonoid contents

Leaf and rhizome extracts contained all four types of hydroxybenzoic acids, namely PCCA, *p*-HBA, GA and VA (Table 1). The highest *p*-HBA content was detected in the leaf extract, which accounts for approximately 0.05% of plant DM by weight. PCCA contents were similar between leaf and rhizome extracts, which were about 1.6-fold higher compared with root extract. GA and VA were present in all extracts but both were the most abundant in the leaf extract.

Among the six hydroxycinnamic acids analyzed, SA was not detected in any of the extracts (data not shown). Leaf extract also had the highest CFA, SNA and ChA contents (Table 1). FA was only found in leaf extract, which accounts for 0.012% of plant DM by weight.

The abundance of myricetin, rutin and quercetin in the plant extracts is presented in Table 1. Myricetin was present in all three extracts. Leaf extract had the highest myricetin content, which was 8.8-fold and 3.2-fold higher compared with the rhizome and root extracts, respectively. Rutin was detected only in the leaf extract at about 0.25% of plant DM by weight. Leaf extract also had the highest quercetin content, which was 5.6-fold greater than that in the root extract.

Anti-LOX and antioxidant activities

Only leaf and root extracts exhibited anti-LOX activity within the range of extract concentration tested. Anti-LOX activities of the leaf and root extracts increased in an extract concentration-dependent manner at 0-10 mg/mL and 0-50 mg/mL, respectively (data not shown). The EC_{50} of leaf extract was 4.5-fold lower than that of root extract (Table 2). The EC_{50} values of both of the extracts were higher compared with nordihydroguaiaretic acid.

All three extracts had iron chelating activity, showing concentration-dependent increases at 0-10 mg/mL (data not shown). The EC_{50} value of the leaf extract was approximately 64% lower than those of rhizome and root extracts (Table 2). The EC_{50} values of rhizome and root extracts were similar. All the extracts had significantly higher EC_{50} values compared with disodium EDTA.

DPPH radical scavenging activity in all three extracts increased in an extract concentration-dependent manner at 0-50 mg/mL (data not shown). The EC_{50} values of the rhizome and root extracts were about 9-fold higher than that of the leaf extract (Table 2). The EC_{50} value of the leaf extract was 51-fold greater compared with ascorbic acid.

All three extracts of *L. flava* also showed NO radical scavenging activity, which was observed to be concentration-dependent at 0-10 mg/mL (data not shown). Leaf extract had the lowest EC_{50} value, which was 78% lower than those of rhizome and root extracts (Table 2). The EC_{50} value of leaf extract was 25-fold higher compared with ascorbic acid. The rhizome and root extracts had similar EC_{50} values.

Table 1. Hydroxybenzoic acid, hydroxycinnamic acid, and flavonoid contents of *L. flava* extracts.

Extracts*		Leaf	Rhizome	Root
Hydroxybenzoic acids (nmol/g DM)	PCCA	1298.4 ± 7.3 [a]	1327.7 ± 20.7 [a]	805.3 ± 25.2 [b]
	p-HBA	3861.2 ± 64.9 [a]	83.1 ± 2.0 [b]	N.D.
	GA	234.0 ± 7.8 [a]	117.7 ± 3.6 [b]	66.1 ± 3.3 [c]
	VA	206.0 ± 4.8 [a]	65.8 ± 1.4 [b]	71.1 ± 1.8 [b]
Hydroxycinnamic acids (nmol/g DM)	FA	648.8 ± 9.5 [a]	N.D.	N.D.
	CFA	332.0 ± 1.6 [a]	N.D.	155.0 ± 3.7 [b]
	p-CA	61.5 ± 0.7 [a]	N.D.	103.3 ± 2.0 [b]
	SNA	86.6 ± 1.9 [a]	4.3 ± 0.1 [b]	36.8 ± 0.2 [c]
	ChA	54.8 ± 4.2 [a]	15.8 ± 0.4 [b]	27.6 ± 1.1 [c]
Flavonoids (nmol/g DM)	Myricetin	631.2 ± 15.4 [a]	71.8 ± 1.1 [b]	196.6 ± 5.8 [c]
	Rutin	4110.7 ± 67.1 [a]	N.D.	N.D.
	Quercetin	25.4 ± 0.7 [a]	N.D.	4.5 ± 0.1 [b]

Data are presented as the mean ± standard errors (n = 3). Values in the same row that are followed by different superscript letters ([a-c]) are significantly different (p < 0.05), as determined by Fisher's LSD test. * Abbreviations - see text. N.D. = not detectable.

Table 2. EC$_{50}$ values for bioactivities of *L. flava* extracts.

Extracts	EC$_{50}$ values (mg/mL)			
	Anti-LOX activity	Iron chelating activity	DPPH scavenging activity	NO scavenging activity
Leaf	6.47 ± 0.05 [a]	6.65 ± 0.04 [a]	15.82 ± 0.21 [a]	3.80 ± 0.02 [a]
Rhizome	N.D.	24.88 ± 0.17 [b]	137.98 ± 1.03 [b]	16.89 ± 0.07 [b]
Root	29.14 ± 0.02 [b]	22.92 ± 0.99 [b]	148.59 ± 0.41 [c]	17.98 ± 0.17 [c]
Positive control	0.12 ± 0.00 [c] (Nordihydroguaiaretic acid)	0.02 ± 0.00 [c] (Disodium EDTA)	0.31 ± 0.01 [d] (Ascorbic acid)	0.15 ± 0.00 [d] (Ascorbic acid)

Data are presented as the mean ± standard errors (n = 3). In each column, values followed by different superscript letters ([a-d]) are significantly different (p < 0.05), as determined by Fisher's LSD test. N.D. = not detectable.

Among the three extracts, leaf extract had the highest FRAP value (79.9 µmol/g), followed by rhizome extract (12.3 µmol/g), and root extract (10.4 µmol/g). The FRAP value of the leaf extract was approximately half of that of BHT (173.3 µmol/g).

DISCUSSION

HPLC analysis of the leaf, rhizome and root extracts of *L. flava* revealed different phytochemical compositions. Leaf extract was the most abundant source of three hydroxybenzoic acids (*p*-HBA, GA, and VA), four hydroxycinnamic acids (FA, CFA, SNA, ChA) and three flavonoids (myricetin, rutin and quercetin). The results point to the leaves being the richest source of health-promoting phenolic acids and flavonoids in the *L. flava* plant. This highlights the potential health benefits of *L. flava* leaves when consumed as vegetable.

The flavonoid profile of *L. flava* plant has only been investigated in one previous study (26), whereas phenolic acid composition of the plant has not been reported in the literature. Hence, this study is the most comprehensive profiling of phenolic constituents of *L. flava* to date, encompassing not only flavonoids, but also hydroxybenzoic and hydroxycinnamic acids. Yang et al. (26) did not detect any myricetin and quercetin in *L. flava* leaf extract. In this study, these two flavonoids occurred in the leaf and root extracts. Such discrepancy may have arisen from the different extraction strategies used in their and this studies. In this study, we have extracted *L. flava* with hot water. In comparison with organic solvent extraction, hot water extraction should resemble more closely the way *L. flava* leaves are likely to be prepared for human consumption. Thus, the HPLC profiling of phenolic acids and flavonoids is likely to give a relatively realistic representation of the phytochemical contents available in *L. flava*

when it is consumed as cooked vegetable.

The contents of some phenolic constituents in the *L. flava* leaf extract were higher compared with some popularly consumed vegetables and food plants. For example, *p*-HBA content of *L. flava* leaf extract (3861.2 nmol/g DM, or 533.3 µg/g DM), by itself, was higher than the total contents of hydroxybenzoic acids in green lettuce (264.7 µg/g dry weight), swiss chard (68.9 µg/g dry weight), spinach (219.9 µg/g dry weight) and pea shoots (409.8 µg/g dry weight) (27). On the other hand, CFA content of *L. flava* leaf extract (332.0 nmol/g DM, or 59.8 µg/g DM) was higher than that of green lettuce (9.3 µg/g dry weight) (27). In this study, we report for the first time the detection of rutin and its high abundance in the leaf extract of *L. flava*. The rutin content of *L. flava* leaf extract (4110.7 nmol/g DM, or 2509.7 µg/g DM) surpassed that of raw buckwheat groats (230.1 µg/g dry weight) (28).

To the best of our knowledge, this is the first report of anti-LOX activity in *L. flava*. In this study, hot water extracts of *L. flava* exhibited anti-LOX activity. This suggests that *L. flava*, especially its leaves, is a potential source of water-soluble and thermal-stable LOX inhibitor. Several phenolic acids and flavonoids, such as protocatechuic acid (29), quercetin (30), caffeic acid and *p*-coumaric acid (31), have been reported to exhibit anti-LOX activity. Quercetin also acts synergistically with other active ingredients in spices to inhibit human 5-lipoxidase (30). Thus the higher anti-LOX activity of the *L .flava* leaf extract may be attributed to its higher levels of PCCA, CFA, *p*-CA and quercetin, when compared to rhizome and root extract. Suppression of LOX activity is associated with mitigation of inflammation-related diseases (22). Our finding therefore implies that *L. flava* may be exploited as a novel source of functional food ingredients for the prevention and/or management of

inflammation-related disorders. In addition, *L. flava* leaf extract may serve as a source of vegetable-derived LOX inhibitors which can be developed further as therapeutic agents. High level of NO produced during the course of inflammation can lead to tissue injuries, which are responsible for diseases such as rheumatoid arthritis, asthma, atherosclerosis, autoimmune disease, Alzheimer's disease and diabetes (32). Scavenging of NO is an option as treatment to attenuate the severity of such diseases (33, 34). Hence, *L. flava* leaf extract, with both its NO scavenging and anti-LOX activities may offer greater protective effects against inflammation-related diseases when compared with other natural products possessing only one of these bioactivities.

Leaf extract had the highest antioxidant activities compared with extracts of other parts of the *L. flava* plant. Besides NO scavenging activity, the iron chelating, DPPH scavenging and ferric reducing activities were all at the highest levels in the leaf extract. Such a trend was also observed in other vegetables and edible plants, such as the indigo plant (*Polygonum tinctorium Lour.*) (35), castor plant (*Ricinus communis* L.) (36), and *Acmella oleraceae* Murr. (37). In this study, the iron chelating activity of *L. flava* leaf extract is reported for the first time. Iron chelating activity of vegetables may have certain significance to human health. Iron is vital to the human body and its functions involve oxygen delivery to tissues, gene regulation, and electron transfer reaction (38). Iron deficiency may result in development of diseases such as anemia, glossitis and blue sclera (39). However, iron overload may also lead to iron accumulation and resulting toxicity in the body. Iron may catalyze the formation of reactive oxygen species (ROS), which subsequently can have detrimental effects on the cardiovascular and neurological systems, leading to atherosclerosis, Parkinson's and Alzheimer's diseases (40). Desferriferrioxamine-B is clinically used to keep the level of iron in the body in control, but it will compromise the renal function (41). The discovery of vegetable sources exhibiting iron chelating activity, such as the *L. flava* leaf extract, could contribute to future development of functional food and/or isolation of natural metal chelators to be used for the control of body iron status, potentially with minimal or no side effects.

DPPH radical scavenging activity has been reported for the ethanolic leaf extract (42), ethanolic extract of bud and flower (19), and 70% ethanol extract of the leaves of *L. flava* (43). Hence, our observation agrees with previous finding of the ability of *L. flava* to scavenge DPPH radicals. The FRAP assay is commonly used to evaluate the antioxidant potential of food samples (44). The FRAP value of *L. flava* leaf extract obtained in this study is comparable to those reported for 70% ethanol extract of *L. flava* leaves (43) and 70% ethanol extract of *L. flava* stem (45). In this study, DPPH and NO radical scavenging assays as well the FRAP assay were employed to demonstrate the antioxidant activity of *L. flava* extracts. Natural products may exert their antioxidant activity by more than one mechanism. Thus multiple antioxidant tests were performed in this study as was previously recommended (46). Correlation analysis was not carried out between phytochemical contents and the bioactivity parameters in this analysis due to the small number of extracts analyzed. Nevertheless, to some extent, the trends of relative abundance of VA, GA, SNA, ChA and myricetin in the extracts appear to correspond with the trends of their relative levels of anti-LOX, iron chelating, radical scavenging and ferric reducing activities. Previous reports of antioxidant properties of VA and ChA (47) as well as myricetin (48) lend further support to our proposal that the antioxidant activities detected in *L. flava* can be accounted for at least partially by their hydroxybenzoic acids, hydroxycinnamic acids and flavonoids. In any case, we cannot rule out the possibility that phytoconstituents not analyzed in this study could also have contributed to the detected bioactivities.

CONCLUSION

In conclusion, this study demonstrated that *L. flava* is vegetable that is rich in health-promoting phenolics, with anti-LOX, iron chelating and radical scavenging activities. HPLC analysis revealed the presence of PCCA, GA, VA, SNA, ChA and myricetin in all *L. flava* extracts tested. Overall, leaf extract of *L. flava* was the most abundant source of health-promoting phenolics and exhibited the strongest anti-LOX, iron chelating and radical scavenging activities when compared to rhizome and root extracts.

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