

# Chemical composition, angiotensin-converting enzyme-inhibitory activity and antioxidant activities of few-flower wild rice (*Zizania latifolia* Turcz.)

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## Abstract

**BACKGROUND:** The chemical compositions of the stem and leaf sheath of few-flower wild rice were analysed. In addition, their extracts were evaluated for diphenylpicrylhydrazyl (DPPH) free radical-scavenging activity, ferric-reducing antioxidant power and angiotensin-converting enzyme (ACE)-inhibitory activity, since these are important properties of sources of nutraceuticals or functional foods.

**RESULTS:** The stems contained more ascorbic acid (0.06 g kg<sup>-1</sup> fresh weight), protein (28.18 g kg<sup>-1</sup> dry weight (DW)), reducing sugars (308.54 g kg<sup>-1</sup> DW), water-soluble pectin (20.63 g kg<sup>-1</sup> DW), Na<sub>2</sub>CO<sub>3</sub>-soluble pectin (44.14 g kg<sup>-1</sup> DW), K (8 g kg<sup>-1</sup> dry matter (DM)), S (6 g kg<sup>-1</sup> DM) and P (5 g kg<sup>-1</sup> DM) but less starch, total dietary fibre, Si, Na and Ca than the leaf sheaths. The DPPH free radical-scavenging IC<sub>50</sub> values of the stem and leaf sheath extracts were 19.28 and 21.22 mg mL<sup>-1</sup> respectively. In addition, the ACE-inhibitory IC<sub>50</sub> value of the stem extracts was 38.54 mg mL<sup>-1</sup>.

**CONCLUSION:** Both the stem and leaf sheath extracts exhibited good antioxidant properties, while good ACE-inhibitory activity was detected only in the phosphate buffer solution extracts of the stem. Few-flower wild rice could be processed into formula feeds for fish, poultry, etc. or functional foods for persons with high blood pressure.

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**Keywords:** antihypertensive; antioxidant; chemical composition; few-flower wild rice

## INTRODUCTION

Few-flower wild rice (*Zizania latifolia* Turcz.) is a fascicular plant grown in fresh water and is mainly distributed in southern China, particularly south of the Yellow River where water, warmth and light are abundant.<sup>1</sup> The edible organ of few-flower wild rice is the swollen gall induced by the parasitic smut fungus (*Ustilago esculenta* P. Henn.), which is usually harvested in May–June and September–October. The edible stems of few-flower wild rice can be consumed fresh or in processed form as a powder or flour and are considered a good functional food resource.<sup>1</sup> As a result of a significant increase in the cultivation area of few-flower wild rice in China, a large quantity of leaf sheath ends up being discarded, and this has become a source of environmental pollution at the cultivation site. As a by-product of few-flower wild rice, leaf sheaths continue to be produced in high amounts. At a rough estimate, the output of dried leaf sheaths is 100 kg per acre of cultivated few-flower wild rice.<sup>2</sup> To date, few authors have investigated the commercial value and functional properties of these leaf sheaths. Cai<sup>3</sup> suggested using them in the cultivation of edible mushrooms. The leaf sheaths are also used as knitting materials after hydrolysis by proteases.<sup>2</sup> In addition, Min<sup>4</sup> and Jin<sup>2</sup> found that the leaf sheaths were rich in dietary fibre and developed a method for extracting this dietary fibre. Furthermore,

high amino acid contents were observed in the leaf sheaths by Wang and Feng.<sup>5</sup> In summary, the functional properties of the leaf sheaths of few-flower wild rice have not yet been completely examined or fully exploited.

Increasingly, the global population is facing challenges from chronic diseases, including hypertension, dyslipidaemia, type II diabetes mellitus, cardiovascular diseases and some cancers. Evidence has shown the health benefits of high levels of antioxidant compounds in fruits and vegetables in reducing the

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risk of hypertension, diabetes mellitus and cancer.<sup>6</sup> Hypertension has become a global threat to public health in recent years. Based on a pooled analysis of available national and regional data, Kearney *et al.*<sup>7</sup> reported the overall prevalence of hypertension in 2000 to be 26.4% of the world's population and also predicted that the burden of hypertension would increase by 60% to approximately 1.56 billion in the year 2025. Previous studies have shown a relationship between blood pressure and several nutrients, including proteins, fibre, potassium, magnesium and calcium.<sup>8</sup> Therefore, to obtain a broad understanding of their functional health benefits and nutritional value, it is necessary to conduct a comprehensive analysis of the nutrient compositions of the leaf sheaths and stems of few-flower wild rice.

Treatment with an angiotensin I-converting enzyme (ACE) inhibitor can offer a clinical advantage in hypertension via two different reactions that it catalyses: the conversion of inactive angiotensin I into a powerful vasoconstrictor and promoter of sodium retention, angiotensin II, and inactivation of the vasodilator bradykinin, which is conducive to lowering blood pressure.<sup>9</sup> Although many different protein hydrolysates from milk and plants exhibit ACE-inhibitory activity,<sup>10,11</sup> more R&D is necessary to identify safer, innovative and economical ACE inhibitors for the control of hypertension.

Few-flower wild rice stems are widely consumed as a health food throughout the world, particularly in Southeast Asia, North America and the European Union. However, information on their chemical composition is scant. Furthermore, there is little information regarding their ACE-inhibitory and antioxidant activities. Therefore the objective of this study was to determine the chemical compositions of few-flower wild rice leaf sheaths and stems and to characterise the antioxidant and ACE-inhibitory activities of their extracts.

## MATERIALS AND METHODS

### Materials

Few-flower wild rice was grown in the vineyard of Shanghai Qingpu Liantang Yelv Jiabai Co., Ltd (Shanghai, China), harvested on 23 September 2010, transported to the Laboratory of Cold Chain Research under refrigerated conditions within 2 h and stored at  $0 \pm 1^\circ\text{C}$  for 3 days. The leaf sheaths and edible stems were subsequently removed, cut into small pieces and dried in a freeze-drier (YRD1501A, Shanghai Yucheng Dryer Equipment Co., Ltd, Shanghai, China). After drying, the samples were pulverised, placed in plastic bags and maintained in a desiccator until further extraction and analysis.

The reagents stannic chloride, L-ascorbic acid, 2,4-dinitrophenylhydrazine, gallic acid, ethylene diamine tetraacetic acid (EDTA), sodium borohydride ( $\text{NaBH}_4$ ), methanol,  $\text{H}_2\text{SO}_4$ , HCl, vitamin C, 2,6-di-*tert*-butyl-4-methylphenol (butylated hydroxytoluene, BHT), potassium ferricyanide ( $\text{K}_3\text{Fe}(\text{CN})_6$ ) and ferric trichloride ( $\text{FeCl}_3$ ) were procured from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Dihydrate calcium chloride was purchased from Si-lian Chemicals Co., Ltd (Shanghai, China).  $\alpha$ -Tocopherol (vitamin E) was purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH\*) was purchased from A. Johnson Matthey Company (Malvern, PA, USA). Hippuryl-L-histidyl-L-leucine (HHL) and rabbit lung ACE were purchased from Sigma-Aldrich (St Louis, MO, USA). All reagents were of analytical grade.

### Chemical composition analysis

Moisture, crude fat, ash, ascorbic acid and dietary fibre contents of leaf sheaths and edible stems were determined by AOAC methods.<sup>12</sup> Total and reducing sugar concentrations were estimated by the method of Luo *et al.*<sup>13</sup> Starch content was estimated by the polarimetric method of Wang *et al.*<sup>14</sup> Briefly, 2 g of dried sample was dispersed in 70 mL of calcium chloride solution (pH 2.3), boiled for 20 min with magnetic stirring and then rapidly cooled to room temperature. A 5 mL aliquot of stannic chloride solution (2.5 g of stannic chloride hydrate plus 97.5 g of calcium chloride solution (pH 2.3)) was added to precipitate the proteins. After filtration the filtrate was collected for the determination of starch content with an automatic spectropolarimeter (WZZ-1, Shanghai Precision & Scientific Instrument Co., Ltd, Shanghai, China). Calcium chloride solution (pH 2.3) was used as control. Protein content was evaluated according to the dye-binding method of Bradford<sup>15</sup> using bovine serum albumin as standard.

Total phenolic content was measured spectrophotometrically using a modified version of the method described by Pirie and Mullins.<sup>16</sup> Briefly, samples (5 g) were extracted three times with 10 mL of 10 mL  $\text{L}^{-1}$  HCl in methanol. The extracts were pooled and centrifuged (Hermle Z-233 MK-2, Wehingen, Germany) at  $14\,000 \times g$  for 40 min at  $4^\circ\text{C}$ . The absorbance of the supernatant was measured at 280 nm using a spectrophotometer (UNIC UV-2100, UNIC (Shanghai) Equipment Co. Ltd, Shanghai, China). Total phenolic content ( $\text{g kg}^{-1}$  fresh weight (FW)) was calculated from a standard curve constructed using gallic acid.

Lignin content was determined by the method of Liu *et al.*<sup>17</sup> Approximately 5 g of frozen tissue was extracted four times with 500 mL  $\text{L}^{-1}$  HCl in methanol with stirring for 1 h and centrifuged at  $4000 \times g$  for 10 min. The final residue was dissolved in 12 mol  $\text{L}^{-1}$   $\text{H}_2\text{SO}_4$  (1:9 w/v) and hydrolysed for 3 h at  $20^\circ\text{C}$  with stirring. The solution was then diluted to 1 mol  $\text{L}^{-1}$   $\text{H}_2\text{SO}_4$  with deionised water. After heating for 2.5 h at  $100^\circ\text{C}$  with continuous shaking, the solution was cooled, vacuum filtered through an acid-treated 0.45  $\mu\text{m}$  Millipore HVLP filter and washed with  $100^\circ\text{C}$  deionised water. The filtrate, containing Klason lignin, was air dried at  $60^\circ\text{C}$  overnight and weighed. Lignin content was expressed as  $\text{g kg}^{-1}$  FW. Pectin (water-soluble pectin (WSP),  $\text{Na}_2\text{CO}_3$ -soluble pectin (SSP) and cyclohexane-diamine-tetraacetic acid (CDTA)-soluble pectin (CSP)) contents were determined according to the method of Deng *et al.*<sup>18</sup>

Mineral contents were measured according to the method of Mendes *et al.*<sup>19</sup> using an inductively coupled plasma optical emission spectrometer (OPTIMA-7000DV, Perkin-Elmer, Waltham, MA, USA). Microwave digestion was applied to digest the samples. Approximately 0.4 g of dried sample was weighed and digested with a reagent mixture of 5 mL of  $\text{HNO}_3$  and 1 mL of  $\text{H}_2\text{O}_2$ . After microwave digestion the contents were diluted to 20 mL with deionised water. Instrument operational conditions were as follows: power, 1300 W; coolant flow, 15  $\text{L min}^{-1}$ ; auxiliary flow, 0.8  $\text{L min}^{-1}$ ; nebuliser flow, 0.2  $\text{L min}^{-1}$ ; read time, 0.2–1 s; sample aspiration rate, 0.2  $\text{mL min}^{-1}$ .

### Extraction of samples for antioxidant and ACE-inhibitory analysis

Extraction was performed using the method described by Ünver *et al.*<sup>20</sup> with some modification. Briefly, 10 g of powder sample was extracted with 500 mL of solvent mixture (methanol : water : acetic acid = 90:9:1, v/v/v) at room temperature for 24 h (three replicates). After filtration the filtrate was evaporated in a rotary vacuum evaporator (W201 SENCO, Shanghai Shensheng

Technology Co., Ltd, Shanghai, China) at 35 °C. The concentrated extract was centrifuged at 7000 × *g* for 10 min and the supernatant was stored at 4 °C before use in the subsequent antioxidant experiments.

The lyophilised few-flower wild rice powder was dissolved in phosphate buffer solution (PBS, pH 7) and treated by sonication under the following conditions: power, 400 W; working time, 5 s; intermission time, 5 s (30 times). Different dilutions were made from the supernatant obtained after centrifugation at 4 °C for the examination of ACE-inhibitory activity.

#### DPPH radical-scavenging activity

Radical-scavenging activity was determined using a DPPH•-scavenging assay with slight modifications.<sup>21</sup> A 1 mL aliquot of each sample of few-flower wild rice extract (serial dilution concentrations of 350, 175, 87.5 and 43.75 mg mL<sup>-1</sup>) was mixed with 4 mL of 75 μmol L<sup>-1</sup> DPPH• dissolved in pure methanol. The mixture was vortexed for 30 s for homogenisation and left to react sufficiently for 60 min at room temperature in the dark. Finally, the absorbance of the resulting solution at 516 nm was measured using 75 μmol L<sup>-1</sup> methanolic DPPH• solution as reference. The free radical-scavenging activity (%) was expressed as  $100 \times (A_{\text{reference}} - A_{\text{sample}})/A_{\text{reference}}$ , where  $A_{\text{reference}}$  is the absorbance of the methanolic DPPH• reference and  $A_{\text{sample}}$  is the absorbance of the few-flower wild rice extract. The results were compared with the activities of BHT and vitamin E as positive antioxidant controls and water as negative control. Each sample was measured in triplicate. The IC<sub>50</sub> value was calculated as the concentration of sample (mg mL<sup>-1</sup>) required to scavenge 50% of DPPH free radicals.

#### Ferric-reducing antioxidant power

The reducing ability of each methanolic extract of few-flower wild rice was measured using the ferric-reducing antioxidant power (FRAP) assay.<sup>22</sup> A 0.5 mL aliquot of freshly prepared 1 g L<sup>-1</sup> K<sub>3</sub>Fe(CN)<sub>6</sub> was mixed with 0.5 mL of each sample of few-flower wild rice methanolic extract and incubated at 50 °C for 20 min. The mixture was then cooled as quickly as possible in an ice bath, followed by the addition of 0.5 mL of 100 g L<sup>-1</sup> trichloroacetic acid and centrifugation at 4200 × *g* for 10 min. Finally, 1.4 mL of the upper layer was mixed with 4 mL of distilled water and 0.8 mL of 1 g L<sup>-1</sup> FeCl<sub>3</sub> dissolved in 35 mL L<sup>-1</sup> HCl, and the absorbance of the mixture at 700 nm was measured as the reducing power. An increase in absorbance of the reaction mixture was interpreted as an increase in reducing activity of the extract. BHT and vitamin C at different concentrations were used as positive controls and water was used as negative control. Each sample was measured in triplicate.

#### ACE-inhibitory activity

ACE-inhibitory activity was determined using a modified version of the spectrophotometric method described by Mugaruma *et al.*<sup>23</sup> Rabbit lung ACE was dissolved in 100 mM sodium borate buffer (BBS) (pH 8.3) to a concentration of 4 mU μL<sup>-1</sup> according to the manufacturer's recommendations. The reaction system comprised 200 μL of the following solution: 95 μL of 100 mmol L<sup>-1</sup> Na-boric acid (pH 8.3) containing 300 mmol L<sup>-1</sup> NaCl, 2 mU ACE and 100 μL of 5 mmol L<sup>-1</sup> HHL. To determine the inhibition rate, different dilutions were prepared from extracts of the 35 mg mL<sup>-1</sup> sample of lyophilised few-flower wild rice powder dissolved in PBS. After the mixture had been incubated at 37 °C for 40 min, 200 μL of 1 mol L<sup>-1</sup> HCl was added to stop the reaction. Then 1.2 mL of

ethyl acetate was added and the sample was vortexed for 2 min to extract the hippuric acid (HA) liberated by ACE. After centrifugation at 7000 × *g* for 10 min, 1 mL of the ethyl acetate layer was collected and dried at 95 °C for 30 min. The liberated HA was dissolved in deionised water and its absorbance at 228 nm was measured using a spectrophotometer. A control reaction was performed with an equal volume of 100 mmol L<sup>-1</sup> Na-boric acid (pH 8.3) containing 300 mmol L<sup>-1</sup> NaCl as sample substitute, and a blank experiment was conducted by first mixing 200 μL of 1 mol L<sup>-1</sup> HCl with the ACE solution to inhibit the activity of ACE before adding HHL. Each sample was measured in triplicate. The concentration of ACE inhibitor required to inhibit the ACE activity by 50% was defined as the IC<sub>50</sub> value. The ACE-inhibitory activity (%) was calculated as  $100 \times (A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}} - A_{\text{blank}})$ .

#### Statistical analysis

Experimental results are presented as mean ± standard deviation (SD) of three parallel measurements. Data were subjected to analysis of variance ( $P < 0.05$ ). Mean differences were determined using Duncan's multiple range test. SAS 8.0 software (SAS Institute, Cary, NC, USA) was used for data analysis.

## RESULTS AND DISCUSSION

### Chemical composition

The chemical compositions of the edible stems and leaf sheaths of few-flower wild rice are shown in Table 1. The moisture content in the stems was 920.01 g kg<sup>-1</sup> FW, giving rise to a tender and crisp texture. Since a high moisture content can lead to increased microbial activity, postharvest few-flower wild rice is highly perishable. The protein content was approximately twofold higher in the stems (28.18 g kg<sup>-1</sup> dry weight (DW)) than in the leaf sheaths (11.71 g kg<sup>-1</sup> DW). The stems contained significantly ( $P < 0.05$ ) more fat, total soluble sugars and reducing sugars but less starch and ash than the leaf sheaths. The stems also contained more polyphenols than the leaf sheaths. Finally, the stems were six times richer in ascorbic acid (0.06 g kg<sup>-1</sup> FW) than the leaf sheaths (0.01 g kg<sup>-1</sup> FW). In a previous study the content of ascorbic acid in the stems was reported to be 0.06 g kg<sup>-1</sup> FW, which agrees with the present findings.<sup>24</sup>

The results presented in Table 2 show that the amount of total dietary fibre in few-flower wild rice was high, with insoluble dietary fibre accounting for about 83 and 73% of the total in the stems and leaf sheaths respectively. Furthermore, the amounts of total dietary fibre, soluble dietary fibre and insoluble dietary fibre in the leaf sheaths were approximately 2.5, 4.0 and 2.2 times higher than those in the stems respectively. The lignin content in the stems was 5.67 g kg<sup>-1</sup> FW, while it was 8.19 g kg<sup>-1</sup> FW in the leaf sheaths. The SSP content (44.14 g kg<sup>-1</sup> FW) in the stems was high, the WSP content (20.63 g kg<sup>-1</sup> FW) was moderate and the CSP content was low (14.44 g kg<sup>-1</sup> FW). In contrast, the CSP content (34.32 g kg<sup>-1</sup> FW) was highest in the leaf sheaths, followed by SSP and WSP. Furthermore, the stems contained more WSP and SSP than the leaf sheaths, whereas the CSP content was higher in the leaf sheaths. Several large epidemiological studies have indicated that there is an inverse relationship between dietary cereal fibre intake and risk of cardiovascular diseases.<sup>25</sup> Furthermore, results from clinical trials have shown that higher intake of fibre can reduce blood pressure and total and low-density lipoprotein cholesterol levels.<sup>8,26</sup> Without doubt, the high dietary fibre content of few-flower wild rice could make it ready source of nutraceuticals or functional foods.

**Table 1.** Chemical composition of edible stems and leaf sheaths of few-flower wild rice

Plant part	Moisture (g kg <sup>-1</sup> FW)	Protein (g kg <sup>-1</sup> DW)	Fat (g kg <sup>-1</sup> FW)	Starch (g kg <sup>-1</sup> DW)	Total soluble sugars (g kg <sup>-1</sup> DW)	Reducing sugars (g kg <sup>-1</sup> DW)	Ascorbic acid (g kg <sup>-1</sup> FW)	Polyphenols (g kg <sup>-1</sup> FW)	Ash (g kg <sup>-1</sup> DW)
Stem	920.01 ± 9.00a	28.18 ± 0.19a	22.58 ± 0.71a	14.29 ± 0.01b	357.19 ± 11.44a	308.54 ± 0.67a	0.06 ± 0.00a	10.50 ± 0.00a	5.31 ± 0.01a
Leaf sheath	870.00 ± 0.00b	11.71 ± 0.56b	18.10 ± 1.49b	25.86 ± 0.02a	220.53 ± 2.57b	182.73 ± 5.89b	0.01 ± 0.00b	6.30 ± 0.00b	10.10 ± 0.18a

 Values within a column followed by the same letter are not significantly different ( $P < 0.05$ ) according to Duncan's multiple range test.

Data on the mineral contents (dry matter (DM) basis) in the edible stems and leaf sheaths of few-flower wild rice are presented in Table 3. The main minerals in few-flower wild rice are K, S and P. The stems had significantly higher K, S and P levels but lower Na, Si and Ca levels than the leaf sheaths ( $P < 0.05$ ), whereas the Mg and Al contents did not show statistically significant differences between the stems and the leaf sheaths ( $P > 0.05$ ). To avoid hypertension from food sources, the Na/K ratio should be about 1:0.60.<sup>27</sup> This study showed that the Na/K ratios in the stem and leaf sheath of few-flower wild rice were about 1 : 13.33 and 1 : 2.50 respectively, which implies that they would not promote the development of hypertension if consumed by humans or other animals. In addition, the stem would maybe perform better than the leaf sheath, since cellular K deficiency contributes directly to hypertension-associated diseases.<sup>28</sup>

### DPPH radical-scavenging activity

The extracts of both stems and leaf sheaths of few-flower wild rice dissolved in mixture solution (methanol:water:acetic acid = 90:9:1, v/v/v) exhibited good DPPH free radical-scavenging activity, with values reaching 97.30 and 94.16% respectively at an extract concentration of 175 mg mL<sup>-1</sup> (Fig. 1), indicating that the antioxidant activity (AOA) was good. In addition, the results show that the activity was correlated with the concentration of the extracts. The extracts from the stems showed better AOAs than those from the leaf sheaths, but the difference was not significant ( $P > 0.05$ ). These AOAs can probably be attributed to the polyphenols and vitamins in the stem and leaf sheath tissues. Kaur *et al.*<sup>6</sup> reported that the AOA of ethyl acetate extracts of the leaves of *Chukrasia tabularis*, which is rich in phenols, is approximately 93.41%. Park and Jhon<sup>11</sup> showed that extracts of bamboo shoot (*Phyllostachys pubescens* and *Phyllostachys nigra*) also exhibit good AOAs; the DPPH radical-scavenging capacities of the extracts were highly correlated with their total phenol contents. Phenols in tomato showed moderate AOAs (57–71%) and have been demonstrated to synergistically promote the antioxidation capacities (81–100%) of tomato carotenoids.<sup>29</sup> The IC<sub>50</sub> values of the methanolic extracts of the stems and leaf sheaths of few-flower wild rice were 19.28 and 21.22 mg mL<sup>-1</sup> respectively. These results indicate that, in addition to the edible stem tissues, the leaf sheaths, which are usually discarded, could be a cheap source of important bioactive phytochemicals with antioxidant properties.

### Ferric-reducing antioxidant power

Our results indicate that the reducing power of the methanolic extracts of the stems and leaf sheaths increased steadily with increasing sample concentration (Fig. 2). In contrast to their DPPH free radical-scavenging activity, the FRAP of the stems was slightly lower than that of the leaf sheaths, but the difference was not significant ( $P > 0.05$ ). The FRAP of both leaf sheaths and stems was close to that of vitamin C (corresponding to a concentration series of 25, 50, 100 and 200 µg mL<sup>-1</sup>) but clearly lower than that of BHT (corresponding to a concentration series of 0.44, 0.88, 1.75 and 3.5 mg mL<sup>-1</sup>). The reducing power of antioxidant candidates may serve as a significant indicator of their antioxidant activity. Kaur *et al.*<sup>6</sup> reported that crude extracts from *C. tabularis* leaves exhibited good FRAP, which increased steadily with increasing sample concentration. Similar results were also obtained with extracts from *Cyperus rotundus* rhizomes.<sup>30</sup> The results of the FRAP assay in this study are consistent with those of the DPPH free radical-scavenging activity assay, which might be attributed to

**Table 2.** Dietary fibre, lignin and pectin contents in edible stems and leaf sheaths of few-flower wild rice

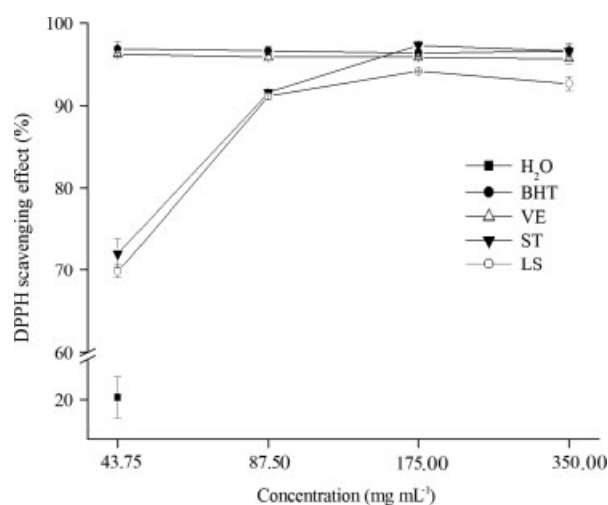
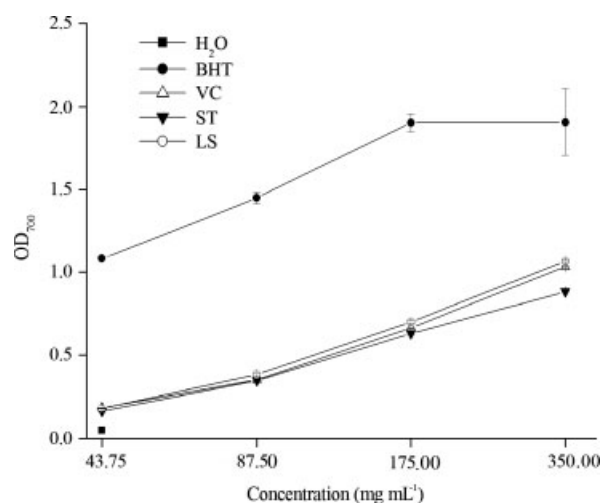
Plant part	Total dietary fibre (g kg <sup>-1</sup> FW)	Soluble dietary fibre (g kg <sup>-1</sup> FW)	Insoluble dietary fibre (g kg <sup>-1</sup> FW)	Lignin (g kg <sup>-1</sup> FW)	Water-soluble pectin (WSP) (g kg <sup>-1</sup> DW)	Na <sub>2</sub> CO <sub>3</sub> -soluble pectin (SSP) (g kg <sup>-1</sup> DW)	CDTA-soluble pectin (CSP) (g kg <sup>-1</sup> DW)
Stem	42.20 ± 0.06b	7.00 ± 0.05b	35.10 ± 0.03b	5.67 ± 0.001b	20.63 ± 0.33a	44.14 ± 0.08a	14.44 ± 0.17b
Leaf sheath	106.20 ± 0.03a	28.90 ± 0.05a	77.40 ± 0.04a	8.19 ± 0.003a	13.68 ± 0.04b	24.24 ± 0.001b	34.32 ± 0.01a

Values within a column followed by the same are not significantly different ( $P < 0.05$ ) according to Duncan's multiple range test.

**Table 3.** Mineral composition (g kg<sup>-1</sup> DM) of edible stems and leaf sheaths of few-flower wild rice

Plant part	K	S	P	Na	Si	Mg	Ca	Al
Stem	8.0 ± 0.8a	6.0 ± 0.3a	5.0 ± 0.5a	0.6 ± 0.2b	0.3 ± 0.0b	0.3 ± 0.1a	0.2 ± 0.1b	0.2 ± 0.2a
Leaf sheath	5.0 ± 0.8b	5.0 ± 0.1b	2.0 ± 0.1b	2.0 ± 0.7a	0.6 ± 0.4a	0.3 ± 0.2a	0.5 ± 0.1a	0.2 ± 0.0a

Values within a column followed by the same letter are not significantly different ( $P < 0.05$ ) according to Duncan's multiple range test.

**Figure 1.** Free radical-scavenging effect of methanolic extracts of stem (ST) and leaf sheath (LS) of few-flower wild rice at different concentrations. Values are mean ± SD. VE, vitamin E; BHT, 2,6-di-*tert*-butyl-4-methylphenol.**Figure 2.** Reducing power of methanolic extracts of stem (ST) and leaf sheath (LS) of few-flower wild rice at different concentrations. Values are mean ± SD. VC, vitamin C; BHT, 2,6-di-*tert*-butyl-4-methylphenol.

the presence of polyphenolic compounds in the stems and leaf sheaths (Table 1).

### ACE-inhibitory activity

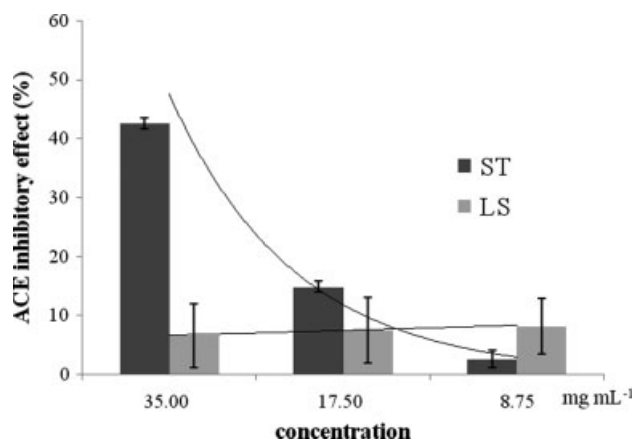
To investigate the antihypertensive activity of the stems and leaf sheaths of few-flower wild rice, their PBS extracts were subjected to an ACE inhibition assay, since PBS can be used to represent a physiological environment. Our results revealed that the inhibitory effect on ACE was correlated with the concentration of few-flower wild rice stem extracts at concentrations of 8.75, 17.5 and 35 mg mL<sup>-1</sup> (Fig. 3). The inhibitory activity increased proportionally to the extract concentration, and the ACE IC<sub>50</sub> value of the extracts of few-flower wild rice stems was approximately 38.54 mg mL<sup>-1</sup>. However, the same treatment of the leaf sheaths did not result in detectable ACE-inhibitory activity; we did not find a significant difference among the three dilutions ( $P > 0.05$ ). From the data in Table 3, we found that the content of Na in the leaf sheath was about 3.33 times higher than that in the stem, which may result in promoting ACE activity to balance the inhibitory effect of other materials in the leaf sheath.<sup>31</sup>

### CONCLUSION

Although most of the nutritional components of the stem tissues were significantly different from those of the leaf sheaths, both the stems and leaf sheaths exhibited good antioxidant properties. However, good ACE-inhibitory activity was detected only in the PBS extracts of the stem. These results showed that the edible stems and the usually discarded leaf sheaths of few-flower wild rice could be processed into formula feeds for fish, poultry, etc. or functional foods for persons with high blood pressure. More research in the future is required to determine the correlation between the chemical composition of the tissues and the antioxidant capacity (or ACE-inhibitory activity) and to identify the active mechanisms.

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**Figure 3.** ACE-inhibitory effect of PBS extracts of stem (ST) and leaf sheath (LS) of few-flower wild rice at different concentrations.

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