Nutritional profile and DNA damage protective activity of *Ottelia acuminata*, an endemic plant from southwestern China

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**ABSTRACT**

*Ottelia acuminata* (*O. acuminata*) is an edible and medicinal submerged plant endemic to southwestern China, yet there is little information available concerning its nutritional or bio-active composition. This study aimed to evaluate in detail the nutritional profile and phenolic composition of *O. acuminata* (inflorescence, peduncle, and leaf) and investigate a protective activity in phenolic extracts on DNA damage induced by -OH and ROO• radicals. *O. acuminata* was excellent sources of protein (17.7–24 g/100 g DW), TPC (11.43–19.00 mg GAE/g DW), ascorbic acid, and minerals (especially potassium, calcium, and iron). Interestingly, the protein contained a high amount of EAA with a ratio (EAA11/TAA) in the range of 47.79–50.93%, BCAA and MSG-like AA. The inflorescences and leaves of *O. acuminata* contained abundant UFA with a ratio of UFA/TFA more than 50%. Five phenolic compounds, with a predominance of luteolin, luteolin-7-O-glucoside and chlorogenic acid, were identified by HPLC. Phenolic extracts of *O. acuminata* displayed a significant protective effect on DNA damage, which effect was comparable to that of the positive control (trilox). It was concluded that *O. acuminata* is an excellent vegetable with a high nutritional value and a great potential as a natural antioxidant.

**INTRODUCTION**

*Ottelia acuminata* (*O. acuminata*), a freshwater perennial member of the Hydrocharitaceae family, is endemic to the Yunnan-Guizhou Plateau in southwestern China, and mostly distributed in Yunnan, Guizhou, and Guangxi provinces. *O. acuminata* has been listed as an endangered species by the Chinese authorities since 1987. This plant has a variety of applications, including the consumption of its inflorescence, peduncle, and leaf as a fresh vegetable since the Qing Dynasty, the use of whole plant in the treatment of unfavorable urination, constipation, fever cough, hemoptysis, asthma and edema in traditional Chinese medicine, serving as a landscape plant and as a biological indicator to monitor changes in water quality since it usually grows in clean freshwater and is highly sensitive to water pollution.

Currently, there has been a growing interest in the health potential of bioactive compounds from plant-based foods. Some naturally occurring phytoconstituents (e.g. phenolic compounds, dietary fiber, vitamins, essential amino acids, branched-chain amino acid, unsaturated fatty acids) in plant foods have health benefits beyond nutritive value and possibly reduce the risk of diseases. Researches concerning phytoconstituents and biological activity of *O. acuminata* are scarce. Our previous study revealed that *O. acuminata* was a rich source of phenolic compounds with great potential as natural antioxidants and enzyme inhibitors. In this study, we aim to investigate the nutritional profile,
phenolic composition and DNA damage protective activity of phenolic extracts from the inflorescences, peduncles and leaves of *O. acuminata* which has been widely cultivated in Dali Yunnan province for its economic importance as a tasty vegetable consumed year-round since 2003. This study is likely to highlight the potential of this little-known plant species, while also contributing to the conservation of the Chinese flora.

**Materials and methods**

**Reagent and chemicals**

Amino acid standard was obtained from Wako Pure Chemical Industries, Ltd (Japan). HPLC-grade standards (luteolin, luteolin-7-O-glucoside, caffeic acid, ferulic acid, and chlorogenic acid), fatty acid methyl ester, four tocopherol standards (α, β, γ, and δ), trolox, Gallic acid, 2,2’-Azobis (2-amidino-propane hydrochloride) (AAPH) and Folin-Ciocalteu phenol reagent were purchased from Sigma-Aldrich Ltd (USA). Vitamin B2 (≥98% purity) and L-ascorbic acid (≥99.9% purity) were purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD (Shanghai, China). Standards of Ca, Fe, Zn, K, Mg, Mn, Cu, Pb, and Cd were obtained from Beijing General Research Institute for Nonferrous Metals Analysis and Testing Institute (Beijing, China). Methanol, acetonitrile and all other reagents for HPLC mobile phase, purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD (Shanghai, China), were HPLC-grade. All other chemicals and reagents used in the experiments were of analytical grade.

**Samples**

*O. acuminata* var. *acuminata* was selected as the experimental material. The plants were transplanted on March 1 2017 in Denchuan Town Eryuan County Dali Prefecture (Yunnan, China; latitude, 100° 5’N; longitude, 25°59’ E; altitude, 1980 m). The samples were collected at the flowering stage on May 5 2017. The herbarium of this plant was deposited at the Herbarium of Medicinal Plants and Crude Drugs of the College of Pharmacy and Chemistry, Dali University and the voucher number was LYH17050501. In the laboratory, plant materials were washed, cleaned up, and separated into three parts including the inflorescences, peduncles and leaves. Then, the samples were freeze-dried, milled to a particle size of less than 1.0 mm and stored at 4°C until further analysis.

**Moisture and carbohydrate**

The moisture of fresh sample was performed according to the method of Chinese standard GB/T 5009.3 2016. Determination of crude fiber was carried out by the method of Chinese standard GB/T 5009.10 2003. Reducing sugar and total sugar were determined colorimetrically using the method of 3,5-dinitrosalicylic acid (DNS) described by previous report.

**Crude protein and amino acid composition**

The crude protein content was analyzed by AOAC approved method (N × 6.25). The analysis of amino acid composition was carried out according to the method described by, with slight modifications. Firstly, the protein of *O. acuminata* was extracted by isoelectric precipitation. Subsequently, Hydrolysis of protein powder was performed using 6 M HCl at 110°C for 24 h under nitrogen atmosphere. Then, the protein hydrolyzate was evaporated to dryness under vacuum at 40°C. Finally, the dried hydrolyzate was dissolved and diluted using sodium citrate buffer (pH 2.2), filtered and then injected into an L-8900 automatic amino acid analyzer (Hitachi, Tokyo, Japan) for analysis. Tryptophan was not analyzed for the samples. The content of amino acid was expressed as mg/g protein.
Crude fat and fatty acid composition

Crude fat was determined using soxhlet extraction method. Fatty acid composition was determined by Gas chromatography-Mass spectrometry (GC-MS) using the method of Chinese standard GB5009.168–2016. Briefly, extracted lipids were mixed with boron trifluoride-methanol reagent and fatty acids were then converted into the methyl ester derivatives. The fatty acid methyl esters analysis was carried out on Agilent GC-MS (7890B-5977). The peaks of fatty acid methyl ester were identified by comparing their retention times and spectra with standard compounds.

Vitamins

Vitamin B2 was determined using the standard method of Chinese standard GB5009.85–2016, which is briefly specified below. Sample was extracted in an autoclave at 121°C for 30 min, using a 0.1 mol/L hydrochloric acid solution, then adjusted to pH 4.7 using 1 mol/L sodium hydroxide solution, and finally hydrolyzed by adding the mixture of papain and amylase in a water bath at 37°C for overnight. The hydrolyzate was filtered through a Millipore membrane of 0.45 µm and then injected into HPLC for analysis. Agilent 1260 HPLC chromatograph system (Agilent, California, USA) was equipped with a reversed-phase C18 column (Agilent, ZORBAX SB-C18, 5 µm, 250 × 4.6 mm) and a fluorescence detector. The mobile phase consisted of solvent A (0.05 mol/L sodium acetate) and solvent B (100% methanol). Detection was carried out with an excitation wavelength of 462 nm and an emission wavelength of 522 nm.

Ascorbic acid content was measured by HPLC system (Agilent 1260, California, USA) using the standard method of Chinese standard GB5009.86–2016. Briefly, sample was extracted using 3% meta-phosphoric acid in 8% acetic acid and then filtered through a Millipore membrane (0.45 µm) before injection. The HPLC system consisted of a column of Grom Sil 120 ODS-5 ST (5 µm, 150 × 4 mm), a mobile phase of 100 mM ammonium acetate, a flow rate of 0.4 mL/min, and a diode array detector. The detection wavelength was set at 254 nm. Ascorbic acid was identified by comparing retention time and spectra with standard.

The quantification of tocopherols were implemented by HPLC system (Agilent 1260, USA) using the standard method of Chinese standard GB5009.82–2016. Briefly, after saponification, extraction, purification, and concentration, the sample was separated by reverse phase liquid chromatography column, detected by a fluorescence detector, and then quantified by external standard method. The HPLC system consisted of a column of Athena C30 (250 × 4.6 mm, 5 µm, CNW, Germany), a mobile phase of water (A) and methanol (B), a flow rate of 0.8 mL/min, and a fluorescence detector. Detection was carried out with an excitation wavelength of 294 nm and an emission wavelength of 328 nm. The tocopherol isomers content were quantified by contrasting with four external standards on the basis of retention time.

Minerals

For main minerals analysis, 1.0 g of sample was firstly ashed in muffle furnace at 500 ± 50°C for 4.5 h and then dissolved in 10 mL 10% nitric acid (v/v) and then diluted with deionized water. Finally, the contents of Fe, Mn, Mg, Ca, Cu, Zn, K, Pb, and Cd were analyzed using a flame atomic absorption spectrophotometer (Shimadu, Tokyo, Japan).

Total phenolic content (TPC), phenolic composition and DNA damage protection activity

The phenolics was extracted with methanol-water (80:20, v/v) solution by an ultrasonic instrument according to a method described previously. The phenolics extracted were then purified using X-5 macro-porous resin provided by the Bonchem Co., Ltd. (Cangzhou, Hebei, China). The Folin-Ciocalteu method was adopted to measure TPC which was expressed as mg Gallic acid equivalent (GAE) per gram dry weight (DW).
The analysis of phenolic composition was performed using HPLC (Agilent 1260, California, USA) supported by a binary pump, an auto-sampler and a DAD detector. Separation was carried out using a reversed-phase C18 column (Agilent, ZORBAX SB-C18, 5 μm, 4.6 mm × 250 mm). The detection wavelengths were set at 280, 320, and 354 nm. The mobile phases were 1% (v/v) acetic acid (A) and acetonitrile (B). The gradient conditions were set as follows: 98–83% A in 0–11 min; 83–76% A in 11–15 min; 76–76% A in 15–17 min; 76–74% A in 17–19 min; 74–74% A in 19–22 min; 74–72% A in 22–28 min; 72–82% A in 28–33 min; 82–90% A in 33–35 min; and 90–98% A in 35–38 min. The column temperature was kept at 37°C, and the injection volume was 10 μL. The flow rate was set at 0.8 mL/min throughout the gradient run. The content of individual phenolic compounds was quantified by contrasting the corresponding standards on the basis of retention time. Standard curves (R² ≥ 0.999 in all cases) were obtained by injection of ferulic acid, chlorogenic acid, caffeic acid, luteolin, and luteolin-7-O-glucoside standard solutions in methanol/water (1:1) with six different concentrations ranging from 10 to 100 μg/mL. The peak area was used as the analytical signal for compound quantification.

DNA damage protective activity of phenolic extracts was assessed by the methods of hydroxyl (•OH) radical-induced DNA damage as depicted by Jeong et al. [18] and peroxyl (ROO•) radical-induced DNA damage as reported by Spanou et al. [19] •OH radical was generated from the mixture reaction of FeSO₄ and H₂O₂. ROO• radical was produced from thermal decomposition of AAPH.

Statistical analysis

All the experiments were carried out in triplicate and data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey’s multiple-range test were applied to determine significant differences (p < 0.05) between the means by SPSS (version 17.0).

Results and discussion

Moisture and carbohydrate

Moisture and carbohydrate contents of O. acuminata are shown in Table 1. From the table moisture content of O. acuminata was in the range of 91.9–95.5 g/100 g fresh weight with the highest in the peduncles and lowest in the leaves (p < 0.05). The content of crude fiber was in the range of 10.2–13 g/100 g dry weight (DW). The peduncles showed the highest content of crude fiber, followed by the inflorescences and leaves (p < 0.05). The peduncles have supporting function that require tough lignin and cellulose, which lead to their higher crude fiber content than that of inflorescences and leaves. The relatively high content of crude fiber of O. acuminata could be related to its traditional medicinal use for the treatment of constipation. [5,6]

The contents of reducing sugar and total sugar were in the range of 3.66–7.37 g/100 g DW and 9.9–19.9 g/100 g DW, respectively, which varied significantly with different morphological parts. Generally, the inflorescences presented the higher sugar contents than the peduncles and leaves, which could be attributed to the different sunlight exposure time since the inflorescences always float on the water surface and therefore get sufficient photosynthesis, while the peduncles and leaves usually remain below the water surface. In addition, this could also be related to the inflorescences’ reproductive activities, such as blooming and fruiting which require more energy in the production phase.

Protein content and amino acid composition

Protein content are presented in Table 1. The protein content of O. acuminata varied significantly with different morphological parts between 17.7 g/100 g DW in the peduncles and 24 g/100 g DW in the leaves (p < 0.05) which was in agreement with previous report published by other authors. [20] The
protein content of *O. acuminata* was comparable to that of the common beans like purple *Phaseolus coccineus* (17.2%), *Dolicho Lablab L.* (19%), *Vigna radiata* (*Linn.*) Wilczek (21.6%), peeled *Vicia faba L.* (24.6%) and higher than that of some grains like oats (13.5%), wheat (14%), corn (9.3%), rice (6.7%).[21]

Therefore, *O. acuminata* is a good source of protein and has a potential use in the formulation of low-cost protein balanced diets as an alternative to current vegetable protein sources, such as legumes and cereals.

**Fig. 1** exhibits the chromatograms of amino acid from *O. acuminata* and standard. Seventeen kinds of amino acid except tryptophan were identified and quantified in *O. acuminata*, which contents are presented in **Table 2**. The results showed total amino acid (TAA) content was in the range of 214–501 mg/g protein. Significant differences were observed in the concentrations of TAA and individual amino acids (IAA) between different morphological parts (*p* < 0.05). Generally, the leaves exhibited the highest TAA and IAA contents, followed by the inflorescences and peduncles, which was in reasonable agreement with the measured protein contents in respective morphological parts. Glutamic acid was the most abundant amino acid in *O. acuminata*, with a level in the range of 30.50–76.71 mg/g protein. The second most abundant amino acid was aspartic acid (38.14–59.66 mg/g protein), while the least abundant is cysteine that was not detected above the limit of detection.

**Table 1. Nutritional profile and phenolic composition of *O. acuminata***.

| Compounds                | Content          |
|--------------------------|------------------|
|                          | Inflorescences   | Peduncles   | Leaves   |
| Moisture (g/100 g FW)    | 94.0 ± 0.3 b     | 95.5 ± 0.7 b | 91.9 ± 0.7 a |
| Protein (g/100 g DW)     | 22 ± 1 ab        | 17.7 ± 0.7 a | 24 ± 1 b   |
| Crude fat (g/100 g DW)   | 9.5 ± 0.3 a      | 9.0 ± 0.4 a  | 10.33 ± 0.06 b |
| Carbohydrate (g/100 g DW)|                 |             |           |
| Crude fiber              | 12.9 ± 1.0 b     | 13 ± 1 b     | 10.2 ± 0.4 a |
| Reducing sugar           | 7.37 ± 0.05 c    | 5.1 ± 0.5 b  | 3.66 ± 0.07 a |
| Total sugar              | 19.9 ± 0.3 c     | 9.9 ± 0.6 a  | 11.4 ± 0.2 b |
| Vitamin (mg/100 g DW)    |                 |             |           |
| Vitamin B<sub>12</sub>   | 0.15 ± 0.01 b    | 0.18 ± 0.02 b | 0.11 ± 0.01 a |
| Ascorbic acid            | 40.6 ± 0.9 a     | 120 ± 2 c    | 81 ± 1 b   |
| α-tocopherol             | 2.28 ± 0.08 c    | 0.72 ± 0.04 b | 0.56 ± 0.04 a |
| β-tocopherol             | nd               | nd           | nd         |
| γ-tocopherol             | 1.6 ± 0.1 b      | 0.77 ± 0.01 a | 0.61 ± 0.06 a |
| δ-tocopherol             | 0.34 ± 0.03 a    | 0.38 ± 0.01 a | 0.35 ± 0.03 a |
| Total tocopherol         | 4.2 ± 0.6 c      | 1.88 ± 0.05 b | 1.52 ± 0.06 a |
| Minerals (mg/100 g DW)   |                 |             |           |
| Fe                       | 58 ± 3 a         | 73 ± 4 b     | 167 ± 3 c  |
| Mn                       | 3.74 ± 0.05 a    | 7.2 ± 0.3 a  | 55 ± 3 b   |
| Mg                       | 5.44 ± 0.01 a    | 5.79 ± 0.02 a | 5.44 ± 0.01 a |
| Ca                       | 95 ± 3 a         | 84 ± 9 a     | 349 ± 21 b |
| Cu                       | 3.1 ± 0.2 b      | 1.9 ± 0.2 a  | 1.64 ± 0.05 a |
| Zn                       | 5.09 ± 0.05 c    | 3.81 ± 0.07 a | 4.31 ± 0.07 b |
| K                        | 1904 ± 83 a      | 3597 ± 131c  | 3113 ± 140 b |
| Pb                       | nd               | nd           | nd         |
| Cd                       | <0.005           | <0.005       | <0.005     |
| TPC (mg GAE/g DW)        | 19.00 ± 0.05 b   | 12.18 ± 0.03 a | 11.43 ± 0.04 a |
| Phenolics (mg/g extract) |                 |             |           |
| Chlorogenic acid         | 66 ± 1 c         | 16.2 ± 0.3 b | 8.19 ± 0.09 a |
| Caffeic acid             | 1.85 ± 0.08 b    | 0.34 ± 0.03 a | 1.50 ± 0.01 b |
| Frueric acid             | 1.39 ± 0.01 a    | 2.27 ± 0.01 b | 3 ± 0 b    |
| Luteolin-7-O-glucoside   | 110 ± 3 c        | 15.4 ± 0.4 a | 24 ± 1 b   |
| Luteolin                 | 167 ± 4 b        | 0.03 ± 0.04 a | nd         |
| TIPC(mg/g extract)       | 346 ± 5 b        | 34.3 ± 0.9 a | 37.4 ± 0.9 a |

Data are expressed as mean values of three independent replicates ± SD. Values in the same row with different lowercase letters are statistically different at *p* < 0.05. FW, fresh weight; DW, dry weight; TPC, total phenolic content; GAE, gallic acid equivalent; nd, not detected.
The nutritional quality of a protein is determined by the content, proportion and availability of essential amino acids (EAA). The percentage ratios of EAA8 to TAA and EAA11 to TAA were in the range 39.59–41.57% and 47.79–50.93%, respectively, which were above 36% considered adequate for an ideal protein. The relatively high content of EAA in *O. acuminata* is favorable for its use as a substitution for meat-and-bone food/feed. The most concentrated EAA in *O. acuminata* was leucine with a value between 17.3 mg/g protein and 42 mg/g protein, followed by phenylalanine (20–39.31 mg/g protein) and lysine (10.49–27.3 mg/g protein). The relatively high concentration of lysine is of particular interest, because this essential amino acid is the first limiting amino acid in main cereal proteins.
Amino acids were classified into several classes, which were monosodium glutamate-like (MSG-like), sweet, and bitter based on their taste properties as described by Komata.[24] MSG-like amino acids, including aspartic acid and glutamic acid, were in the range of 69–136 mg/g protein in *O. acuminata* and accounted for approximately 30% of the total amino acid. The high level of MSG-like amino acids could explain the palatable taste of *O. acuminata* and make it to be a potential raw material for the food spice industry.

The content of branched-chain amino acid (BCAA), including valine, leucine, and isoleucine, was 37–91 mg/g protein and amounted to about 45% of EAA. The richness of BCAA content endows *O. acuminata* with high medicinal and nutritional value since BCAA plays very important roles in human body such as participation in muscle protein synthesis, insulin secretion, brain amino acid uptake, and a large number of regulatory functions.[23] Moreover, BCAA has been used in the management of hepatic encephalopathy,[25] the prevention of muscle fatigue and the recovery of fatigue.[26,27]

**Crude fat and fatty acid composition**

Crude fat content of *O. acuminata* is presented in Table 1. Crude fat content was in the range of 9–10.33 g/100 g DW with the highest content in the leaves, which is significantly higher than that of the common vegetables like Chinese cabbage (0.8 g/100 g DW), spinach (0.6 g/100 g DW) and broccoli (0.6 g/100 g DW).[21]
Fatty acid composition of *O. acuminata* is shown in Table 3. Fourteen different fatty acids were detected in three plant parts and the amount of total fatty acid (TFA) ranged from 1976 to 3674 µg/g DW. Palmitic acid (51.76–60.90% of SFA) was the dominant saturated fatty acid, followed by behenic acid (13.03–18.09%) and lignoceric acid (12.42–15.72%); while the main unsaturated fatty acid were palmitoleic acid (39.13–46.21% of UFA), linolenic acid (10.58–24.92%), and oleic acid (2.40–18.16%). Other fatty acids were presented in a small amount. Significant difference was observed in the contents of different types of fatty acid among three morphological parts (*p* < 0.05). In general, the leaves displayed the highest content, followed by the inflorescences and peduncles, which was in agreement with the measured crude fat content in respective morphological part. The ratio of unsaturated fatty acids (UFA)/TFA was higher than the ratio of saturated fatty acids (SFA)/TFA in the inflorescences and leaves. Fatty acid composition and especially the ratio of UFA/TFA are of major importance for nutritional value of vegetables and their contribution in a balanced diet. Our result suggested the inflorescences and leaves had a more balanced fatty acid composition than the peduncles. The inflorescences and leaves possessing higher unsaturated fatty acid contents are also nutritionally important since UFA has been reported to play an important role in preventing the development of cardiovascular disease and slowing aging, among others.\(^{[28]}\)

Lipid content and fatty acid synthesis of plant were influenced by environmental factors including light, temperature, water stress, atmospheric composition, and so on.\(^{[29]}\) Lipid could help store energy and enhance the plant’s tolerance toward cold environment.\(^{[30,31]}\) The relative high contents of crude fat and fatty acid found in the leaves could probably be related to the lower temperature and less sunlight the leaves suffered than the peduncles and inflorescences since the leaves always grow below the surface of the water.

### Vitamins

Table 1 lists the contents of vitamin B\(_2\), ascorbic acid, and tocopherols from *O. acuminata*. The content of vitamin B\(_2\) was in the range of 0.11–0.18 mg/100 g. The peduncles and inflorescences exhibited higher content of vitamin B\(_2\) than that of the leaves (*p* < 0.05).
The content of ascorbic acid varied significantly with the different morphological parts within the range of 40.6 to 120 mg/100 g. The peduncles presented the highest content of ascorbic acid, which is more abundant than that of broccoli (82 mg/100 g), followed by the leaves and inflorescences (p < 0.05). This divergence was probably caused by a combination of varying temperature and sunlight intensity conditions three plant parts had gotten. Ascorbic acid is not only an essential vitamin for normal growth and development, but also one of the most important water-soluble antioxidants that could provide antioxidant protection against a number of chronic diseases for humans. The high concentration of ascorbic acid endows *O. acuminata* with the high nutritional and functional values.

The total concentration of tocopherols in *O. acuminata* varied in the range of 1.52 to 4.2 mg/100 g. The highest content of total tocopherols was found in the inflorescences. Three kinds of tocopherols (α-, γ-, and δ-homologues) were detected with α and γ-homologues predominant, while β-tocopherol was not detected in the samples. The content of tocopherols and the relative proportion of their homologues are species-related. Our study is in line with the other authors’ findings that α- and γ-tocopherols are the predominant forms of tocopherol homologues, while β-tocopherol is only a minor component (even not occurring at all) in many plants. Tocopherol, consisting of four homologs (α-, β-, γ-, δ-), is a main form of vitamin E and has many health benefits for human, including neuroprotective, cardioprotective, and anti-inflammatory activities, among others. The richness of tocopherols in *O. acuminata* makes it to be a vegetable with a high health-care value.

Minerals

The main minerals including Fe, Mn, Mg, Ca, Cu, Zn, K, Pb, and Cd were analyzed for different morphological parts of *O. acuminata* and the results are shown in Table 1. Potassium was found to be the most abundant mineral in *O. acuminata* with a level ranging from 1984 to 3597 mg/100 g, followed by calcium (84–349 mg/100 g), iron (58–167 mg/100 g), manganese (3.74–55 mg/100 g), magnesium (5.44–5.79 mg/100 g) and zinc (3.81–5.09 mg/100 g), while copper content was the lowest with a level ranging from 1.64 to 3.1 mg/100 g among the measured minerals. The heavy metal lead was not detected in this study, while the cadmium level in three plant parts was below 0.005 mg/100 g.

Our study indicated that *O. acuminata* is a good source of potassium, calcium, iron, and manganese, especially for iron, which level is 4–12 times, 2–6 times and 9–26 times higher than that of Chinese cabbage (13.8 mg/100 g), spinach (25.9 mg/100 g), and broccoli (6.4 mg/100 g), respectively, when compared to three common vegetables.

On the other hand, content of the same mineral varied greatly in different morphological parts of *O. acuminata*. Generally, the contents of iron, manganese, and calcium in the leaves were significantly higher than that in the peduncles and inflorescences (p < 0.05). The contents of zinc and copper in the inflorescences were significantly higher than that in the peduncles and leaves (p < 0.05) while potassium content in the peduncles was significantly higher than that in the inflorescences and leaves (p < 0.05). These divergences could be attributed to the differences in absorption and metabolism of minerals in different morphological parts.

TPC, phenolic composition and DNA damage protective activity

Table 1 displays TPC and phenolic composition from three plant parts of *O. acuminata*. The inflorescences presented the highest TPC of 19.00 mg GAE/g DW, followed by the peduncles (12.18 mg GAE/g DW) and leaves (11.43 mg GAE/g DW). Five phenolic compounds, including three hydroxycinnamic acids (chlorogenic acid, caffeic acid, and ferulic acid) and two flavones (luteolin, luteolin-7-O-glucoside), were qualified using HPLC from phenolic extracts of *O. acuminata*. Quantitative results showed that luteolin was the most abundant phenolic compound in the inflorescences with a level of 167 mg/g extract, followed by luteolin-7-O-glucoside (110 mg/g extract) and chlorogenic acid (66 mg/g extract) (p < 0.05), which was in line with our previous results measured by HPLC-MS. Meanwhile, the total content of five individual phenolics (TIPC) measured in the inflorescences (346 mg/g extract) was significantly higher than those in the leaves (37.4 mg/g extract).
extract) and peduncles (34.3 mg/g extract) (p < .05), which followed the same trend as determined by Folin-Ciocalteu method. Luteolin is a natural flavonoid, extensively presents in many fruits, vegetables, and medicinal herbs, such as apple skins, broccoli, carrots, onion leaves and Chrysanthemum flowers, and was reported to have multiple biological effects such as anti-oxidant, anti-inflammatory, anti-allergy, and anticancer. The use of O. acuminata in traditional Chinese medicine was probably related to the richness of luteolin and luteolin-7-O-glucoside in the inflorescences.

Protective effect of phenolic extracts on the oxidative DNA damage induced by •OH and ROO• radicals was evaluated by in vitro DNA strand break assay using pBR322 plasmid DNA damage assay. Figs 2 and 3 show protective activity of phenolic extracts from different plant parts of O. acuminata on pBR322 plasmid DNA damage induced by •OH and ROO• radicals, respectively. Conversion of the supercoiled conformation of DNA to the open circular or further linear conformations has been taken as an index of DNA damage.

From the Figs 2 and 3, it was found that DNA was mostly of the super-coiled forms possessing nearly 100% in the absence of •OH radical and ROO• radical (lane 1, control), while only a few super-coiled forms (less than 5%) was found and open circular and linear forms were predominant in the presence of Fe²⁺ and H₂O₂ or in the presence of AAPH (lane 2). During the addition of phenolic extracts or trolox, the supercoiled forms of DNA increased (lanes 3–8), as exhibits in Fig 2II (A) the percentage of supercoiled form amounted to the highest with a value at 95.91%.

Figure 2. Protective activity of phenolic extracts on DNA damage induced by •OH radical: (I) DNA electrophoresis; (II) Semi-quantitative analysis of DNA electrophoresis. A: inflorescences; B: peduncles; C: leaves; D: trolox (the positive control); Lane 1: pBR322plasmidDNA; Lane 2: FeSO₄+ H₂O₂+ pBR322 plasmid DNA; Lanes 3–8: FeSO₄+ H₂O₂+ pBR322 plasmid DNA in the presence of 25, 50, 100, 200, 400, 800 µg/mL phenolic extracts or trolox, respectively. In figure II control corresponds to lane 1 treated with nothing; the histograms in the same row with different lowercase letters are statistically different at p < 0.05.
which was comparable to that of the control (in the absence of radicals) and trolox (the positive control) when the concentration of phenolic extract from the inflorescences was 800 μg/mL. Fig 3II shows a significant dose-dependent increase in the supercoiled forms of DNA when the concentration of phenolic extracts from three plant parts was in the range of 25–200 μg/mL ($p < .05$). On the other hand, protective effect of phenolic extracts on the DNA damage significantly varied with different parts of the plant ($p < .05$), which could be attributed to the differences in the composition and content of phenolic compounds between three plant parts of *O. acuminata*. The significant protective activity against DNA damage is probably related to the predominant content of luteolin, luteolin-7-O-glycoside and chlorogenic acid that were widely reported to possess a strong antioxidant activity.[42,43,45] Therefore, phenolic compounds from *O. acuminata* are natural radical scavengers and could be used in preventing oxidative stress and damage caused by reactive oxygen species.
Conclusion

This is the first detailed report on the nutritional profiles and DNA damage protection of phenolic extracts present in the inflorescences, peduncles, and leaves of *O. acuminata*. This plant was highly rich in various nutrients, such as phenolics (luteolin, luteolin-7-O-glycoside, and chlorogenic acid), protein, dietary fiber, ascorbic acid, and minerals, especially for the protein which content is comparable to those found in some common beans. What’s more, this plant was an excellent source of protein, which contained high levels of EAA, BCAA, and MSG-like amino acids. On the other hand, phenolic extract of *O. acuminata* exhibited a significant protective effect on DNA damage induced by •OH and ROO• radicals. Therefore, this vegetable should be widely promoted for consumption due to its richness in nutrients and bio-active compounds. Moreover, efforts should be devoted to the development of novel natural health products from *O. acuminata*.

Abbreviations

AAPH, 2,2'-Azobis (2-amidino-propane hydrochloride); •OH, hydroxyl; ROO•, peroxyl; MEs, methanol extracts; TPC, total phenolic content; GAE, gallic acid equivalent; EAA, essential amino acids; BCAA, branched-chain amino acids; MSG-like AA, monosodium glutamate-like amino acids; TFA, total fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids.

Disclosure statement

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