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# Antioxidant capacity and α-glucosidase inhibitory activity of leaf extracts from ten ramie cultivars

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ABSTRACT

Ramie (*Boehmeria nivea* L. Gaud) is widely cultivated as a raw material for producing natural textiles due to its fine and strong fibers. Ramie leaves are by-products and rich in bioactive compounds that contribute to the medicinal properties of its extracts. This study was conducted to identify the phenolic compounds and evaluate the antioxidant capacity and α-glucosidase inhibitory activities of ramie leaves extracts, obtained from ten cultivars widely growing in China. The phenolic compounds were identified using advanced analytical techniques such as ultra-high performance liquid chromatography coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOF-MS) and quantified by UPLC-DAD analysis. The antioxidant activity and α-glucosidase inhibitory properties were evaluated using rapid in vitro assays. The content of total phenolic compounds ranged from 0.52 to 2.41 mg/g DW, and the content of total flavonoids varied from 0.40 to 2.50 mg/g DW. UPLC-ESI-QTOF-MS analysis enabled to tentatively identify 15 compounds in ten cultivars, mainly chlorogenic acid, rutin, *p*-coumaroylmalic acid, caffeoylmalic acid, feruloylquinic acid, hyperoside and isoquercetin. The highest contents of chlorogenic acid, rutin and *p*-coumaroylmalic acid were found in cultivar Luzhuqing (LZQ) with 439.49 μg/g DW, 339.97 μg/g DW and 345.93 μg/g DW, respectively. All cultivars performed differently on the antioxidant capacity and α-glucosidase inhibitory ability. Principle component analysis revealed that Qianjiangxianma (QJX) and Luzhuqing (LZQ) were the top two cultivars. This study indicates that ramie leaves can be considered as potentially new source of antioxidants and antidiabetic agents, and it gives insights into cultivar selection in the ramie planting and production.

## 1. Introduction

Ramie (*Boehmeria nivea* L. Gaud) is a perennial herbaceous plant of Urticaceae family native to China, Japan and Malay Peninsula, where it has been cultivated as a fiber crop for many centuries (Hester and Yuen, 1989). In China, ramie is the second largest fiber crop with a production of 114,080 ton of fibers in 2010, participating up to 90% of the world production (Liu et al., 2013). However, being cultivated especially for its fibrous stem, ramie leaves are crop residues with great potential for reutilization and product development. Furthermore, the leaves of this plant has been used as sources of animal feeds (Kipriotis et al., 2015), herbal medicines, tea and foods (Lee et al., 2015). It has been showed that ramie leaves are rich in bioactive compounds with antioxidant (Chen et al., 2014), antibacterial (Lee et al., 2014), anti-inflammatory (Sung et al., 2013) and anti-obesity activities (Lee et al., 2016). The bioactivity was mainly attributed to phenolic compounds, such as phenolic acids and flavonoids, which exist ubiquitously in plants as secondary metabolites (Akter et al., 2018). Hence, phenolic profile has become a desirable and essential quality characteristic of ramie and a predictor for selecting ramie cultivars. Yet, few studies have evaluated chemical fractions of the leaf extracts for industrial applications among different cultivars.

Type 2 diabetes epidemic is rising rapidly worldwide and originates from the imbalance of hormones. Inhibition of α-glucosidase in mammals, which could reduce the hydrolyzation of starch into glucose, decrease glucose uptake and normalize postprandial blood glucose concentration. Phenolic bioactives of herbs and medicinal plants with higher antioxidant activity can be targeted for chronic disease management including type 2 diabetes (Saleem et al., 2017). However, there is still no report on the α-glucosidase inhibitory activity of leaf extracts of various ramie cultivars.

This study aimed to investigate the phenolic profile and the antioxidant capacity as well as α-glucosidase inhibitory activity of leaf extracts from ten ramie cultivars which are widely planted in China. Thus phenolic compounds were identified by an ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry

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(UPLC-ESI-QTOF-MS) system and quantified by an UPLC-DAD method. Antioxidant activity and α-glucosidase inhibitory properties were evaluated using rapid in vitro assays. Results indicated that ramie leaves could be a potential source for nutraceuticals.

## 2. Materials and methods

### 2.1. Plant materials and leaf extracts

Ten ramie (*Boehmeria nivea* L. Gaud) cultivars, named as Qianjiangxianma (QJX), Luzhuqing (LZQ), Lubandou (LBD), Douchanghema (DCH), Linshuizhuma (LSZ), Dazhuhuangbaima (DZB), Xingwenxiaoma (XWX), Libodadouma (LBM), Dazhuhongma (DZH), Zhongsizhu No.1 (ZSZ) were planted in March 2017 at the research fields of Huazhong Agricultural University, Wuhan, China. Leaves of each cultivar were harvested at the plant height of 70 cm in September 2017. The harvested leaves were dried in a hot-air oven at 45 °C to constant weight. After ground to a fine powder using an electric mill, samples were stored in sealed polyethylene bags in the dark at room temperature until analyses.

An adaptation of the extraction method used by (Pascoal et al., 2015) were applied. For each cultivar, 1.0 g of dried leaf powder was mixed with 15 mL of 70% ethanol and maintained for 20 min in the ultrasonic bath at 60 °C. Then, samples were centrifuged for 10 min at 1800 × *g* using a centrifuge (TDL-5-A, Anke, Shanghai, China), the supernatant was removed, and the extraction was repeated two more times. The supernatants were collected and filled with 70% ethanol to a final volume of 50 mL. After concentration by rotary evaporation under 40 °C, the supernatants were collected and volume to 10 mL with distilled water and then discolored by hexane solvent. Finally, the extracts were filtered through 0.45 μm syringe filters and stored at −20 °C until analysis. Three replicated extracts were prepared for each cultivar and all analysis for each extracts was conducted in triplicate.

### 2.2. Total phenolics and flavonoids

The total phenolic content (TPC) and total flavonoid content (TFC) of extracts were determined according to the procedures described by (Kaur et al., 2014). The TPC was evaluated by Folin–Ciocalteu's method and expressed as mg of gallic acid equivalents per gram of dry leaves (mg GAE/g) calculated from the calibration curve plotted with gallic acid (0–250 μg/mL). The TFC was evaluated by $NaNO_2$-$AlCl_3$-NaOH method and expressed as mg rutin equivalents per gram of dry leaves (mg RTE/g) according to a calibration curve plotted with rutin as standard (0–300 μg/mL).

### 2.3. UPLC-DAD and UPLC-ESI-QTOF-MS analysis of phenolic compounds

UPLC-DAD analysis were performed on a Waters Acquity UPLC system (Waters Corp., Milford, MA, USA) equipped with a pump, an auto sampler, a column compartment and a diode array detector (DAD). Chromatographic separation was carried out on an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm, Waters Corp., Milford, MA, USA) at a column temperature of 25 °C, with a flow rate of 0.3 mL/min. Gradient elution of 0.1% formic acid solution (solvent A) and acetonitrile (solvent B) was employed: 0–15 min, 5–25% B; 15–19 min, 25–50% B; 19–21 min, 50–5% B; 21–23 min, 5% B. The injection volume was 3 μL, and the detection wavelength was set at 280 nm.

UPLC-ESI-QTOF-MS analysis was carried out on an Acquity UPLC system connected to a Xevo G2Q-TOF mass spectrometer via ESI interface (Waters Corp., Milford, MA, USA). The mobile phase was same to UPLC-DAD analysis, as well as the flow of rate and the injection volume was 1 μL. The tandem mass experiment was performed in negative ESI ionization modes with the data acquisition ranging of *m/z* 50-1200. The capillary voltage was optimized to 2.6 kV, and the cone voltage was 40 V. Source and desolvation temperatures were set at 120 and 350 °C, respectively. The desolvation gas flow was set to 600 L/h, and the cone gas flow was set at 50 L/h. Finally, the instrument was controlled by the Waters Masslynx 4.1 software.

Phenolic compounds were identified by comparing retention time and UV absorption spectra with available external standards and confirmed by LC–MS. Compounds without standard reference materials were tentatively identified by UV spectrum, MS data and by matching with published data. Quantification was performed with standard curves of external standards generated by plotting UPLC peak areas at 280 nm against the concentrations. For compounds without standards, quantification was based on calibration curves of similar compounds of the same phenolic subgroup. For instance, chlorogenic acid isomer was expressed as chlorogenic acid equivalent; caffeoylmalic acid and sinapic acid hexoside were quantified with caffeic acid and sinapic acid, respectively; feruloylquinic acid and feruloylmalic acid were expressed as ferulic acid equivalents; *p*-coumaroylhexose, *p*-coumaroylquinic acid, *p*-coumaroylmalic acid and *p*-coumaroylmalic acid isomer were expressed as *p*-coumaric acid equivalents; isoquercetin was quantified with the calibration curve of hyperoside.

### 2.4. Antioxidant activity in vitro

#### 2.4.1. DPPH assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the leaf extracts was determined spectrophotometrically on a UV–vis plate reader according to the method described by Bing et al. (2015) with slightly modifications. Briefly, aliquots of 280 μL of 65 μM DPPH in methanol were mixed with 20 μL of ascorbic acid standard solutions (0–300 μM) or leaf extracts in a 96-well plate. The mixtures were reacted in the dark at room temperature for 30 min. The absorbance of DPPH radicals was read at 540 nm. All samples were tested in triplicate. The scavenging of DPPH radical was calculated as equivalent units of ascorbic acid per gram dry weight leaves (μmol AAE/g DW).

#### 2.4.2. FRAP assay

The ferric reducing antioxidant power (FRAP) assay followed a previously reported procedure (Bing et al., 2015). Briefly, 10 μL of ascorbic acid standard (25–800 μM) or leaf extracts were mixed with 300 μL of ferric-TPTZ (2,4,6-tripyridyl-s-triazine) reagent (prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ at a ratio of 10:1:1 (v/v/v)) and allowed to react at room temperature for 30 min. The absorbance was read at 593 nm using microplate reader. The antioxidant activity was expressed as micromole ascorbic acid equivalents per gram dry weight leaves (μmol AAE/g DW).

#### 2.4.3. ABTS assay

The free radical scavenging activity was also determined by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method using a commercial kit (Beyotime Biotechnology, Shanghai, China). In brief, $ABTS^{\cdot+}$ was prepared by mixing equal quantities of 7 mM ABTS solution with 2.4 mM potassium persulfate. Mixture kept for 12 h at room temperature in dark for reaching a stable oxidative state. Solution was diluted by mixing 1 mL ABTS solution with 60 mL methanol to obtain an absorbance of 0.70 ± 0.05 units at 734 nm. Leaf extracts (10 μL) or Trolox (0.15–1.5 mM) was added to the 200 μL of ABTS solution and absorbance was taken after 7 min of incubation at 734 nm using the microplate reader. The ABTS anti-radical activity of samples was calculated as micromole trolox equivalent per gram dry weight leaves (μmol TE/g DW).

### 2.5. α-Glucosidase inhibition assay

The α-glucosidase enzyme inhibitory activity was determined by an assay modified from Saleem et al. (2017). α-Glucosidase was assayed by using 50 μL of five times diluted sample extracts and adding 100 μL of α-

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