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Metabolic profiling of antioxidants constituents in *Artemisia selengensis* leaves

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3,5-Dicaffeoylquinic acid (PubChem CID: 6474310)
3-Caffeoylquinic acid (PubChem CID: 1794427)
Protocatechualdehyde (PubChem CID: 8768)
Ferulic acid (PubChem CID: 445858)
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ABSTRACT

This study aimed to evaluate the antioxidant potential of *Artemisia selengensis* Turcz (AST) leaves, a byproduct when processing AST stalk, and identify the antioxidant constituents by using HPLC-QTOF-MS^2. The total phenolics content (TPC), total flavonoids content (TFC) and antioxidant abilities of fractions resulted from the successively partition of chloroform, ethyl acetate and *n*-butanol were compared. Ethyl acetate fraction (EAF) exhibited the highest TFC (65.44 mg QuE/g fraction), *n*-butanol fraction (*n*BuF) showed the highest TPC (384.78 mg GAE/g fraction) and the best DPPH$^{\cdot}$ scavenging ability, ABTS$^{+\cdot}$ scavenging ability and reducing power. Totally, 57 compounds were identified or tentatively identified in *n*BuF and EAF, 40 of them were reported in AST for the first time. The major constituents in EAF were flavonoids, and the major constituents in *n*BuF were phenolic acids and organic acids. Thus, AST leaves might be a potential low-cost resource of natural antioxidants.

1. Introduction

Antioxidants are substances that directly scavenge reactive oxygen species (ROS) or indirectly act to up-regulate antioxidant defense or inhibit the ROS production (Halliwell, 1990). In recent years, natural antioxidants, mainly from vegetables and fruits have received a rising attention based on the positive reports of their prevention role against various human diseases (Boeing et al., 2012; Leopoldini, Russo, & Toscano, 2011). Numerous reports indicated a positive correlation between increased consumption of fruits and vegetables and decreased risk of developing several

Abbreviations: AST, *Artemisia selengensis* Turcz; HPLC-QTOF-MS^2, high-performance liquid chromatography equipped with diode array detector, electrospray ionization and quadruple/time-of-flight tandem mass spectrometry; TPC, total phenolics content; TFC, total flavonoids content; CE, crude extract; CCF, chloroform fraction; EAF, ethyl acetate fraction; *n*BuF, *n*-butanol fraction; WF, water fraction; HCAs, hydroxycinnamic acids; HBAs, hydroxybenzoic acids; CQA, caffeoylquinic acid; diCQA, dicaffeoylquinic acid; triCQA, tricaffeoylquinic acid; CoQA, coumaroylquinic acid; Fr., fraction.

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chronic diseases, such as cardiovascular disease, obesity and cancer (Dauchet, Amouyel, Hercberg, & Dallongeville, 2006; Kushi, Meyer, & Jacobs, 1999). These beneficial effects are considered mainly due to the polyphenols, the most abundant secondary plant metabolites in human diets in the dietary antioxidants (Robbins, 2003).

Artemisia selengensis Turcz (AST), belonging to *Artemisia* family is a popular vegetable widely planted in South China. It contains high level of vitamins, proteins, polyphenols, minerals and dietary fibers. Phytochemical investigations on AST showed the presence of essential oils, phenolic acids, flavonoids, fatty acids and saponins (Ming, 2008). In ancient, AST was used to treat many ailments such as fever, rheumatism, cough, dysentery and hepatitis (Ming, 2008). Water and aqueous ethanol extracts of AST were evidenced to show effective antioxidant, antibacterial, antihypertensive, anticancer and immunity enhancement effects (Shao, Huang, & Gao, 2010; Zhang et al., 2014; Shen, Wang, & Jiang, 1999). The stalks have been consumed as a vegetable due to its distinctive smell, delicious flavor, crisp and tender taste since Ming Dynasty. AST leaves are usually discarded after the processing of the stalks, thus leading to serious environmental pollution. Therefore, strong evidence is needed to prove whether AST leaves are potential antioxidant resource. Screening and identification of bioactive constituents in AST leaves, specially referring to the antioxidant compounds, are also necessary for its further investigation and usage.

High performance liquid chromatography equipped with diode array detector, electrospray ionization and quadruple/time-of-flight tandem mass spectrometry (HPLC-QTOF-MS^2) is a significant analytical tool in screening the phytochemical profile of plant extract. It is considered to be a convenient, effective, precise and comprehensive technique in the on-line analysis of complex samples. It combines the high efficient separation of HPLC and high selectivity, sensitivity and high-resolution of QTOF-MS. The latter can determine the accurate mass of parent and fragment ions and provide the possible elemental compositions, which is very useful for structural confirmation when the standards are not available (Abu-Reidah, Arráez-Román, Segura-Carretero, & Fernández-Gutiérrez, 2013; Abu-Reidah, del Mar Contreras, Arráez-Román, Fernández-Gutiérrez, & Segura-Carretero, 2014; Gu, Yang, Abdulla, & Aisa, 2012).

Previous research (Zhang et al., 2014) had identified or tentatively identified 11 organic acids, 1 amino acid and 15 fatty acids from AST, but it did not tell us whether AST leaves could be a potential antioxidant resource due to the use of whole herbs. In additional, the extract was enriched by macroporous resin with 50–100% ethanol as eluent, which may lead to the misdetection of compounds at relatively low levels, as well as those with weak or high polarity. Therefore, The current research was designed to evaluate whether AST leaves could be used as a potential antioxidant resource by investigating the antioxidant activities and identifying the antioxidant components in AST leaves. AST leaves extract was successively partitioned with chloroform, ethyl acetate and *n*-butanol. The content of total phenolics and total flavonoids and antioxidant activities (DPPH· and $ABTS^{+\cdot}$ scavenging ability and reducing power) of the obtained fractions were compared. HPLC-QTOF-MS^2 was applied to characterize the antioxidant components. We believe that this study will provide sufficient experimental evidences for the further study and utilization of AST leaves.

2. Materials and methods

2.1. Chemicals and material

Kaempferol-3-*O*-glucoside, 4-caffeyolquinic acid (4-CQA), 3,4-dicaffeyolquinic acid (3,4-diCQA), 3,5-dicaffeyolquinic acid (3,5-diCQA), 4,5-dicaffeyolquinic acid (4,5-diCQA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, USA). Formic acid and acetonitrile were of HPLC grade and purchased from Waters (Waters, USA). Distilled water used for HPLC analysis was purified using a Milli-Q system from Millipore (Bedford, MA, USA). All other reagents were of analytical grade and purchased from Aladdin (Shanghai, China).

2.2. Plant material

The aerial parts of AST were manually harvested from Poyang Lake (Jiangxi, China) in Dec., 2013, and identified by experts in Agricultural Science Research Institute of Jiangxi. Then, the fresh AST were cleaned with tap-water to remove the impurity parts and cut into small pieces. After drying in a constant temperature oven at 40 °C, they were milled into powder using a disintegrator and sieved through a 100 mesh screen. Finally, the prepared powders were kept in a sealed polyethylene bag and stored in a drier at −20 °C.

2.3. Preparation of fractions

AST leaves powder (10.00 g) was extracted with 50% ethanol according to a previously optimized condition (Zhang et al., 2014): ultrasonic power of 160 W, ultrasonic time of 80 min, liquid/solid ratio of 20:1 (v/m, mL/g) and ultrasonic temperature of 40 °C. The mixture was filtered under reduced pressure, and the residue was then re-extracted following the same conditions. The supernatants were combined and concentrated with a vacuum rotary evaporator at 45 °C and lyophilized to obtain 3.03 g crude extract (CE). The CE was suspended in 50 mL distilled water and partitioned sequentially with chloroform (5 × 150 mL), ethyl acetate (5 × 150 mL) and *n*-butanol (5 × 150 mL). The resulted four fractions were then evaporated to dry under reduced pressure to give chloroform fraction (CCF, 0.05 g), ethyl acetate fraction (EAF, 0.17 g), *n*-butanol fraction (*n*BuF, 0.37 g) and water fraction (WF, 2.59 g), respectively. These fractions were kept in brown bottle and stored at −20 °C.

2.4. Determination of phenolics and flavonoids

The total phenolics content (TPC) was determined by the Folin–Ciocalteu's method following previously reported procedures (Zhang et al., 2014) with gallic acid (10–60 μg/mL) as standard ($Y = 0.0161X$, $R^2 = 0.9980$). The TPC was expressed as milligram of gallic acid equivalent per gram of fraction (mg GAE/g Fr.). The total flavonoids content (TFC) in fractions was measured as reported by Liao, Lai, Yuan, Hsu, and Chan (2011). Quercetin (2.5–12.5 μg/mL) was used as standard ($Y = 0.0338X$, $R^2 = 0.9995$), and the TFC was expressed as milligram of quercetin equivalent per gram of fraction (mg QuE/g Fr.). All tests were performed in triplicate.

2.5. Radical scavenging ability assays

2.5.1. DPPH· scavenging ability

The DPPH· scavenging ability was determined following the procedure of Yuan, Gao, Xiao, Tan, and Du (2012). Exactly 2.0 mL of diluted sample was mixed with 2.0 mL of freshly prepared DPPH· solution (0.1 mM in methanol). After being incubated in darkness for 30 min, absorbance (A_i) at 510 nm was measured against a blank (2.0 mL of 50% ethanol + 2.0 mL of methanol). BHA was used as a positive control. The DPPH· inhibition was calculated as follows:

$$\text{Inhibition } (\%) = \left[\left(A_{\text{control}} - \left(A_i - A_j\right)\right)/A_{\text{control}}\right] \times 100 \quad (1)$$