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The free, esterified, and insoluble-bound phenolic profiles of *Rhus chinensis* Mill. fruits and their pancreatic lipase inhibitory activities with molecular docking analysis

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ABSTRACT

This study investigated the free, esterified, and insoluble-bound phenolics in *Rhus chinensis* Mill. fruits and their pancreatic lipase inhibitory activities with molecular docking analysis. Results showed that the free phenolic fraction displayed the highest total phenolic content and the strongest lipase inhibitory activity. A total of 12, 11, and 8 free, esterified, and insoluble-bound phenolics were identified and quantified, respectively. Myricitrin and quercitrin were the major phenolics in all fractions with good dose-dependent lipase inhibitory effects, and myricitrin had a stronger inhibitory effect. Molecular docking analysis showed that myricitrin bound more tightly than quercitrin to the lipase with more number and shorter distance of hydrogen bonds, which indicated myricitrin had stronger lipase inhibitory activity and was consistent with their experimental results. The present study demonstrated that the free phenolics fraction of *R. chinensis* Mill. fruits had a strong lipase inhibitory activity, and can potentially prevent obesity-related problems.

1. Introduction

Obesity has recently become a research hotspot as it is linked to some metabolic syndromes, such as cardiovascular disease, hypertension, and diabetes (Jais & Brüning, 2017; Landsberg et al., 2013). In addition to these physical illnesses, mental and spiritual traumas are being investigated to be caused by obesity. Obesity and high-fat profiles are prevalent among children in western countries, and these conditions not only affect these children's health but also their self-esteem; such correlation is a very important issue (Strauss, 2000). Effective control of obesity can therefore reduce the onset of diseases and related concerns.

Studies have reported that polyphenols prevent obesity by inhibiting pancreatic lipase activity and ultimately reducing fat absorption. For example, polyphenols extracted from berry and tea inhibit pancreatic lipase activity (Gondoin, Grussu, Stewart, & McDougall, 2010; McDougall, Kulkarni, & Stewart, 2009). Therefore, phenolic compounds from plant extracts are potential anti-obesity agents (Sergent, Vanderstraeten, Winand, Beguin, & Schneider, 2012). *Rhus chinensis* Mill. (commonly called Chinese sumac) belongs to the genus *Rhus*. This plant family has been extensively investigated; for instance, the antioxidant activities of *R. hirta* L. (Wu et al., 2013), the antimicrobial activities of *R. typhina* L. (Kossah, Zhang, & Chen, 2011), and the anti-inflammatory activities of *R. hirta* L. (Peng et al., 2016) have been reported. *R. chinensis* Mill. has recently drawn attention because a study found that *Galla chinensis*, the infestation of this plant, contains abundant gallotannins (Tian, Li, Ji, Zhang, & Luo, 2009), and the fruits of *R. chinensis* Mill. are often used as the main ingredient of a Chinese medicine used to treat night sweats, diarrhea, coughs, and uterine bleeding (Djakpo & Yao, 2010). Moreover, local people use this fruit to produce natural vinegar and spice. However, little information about the phytochemistry and bioactivity of *R. chinensis* Mill. fruits is available. The compositions of the free, esterified, and insoluble-bound phenolic fractions of *R. chinensis* Mill. fruit and their inhibitory properties on pancreatic lipase have not yet been reported.

This work is the first to investigate the free, esterified, and insoluble-bound phenolic profiles of *R. chinensis* Mill. fruits and the inhibitory properties of their components against pancreatic lipase. This study analyzed the characteristics of the free, esterified, and insoluble-bound phenolic fractions of *R. chinensis* Mill. fruits, including their total phenolic contents (TPCs), their phytochemical contents, and their pancreatic lipase inhibitory activities. Moreover, this study used

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molecular docking to reveal the pancreatic lipase inhibitory mechanisms of the predominant phenolic compounds in the fruits.

2. Materials and methods

2.1. Chemicals and reagents

Folin–Ciocalteu reagent, methanol, and acetonitrile were purchased from Merck (Darmstadt, Germany). Porcine pancreatic lipase (from porcine pancreas, 127 U/mg, EC: 3.1.1.3), orlistat and *p*-nitrophenyl laurate were purchased from Sigma (Sigma-Aldrich, Shanghai, China). The standard samples of quercitrin and myricitrin (purity ≥ 98.0%) were obtained from Chengdu Must Bio-technology Co., Ltd. (Chengdu, Sichuan, China). Other reagents used were of analytical grade.

2.2. Sample preparation

R. chinensis Mill. fruits were purchased from Kunming plant-classification biotechnology Co., Ltd. (Kunming, Yunnan, China) in October 2016 and stored at −20 °C until use. The fruits were manually picked and then lyophilized (Alpha 1-2 LD plus, Christ, Germany). The dried fruits were pulverized by a high-speed grinder (Lingdan LD-T300, Shanghai, China), passed through a 60-mesh sieve, and defatted by *n*-hexane (1:5 ratio w/v; three times for total of 60 min) in a shaker (0.21 *g*; TS-200B, Tensuc, Shanghai, China) at room temperature. The defatted sumac powder was stored at −20 °C for further extractions.

2.3. Extraction of soluble phenolic compounds

Extraction was performed as previously described (Alshikh, de Camargo, & Shahidi, 2015; Krygier, Sosulski, & Hogge, 1982) with slight modification. Defatted sumac powder (30 g) was extracted with 150 mL of 70% methanol and 70% acetone (1:1 v/v) in an ultrasonic bath (200 W) for 30 min. The mixture was filtered, and the filtrate was collected. This procedure was performed twice and the total combined filtrate was evaporated using a rotary evaporator (Hei-VAP, Heidolph, Germany) to remove the organic reagent. The aqueous phase was finally collected and used in extracting the free and esterified fractions.

For the extraction of the free phenolic fraction, the aqueous phase was first adjusted to pH 2 using 6 M HCl and then extracted five times with diethyl ether and ethyl acetate (1:1 v/v). The diethyl ether-ethyl acetate extract was subsequently evaporated, lyophilized, and stored at −20 °C. For the extraction of the esterified phenolic fraction, the aqueous phase was added with 4 M NaOH (1:10 v/v) and then hydrolyzed for 4 h at room temperature. The remaining procedures were similar to those for the extraction of the free fraction.

2.4. Extraction of insoluble-bound phenolic compounds

After the free and esterified phenolic fractions were extracted, the remaining solid residues were hydrolyzed for 4 h with 4 M NaOH (1:10 v/v) at room temperature. The mixture was filtered, and the filtrate was adjusted to pH 2 using 6 M HCl. The acidified solution was extracted three times with petroleum ether for defatting and then extracted five times with diethyl ether and ethyl acetate (1:1 v/v). The diethyl ether-ethyl acetate extract was dealt with using the procedure described above.

2.5. Identification and quantification of phenolics by UHPLC-ESI-HRMS/MS

A Thermo Fisher Ultimate 3000 UHPLC System (Thermo Fisher Scientific, Germany) equipped with a Reprospher 100 C18 column (1.8 × 100 mm, 2 μm, Dr. Asp 80Maisch, Germany) was used to separate the main phenolic compounds in the three phenolic fractions of *R. chinensis* Mill. fruits. Acidified water (0.1% formic acid, phase A) and acetonitrile (phase B) were used as follows: 0–3 min, 5–20% B; 3–16 min, 20–23% B. The injection volume was 3 μL, the column temperature was set at 30 °C, and the flow rate was 0.2 mL/min.

For the HRMS/MS, a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) was used to obtain mass data in the negative mode (3.3 kV). The relative parameters were as follows: resolution, 70,000; full MS scan range, 50–1000 *m/z*; auxiliary gas flow, 8 L/min; sheath gas flow rate, 32 L/min; sweep gas, 4 L/min; S-lens RF level, 50%; spray voltage, 3.3 kV, capillary temperature, 320 °C; and heater temperature, 350 °C. The corresponding standards (or at least with the same aglycone) were used to quantify all the phenolic compounds identified in the three phenolic fractions under the same conditions. The identified phenolic compounds in the three phenolic fractions were quantified based on their standard curves.

2.6. Determination of the total phenolics content (TPC)

The TPCs of the three phenolic fractions were detected as previously described (Gámez-Meza et al., 1999) with a minor modification. In brief, the three phenolic fractions were dissolved in methanol and served as the sample solutions. Subsequently, 1.0 mL of each sample solution was added into 0.5 mL of Folin–Ciocalteu reagent, and the mixture was allowed to react for at least 1 min. Na_2CO_3 (m/v: 20%; 1.5 mL) and distilled water (7.5 mL) were successively added into the mixture for a total volume of 10 mL. The entire reaction system was placed in water bath at 70 °C for 10 min. Once the temperature cooled to room temperature, the absorbance of the reaction mixture was measured at 765 nm by using a microplate reader (SpectraMax M5, Molecular Device, Sunnyvale, CA, USA).

2.7. Determination of the total flavonoids content (TFC)

The TFCs of the three phenolic fractions were measured as previously described (Cai et al., 2011). In brief, 0.15 mL of 5% $NaNO_2$ (m/v) was added into 1 mL of the prepared sample solutions, thoroughly mixed, and allowed to stand for 5 min. Subsequently, 0.15 mL of 10% $Al(NO_3)_3$ (m/v) was added into the mixture followed by addition of 1.0 mL of 1 M NaOH after 6 min. Finally, 2.5 mL of 70% ethanol (v/v) was added. The mixture was stored at room temperature for 30 min, and the absorbance was measured at 500 nm by using a SpectraMax M5 microplate reader.

2.8. Determination of pancreatic lipase inhibition

The inhibitory effects of the three phenolic fractions and two predominant phenolic standards against pancreatic lipase activity were evaluated as previously described (Cai et al., 2012). Porcine pancreatic lipase solution (150 mg/mL) was first centrifuged at 10,000*g* at 4 °C (Heraeus Multifuge X1R, Thermo Electron LED GmbH, Osterode, Germany) for 5 min to obtain the supernatant enzyme. The substrate solution consisted of *p*-nitrophenyl laurate dissolved in 5 mM (pH 5.0) sodium acetate (1:100 m/v), as well as Triton X-100 (1:100 v/v). The substrate solution was placed in boiling water for 2 min to fully dissolve the reaction substrate. The three phenolic fractions were dissolved in dimethyl sulfoxide (DMSO) and diluted with Tris-buffer (100 mM, pH 8.2) to appropriate (different) concentrations, and the concentration of DMSO was no more than 5% in the final reaction cocktail because of different dilution ratio. Orlistat was used as a positive control.

The control consisted of 400 μL of Tris buffer, 350 μL of prepared reaction substrate solution, 100 μL of distilled water, and 150 μL of enzyme. The treatment samples (three phenolic fractions and three standards) consisted of 400 μL of Tris buffer, 350 μL of prepared reaction substrate solution, 100 μL of sample solution, and 150 μL of enzyme. A blank was needed for each group. The last step involved the incubation of the entire reaction mixture at 37 °C for 2 h followed by centrifugation at 10,000*g* for 3 min. The absorbance (OD) of the