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Protective effects of rambutan (*Nephelium lappaceum*) peel phenolics on H_2O_2-induced oxidative damages in HepG2 cells and D-galactose-induced aging mice

Yongliang Zhuang, Qingyu Ma, Yan Guo, Liping Sun*

Yunnan Institute of Food Safety, Kunming University of Science and Technology, No. 727 South Jingming Road, Kunming, Yunnan 650500, China

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ABSTRACT

Rambutan peel phenolic (RPP) extracts were prepared via dynamic separation with macroporous resin. The total phenolic content and individual phenolics in RPP were determined. Results showed that the total phenolic content of RPP was 877.11 mg gallic acid equivalents (GAE)/g extract. The content of geranin (122.18 mg/g extract) was the highest among those of the 39 identified phenolic compounds. RPP protected against oxidative stress in H_2O_2-induced HepG2 cells in a dose-response manner. The inhibitory effects of RPP on cell apoptosis might be related to its inhibitory effects on the generation of intracellular reactive oxygen species and increased effects on superoxide dismutase activity. The *in vivo* anti-aging activity of RPP was evaluated using an aging mice model that was induced by D-galactose (D-gal). The results showed that RPP enhanced the antioxidative status of experimental mice. Moreover, histological analysis indicated that RPP effectively reduced D-gal-induced liver and kidney tissue damage in a dose-dependent manner. Therefore, RPP can be used as a natural antioxidant and anti-aging agent in the pharmaceutical and food industries.

1. Introduction

Natural aging, an aspect of life, is a highly complex, multifactorial process. Excessive reactive oxygen species (ROS) are formed and the antioxidant defense system declines as aging progresses (Kawakami et al., 1999). ROS formation causes oxidative stress that damages cell membranes and DNA. In addition to cell damage and death, oxidative damage may exacerbate several age-related chronic diseases, including cancer, Alzheimer's disease, Parkinson's disease, and heart disease (Zitka et al., 2012). Therefore, studies on antioxidant and anti-aging compounds are major public issues given that the elderly population continues to increase worldwide.

Phenolic compounds are important secondary plant metabolites that determine the sensory and nutritional qualities of fruits, vegetables, and other plant products (de Camargo et al., 2014). Phenolics have received attention in recent years because of their antioxidant (Xiao et al., 2016), antimicrobial (Alkan and Yemenicioğlu, 2016), antiradiation (Zhou et al., 2016), anti-diabetic (Xiao and Högger, 2015; Xiao et al., 2015), hepatoprotective (Ma et al., 2015) and anti-inflammatory effects (Wong et al., 2014; Xiao, 2017). Phenolic compounds are strong antioxidants due to their redox properties, hydrogen donors, and singlet oxygen quenchers (Rajendran et al., 2014). Dietary phenolics are viable alternative treatment strategies for various free radical-mediated diseases. Previous researches have studied that phenolics were the major contributors to the total antioxidant capacity of the plant and the total phenolic content and its antioxidant activity mostly was linear relation (Isabelle et al., 2010). However, some studies showed the different phenolic compounds had different antioxidant activities that were directly related to phenolic profiles (Jacobo-Velázquez and Cisneros-Zevallos, 2009). Therefore, the total antioxidant capacity of a plant extract is related to its phenolic content and profile.

Rambutan (*Nephelium lappaceum*) is an importantly commercial crop, which belongs to the subtropical fruits. It is appreciated for its refreshing flavor and exotic appearance. The consumption of fresh, canned, or processed rambutan produces seeds and peels as major residues. These residues are rich in nutrients and bioactive constituents (Lourith et al., 2016; Yuvakkumar et al., 2015). In our previous study, we optimized the parameters for the extraction of

* Corresponding author.
E-mail address: lpsun@kmust.edu.cn (L. Sun).

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soluble phenolics from rambutan peels. We then prepared free, soluble conjugate, and insoluble-bound phenolic compounds from rambutan peels and evaluated the *in vitro* antioxidant activities and scavenging ROS abilities of the isolated compounds (Sun et al., 2012). Moreover, the rambutan peel phenolic extract was purified by macroporous resin adsorption technology.

Since the potent antioxidant activity of rambutan peel extracts *in vitro* was studied, the protective effects of phenolic extracts on H_2O_2-induced oxidative stress in HepG2 cells and D-gal-induced mice aging were evaluated in this study. The objective of this study was to provide methods and data for the potential applications of rambutan peel phenolics (RPP) as pharmaceutical and food ingredient.

2. Material and methods

2.1. Chemicals and animals

HepG2 cell was purchased from Kunming Institute of Zoology (Kunming, China). DMSO and FBS were purchased from ScienCell (San Diego, California). GIBCO®DMEM, Annexin V-FITC and propidium iodide (PI) apoptosis and necrosis assay kits were purchased from 7 Sea Pharmatech Co. Ltd (Shanghai, China). 2′,7'-dichlorodihydrofluorescein diacetate (DCFH-DA) reactive oxygen species assay kits were purchased from Beyotime Biotechnology Co. Ltd (Shanghai, China). Total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) commercial kits were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). D-galactose was purchased from Shanghai Yuanju Biotechnology Company (Shanghai, China). Acetonitrile and acetic acid were purchased from Merck & Co. Inc (Darmstadt, Germany). Other chemicals and reagents used were of analytical grade commercially available.

50 male ICR mice (18–25) g were purchased from Kunming Medical College (License number SYXK, 2011–0004). All animal treatments were strictly in accordance with international ethical guidelines and the national institutes of health guide on the care and use of laboratory animals.

2.2. Materials

RPP extract was prepared using a previously reported method (Sun et al., 2012). NKA-9 macroporous resin was wet-packed in a glass column (1.6 cm × 20 cm). The bed volume (BV) of the resin was set to 30 mL. The flow rate of dynamic adsorption for the phenolic extract was 2 BV/h. The flow rate of dynamic desorption was 2 BV/h with 60% (v/v) ethanol. The resin-purified RPP was lyophilized to use.

2.3. Total phenolic content and phytochemical compound analysis of RPP

Total phenolic content of RPP was determined via Folin-Ciocalteu method and expressed as mg GAE/g extract.

UPLC-Q-Orbitrap-MS^2 analysis was performed with an Ultimate 3000 Series UPLC systems (Thermo Scientific) equipped an Q Exactive hybrid quadrupole-orbitrap mass spectrometer (Thermo Fisher, MA, Germany). The column temperature was fixed at 35 °C. Mobile phase, consisting of 0.1% aqueous formic acid (A) and acetonitrile (B), was delivered at a flow rate of 0.2 mL/min in gradient mode (0–1 min, 5% B; 1–5 min, 15% B; 5–10 min, 38% B; 10–15 min, 65% B; 15–18 min, 80% B; 18–20 min, 100% B; 20–22min, 100% B). An ESI source was used in negative ion mode for accurate and tandem mass measurements. The resolution was set to 70,000 for accurate mass measurement and 35,000 for MS^2 measurement. The mass data was acquired in profile mode. Data analysis was performed with XCalibur™ software (Thermo Fisher, MA, Gemerny).

2.4. Analysis of RPP effects on H_2O_2-induced oxidative damage in HepG2 cells

2.4.1. Cell culture

HepG2 cells were maintained in DMEM medium supplemented with 10% FBS. Cells were incubated in a 5% CO_2 humidified atmosphere at 37 °C. The cell culture medium was replaced every other day. Cells were subcultured at 3–5 day intervals before reaching 90% confluence.

2.4.2. Cell viability assay

To determine the cytotoxic effects of RPP, HepG2 cell viability was evaluated with the MTT assay. Briefly, HepG2 cells were pre-incubated for 24 h in a 96-well plate at a density of 5×10^4 cells/mL. The medium was removed and the cells were treated for 24 h with serum-free medium that contained 5–80 μg/mL of RPP. Cells were then treated with 150 μL MTT reagents (0.5 mg/mL) for 4 h at 37 °C. Subsequently, the MTT reagent was removed and the amount of MTT formazan that was dissolved in 150 μL DMSO was measured using ELISA (Spectra Max M5; Molecular Devices, USA) at 570 nm. The cell viability percentage was determined based on comparison with the formazan level of the control.

2.4.3. Apoptosis assay

Double-straining with Annexin V-FITC and PI and flow cytometry assay were used to determine the protective effects of RPP on H_2O_2-induced apoptosis of HepG2 cells (Zhuang et al., 2016). HepG2 cells were pre-incubated in a 6-well plate at a density of 5×10^4 cells/mL for 24 h and treated with 5, 10 and 20 μg/mL of RPP and 5 μg/mL Vc for a further 24 h. The medium was removed and the cells were treated with H_2O_2 (0.9 mM) for 6 h. Then, cells were collected, rinsed with cold PBS, and resuspended in 400 μL binding buffer that contained 5 μL Annexin V-FITC. The cells were then incubated at room temperature in the dark for 15 min. Finally, 10 μL PI was added to cells, which were then incubated in an ice bath in the dark for 5 min. The cells were immediately examined using a flow cytometer (Guava easyCyte 6-2L; EMD Millipore, Hayward, CA, USA).

2.4.4. Determination of generated intracellular ROS

Levels of intracellular oxidative stress were determined via a fluorescence assay using a DCFH-DA ROS assay kit (Yarnpakdee et al., 2015). HepG2 cells were pretreated with RPP, Vc, and H_2O_2 as in the apoptosis assay. The collected cells were resuspended in freshly prepared, serum-free medium that contained 10 μM DCFH-DA at 37 °C in the dark for 20 min. Cells were then harvested and washed with PBS buffer. The formation of 2′,7'-dichlorofluorescin due to oxidation of DCFH-DA by intracellular ROS was immediately examined at λex of 485 nm and λem of 535 nm using a flow cytometer (Guava easyCyte 6-2L; EMD Millipore, Hayward, CA,USA).

2.4.5. Analysis of intracellular superoxide dismutase activity

Antioxidant enzyme activities of intracellular were measured with the level of superoside dismutase (SOD). Enzymatic activities were measured using a SOD assay kit. HepG2 cells were pretreated with RPP, Vc, and H_2O_2 as with the apoptosis assay. The collected cells resuspended in PBS and lysates were prepared under ultrasonic and centrifuged. The supernatants were quantified using BCA protein assay and SOD assay kits.

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