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Antioxidative characteristics of aqueous and ethanol extracts of glossy privet fruit

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ABSTRACT

Non-enzymatic antioxidant activities of aqueous extract, 50% ethanol extract and 75% ethanol extract of glossy privet fruit were examined. Aqueous and ethanol extracts contained various concentrations of phenolic acids, flavonoids, oleanolic acid and ursolic acid. Each extract scavenged superoxide anion, hydroxyl radical and nitric oxide ($P < 0.05$) in a concentration-dependent manner and the effect of 75% ethanol extract was significantly greater than other extracts ($P < 0.05$). Each extract showed a concentration-dependent effect on chelating effect, xanthine oxidase inhibition activity and reducing power ($P < 0.05$). Compared with controls, each extract significantly decreased malondialdehyde formation in low density lipoprotein (LDL) and 8-epi-$PGF_{2\alpha}$ formation in plasma ($P < 0.05$). Aqueous extract exerted a greater effect than ethanol extract on increasing catalase and glutathione peroxidase activities in plasma ($P < 0.05$). These data suggest that using glossy privet fruit extracts may enhance lipid stability in food systems, and provide antioxidative protection for LDL and plasma.

1. Introduction

Glossy privet fruit (*Ligustrum lucidum* Ait.) is a common edible plant in Asian countries such as China, Japan, Korea, and Taiwan. It is used as a spice for meat cooking; an ingredient of tea-like beverage (locally called flower tea) consisting of mint, rose, rosemary, chrysanthemum and this fruit; and an herb for traditional Chinese medicine (Wang, 1983). It has been reported that this fruit possesses anti-inflammatory, hepatoprotective, and anti-aging activities (He et al., 2001). The antioxidative effects of this fruit has been observed (Lin, Yen, Ng, & Lin, 2007; Yim, Wu, Pak, & Ko, 2001), and these authors indicated that its antioxidative activity was ascribed to its triterpenes and/or glucosides components such as oleanolic acid and ursolic acid. Our previous study (Yin & Chan, 2007) also found that oleanolic acid and ursolic acid possessed superoxide anion scavenging activity, chelating effect, xanthine oxidase inhibition activity and reducing power; thus, we proposed that these two compounds may contribute to the antioxidative protection of glossy privet fruit. However, based on the safety and economic consideration, using or taking this fruit directly for food systems and for consumers may be more practical than using its components. So far, it remains unknown that the extract of this fruit could contribute to the protection of food systems. Furthermore, information on the effect of this fruit and oleanolic acid or ursolic acid upon low density lipoprotein (LDL) and/or plasma stability against oxidative damage is also lacking.

In order to evaluate the possibility of using glossy privet fruit in food systems via exogenous addition for antioxidant protection, this study was designed to examine the non-enzymatic antioxidant capabilities of aqueous and ethanol extracts from this edible plant. The possible non-enzymatic antioxidant activities include reducing power, metal ion chelating effect, and scavenging activity for radicals such as superoxide anion and nitric oxide.

It is well known that low density lipoprotein (LDL) oxidation is strongly related to diabetic complications, atherosclerosis and cardiovascular diseases (Krentz, 2003; Mertens & Holvoet, 2001). Thus, use of supplements with antioxidative protection may benefit LDL stability, and prevent or alleviate LDL oxidation-associated diseases. In order to further understand the antioxidative protection of this fruit for human health, our present study also investigated the antioxidative effects of oleanolic acid, ursolic acid, aqueous and ethanol extracts from this fruit on human LDL and plasma.

In this study, the content of phenolic acids, flavonoids, oleanolic acid and ursolic acid in aqueous and ethanol extracts of glossy privet fruit was determined. Non-enzymatic antioxidant activities of these extracts were also examined. Furthermore, the antioxidative effects of these extracts, oleanolic acid and ursolic acid on human LDL and plasma were evaluated.

2. Materials and methods

2.1. Materials

Fresh glossy privet fruit (GPF) was obtained from farms in Nantou County (Taiwan). A 50 g edible portion of GPF was

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chopped and mixed with 100 ml sterile distilled water, 50% ethanol or 75% ethanol at 25 °C for 12 h, and followed by homogenizing in a Waring blender. After filtration through Whatman No. 1 filter paper, the filtrate was further freeze-dried to a fine powder. Pure standards such as gallic acid, caffeic acid, ferulic acid, ellagic acid, myricetin, quercetin, rutin and epicatechin were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Determination of phenolic acids and flavonoids content in aqueous and ethanol extracts of GPF

The content of four phenolic acids including gallic acid, caffeic acid, ferulic acid, ellagic acid, and four flavonoids including myricetin, quercetin, rutin, epicatechin in aqueous and ethanol extracts of GPF were determined by an HPLC method described by Sellappan, Akoh, and Krewer (2002). Briefly, 2.5 g aqueous or ethanol extracts of GPF were mixed with 25 mg of ascorbic acid, and 5 ml of 6 M HCl. Methanol was added to make total volume to 25 ml. Sample was refluxed at 95 °C for 2 h to hydrolyze the flavonoid glycosides to aglycons, followed by cooling in the dark and filtering through a micron syringe nylon filter. HPLC equipped with a diode array UV–visible detector and a Phenomenex Prodigy 5-μ, ODS-2, RP C18 column was used, and UV spectra were recorded from 220 to 450 nm. Quantification was performed based on external standards (gallic acid, caffeic acid, ferulic acid, ellagic acid, myricetin, quercetin, rutin, and epicatechin) with known concentrations. Calibration curves of these standards ranging from 10 to 200 ng/ml were used with good linearity and R^2 values exceeding 0.98 (peak areas vs concentration), and peak areas were used to quantify the content of each phenolic acid or flavonoid compound in the sample.

2.3. Determination of oleanolic acid and ursolic acid content

The content of oleanolic acid and ursolic acid in aqueous and ethanol extracts of GPF was analyzed by an HPLC method (Liu et al., 2003).

2.4. Superoxide anion production assay

The production of superoxide anion was assayed by monitoring the reduction of cytochrome *c* (Beissenhirtz et al., 2004). Three GPF extracts at 2.5 or 5% was prepared in 50 mM phosphate buffer (PBS, pH 7.0). The control group contained no test agent. Then, 1 ml sample was mixed with 1 ml solution containing 0.07 U/ml xanthine oxidase, 100 μM xanthine and 50 μM cytochrome *c*. After incubation at room temperature for 3 min, the absorbance at 550 nm was determined spectrophotometrically. Lower absorbance of the reaction mixture indicated greater superoxide anion scavenging activity. The inhibition percentage of superoxide anion generation was calculated according to the following formula: % inhibition = $(A_{control} - A_{sample})/A_{control} \times 100$.

2.5. Nitric oxide scavenging activity

The method of Green et al. (1982) was used to assay the scavenging activity of GPF extracts on nitric oxide. The reaction solution (1 ml) containing 10 mM sodium nitroprusside in PBS (pH 7.0) was mixed with GPF extracts at 2.5 or 5% and followed by incubation at 37 °C for 1 h. A 0.5 ml aliquot was then mixed with 0.5 ml Griess reagent. The absorbance at 540 nm was measured. Percent inhibition of nitric oxide generated was measured by comparing with the absorbance value of negative control (10 mM sodium nitroprusside and PBS).

2.6. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was assayed according to the method of Lopes, Schulman, and Hermes-Lima (1999). Briefly, each GPF extract at 2.5 or 5% was mixed with a solution containing 5 mM 2-deoxyribose, 100 mM H_2O_2, and 20 mM PBS (pH 7.2). Then, reaction was started by the addition of Fe^{2+} (6 μM final concentration) to this mixture. The reaction was carried out for 15 min at room temperature and stopped by adding 4% phosphoric acid (v/v) and 1% thiobarbituric acid (TBA, w/v, in 50 mM NaOH). After boiling for 15 min at 95 °C, sample was cooled to room temperature and the absorbance was read at 532 nm.

2.7. Xanthine oxidase inhibition assay

Xanthine oxidase activity was determined by measuring the formation of uric acid from xanthine. Each GPF extract at 2.5 or 5% was prepared in 50 mM PBS (pH 7.0). The control group contained no test agent. Then, 1 ml sample was mixed with 1 ml solution containing 0.4 U/ml xanthine oxidase and 100 μM xanthine. After incubating at room temperature for 3 min, uric acid production was determined by measuring the absorbance at 295 nm. Lower absorbance of the reaction mixture indicated greater xanthine oxidase inhibitory activity. The inhibition percentage of xanthine oxidase activity was calculated according to the formula: % inhibition = $(A_{control} - A_{sample})/A_{contro1} \times 100$.

2.8. Chelating effect on ferrous ions

The method of Shimada, Fujikawa, Yahara, and Nakamura (1992) was used to determine the chelating effect of GPF extracts on ferrous ions. Each extract in methanol (2 mg/ml) was mixed thoroughly with 200 μl of 1 mM tetramethyl murexide and 2 ml of a solution consisting of 30 mM hexamine, 30 mM potassium chloride, and 9 mM ferrous sulfate. The control group contained no test agent. Absorbance at 485 nm was measured after 3 min incubation at 25 °C. Lower absorbance indicated higher iron-chelating effect. In this study, the iron-chelating ability of test agent was compared with that of EDTA, and was expressed in percentage.

2.9. Reducing power

The method of Oyaizu (1986) was used to determine the reducing power of GPF extracts. Each extract was dissolved in methanol (2 mg/ml), and then was mixed with a solution containing 2.5 ml of PBS (pH 6.6, 200 mM) and 2.5 ml of 1% potassium ferricyanide. After the mixture was incubated at 50 °C for 20 min, 2.5 ml of 10% trichloroacetic acid (TCA) were added. Then, the resulting suspension was centrifuged at 650*g* for 10 min. The supernatant was mixed thoroughly with 5 ml of deionized water and 1 ml of 0.1% ferric chloride. Absorbance at 700 nm was measured and directly used to express reducing power. Higher absorbance indicated greater reducing power.

2.10. LDL preparation and oxidation

Informed consent for study participation was obtained from ten graduate students in Chung Shan Medical University (Taichung City, Taiwan). Blood was drawn from these subjects after an overnight fast. LDL fractions with densities of 1.006–1.063 g/ml were isolated from plasma by sequential ultracentrifugation (Esterbauer, Striegl, Puhl, & Rotheneder, 1989). The isolated LDL was dialyzed against 1.5 mM PBS (pH 7.2) and sterilized with a 0.22 μM filter. The protein concentration of LDL was determined according to Lowry, Rosebrough, Farr, and Randall (1951) using bovine serum albumin as a standard. The LDL fraction was diluted to