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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

a final concentration of 500 μg protein/ml using PBS. Each GPF extract at 2.5 or 5%, as well as oleanolic acid and ursolic acid at 2.5 or 5 μM was added into the LDL suspension, and oxidation was initiated with 50 mM glucose at 37 °C; the oxidation level was determined after 72 h incubation. On the basis of lipid solubility, oleanolic acid and ursolic acid was first dissolved in methanol and then added into the LDL suspension or plasma for final concentration preparation. The influence of methanol residue on lipid oxidation in these suspensions was examined, and found to be insignificant (data not shown). The method of Jain and Palmer (1997) was used to measure malondialdehyde (MDA) formation in LDL. Briefly, 0.2 ml LDL solution was suspended in 0.8 ml PBS. Then, 0.5 ml TCA (30%) was added. After vortexing and standing in ice for 2 h, samples were centrifuged at 1000g for 15 min. The supernatant at 1 ml was mixed with 0.25 ml 1% TBA (w/v, in distilled water), and the mixture was kept in a boiling water bath for 15 min. The concentration of MDA–TBA complex was assayed using an HPLC equipped with a reverse-phase Shodex KC-812 column (id, 4.6 mm; od, 250 mm; Shodex Asahipak, Tokyo, Japan) with the UV–vis detector at 532 nm.

### 2.11. Plasma oxidation

Each GPF extract at 2.5 or 5%, as well as oleanolic acid and ursolic acid at 2.5 or 5 μM were added into plasma, and oxidation was initiated by adding 50 mM glucose at 37 °C; the 8-epi-$PGF_{2\alpha}$ level was determined after 72 h incubation. The plasma level of free 8-epi-$PGF_{2\alpha}$ was measured according to the method of Morrow, Awad, Boss, Blair, and Roberts (1992). Briefly, each plasma sample was acidified to pH 3 and applied to a C18 Sep-Pak column, in which 8-epi-$PGF_{2\alpha}$ was eluted with ethyl acetate/heptane (50:50, v/v). The final eluate was dried under $N_2$ and reconstituted in 1 ml PBS containing 1% bovine serum albumin. The samples were assayed using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA). Ellman's reagent was added to the well after washing, and the absorbance at 405 nm was measured.

### 2.12. Catalase and glutathione peroxidase (GPX) assay

The protein concentration of plasma was determined according to Lowry et al. (1951) using bovine serum albumin as a standard. Then, plasma was diluted to a final concentration of 1 mg protein/ml using PBS. Catalase and GPX activities in plasma were determined by using commercial kits (Calbiochem, EMD Biosciences, Inc., San Diego, CA, USA).

### 2.13. Statistical analysis

The effect of each treatment was analyzed from ten different preparations ($n = 10$). Data were reported as means ± standard deviation (SD), and subjected to analysis of variance (ANOVA). Differences among means were determined by the Least Significance Difference Test with significance defined at $P < 0.05$.

## 3. Results

The contents of four phenolic acids, four flavonoids, oleanolic acid and ursolic acid in aqueous and ethanol extracts of glossy privet fruit are presented in Table 1. Aqueous extract had more gallic acid and caffeic acid than ethanol extracts; ethanol extracts had more myricetin, quercetin, oleanolic acid and ursolic acid than aqueous extract. Scavenging activity of GPF aqueous and ethanol extracts on superoxide anion, hydroxyl radical and nitric oxide is shown in Table 2. Each extract showed a concentration-dependent scavenging effect on the three radicals tested ($P < 0.05$), in which 75% ethanol extract was significantly greater than other two extracts in scavenging three radicals ($P < 0.05$). Effects of GPF aqueous and ethanol extracts on chelating effect, xanthine oxidase inhibition activity and reducing power are shown in Table 3. Each extract showed a concentration-dependent effect on chelating effect, xanthine oxidase inhibition activity and reducing power ($P < 0.05$). At equal concentration, 50% ethanol extract exhibited a significantly higher activity than the other two extracts in different tests ($P < 0.05$). The effects of GPF extracts and oleanolic acid or ursolic acid against 50 μM glucose-induced MDA formation in LDL and 8-epi-$PGF_{2\alpha}$ formation in plasma are presented in Table 4. Compared with the controls, each extract and oleanolic acid or

**Table 1**
Content of four phenolic acids (gallic acid, caffeic acid, ferulic acid, ellagic acid), four 290 flavonoids (myricetin, quercetin, rutin, epicatechin), oleanolic acid and ursolic acid in GPF 291 aqueous extract (AE), 50% ethanol extract (50EE) and 75% ethanol extract (75EE)

| Compound (mg/100 g) | AE | 50EE | 75EE |
|---|---|---|---|
| *Phenolic acids* | | | |
| Gallic acid | 3.24 ± 0.53$^{b}$ | 1.37 ± 0.36$^{a}$ | 1.87 ± 0.46$^{a}$ |
| Caffeic acid | 5.03 ± 0.68$^{b}$ | 1.54 ± 0.23$^{a}$ | 1.66 ± 0.37$^{a}$ |
| Ferulic acid | 1.07 ± 0.21$^{a}$ | 1.10 ± 0.18$^{a}$ | 0.94 ± 0.29$^{a}$ |
| Ellagic acid | 0.68 ± 0.10$^{a}$ | 0.72 ± 0.14$^{a}$ | 0.81 ± 0.11$^{a}$ |
| *Flavonoids* | | | |
| Myricetin | 0.81 ± 0.17$^{a}$ | 2.46 ± 0.39$^{b}$ | 2.51 ± 0.47$^{b}$ |
| Quercetin | 2.09 ± 0.70$^{a}$ | 5.30 ± 0.85$^{b}$ | 7.25 ± 1.04$^{c}$ |
| Rutin | 0.42 ± 0.11$^{a}$ | 0.68 ± 0.15$^{a}$ | 0.61 ± 0.12$^{a}$ |
| Epicatechin | 0.54 ± 0.16$^{a}$ | 0.49 ± 0.13$^{a}$ | 0.33 ± 0.08$^{a}$ |
| Oleanolic acid | 2.3 ± 0.8$^{a}$ | 6.4 ± 1.3$^{b}$ | 9.1 ± 1.7$^{c}$ |
| Ursolic acid | 1.7 ± 0.5$^{a}$ | 7.6 ± 1.0$^{b}$ | 8.2 ± 1.1$^{b}$ |

Data are expressed as mean ± SD ($n = 10$).
$^{a–c}$ Least square means with a common superscript within a row are not different at the 5% level.

**Table 2**
Scavenging activity of GPF aqueous extract (AE), 50% ethanol extract (50EE) and 75% ethanol extract (75EE) at 2.5 and 5% on superoxide anion (SA), hydroxyl radical (HR) and nitric oxide (NO)

| Agent | Concentration (%) | SA (%) | HR (%) | NO (%) |
|---|---|---|---|---|
| AE | 2.5 | 7.2 ± 0.6$^{a}$ | 10.1 ± 0.6$^{a}$ | 8.8 ± 0.9$^{a}$ |
| | 5 | 13.5 ± 0.8$^{b}$ | 16.8 ± 1.0$^{b}$ | 15.2 ± 1.1$^{b}$ |
| 50EE | 2.5 | 12.7 ± 0.8$^{b}$ | 10.5 ± 0.7$^{a}$ | 14.4 ± 1.1$^{b}$ |
| | 5 | 19.6 ± 1.3$^{c}$ | 17.2 ± 1.2$^{b}$ | 24.2 ± 1.6$^{c}$ |
| 75EE | 2.5 | 21.4 ± 2.1$^{c}$ | 21.3 ± 0.8$^{c}$ | 22.7 ± 1.0$^{c}$ |
| | 5 | 39.5 ± 2.8$^{d}$ | 30.6 ± 1.7$^{d}$ | 36.3 ± 2.3$^{d}$ |

Data are mean ± SD ($n = 10$).
$^{a–d}$ Least square means with a common superscript within a column are not different at the 5% level.

**Table 3**
Effect of GPF aqueous extract (AE), 50% ethanol extract (50EE) and 75% ethanol extract (75EE) at 2.5 and 5% on chelating effect, xanthine oxidase (XO) inhibition activity and reducing power

| Agent | Concentration (%) | Chelating effect (%) | XO inhibition (%) | Reducing power |
|---|---|---|---|---|
| AE | 2.5 | 8.2 ± 0.5$^{a}$ | 15.8 ± 0.3$^{a}$ | 0.20 ± 0.08$^{a}$ |
| | 5 | 13.3 ± 1.0$^{b}$ | 26.3 ± 1.2$^{c}$ | 0.34 ± 0.10$^{b}$ |
| 50EE | 2.5 | 24.6 ± 1.3$^{d}$ | 20.1 ± 1.9$^{b}$ | 0.39 ± 0.09$^{b}$ |
| | 5 | 43.1 ± 3.3$^{f}$ | 32.4 ± 1.6$^{d}$ | 0.58 ± 0.13$^{c}$ |
| 75EE | 2.5 | 19.5 ± 1.4$^{c}$ | 16.5 ± 1.0$^{a}$ | 0.25 ± 0.06$^{a}$ |
| | 5 | 32.5 ± 2.6$^{e}$ | 25.5 ± 2.2$^{c}$ | 0.41 ± 0.11$^{b}$ |

Data are mean ± SD ($n = 10$).
$^{a–f}$ Least square means with a common superscript within a column are not different at the 5% level.

**Table 4**
Effects of GPF aqueous extract (AE), 50% ethanol extract (50EE), 75% ethanol extract (75EE) at 2.5 and 5%, or oleanolic acid (OA) and ursolic acid (UA) at 2.5 and 5 μM against 50 μM glucose-induced MDA formation and 8-epi-$PGF_{2\alpha}$ formation in LDL and plasma after 72 h incubation at 37 °C

| Agent | Concentration | MDA (nmol/mg of LDL protein) | 8-epi-$PGF_{2\alpha}$ (pg/ml) |
|---|---|---|---|
| Control | | $18.4 \pm 1.9^{f}$ | $50.3 \pm 4.3^{e}$ |
| AE | 2.5% | $12.4 \pm 1.4^{d}$ | $38.5 \pm 2.7^{c}$ |
| | 5% | $8.8 \pm 0.7^{b}$ | $30.6 \pm 2.4^{a}$ |
| 50EE | 2.5% | $14.8 \pm 0.9^{e}$ | $43.4 \pm 3.0^{d}$ |
| | 5% | $11.1 \pm 0.7^{c}$ | $37.0 \pm 2.6^{c}$ |
| 75EE | 2.5% | $14.2 \pm 1.2^{e}$ | $42.1 \pm 3.2^{d}$ |
| | 5% | $12.8 \pm 0.8^{d}$ | $34.1 \pm 2.6^{b}$ |
| OA | 2.5 μM | $9.2 \pm 1.1^{b}$ | $37.1 \pm 1.7^{c}$ |
| | 5 μM | $7.3 \pm 0.7^{a}$ | $31.2 \pm 1.0^{a}$ |
| UA | 2.5 μM | $9.0 \pm 1.0^{b}$ | $38.4 \pm 2.0^{c}$ |
| | 5 μM | $6.9 \pm 0.9^{a}$ | $32.5 \pm 1.3^{a}$ |

Data are mean ± SD ($n = 10$).
[a–f] Least square means with a common superscript within a column are not different at the 5% level.

**Table 5**
Effects of GPF aqueous extract (AE), 50% ethanol extract (50EE) and 75% ethanol extract (75EE) at 2.5 and 5% or oleanolic acid (OA) and ursolic acid (UA) at 2.5 and 5 μM on plasma catalase and glutathione peroxidase (GPX) activities after 72 h incubation at 37 °C

| Agent | Concentration | Catalase (unit/mg of protein) | GPX (unit/mg of protein) |
|---|---|---|---|
| Control | | $3.2 \pm 0.3^{a}$ | $4.3 \pm 0.7^{a}$ |
| AE | 2.5% | $4.0 \pm 0.5^{b}$ | $4.9 \pm 0.4^{b}$ |
| | 5% | $4.7 \pm 0.4^{c}$ | $5.6 \pm 0.8^{c}$ |
| 50EE | 2.5% | $3.1 \pm 0.3^{a}$ | $4.2 \pm 0.3^{a}$ |
| | 5% | $3.4 \pm 0.6^{a}$ | $4.4 \pm 0.5^{a}$ |
| 75EE | 2.5% | $3.5 \pm 0.3^{a}$ | $4.8 \pm 0.6^{b}$ |
| | 5% | $4.1 \pm 0.4^{b}$ | $5.0 \pm 0.7^{b}$ |
| OA | 2.5 μM | $3.3 \pm 0.6^{a}$ | $4.3 \pm 0.5^{a}$ |
| | 5 μM | $3.9 \pm 0.3^{b}$ | $4.8 \pm 0.3^{b}$ |
| UA | 2.5 μM | $3.4 \pm 0.5^{a}$ | $4.4 \pm 0.4^{a}$ |
| | 5 μM | $4.1 \pm 0.3^{b}$ | $4.7 \pm 0.5^{b}$ |

Data are mean ± SD ($n = 10$).
[a–c] Least square means with a common superscript within a column are not different at the 5% level.

ursolic acid showed a concentration-dependent effect in decreasing MDA formation in LDL and 8-epi-$PGF_{2\alpha}$ formation in plasma ($P < 0.05$). The effects of GPF extracts and oleanolic acid or ursolic acid upon the activity of catalase and GPX in plasma are shown in Table 5. Aqueous extract was greater than ethanol extracts in increasing catalase and glutathione peroxidase activities in plasma ($P < 0.05$). Oleanolic acid or ursolic acid significantly increased the activity of catalase and GPX only at high dose (5 μM) ($P < 0.05$).

## 4. Discussion

The aqueous and ethanol extracts of glossy privet fruit were found to possess several non-enzymatic antioxidative activities such as metal ion chelating effect, reducing power and xanthine oxidase inhibition activity; and could effectively scavenge superoxide anion, nitric oxide and hydroxyl radical. These findings suggest that glossy privet fruit extracts could be used in food systems to enhance lipid stability. Furthermore, we found that these extracts contain gallic acid, caffeic acid, ferulic acid, ellagic acid, myricetin, quercetin, rutin, epicatechin, oleanolic acid and ursolic acid. Our previous studies and others have reported that oleanolic acid, ursolic acid and phenolic acids or flavonoids identified possessed antioxidative activities including scavenging of free radicals (Kountouri, Mylona, Kaliora, & Andrikopoulos, 2007; Liao & Yin, 2000; O'Dowd et al., 2004; Yin & Chan, 2007). Thus, the presence of these compounds may contribute to the observed non-enzymatic antioxidative activities of glossy privet fruit extracts. Using this fruit for cooking or tea-like beverage might provide antioxidative protection via these components, which might also benefit the preservation of foods or beverage.

Ethanol extracts contained more myricetin, quercetin, oleanolic acid and ursolic acid, which partially explains the greater chelating effect, reducing power and xanthine oxidase inhibition activity of these extracts. On the other hand, 75% ethanol extract contained more oleanolic acid. This finding might also partially explain the greatest scavenging activities of 75% ethanol extract because our previous study indicated that oleanolic acid could effectively scavenge superoxide anion (Yin & Chan, 2007). Based on the greater radical scavenging and chelating effects, ethanol extracts may be more efficient than aqueous extract for food systems to prevent or delay lipid oxidation. Although these ethanol extracts did not markedly increase the activity of catalase and GPX, these extracts effectively decreased lipid oxidation level in LDL and plasma. These results still supported that ethanol extracts so prepared could provide antioxidative protection for LDL and plasma.

Aqueous extract of glossy privet fruit was a mild radical scavenger, and its reducing power and chelating effect were not substantial. Thus, aqueous extract of this plant might provide less protection for food systems. However, this extract contained more gallic acid and caffeic acid, effectively elevated the activity of catalase and GPX, and exhibited greater antioxidative efficiency than ethanol extracts in LDL and plasma. The studies of Pari and Prasath (2008), and Li et al. (2005) reported that oral intake of gallic acid or caffeic acid effectively enhanced hepatic activity of catalase and GPX in animal. Thus, the higher content of these two phenolic acids present in aqueous extract of glossy privet fruit may partially explain their greater effect on increasing antioxidant enzyme activity. Since this aqueous extract effectively reduced LDL and plasma oxidation, it might be more appropriate for human nutrition to prevent or alleviate LDL and plasma oxidation responsible for the development or progression of oxidation-associated diseases such as diabetic complications, atherosclerosis and cardiovascular diseases.

Oleanolic acid and ursolic acid are two triterpenes presented in glossy privet fruit. Our present study further found that these two triterpenes could protect plasma and LDL against glucose-induced oxidation. This finding showed that oleanolic acid and ursolic acid are effective antioxidative agents. It is interesting to find that 50% ethanol extract of glossy privet fruit contained oleanolic acid and ursolic acid, but failed to elevate catalase and GPX activities. It is possible that oleanolic acid and ursolic acid were mild agents to affect catalase and GPX activities because they could increase the activity of these two enzymes only at high dose (5 μM). Further *in vivo* studies are needed to verify the enzymatic antioxidant activity of oleanolic and ursolic acids.

In conclusion, glossy privet fruit aqueous and ethanol extracts contained phenolic acids, flavonoids, oleanolic acid and ursolic acid. Based on the greater radical scavenging and chelating effects, ethanol extracts may be more efficient than aqueous extract for food systems to delay lipid oxidation. However, aqueous extract of this fruit might be more appropriate for human nutrition to prevent or alleviate LDL and plasma oxidation via elevating activity of catalase and GPX.

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