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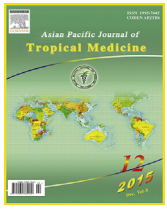

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Antioxidant and antiglycation properties of different solvent extracts from Chinese olive (*Canarium album* L.) fruit

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ABSTRACT

Objective: To evaluate the antioxidant activity, antiglycation property, and bioactive components content of different solvent extracts from Chinese olive (*Canarium album* L.) fruit.

Methods: The dry powder of Chinese olive fruit was extracted with different solvents, *i.e.*, water, water/ethanol (1/1, v/v), ethanol, methanol, acetone and ethyl acetate. The total phenolic, total flavonoids and total triterpenoids contents of various extracts were determined by spectrophotometric methods. Phenolic compounds were identified by high performance liquid chromatography. The assayed antioxidant activity was determined *in vitro* models such as antioxidant capacity by radical scavenging activity using 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picryl- hydrazyl (DPPH) and nitrite oxide methods, chelating activity on metal ions, lipid and protein peroxidation methods. *In vitro* glucose-bovine serum albumin assay was used to evaluate the antiglycation of various extracts.

Results: The water/ethanol extracts of Chinese olive fruit exerted significant scavenging effects on free radicals and strong inhibitory effects on advanced glycation end products formation. The Chinese olive fruit extracts were rich in phenolic compounds and triterpenoids. Gallic acid, ferulic acid and rutin were identified from the water/ethanol extracts. Correlation analysis indicated that there was a linear relationship between the antioxidant potency, free radical scavenging ability and phenolic compounds content of the Chinese olive fruit extracts.

Conclusions: Chinese olive fruit could be a natural candidate for studies of dietary complement to diabetes treatment since it combines antioxidant and antiglycation activities.

1. Introduction

Reactive oxygen species, reactive nitrogen species and free radicals have been implicated in mediating various pathological processes such as cancer, aging, atherosclerosis and diabetic complications [1,2]. In cells, respiratory chain reactions and glycation may be two important sources of radical production [3]. Evidences reveal that many biochemical pathways associated with hyperglycaemia can increase the production of reactive oxygen species and free radicals [1]. In the presence of oxygen and transition metals, glucose can undergo autoxidation (autoxidative glycation) to produce free radicals capable of damaging proteins, lipids and nucleic acids [4]. The oxidative steps are also involved in glycation and the process can therefore be also called glycoxidation [5]. Glycation, the nonenzymatic reaction of reducing sugars with amino groups, is increased in hyperglycemic physiological environments, leading to an acceleration of the formation of advanced glycation end products (AGEs) [6]. Increased accumulation of AGEs and oxidative stress can induce multiple cellular

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changes leading to macro- and micro-vascular complications, such as atherosclerosis, diabetic retinopathy, nephropathy, and neuropathy [7].

It has been reported that antioxidants and radical scavengers inhibit the glycation processes [8,9]. Antioxidants may play a theoretical strategy for preventing diabetic complications [10]. In addition, recent studies have shown that compounds with combined antioxidant and antiglycation properties are more effective in treating diabetes mellitus [11]. Therefore, many efforts have been extended to search dietary plants and fruits which effectively inhibit AGEs formation.

Chinese olive (*Canarium album* L.), a plant in the Burseraceae family, is widely cultivated in Taiwan, the southeast area of China and other Asian regions. Recently, Chinese olive fruit can also be found in speciality shops in Europe and the North America. Different from Mediterranean olive (*Olea europaea* L.), Chinese olive fruits have relatively low oil contents. Mature Chinese olive fruit is a fusiform drupe and yellowish green. The fresh fruit has the organoleptic characteristics of strong bitter and astringent tastes, and then tastes fragrant, sour and sweet after being chewed for a longer time. Some fresh fruits of Chinese olive are edible; however, they are normally pickled before eating, and most of them are generally processed in the food industry to beverages, candy and conserves. Olive tea, the infusion of dried Chinese olive fruit with tea leaves, has recently become popular as a drink in Taiwan. In Asian, Chinese olive fruit is used in folk medicine to relieve sore throats, quench thirst, combat diarrhoea, and promote the production of body fluid and detoxicating. Some pharmacological functions such as hepatoprotective, antimicrobial and antivirus properties of Chinese olive fruits have been demonstrated [12–14]. Zhang and Lin indicated that tannins extracted from the leaves, twigs and stem bark of Chinese olive exhibited very well radical scavenging activity and ferric reducing power [15].

Phytochemical studies have shown that Chinese olive fruit is rich in phenolic compounds and triterpenoids. Gallic acid, brevifolin, hyperin, ellagic acid, 3,3′-di-*O*-methylellagic acid, methyl gallate, ethyl gallate, brevifolin carboxylic acid, sinapic acid, corilagin, kaempferol-3-glucoside, amentoflavone, 3-*O*-galloyl quinic acid butyl ester, scoparone, scopoletin, (E)-3,3′-dihydroxy-4,4′-dimethoxystilbene, urs-12-ene-3α,16β-diol and olean-12-ene-3α,16β-diols have been identified from Chinese olive fruit [12,16–18]. These compounds appear to be responsible for the pharmacological activity of Chinese olive fruit; however, the exact mechanism of protection is not real understood.

As given above, investigations on antioxidants and AGEs inhibitors could present a potential preventive and therapeutic method for lowering the development of diabetic complications. Chinese olive fruit has potential as a functional food or drink. However, information and mechanism concerning the antioxidant and antiglycation activities of Chinese olive fruit are unclear. This study aimed to study the free radicals scavenging effects, antioxidant and antiglycation activities of Chinese olive fruit extracts derived from various solvents using a variety of *in vitro* methods.

2. Materials and methods

2.1. Chemicals

2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), sodium nitroprusside, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferrozine, glucose, bovine serum albumin (BSA) catechin, 5,5′-dithiobis-(2-nitro-benzoic acid) (DTNB), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ferrozine and linoleic acid were purchased from the Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Ammonium thiocyanate and Folin–Ciocalteau reagent were purchased from E. Merck Co. (Darmstadt, Germany). All other reagents were of analytical grade.

2.2. Plant material and extraction

Fresh Chinese olive fruits were collected in Hsinchu, Taiwan. The fruits were stored at −20 °C and the cores were removed before lyophilisation. Lyophilised sample was powdered to pass an 80-mesh sieve. Each sample (10 g) was repeatedly extracted twice with 250 mL water, water/ethanol (1/1, v/v), ethanol, methanol, acetone or ethyl acetate at room temperature for 15 min, respectively. The filtrates were evaporated to dryness in vacuo and weighed to determine the yield of soluble constituents.

2.3. Determination of total phenolic compounds

Total phenolic compounds in the extracts were determined using Folin–Ciocalteu reagent. Extracts (100 μL) were added to 2 mL of 20 g/L Na_2CO_3. After 2 min, 50% Folin–Ciocalteau reagent (100 μL) was added to the mixture which was then left to stand for 30 min. Absorbance was read at 750 nm using a spectrophotometer and compared to gallic acid calibration curves. The content of total phenolics was expressed as gallic acid equivalents (GAE).

2.4. Determination of total flavonoids

The spectrophotometer assay for the quantitative determination of flavonoid content was carried out as described previously [19]. Briefly, the extracts (1 mL, 1 mg/mL) were diluted with 1.25 mL distilled water. At zero time, 75 μL of 50 g/L $NaNO_2$ were added to the mixture. After 6 min, 150 μL of 100/L $AlCl_3$ was added. After another 5 min, 1 mL of 1 mol/L NaOH were added to the mixture. Immediately, the absorbance of the mixture was determined at 510 nm versus prepared water blank. Total flavonoids of fruits were expressed as catechin equivalents.

2.5. Characterization of phenolic compounds

The contents of phenolic compounds in Chinese olive fruit extracts were determined by HPLC, performed on a Hitachi liquid chromatograph (Hitachi, Ltd., Tokyo, Japan) consisting of a model L-2130 pump, and a model L-2455 photo diode array detector set at 280 nm. A reversed phase LiChrosphere RP-18 column (250.0 mm × 4.6 mm, particle size 5 μm, E. Merck Co., Darmstadt, Germany) was used for HPLC analysis. Elution was carried out at room temperature and utilized 2.5% (v/v) acetic acid in water as solvent A and 0.5% acetic acid in water and acetonitrile (50:50, v/v) as solvent B. The elution gradient program is as follows: 0% B to 5.0% B (0–1 min), 5.0% B to 10.0% B (1–10 min), 10.0% B to 25.0% B (10–30 min), 25.0% B to 35.0% B (30–35 min), 35.0% B to 36.5% B (35–55 min), 36.5% B to 40.0% B (55–60 min), 40.0% B to 70.0% B (60–65 min) at a flow rate of 1 mL/min. Phenolic compounds were

identified by comparing their retention times (R_t) and UV-VIS spectra with those of known standards and determined by peak areas from the chromatograms.

2.6. Determination of total triterpenoids

The total content of triterpenoids was determined as described previously [20] with slight modifications. Samples in 0.3 mL, 5% vanillin-acetic acid solution were mixed with 1 mL perchloric acid and incubated at 70 °C for 25 min. After being cooled in ice water bath, 10 mL of acetic acid was added, and the absorbance was determined at the maximum absorption at 550 nm. The content of total triterpenoids was expressed as oleanolic acid equivalents.

2.7. Trolox equivalent antioxidant capacity (TEAC) assay

Antioxidant ability of different solvent extracts of Chinese olive fruit was measured as described previously [21]. Briefly, 10 mmol/L ABTS radical cation ($ABTS^{\bullet+}$) was produced using 2 mmol/L hydrogen peroxide alone in 30 mmol/L acetate buffer (pH 3.6) incubated for 30 min at room temperature. The $ABTS^{\bullet+}$ solution (80 μL) was added to 20 μL of the sample solutions and 0.4 mol/L acetate buffer (pH 5.8, 800 μL), then the absorbance was read at 734 nm after exactly 5 min. A dose-response curve was plotted for Trolox and antioxidant ability was expressed as TEAC value.

2.8. Scavenging effects on DPPH radicals

The scavenging effects of various solvent extracts from Chinese olive fruit on DPPH radicals were estimated according to the method of Chen and Yen [22]. Chinese olive fruit extracts (4 mL) were added to 1 mL solution of DPPH in methanol. The final concentration of DPPH was 0.2 mmol/L. The mixture was shaken vigorously and was allowed to stand for 30 min at room temperature. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (U-3900, Hitachi).

2.9. Scavenging effects on nitrite oxide

The scavenging effects of Chinese olive fruit extracts on nitric oxide were measured according to the method of Marcocci *et al.* [23]. Chinese olive fruit extracts (0.3 mL) were added in the test tubes to 0.1 mL of sodium nitroprusside solution (25 mmol/L), and the tubes incubated at room temperature for 150 min. Griess reagent (0.3 mL of 10 g/L sulfanilamide in 5% H_3PO_4 and 0.3 mL of 1 g/L naphthylethylenediamine dihydrochloride) was then added to the incubation solution. The absorbance of the chromophore formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 570 nm and referred to the absorbance of standard solutions of sodium nitrite salt treated in the same way with Griess reagent.

2.10. Chelating activity on metal ions

The chelating activity of Chinese olive fruit extracts for Fe^{2+} was estimated by the methods of Chen and Yen [22]. The extracts were reacted with 40 μmol/L $FeCl_2$ for 5 min. Ferrozine (200 μmol/L) was then added and the mixture left to stand for another 10 min. The absorbance at 562 nm was determined spectrophotometrically.

2.11. Antioxidant activity in a linoleic acid system

The antioxidant activities of Chinese olive fruit extracts were based on the thiocyanate method described previously by Chen and Yen [22]. Each sample in 0.5 mL of distilled water was mixed with linoleic acid emulsion (2.5 mL, 0.02 mol/L, pH 7.0) and phosphate buffer (2 mL, 0.2 mol/L, pH 7.0) in a test tube and placed in darkness at 37 °C to accelerate oxidation. The linoleic acid emulsion was prepared by mixing an equivalent weight of linoleic acid and Tween 20 in phosphate buffer (0.2 mol/L, pH 7.0). The peroxide value was determined by reading the absorption at 500 nm with a spectrophotometer (Hitachi U-3900) after color development with $FeCl_2$ and thiocyanate at various intervals during incubation.

2.12. Effects on the oxidation of BSA induced by H_2O_2

Thiol groups of native and modified BSA were measured using DTNB. Briefly, samples (0.2 mL) were incubated with 0.4 mL of 20 mmol/L phosphate buffer (pH 7.4), 1 mL BSA (40 mg/mL) and 0.4 mL H_2O_2 (20 mmo/L) at 37 °C for 2 h. DTNB (1 mL, 2 mmol/L) was then added and the mixture left to stand for another 30 min. The absorbance was measured at 410 nm. The free thiol concentration of samples was calculated based on the standard curve prepared by using various concentration of L-cysteine.

2.13. In vitro glycation of BSA

Glycation of BSA was performed by incubating BSA (20 mg/mL) with 0.5 mol/L glucose in PBS (pH 7.4) containing 0.2 g/L sodium azide at 37 °C for 6 weeks under sterile conditions. The degree of BSA glycation was detected by AGEs-specific fluorescence (Ex 370 nm/Em 440 nm).

2.14. Statistical analysis

All analyses were run in triplicate and averaged. Statistical analyses were performed according to the SAS Institute User's Guide. Analyses of variance were performed using the ANOVA procedure. Significant differences ($P < 0.05$) between the means were determined using the Duncan's multiple range test.

3. Results

3.1. Phytochemical analysis

The yield and phytochemical content of crude extracts from the Chinese olive fruits are presented in Table 1. The yield of extraction ranged from 13.7% to 56.3%. Among the six solvents tested, the highest yield was observed with the water/ethanol (1/1, v/v) extracts (WEE), followed by the water extracts (WE), methanol extracts (ME), ethanol extracts (EE), acetone extracts (AE) and ethyl acetate extracts (EAE). The total phenolic contents as determined by Folin–Ciocalteu method were expressed

Table 1
The contents of total phenolic compounds, total flavonoids and total triterpenoids of different solvent extracts from Chinese olive fruits.

Sample	Yield (%)	Total phenolic compounds (mg/g)	Total flavonoids (mg/g)	Total triterpenoids (mg/g)
WE	50.2 ± 6.1[a]	170.9 ± 2.5[b]	14.7 ± 0.4[a]	96.0 ± 5.2[d]
WEE	56.3 ± 5.9[a]	209.4 ± 7.0[a]	14.9 ± 0.2[a]	227.0 ± 9.5[c]
ME	44.7 ± 3.9[b]	93.7 ± 3.8[c]	13.8 ± 0.9[a]	280.8 ± 6.9[b]
EE	37.4 ± 4.1[c]	84.0 ± 2.6[d]	ND	351.5 ± 10.6[a]
AE	15.2 ± 1.8[d]	50.3 ± 3.3[e]	ND	292.3 ± 19.2[b]
EAE	13.7 ± 2.4[d]	49.7 ± 2.7[e]	ND	288.8 ± 2.6[b]

Total phenolic compounds, total flavonoids and total triterpenoids were expressed as gallic acid, catechin and oleanolic acid equivalents, respectively. The data represent the means ± SD of three determinations. Values in a column with the same superscripts are not significantly different. ND: Not detectable.

in mg GAE/g. The presence of total phenolic compounds was observed in all the six extracts analyzed, while flavonoids were absent in the EE, AE and EAE (Table 1). The solvent used resulted to be a significant factor on the total phenols content ($P < 0.05$). A high content of total phenolics was observed in the WEE in comparison with other extracts, followed by the WE, and the EAE extracts which had the lowest ($P < 0.05$). Flavonoids as a part of polyphenols were in lower amounts in comparison to total phenolic. The total triterpenoids of six extracts examined decreased in the order of EE > AE ≥ EAE ≥ ME > WEE > WE.

The phenolic compounds in Chinese olive fruit extracts were also identified by HPLC. The results of HPLC analyses show that over 10 main peaks were found in the WEE from Chinese olive fruits at the absorbance of 280 nm (Figure 1). Gallic acid (R_t = 5.75 min), ferulic acid (R_t = 41.63 min) and rutin (R_t = 45.95 min) were identified by comparison of their retention time values and UV spectra with those of known standards. The contents of gallic acid, ferulic acid and rutin in the WEE were (60.6 ± 5.6), (23.0 ± 2.8) and (38.2 ± 2.9) mg/g extracts, respectively (data not shown).

3.2. Free radical scavenging effects

The total antioxidant capacity of Chinese olive fruit extracts derived from different solvents was evaluated according to the ABTS decolorization method (Figure 2A). Water soluble α-tocopherol analog, trolox, was applied as a standard. The total antioxidant activities of six extracts examined decreased in the order of WEE ≥ WE > EE > ME > AE > EAE. The TEAC values expressing as mmol trolox equivalents per gram extracts for WEE, WE, EE, ME, AE, EAE were (1.93 ± 0.03), (1.76 ± 0.01), (1.78 ± 0.03), (1.14 ± 0.04), (0.19 ± 0.09) and (0.37 ± 0.02) mmol/mg extracts based on a concentration of 20 μg/mL, respectively.

The scavenging effects of Chinese olive fruit extracts on DPPH radicals are summarized in Figure 2B. The scavenging effects of WE, WEE, ME and EE showed a concentration-dependent activity. Among the six extracts examined, the WEE and WE exhibited the strongest efficiency and showed over 70% scavenging effect of DPPH at a concentration of 100 μg/mL. The results were also expressed as EC_{50} value that is the amount of antioxidant necessary to decrease by 50% the initial DPPH radical concentration. EC_{50} values of scavenging DPPH radicals for the WEE, WE, ME and EE were (45.0 ± 0.2), (52.8 ± 0.5), (150.1 ± 1.5) and (185.4 ± 2.0) μg/mL, respectively.

As evident from Table 2, the Chinese olive extracts exerted nitric oxide scavenging activities with an increasing concentration of extract. The WEE showed the highest scavenging effects

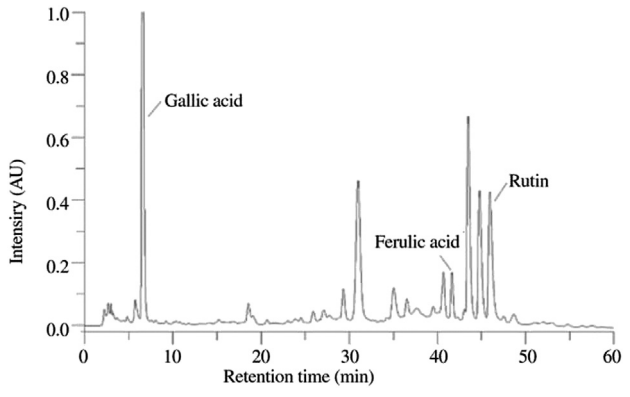

Figure 1. HPLC chromatographs of water/ethanol extracts from Chinese olive fruits.

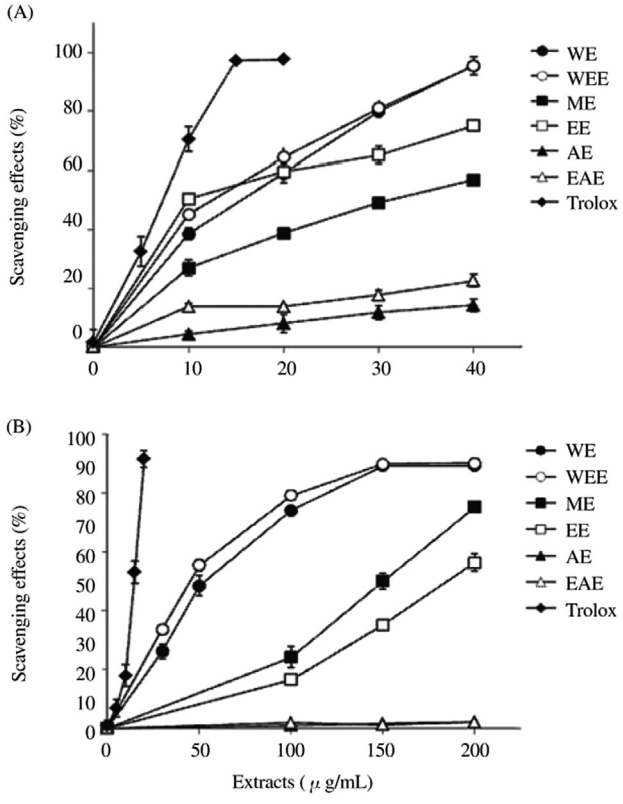

Figure 2. Scavenging effects of Chinese olive fruit extracts on the (A) ABTS radicals and (B) DPPH radicals.

Table 2
Scavenging effects of different solvent extracts from Chinese olive fruits on nitric oxide.

Sample	Scavenging effects (%)	
	100 μg/mL	200 μg/mL
WE	66.9 ± 1.3^{c}	66.8 ± 0.6^{c}
WEE	76.2 ± 1.6^{b}	81.5 ± 0.2^{a}
ME	16.9 ± 0.4^{fe}	18.9 ± 3.7^{e}
EE	61.6 ± 4.0^{d}	74.1 ± 2.6^{b}
AE	1.1 ± 0.8^{g}	1.1 ± 0.6^{g}
EAE	13.9 ± 2.1^{f}	16.1 ± 1.7^{f,e}

The data represent the means ± SD of three determinations. Values with the same superscripts are not significantly different.

on nitric oxide, followed by the WE and EE. However, the ME, EAE and EE displayed lower scavenging value on NO.

3.3. Antioxidant activity of different solvent extracts from Chinese olive fruits in various oxidation systems

The antioxidant activities of Chinese olive fruit extracts were further measured in linoleic acid and albumin systems. The ferric thiocyanate method measures the amount of peroxide produced during the initial stages of oxidation which are the primary products of oxidation. Comparison of antioxidant activity of Chinese olive fruit extracts is shown in Figure 3. Four Chinese olive fruit extracts significantly retarded the formation of peroxides in the linoleic acid emulsion system throughout the incubation period as compared to the control sample ($P < 0.05$). The inhibitory effects of Chinese olive fruit extracts against peroxides formation can be established in the following descending order: ME > WEE ≈ WE > EE (Figure 3A). The ME exhibited over 60% inhibition at 100 μg/mL in linoleic acid peroxidation system. The AE and EAE both showed no inhibition on the peroxidation of linoleic acid (data not shown). Consequently, these results clearly indicate that the Chinese olive fruit extracts, especially the ME, had effective and potent antioxidant activities in the ferric thiocyanate assays.

In this study, the inhibitions of Chinese olive fruit extracts on the oxidation of BSA induced by H_2O_2 were also examined. Exposure of BSA to H_2O_2 results in decreasing free thiol groups level. As shown in Figure 3B, the Chinese olive fruit extracts, in a dose-dependent manner significantly inhibited the oxidation of these thiol groups induced by H_2O_2. Among the four extracts tested, the WE showed the strongest antioxidant activity and prevented 98.4% depletion of protein thiol groups at a concentration of 300 μg/mL. These results indicated that hydrophilic antioxidants in the WE could scavenge H_2O_2 and reduce H_2O_2-induced thiol groups oxidation in BSA. Similar to the results obtained in linoleic acid system, the AE and EAE both exhibited very less protective effects against the oxidation of BSA induced by H_2O_2 (data not shown).

3.4. Chelating activity on metal ions of different solvent extracts from Chinese olive fruits

An important mechanism of antioxidant activity is the ability to chelate/deactivate transition metals, which possess the ability to catalyze hydro peroxide decomposition and Fenton-type reactions. Therefore, it was considered of importance to screen the metal ions chelating ability of samples. The chelating activity of samples on metal ions was determined by measuring the absorption of ferrozine-Fe^{2+} complex at 562 nm. The abilities of extracts from Chinese olive fruit to suppress the formation of ferrozine-Fe^{2+} complex decreased in the order of ME > AE > EE > EAE (Figure 4). In contrast to the free radical scavenging results, it was found that both the WEE and WE exhibited no chelating capability on iron ions.

3.5. Antiglycation of different solvent extracts from Chinese olive fruits

To evaluate the inhibitory effect of Chinese olive fruit extracts on protein glycation, a glycation-inducing reaction system with purified BSA and glucose was used, the fluorescence

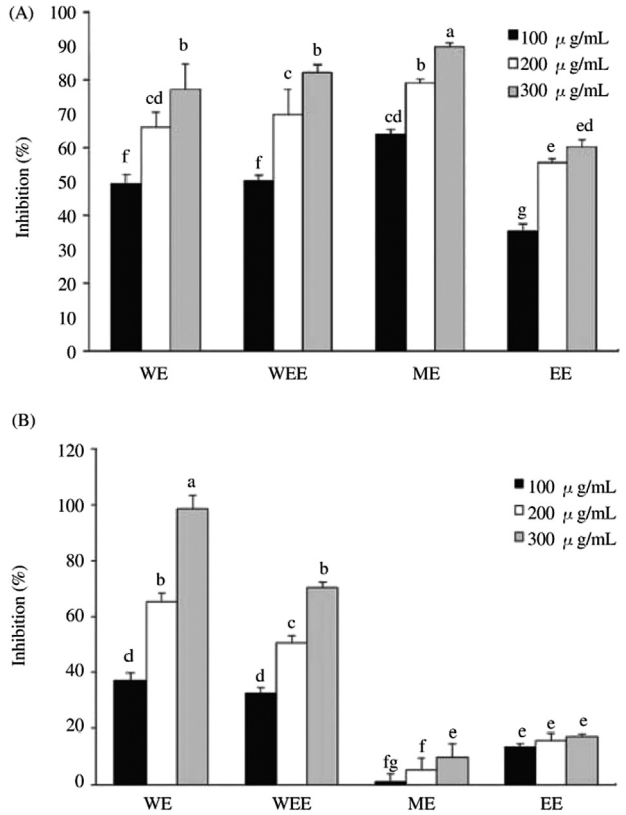

Figure 3. Effects of extracts from Chinese olive fruit on the peroxidation of (A) linoleic acid and (B) BSA induced by Fe^{2+}/H_2O_2.
Values with the same superscripts are not significantly different.

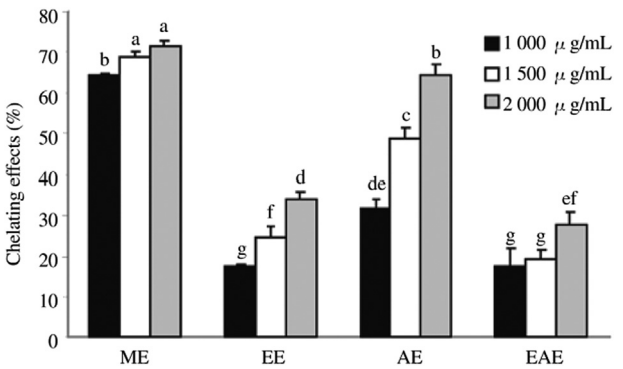

Figure 4. Chelating effects of extracts from Chinese olive fruit on the ferrous ions.
Values with the same superscripts are not significantly different.

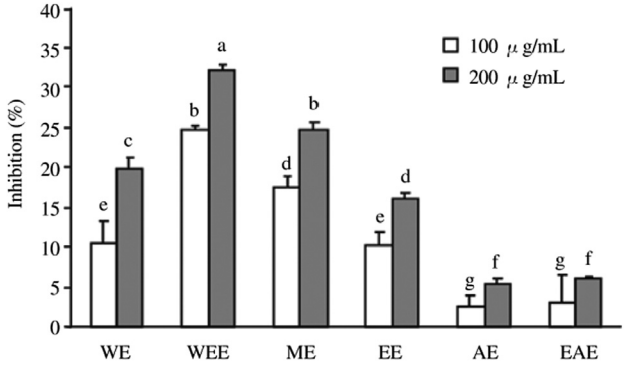

Figure 5. The inhibitory effect of various extracts from Chinese olive fruit on the formation of AGEs.
Values with the same superscripts are not significantly different.

intensity was measured and employed aminoguanidine, a well-known AGEs inhibitor. Chinese olive fruit extracts exhibited significant inhibitory effects on AGEs formation in BSA glycation systems (Figure 5). The ability of Chinese olive fruit extracts to inhibit AGEs generation decreased in the order of WEE > ME > WE > EE > AE ≈ EAE.

3.6. Correlative analysis

The correlations between antioxidant activity, antiglycation, and contents of phytochemicals of Chinese olive fruit extracts are analyzed (Table 3). For scavenging effects on radicals, high correlations (r = 0.825–0.926) were observed among ABTS, DPPH and nitric oxide, indicating that these three methods have satisfactory correlations for the examination of antioxidants. The antioxidant activities of Chinese olive fruit extracts in linoleic acid and BSA oxidation systems were significantly correlated with their scavenging effects on $ABTS^{\bullet+}$ radicals (r = 0.832 and 0.885 for linoleic acid and BSA systems, respectively) and DPPH radicals (r = 0.945 for BSA system). The scavenging effects on free radicals and antioxidant activity in BSA system of Chinese olive fruit extracts were also correlated well with their content of total phenolic compounds (r = 0.839–0.990). However, significantly correlation with the content of flavonoids was only observed for scavenging effects on DPPH radicals (r = 0.846) and inhibition on linoleic acid peroxidation (r = 0.850).

The results obtained from this study also indicated that the antiglycation capacity of Chinese olive fruit extracts well correlated to their scavenging effects on ABTS and DPPH radicals and antioxidant activity in linoleic acid oxidation system (r = 0.800–0.901). Notably, the antiglycation capacity also depended on phenolic composition (r = 0.846 and 0.859 for total phenolic and flavonoids contents, respectively).

4. Discussion

In the present study, we evaluated the phytochemicals content, antioxidant activity and antiglycating effect of various solvent extracts from Chinese olive fruits. Extraction with different solvents is frequently used for isolation and quantification of antioxidant compounds, and both extraction yield and antioxidant activity of the extracts are strongly correlated with the solvent employed. Water and aqueous mixtures of ethanol, methanol and acetone, are commonly used in plant extraction [24]. Therefore, in this work, different solvents including water, methanol, 50% aqueous solutions of ethanol, ethanol, acetone, and ethyl acetate were used for the extraction of Chinese olive fruits, and extraction yield and phytochemical contents of the extracts obtained were compared. Among the six solvents tested, the highest yield and total phenolics content was observed in the WEE, followed by the WE, and the EAE extracts which had the lowest. Variation in the yield and phytochemical contents of various extracts is attributed to polarities of different compounds present in plant, and such differences have been reported in literatures concerning fruit and spice [25,26]. Our results obtained for extraction yield and total phenols content showed that binary-solvent systems were more favorable in the extraction of phenolic compounds from Chinese olive fruit as compared to mono-solvent systems. The contents of total phenolic compounds seem to be dependent on the solvent polarity. The highest values of phenolic compounds were found in the more polar solvents, ethanol/water and water extracts, followed by the middle polar and less polar solvents, methanol, ethanol, acetone and ethyl acetate extracts. On the other hand, middle polar solvent showed better extraction efficiency towards triterpenoids. Ethanol was a good extraction solvent for extracting triterpenoids from Chinese olive fruits. Acetone and ethyl acetate were also the relatively better extraction solvents due to providing relatively higher

Table 3
Correlation between the antioxidant, antiglycatic properties and phytochemicals of extracts from Chinese olive fruit.

Correlation coefficient	Scavenging effects			Fe^{2+} chelating	Antioxidant activity		AGEs formation	Phytochemicals		
	ABTS	DPPH	Nitric oxide		Linoleic acid	BSA		Total phenolic	Total flavonoids	Total triterpenoids
ABTS	1.000									
DPPH	0.887[a]	1.000								
nitric oxide	0.926[b]	0.825[a]	1.000							
Fe^{2+} chelating	–0.435	–0.564	–0.699	1.000						
Linoleic acid	0.832[a]	0.679	0.580	0.120	1.000					
BSA	0.885[a]	0.945[b]	0.911[a]	–0.748	0.535	1.000				
AGEs formation	0.847[a]	0.800[a]	0.686	–0.097	0.901[b]	0.635	1.000			
Total phenolic	0.887[a]	0.990[b]	0.839[a]	–0.553	0.681	0.922[b]	0.846	1.000		
Total flavonoids	0.742	0.846[a]	0.499	–0.060	0.850[a]	0.649	0.859	0.828[a]	1.000	
Total triterpenoids	–0.620	–0.884	–0.548	0.553	–0.423	–0.830	–0.499	–0.825	–0.774	1.000

The concentrations of Chinese olive fruit extracts in the ABTS, DPPH, nitric oxide, Fe^{2+} chelating, linoleic acid oxidation, BSA oxidation, AGEs formation methods used for correlation analysis were 40, 100, 100, 1 000, 100, 100 and 200 μg/mL, respectively.
[a]$P < 0.05$; [b]$P < 0.01$.

triterpenoids compared with the other extraction solvents. These observations are similar to the results of Fan and He [27] which determined the content of total triterpenoids in five different solvent extracts of the leaves of *Diospyros kaki*.

Phenolic compounds, flavonoids, triterpenoids, and tannins are the main chemical constituents responsible for reducing lipid peroxidation and hence act as primary and secondary antioxidants [28–30]. Phenolic compounds are secondary metabolites widely found in fruits, mostly represented by flavonoids and phenolic acids. Studies have shown the importance of the regular consumption of fruits, especially for preventing diseases associated with oxidative stress. As shown by our result, the Chinese olive fruit contained several different kinds of phenolic compounds that could be extracted by polarity and medium polarity solvents. Liu *et al.* indicated that the contents of total phenolics and flavonoids in Chinese Olive fruit were 280.5 mg GAE/g and 130.29 mg rutin equivalents/g, respectively, expressed on a dry weight basis of fruit [31]. However, our results showed that less than 11% of the extracted phenolic substances in the extracts were of flavonoid origin. The phenolic compounds such as quercetin, rutin, naringin, catechins, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents because of their antioxidant activity [32,33]. Gallic acid has been isolated from Chinese olive fruit. He and Xia indicated that gallic acid (166 mg/100 g FW), ellagic acid (87.8 mg/100 g FW) and hexahydroxydiphenoyl hexose (80.9 mg/100 g FW) were the major phenolic compounds [16]. In this study, ferulic acid is reported in Chinese olive fruit for the first time.

It is well known that thorough antioxidant assays should involve several activity studies with complementary mechanisms of action. Antioxidants can snatch the free radical chain of oxidation and form stable free radicals, which would not initiate or propagate further oxidation. Therefore, several *in vitro* tests were adopted to evaluate the free radicals scavenging effects, the protective effects toward linoleic acid and BSA oxidation, and Fe^{2+} chelating ability of various solvents extracts at different concentrations. The WEE and WE with higher amount of total phenolics also got better scavenging effects on free radicals and exhibited potential protective effects against H_2O_2-induced BSA oxidation. Guo *et al.* evaluated the antioxidative capacities of 16 commonly used soup making tonic Chinese medicinal herbs and indicated that the boiling water extracts from Chinese olive fruit had the highest DPPH-scavenging activity and the strongest reducing power amongst the tested herbal samples [34]. Liu *et al.* compared antioxidant activities of 68 common Chinese herbal materials and indicated that Chinese Olive extracts obtained with 60% ethanol contained highest ferric reducing–antioxidant power value (15.85 mmol/g) and exhibited the most DPPH scavenging potential, whose EC_{50} (0.103 μg/μg DPPH) was lower than the EC_{50} of ascorbic acid (0.105 μg/μg DPPH) and TBHQ (0.112 μg/μg DPPH) [31]. Our results indicated that the WEE showed the highest hydrogen-donating capacity towards the DPPH radical, followed by the WE, ME and EE, while the AE and EAE rendered the weaker effect. Therefore, Chinese olive fruit extracts could react with free radicals, converting them to more stable products and terminating the radical chain reaction in the peroxidation of linoleic acids and preventing the oxidation of thiol group in BSA. The antioxidant action of Chinese olive fruit extracts may be attributed, in a significant part, at least, to the phenolic compounds. Unlike other polar antioxidant with free radical scavenger activity in the WE and WEE, our results suggested that specific moderately polar compounds in the ME, which might have contributed to the chelating ability. Moreover, the chelation effects of ME may also contribute to their well antioxidant activity in linoleic acid system.

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons, *etc*., and involved in the regulation of various physiological processes. However, excess concentration of nitric oxide in various types of inflammatory processes is associated with several diseases. Regarding the reactive nitrogen species-scavenging properties, the higher the phenolic compound contents in Chinese olive extracts, such as the WEE and WE, the higher is the efficiency in the scavenging effect of nitric oxide. However, as compared to the ME, EE showed a stronger scavenging activity of reactive nitrogen species, although the EE with less amount of phenolic compounds. This may be related to the high amount of triterpenes in the EE. Several studies demonstrated that triterpenes were effective in scavenging nitric oxide and could be of importance for the antioxidant activity of the plant extracts [35,36]. Chinese olive fruit is rich in phenolic compounds and triterpenes [37], which have diverse biological functions such as anti-inflammatory effects [38]. These functions might be associated with their antioxidant activity [39,40]. Further studies focusing on anti-inflammatory properties of Chinese olive fruit are required and are in progress in our laboratory.

Free radicals have been shown to enhance AGEs formation, moreover, advanced products contribute to oxygen free radical production and can induce oxidant stress *in vivo* and *in vitro* [41]. Interaction of AGEs with cellular targets leads to oxidant stress resulting in changes in gene expression and other cellular properties, potentially contributing to the development of tissue lesions. In this way, the contribution of AGEs generation to diabetes, aging and Alzheimer's disease has received considerable attention in recent years [42–44]. Previous studies have reported that antioxidants inhibit the glycation processes. AGE-induced oxidant stress was also inhibited by pretreatment of animals with antioxidants [45]. As AGEs are known to be involved in the development of the pathological process of various diseases, many efforts have focused on the isolation of AGEs inhibitors from natural resources. In this regard, phenolic antioxidants are increasingly viewed as promising AGEs inhibitors [46]. Except polyphenols which constitute a major group of plant-derived compounds with antiglycation activity, some triterpenes and saponins were shown to decrease the AGEs formation [47,48]. Chinese olive fruit extracts showed protective effects against glucose-induced protein modifications; especially the WEE significantly inhibiting the protein glycation. Moreover, the antiglycation capacity of Chinese olive fruit extracts well correlated to their scavenging effects on ABTS and DPPH radicals and antioxidant activity in linoleic acid oxidation system (r = 0.800–0.901). Notably, the antiglycation capacity also depended on phenolic composition (r = 0.846 and 0.859 for total phenolic and flavonoids contents, respectively). The same observation was also found in herb and medicinal plant extracts [8,49].

According to the data obtained in the study, Chinese olive fruit extracts contain various phenolic compounds and triterpenoids which showed well antioxidant activities and protective effects against glucose-mediated protein modification *in vitro*. These findings suggest that daily consumption of Chinese olive fruit may be beneficial for preventing complications in diabetic

patients. However, further studies on the antiglycation components of Chinese olive fruit extracts and more *in vivo* evidence from diabetic patients are required.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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