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ORIGINAL ARTICLE

Antioxidant and Hemolysis Protective Effects of Polyphenol-Rich Extract from Mulberry Fruits

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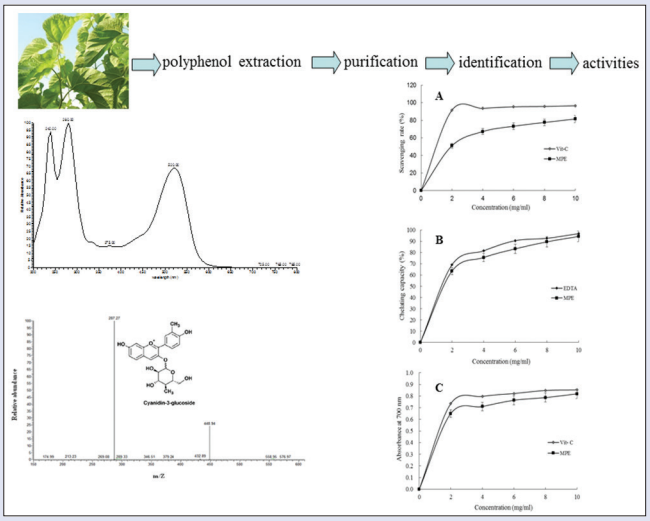
ABSTRACT

Background: Mulberry fruits are a superior source of polyphenol, especially anthocyanins that contribute potentially to the beneficial effects which include reducing the risk of cardiovascular diseases and cancers with antioxidant, anti-inflammatory, and chemoprotective properties. **Objectives:** In this study, purification of the polyphenol-rich extract from mulberry fruit (MPE) was purified and assessed the activities of antioxidant and hemolysis protective *in vivo* and *in vitro*. **Materials and Methods:** Antioxidant activities *in vitro* was measured by quantifying its 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, reducing power and Fe^{2+}-chelating ability. MPE was purified by high-pressure liquid chromatography (HPLC) and analyzed individual polyphenols using liquid chromatography–mass spectrometry (LC-MS)/MS. **Results:** The total polyphenol content was 147.69 ± 0.02 mg gallic acid equivalents (GAE)/g dried weight (DW) in the extract and 403.55 ± 0.02 mg GAE/g DW in the purified extract. Further identification by HPLC-ultraviolet-visible and LC-MS/MS analysis indicated in MPE, an anthocyanin compound, cyanidin-3-*O*-glucoside. With regard to *in vitro* assays, MPE possessed antioxidant effect, especially in Fe^{2+} chelating ability with an IC_{50} value of 1.016 mg/mL. The protective effects on mouse red blood cell hemolysis and lipid peroxidation *ex vivo* were dose and time dependent. **Conclusion:** It indicates that MPE could be a good candidate for future biomedical applications to promote human health with limited side effects.

Key words: Antioxidant activity, hemolysis protective, mulberry fruit, polyphenols, purification

SUMMARY

- Mulberry fruit is an excellent source of polyphenols, in particular, anthocyanins, which has infinite health benefits. This study determined the predominant anthocyanin, cyanidin-3-glucoside, could possibly be the rationale behind the antioxidant and antihemolytic effect of MPE. Results indicate that MPE could be a good candidate for future biomedical applications to promote human health with limited side effects.



Abbreviations used: MPE: Purification of the polyphenol-rich extract from mulberry fruit, LC-MS: Liquid chromatography–mass spectrometry, HPLC: High-pressure liquid chromatography, DPPH: 2,2-diphenyl-1-picrylhydrazyl scavenging activity, RBC: Red blood cell, GAE: Gallic acid equivalent, $FeCl_2$: Ferrous chloride, H_2O_2: Hydrogen peroxide, EDTA-2Na: Ethylenediaminetetraacetic acid disodium salt, PBS: Phosphate-buffered saline, TCA: Trichloroacetic acid, TBA: 2-thiobarbituric acid, $FeSO_4$: Ferrous sulphate, MDA: Malondialdehyde, V_C: Vitamin C, DW: Dried weight.

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INTRODUCTION

Mulberry (*Morus alba*) is a deciduous, fast-growing, medium size tree, which has been historically used in sericulture for 1000 of years. Mulberry tree is native to almost all continents of the world.[1] It is a valued species for landscaping, gardening in urban conditions, street shade, and city embellishment because it withstands high levels of air pollution. All parts of mulberry plant, such as leaves, fruits, bark, and branches, have long been used in Chinese medicine. Nowadays, many mulberry fruit products are in commercial market such as jams, juices, wine, vinegars, ice creams, marmalades, and other food-related products.

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In folk, mulberry fruit is used as a medicine to protect liver from damage, strengthening the joints, lower blood pressure, facilitate discharge of urine, and treatment of premature graying of hair, weakness, fatigue, laxative, and anemia.[2-4] Due to the functional quality characteristics, good taste, and nutritional value, the production and consumption of mulberry fruit have increased rapidly in recent years.[5]

The physiological functions of fruits and vegetables are partly attributed to their abundance of polyphenol compounds.[6] Polyphenols are aromatic compounds containing one or several hydroxyl groups directly attached to the benzene ring. According to the number of hydroxyl groups, polyphenols are classified as dihydric, trihydric, and polyhydric. By the year 2005, 1000 of polyphenol compounds have been isolated from plants.[7] In recent years, much attention has been paid by nutritionists toward the dietary polyphenols due to their potent antioxidative effects and their credible effects in the prevention of various oxidative stress-associated diseases.[8]

Numerous studies have demonstrated that mulberry fruits and leaves exhibited significant scavenging effects on free radicals and possess protective effects against oxidative damage. Fresh fruit extracts are excellent sources of polyphenolic compounds that exhibit antioxidant activity.[9] These medicinal capabilities are attributable to the presence of active ingredients with notable therapeutic functions.[2] Anthocyanin from mulberry fruit can scavenge free radicals, inhibit LDL oxidation, and have beneficial effects on blood lipid levels and atherosclerosis.[10-12] Epidemiological studies shown that the high intake of mulberry fruits has been associated with a reduced risk of chronic diseases such as cardiovascular diseases, diabetes, and cancer.[13]

Mulberry fruit extracts contain high amounts of anthocyanin, which are a group of naturally occurring polyphenolic compounds that are responsible for their color.[14] Compared to the other berries, nowadays, mulberry fruit competitively rocks the market due to its low cost and abundant content of polyphenolic compounds and naturally occurring α-glucosidase inhibitors. To understand the anthocyanin compound-rich extract and its bioactivity, here we purified anthocyanin from mulberry fruit and analyzed the antioxidant and hemolysis protective effects.

MATERIALS AND METHODS

Materials

Mulberry fruit, *M. alba* of J33 variety, was harvested at ripe stage from the Plantation of National Mulberry Orchard (Zhenjiang, Jiangsu province, PR China). Fruits were dried by freeze dryer (EYELA FDU-2100, Japan) and milled into powder for further use. The Experimental Animal Center of Jiangsu University (Zhenjiang, China) provided clean grade female ICR mice weighing 30 g with an age of 6 weeks. Moreover, the experiments were performed in accordance with regional legal regulations (Ethical issue number UJS-LAER-2015080101). HP-20 resin was purchased from Sigma Chemical Co. (St. Louis, MO, USA), whereas sea sand, n-hexane, ethanol, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrozine, potassium ferricyanide, ferrous chloride, and ferric chloride were purchased from Sino pharm Chemical Reagent Co. Ltd. (Shanghai, China). All other reagents used in this study were of analytical grade.

Extraction of polyphenolic compound

Lyophilized mulberry fruit powder was used for the extraction of polyphenolic compounds. Two grams mulberry fruit powder was mixed with hexane (1:10 w/v) for 1 h with continuous shaking in an ultrasonic bath at 55°C to removal of lipids and fatty acids. The pellet was sonicated by adding 80% ethanol (1:3 w/v) under the same condition to prepare the polyphenol-rich extract. Subsequently, the extract was kept at 4°C overnight and sonicated again for 25 min followed by filtration through Whatman No. 1 filter paper. The residue was re-extracted in the same manner and the three filtrates (5 ml each) were combined.[15] The three filtrates of ethanol were combined and evaporated under vacuum at 40°C to obtain dry extract. The extracts were placed in a plastic bottle and then stored at −20°C until used.[16]

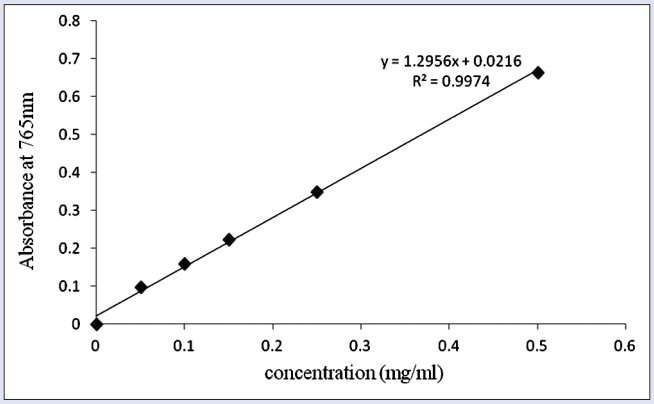

Figure 1: Standard gallic acid curve

Determination of total polyphenols

Total polyphenol content of ethanol extract was determined by the Folin–Ciocalteu method[17] and was expressed as gallic acid equivalents (GAEs) as shown in Figure 1.

Purification of the polyphenol-rich extract from mulberry fruit

The solid-phase extraction technique was used to separate the anthocyanins from polyphenol compound. Extracts were evenly dissolved in deionized water and this concentrated solution was chromatographed through a 40 cm × 2.5 cm Diaion HP-20 column and eluted with different proportions of ethanol: water as shown in Figure 2.[18] In total, fifty fractions were collected, and the solitary peak obtained was selected for further processes. The Dionex collected was performed to ultraviolet (UV)-visible spectrophotometer to determine the visible spectra by scanning the absorbance between 200 and 750 nm.[19,20]

Liquid chromatography–mass spectrometry/mass spectrometry analysis

It was carried out on a mass spectrometer (Finnigan LCQ DECA XP plus, America) equipped with a quaternary solvent delivery system, a column oven, UV detector, and an autosampler. The gradient method was followed with the mobile phase consists of methanol and water (0.1% formic acid) by 50:50 ratio solutions with the run time of 60 min. The flow rate used as 0.8 ml/min at a maximum pressure of 431 bars using the wavelength at 280 nm. The Dionex C18 column (150 mm × 4.6 mm) was used in this analysis. Subsequently, in surveyor AS method, the injection volume was 10 μl and column oven temperature maintenance at 30°C. The MS detector setup was fixed with acquire time of 60 min time, collision energy of 15.0 (v), and mass range between 50.00 and 2000.00 using both positive and negative modes using multimode reaction method (MRM).[21]

Determination of the antioxidant activity

2,2-diphenyl-1-picrylhydrazyl scavenging activity

DPPH scavenging activity was determined according to a previously described method.[22] Briefly, various concentrations of (2, 4, 6, 8, and 10) mg/ml ethanol extract were prepared and 0.1 ml

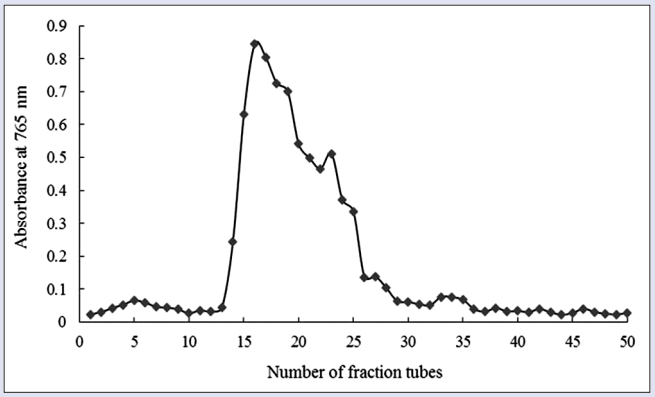

Figure 2: Elution profiles of polyphenolic compounds extracted from mulberry fruit by non-ion exchange chromatography. Tube No. 1–10, distilled water; No. 11–20, 30% ethanol; tube No. 21–30, 50% ethanol; No. 31–40, 70% ethanol; tube No. 41–50, 90% ethanol

of the purification of the polyphenol-rich extract from mulberry fruit (MPE) sample was mixed to 3.9 ml of a 6×10^{-5} M solution of DPPH in methanol. The mixture was shaken vigorously and allowed to stand at 25°C for 30 min in dark condition and centrifuged at 1500 g for 10 min. The absorbance of the supernatant was measured at 517 nm using ascorbic acid as a control. A control sample containing the same volume of ethanol in place of extract was used to measure the maximum DPPH absorbance. The activity was expressed as percentage of DPPH scavenging relative to control (0.1 ml ethanol and 3.9 ml DPPH solution). Ascorbic acid was used as the control. The DPPH radical scavenging capacity was calculated using the following formula:

$$\text{Scavenging capacity (\%)} = \frac{1-(A_1-A_2)}{A_0} \times 100$$

Where, *A*0 is the absorbance of the control (without extract), *A*1 is the absorbance in the presence of the extract, and *A*2 is the absorbance without DPPH.

Ferrous ion chelating capacity

The ferrous ion chelating capacity was determined following the method.[22] Various concentrations of (2, 4, 6, 8, and 10) mg/ml ethanol extract were prepared and the reaction mixture, containing 3 mL of MPE (0.1/ml), 0.05 ml of 2 mmol/L ferrous chloride ($FeCl_2$) solution, and 0.2 mL of 5 mmol/L ferrozine solution, was shaken vigorously and incubated at 25°C for 10 min. The absorbance of the mixture was then measured at 562 nm. Ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) was used as a control. The ferrous ion chelating capacity of the sample was calculated as follows:

$$\text{Chelating capacity (\%)} = \frac{A_0-(A_1-A_2)}{A_0} \times 100$$

Where, *A*0 is the absorbance of the control (without extract), *A*1 is the absorbance in the presence of the extract, and *A*2 is the absorbance without EDTA.

Reducing power

Reducing power was determined based on the method.[23] Briefly, various concentrations of (2, 4, 6, 8, and 10) mg/ml ethanol extract were prepared and 1 ml of MPE in phosphate buffer (0.2 mol/L, pH 6.6) was mixed with 2 ml potassium ferricyanide (1%) and incubated at 50°C for 20 min. Then, 2 ml of 10% trichloroacetic acid was added to the mixture to stop the reaction. After centrifugation at 3000 g for 10 min, 2.5 ml of supernatant was mixed with 2.5 ml distilled water and 0.5 ml 0.1% ferric chloride. The mixture was allowed to rest for 10 min at room temperature in dark condition. The blank

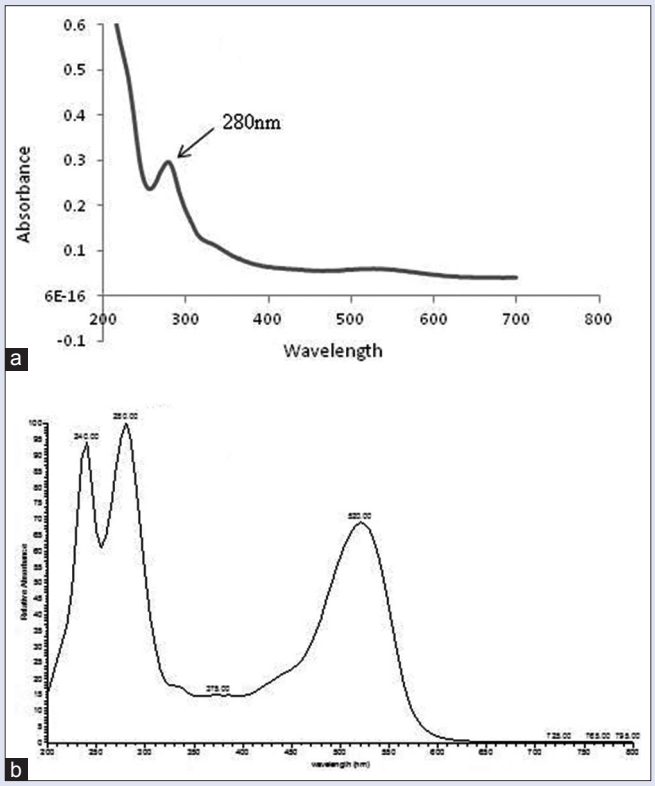

Figure 3: Ultraviolet/visible spectral characteristic of purification of the polyphenol-rich extract from mulberry fruit. (a) Dionex subjected to ultraviolet/visible spectral characteristic after purification by non-ion exchange chromatography showed maximum absorbance of 280 nm. (b) ultraviolet/visible spectral characteristics of identified cyanidin-3-glucoside peak recorded at 520 nm

was prepared using ethanol by replacing the extract and absorbance was measured at 700 nm. Ascorbic acid was used as a control.

Red blood cell hemolytic assay

Whole blood was collected from adult mice and centrifuged at 1000 g for 10 min at 4°C. The plasma and buffy coat layers were discarded, and an equal volume of phosphate-buffered saline (PBS) (pH 7.4) was added. After repeating three times, 4% suspension of red blood cells (RBC) was obtained with PBS dilution.

Erythrocytes were hemolyzed following the modified method.[24] One milliliter of erythrocyte suspension (4%) was mixed with 1 ml of the different concentrations of MPE (2, 4, 6, 8, and 10) µg/ml ethanol extract was prepared and then added 1 ml of H_2O_2 (100 mmol/L in PBS). The blank control consisted of 2 ml of PBS and 1 ml RBC suspension, and the induced control consisted of 1 ml of PBS, 1 ml RBC suspension, and 1 ml of H_2O_2. The mixture was incubated in a shaking water bath at 37°C for varying time intervals (30, 60, 90, 120, and 150 min) and centrifuged at 1000 g for 10 min at 4°C. The RBC-free supernatant solution from each tube was transferred to cuvettes. Absorbance was measured at 415 nm in a spectrophotometer (Shimadzu UV-VIS 1650, Tokyo, Japan). Each sample was measured in triplicate.[25] The percentage of hemolysis was calculated by the following equation:

$$\text{Hemolysis percentage (\%)} = (\text{Abs}_{[\text{sample}]}/\text{Abs}_{[\text{induced control}]}) \times 100\%.$$

Lipid peroxidation

The inhibition effect of fractionation on lipid peroxidation was determined according to the 2-thiobarbituric acid method (TBA).[25] Briefly, 1 mL

of the different concentrations of MPE (2, 4, 6, 8, and 10) μg/ml was mixed with 1 mL of 0.5% mice liver homogenate. Then, 100 μL of 15 mM $FeSO_4$ and 50 μL of 0.1 mmol/L ascorbic acid were added, and the mixture was incubated at 37°C for 1 h in dark condition. The blank control consisted of 1.15 mL of PBS and 1 mL liver homogenate, and the induced control consisted of 1 mL of PBS, 1 mL liver homogenate, and 150 μL of $FeSO_4$-Vc. Subsequently, 1 mL of 15% TCA and 1 mL of 0.67% TBA were added to stop the reaction. The mixture was incubated in boiling water for 15 min. After it had cooled down, the absorbance was measured spectrophotometrically at 532 nm.

Inhibition ratio = $([Abs_{[induced\ control]} - Abs_{[sample]}]/Abs_{[induced\ control]} - Abs_{[blank\ control]}) \times 100\%$.

Data analysis

Data of all assays were run in triplicate. The statistical significance was analyzed by Student's *t*-test and regression analysis. The data were fitted using the Expert Design 7.1.3 for Windows software (SPSS Inc., IBM, Co.Ld., USA) and IC_{50} value was calculated using statistical software solution version 20.

RESULTS AND DISCUSSION

Polyphenolic compounds are beneficial toward health because of their antioxidant property.[26] They act as free radical scavengers and can chelate metals resulting in various biological effects.[27] Therefore, it is important to quantify the polyphenol contents of plant extracts to assess their contribution toward antioxidant activity.[28] In this study, polyphenolic compounds from mulberry fruit powder were extracted by ultrasonic-assisted ethanol. After filtration and centrifugation to removal of lipids and fatty acids, the total polyphenol content in the extracts was 147.69 ± 0.02 mg GAE/g dried weight (DW).

Polyphenolic compounds content in mulberry fruit is much higher than that of other berries including gooseberry (5.2 mg/g), elderberry (19.5 mg/g), chokeberry (20.1 mg/g), red currant (5.4 mg/g), and black currant (13.3 mg/g)[29] and even higher than in grapes (731.7 mg/kg).[30]

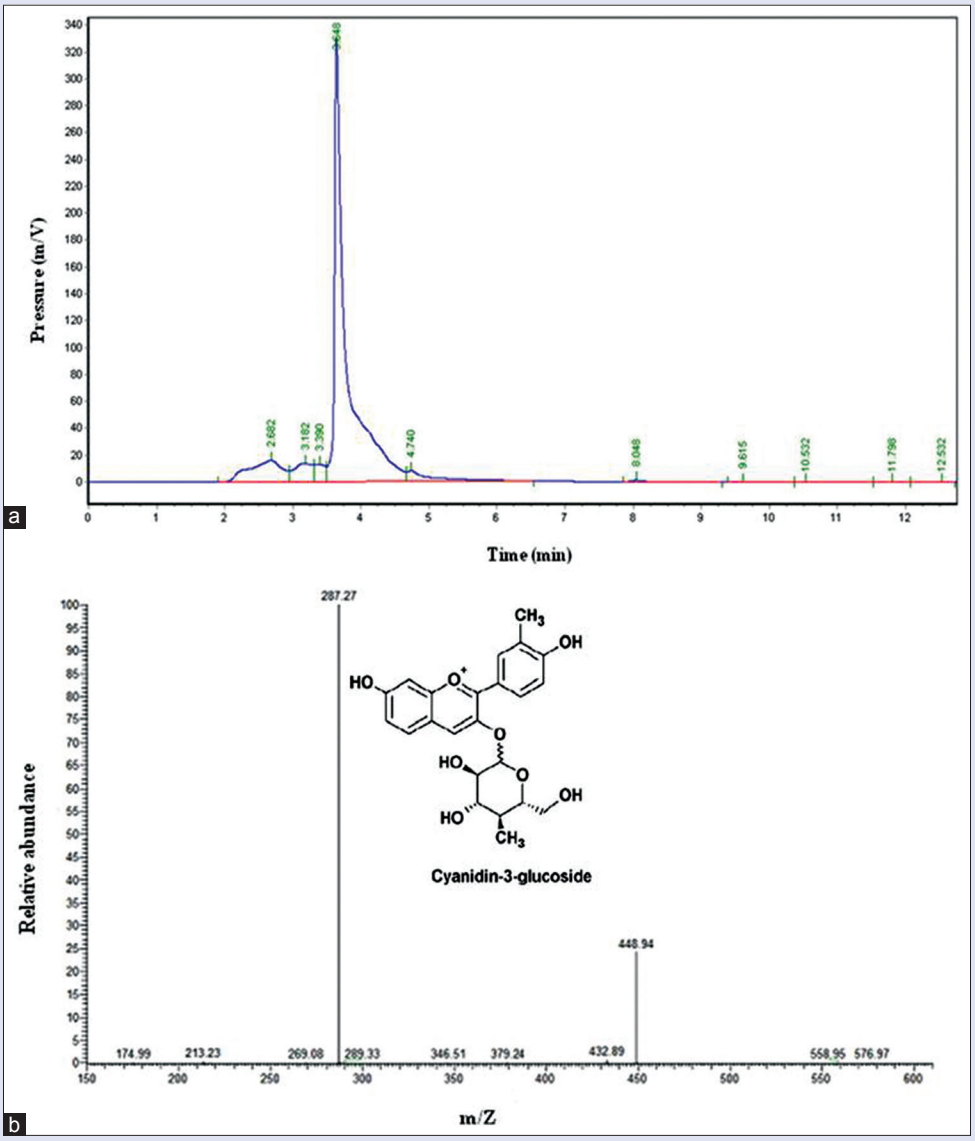

Figure 4: High-pressure liquid chromatography and mass spectrometry/mass spectrometry profiles of purification of the polyphenol-rich extract from mulberry fruit. (a) High-pressure liquid chromatography profile of purified polyphenol extract from mulberry fruit. (b) Liquid chromatography–mass spectrometry/mass spectrometry spectra of cyanidin-3-glucoside of mulberry fruit polyphenol extract

Many studies showed that the polyphenol content of *Morus* species fruits is difference with varieties and plant locations. It was reported that the total polyphenol content was 14.22 mg/g in fresh fruits of *Morus nigra* L. and 19.43-22.23 mg/g in fresh fruits of *M. alba* L.[2] It was 17.66–34.88 mg/g in fresh fruits at Turkey[9] and 15.16 mg/g in fresh fruits at Taiwan.[6] Our study showed that the total polyphenol content of mulberry fruits ranged from 230.05 ± 0.05–23.59 ± 0.06 mg GAE/g DW according to their varieties. This result is consistent with the previous report.

Here, we used ethanol as a solvent since it is efficient for polyphenol extraction.[31] Figure 2 showed that 30% ethanol was the most suitable combination for extraction of phenol-rich extract. To identify the possible phytochemical present, HP-20 column chromatography was used for separation of polyphenol-rich extract. The polarity of the extracting solvent and solubility of the compound in the solvent are key issues in recovery of polyphenol compounds.[32] The Dionex collected during purification was subjected to UV-VIS spectrophotometer showed that the polyphenol extracts of mulberry fruits have characteristic UV/visible spectra with maximum absorbance of 280 nm [Figure 3a]. The extract sample purified by HP-20 column chromatography was 403.55 ± 0.02 mg/g, 2.73 folds higher than that of the extract. HP-20 column chromatography could efficiently increase the polyphenol content of the extract.

To understand the particular compound in the polyphenol extract, the purified polyphenol extract was chromatographed [Figure 4a], and spectral characteristics including mass spectrum were carried out. Vis_{max} was observed the characteristic peak at 520 nm [Figure 3b]. Vis_{max} (%) was 30.83 which suggested that a sugar moiety was linked to the anthocyanidin (aglycone) through C3 position. There were no characteristic peaks around 310 nm, which elicits that there was no acylation with aromatic organic acids. Since there was a single prominent peak in the visible range, the anthocyanin could be a monomer. The mass spectrum showed [M + H] + at 449 m/z, which established the molecule as cyanidin 3-glucoside [Figure 4b]. A fragment ion at 287 m/z corresponding to aglycone cyanidin was observed. From these liquid chromatography–mass spectrometry (LC-MS)/MS observations, the predominant anthocyanin in *Morus* berries was confirmed as cyanidin 3-glucoside.[33-36]

The antioxidant functions of anthocyanins have been ascribed to the aglycone moiety, and this was demonstrated for cyanidin and some of its glycosides, but the number of sugar residues at the 3-position, the oxidation state of the C ring, the hydroxylation and methylation pattern, as well as the acylation by phenolic acids are considered crucial factors for the expression of antioxidant effects.[16] Since there are complexes of natural phytochemicals, multiple assays are necessary in determining the free radical scavenging ability.[37] The DPPH radical has been widely used to evaluate the free radical scavenging ability of various natural products and has been accepted as a model compound for free radicals.[38] The antioxidant effect of polyphenols on DPPH is due to their hydrogen-donating ability, and it has been used to determine the antioxidant effect of numerous phytochemicals.

In this study, MPE exhibited DPPH radical scavenging activity in a concentration-dependent manner with an IC_{50} value of 1.79 mg/mL [Figure 5a]. The degree of discoloration denotes the strong antioxidant activity of the extract.[39] Scavenging abilities on DPPH radicals were excellent for ethanolic extract in *Morus Pachungsipyung, Morus Whazosipmunja, Morus Suwonnosang, Morus Jasan, Morus Mocksang,* and IC_{50} value of *M. Whazosipmunja* were 138.4 μg/mL, which was relatively high compared to the other varieties.[16] In the other study, the extract of *M. alba* showed highest radical scavenging activity than the other fruits, followed by *M. laevigata* (large white fruit), *M. laevigata* (large black fruit), and *M. nigra*.[40] This proves radical scavenging activity for *M. alba* was significantly higher than that of the other mulberry fruits and the ethanolic extract from mulberry fruit shows the increase of antioxidant activity.

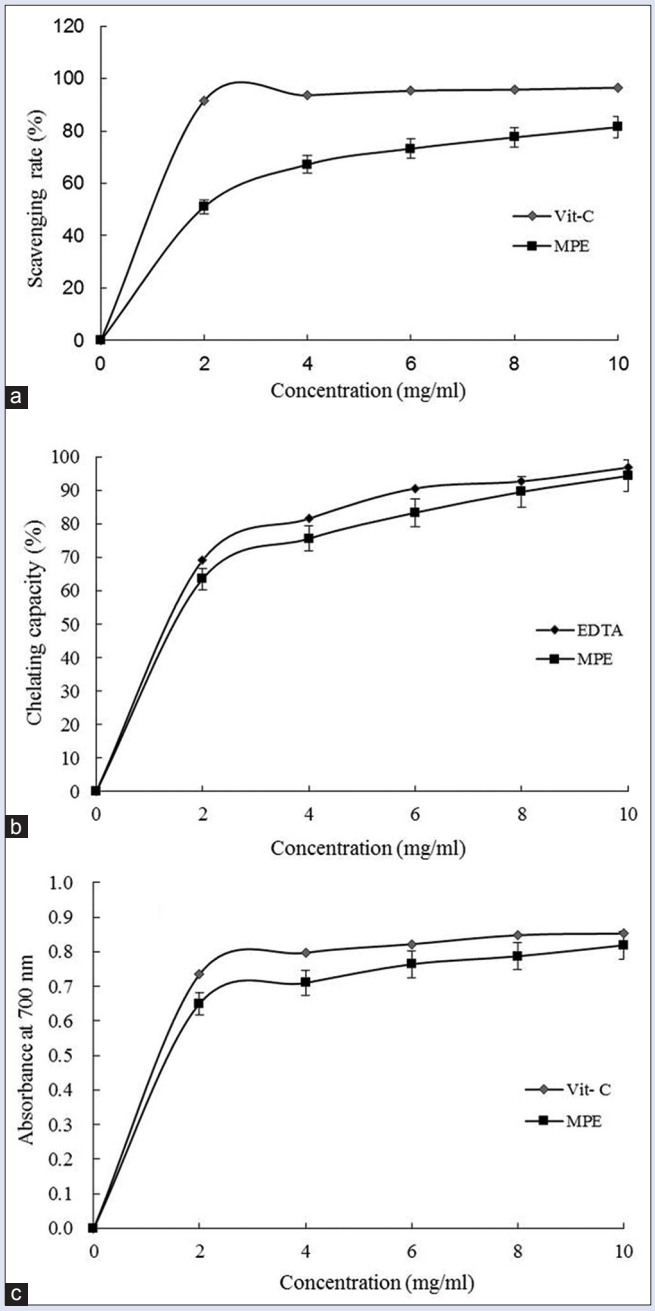

Figure 5: Antioxidant activities of purified polyphenol extract from mulberry fruit. (a) 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, (b) Fe^{2+}-chelating capacity, and (c) Reducing power

Iron participates in various cellular functions. Excess iron can generate reactive oxygen species. The iron chelating capacity would prevent transition metals from participating in the commencement of oxidative stress. Here, Fe^{2+}-chelating activity reached a level of 94.36% at high concentrations (10 mg/mL). The IC_{50} value of the MPE was 1.016 mg/mL [Figure 5b]. Reducing power is an index of a compound's antioxidant activity. The antioxidant action of the reductants present in the extract was based on the splitting up of free radical chain by donating a hydrogen atom. The resultant reducing power of MPE showed similar

Table 1: Effect of the purified polyphenol from mulberry fruit on hemolysis and inhibition of red blood cell from mice induced by H_2O_2

Group	$A_{415\ nm}$	Hemolysis (%)	Inhibition rate (%)
Normal	0.195	39.87±0.01	-
Control + H_2O_2	0.489	100	-
MPE 10 μg/mL			
30 min	0.403	82.41±0.04	29.25±1.39
60 min	0.396	80.98±0.31	31.63±2.62
90 min	0.393	80.36±0.13	32.65±0.09
120 min	0.388	79.34±0.29	34.35±0.37
150 min	0.387	79.14±0.08	34.69±1.18
MPE 30 μg/mL			
30 min	0.396	80.98±0.46	31.63±0.03
60 min	0.391	79.95±0.33	33.33±0.22
90 min	0.381	77.91±0.51	36.73±0.07
120 min	0.380	77.70±0.85	37.07±0.43
150 min	0.379	77.50±1.26	37.41±1.51
MPE 55 μg/mL			
30 min	0.348	71.16±0.39	47.95±0.92
60 min	0.346	70.75±0.81	48.63±0.16
90 min	0.335	68.50±0.01	52.38±0.25
120 min	0.332	67.89±0.12	53.40±0.76
150 min	0.330	67.48±0.21	54.08±0.41
MPE 80 μg/mL			
30 min	0.308	62.98±0.19	61.56±0.23
60 min	0.300	61.34±2.40	64.28±2.01
90 min	0.296	60.53±0.10	65.64±1.13
120 min	0.286	58.48±0.29	69.04±0.76
150 min	0.285	58.28±2.29	69.38±0.81
MPE 100 μg/mL			
30 min	0.278	56.85±0.01	71.76±1.22
60 min	0.274	56.03±0.20	73.12±0.65
90 min	0.270	55.21±4.65	74.48±0.01
120 min	0.264	53.98±0.22	76.53±0.87
150 min	0.264	53.98±0.06	76.53±0.09

Table 2: Effect of the purified polyphenol from mulberry fruit on lipid peroxidation of mice liver

Group	Lipid peroxidation	
	A_{532} nm	Inhibition rate (%)
Normal control	0.323	-
Induced control	0.622	-
MPE 10 μg/mL	0.576	15.38±0.23
MPE 30 μg/mL	0.523	33.11±0.71
MPE 55 μg/mL	0.483	46.48±0.56
MPE 80 μg/mL	0.414	69.56±0.07
MPE 100 μg/mL	0.352	90.30±0.04

activity to ascorbic acid, which was equivalent to the reducing power of the control, ascorbic acid [Figure 5c]. This shows that the extract can turn free radicals into more stable products by acting as an electron and hydrogen donor.[37] The overall results of antioxidant activity show that MPE was an efficient antioxidant *in vitro*. This may be possibly due to the presence of polyphenols such as cyanidin-3-glucoside, which is a strong antioxidant.[41] In addition, antioxidant activities of five Brazilian plants (*Hyptis martiusii, Mentha arvensis, Momordica charantia, Eugenia jambolana*, and *Eugenia uniflora*) result indicates that the ethanol extract of all the plants containing phenolic compounds possess strong antioxidant activity and are widely distributed in the plant kingdom.[42]

Oxidative damage is mediated by peroxidation of erythrocyte membranes by reactive oxygen species. Oxidant damage of the cell membrane, induced by H_2O_2, can result in increased erythrocyte hemolysis and inhibition rate.[43] Our result showed that the RBC hemolysis inhibition rate was time and dose dependent with MPE treatment. It was 76.53% by 100 μg/mL MPE for 150 min [Table 1]. The binding of polyphenols to RBC membrane matrix, in close proximity to tryptophan residues, results in inhibition of lipid peroxidation and subsequent antihemolytic activity.[37] Lipid peroxidation of mice liver induced by $FeSO_4$-ascorbic acid created membrane damage. The effects of MPE on lipid peroxidation in liver cells are shown in Table 2. The content of malondialdehyde (MDA) was high in all treatment groups. MPE decreased the amount of MDA in a dose-dependent manner. The percentage of inhibition at the highest concentration (100 μg/mL) of MPE was 90.3% with an IC_{50} value of 40.91 μg/mL. This shows that MPE has a protective role on lipid peroxidation. The antioxidant activity of cyanidin-3-glucoside, an anthocyanin of the family flavonoids, could possibly have an effect on the observed antihemolytic activity.[37] Similar findings have been reported in our previously work. The effect of flavonoids from mulberry fruit accelerates the repair of mitochondrial and microsome membrane injury induced by Fe^{2+}-Vc.[25]

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Conflicts of interest

There are no conflicts of interest.

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