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Analytical Methods

Identification of phenolics in the fruit of emblica (*Phyllanthus emblica* L.) and their antioxidant activities

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Abstract

An activity-directed fractionation and purification process was used to identify the antioxidative components of emblica fruit. Dried fruit of emblica was extracted with methanol and then partitioned by ethyl ether, ethyl acetate, butanol and water. The ethyl acetate fraction showed the strongest 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity among four fractions. The ethyl acetate fraction was then subjected to separation and purification using Sephadex LH-20 chromatography and reverse-phase high-performance liquid chromatography (HPLC). Six compounds were identified to be geraniin (1), quercetin 3-β-D-glucopyranoside (2), kaempferol 3-β-D-glucopyranoside (3), isocorilagin (4), quercetin (5), and kaempferol (6), respectively, by spectral methods, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, ultraviolet–visible (UV–Vis) spectrophotometry and mass spectroscopy (MS), and comparison with literatures. Compounds 2–4 and 6 were identified from emblica fruit for the first time. Furthermore, the antioxidant activities of purified compounds were screened for their antioxidative potential using lipid peroxidation and DPPH systems. All the purified compounds showed strong antioxidant and radical scavenging activities. Amongst, geraniin showed the highest antioxidant activity (4.7 and 65.7 μM of IC_{50} values for DPPH and lipid peroxidation assay, respectively) than other purified compounds.

Keywords: *Phyllanthus emblica* L.; Phenolic; Antioxidatant activity; Identification

1. Introduction

Emblica (*Phyllanthus emblica* L.), an euphorbiaceous plant, is widely distributed in subtropical and tropical areas of China, India, Indonesia and Malay Peninsula, and used in many traditional medicinal systems, such as Chinese herbal medicine, Tibetan medicine and Ayurvedic medicine (Zhang, Tanaka, Iwamoto, Yang, & Kouno, 2000). Emblica fruit is reported to have antioxidant (Anila & Vijayalakshmi, 2003), hypolipidemic (Anila & Vijayalakshmi, 2000) and hypoglycemic activities (Abesundara, Matsui, & Matsumoto, 2004), and acts as an important constituent of many hepatoprotective formulas available (Panda & Kar, 2003). It is also used as antimicrobial agent (Rani & Khullar, 2004), antitumor (Jeena, Kuttan, & Kuttan, 2001) or anti-inflammatory agent (Perianayagam, Sharma, Joseph, & Christina, 2004) and can improve the metal-induced clastogenic effects (Biswas, Talukder, & Sharma, 1999). Earlier work on this plant showed the occurrence of tannins, lignans, flavonoids and alkaloids (Anila & Vijayalakshmi, 2003; Houghton, Woldemariam, O'Shea, & Thyagatajan, 1996; Zhang, Abe, Tanaka, Yang, & Kouno, 2001).

In the present study, isolation and purification of phenolic constituents from emblica fruit were done using Sephadex LH-20 chromatography and reverse-phase HPLC. The structures of these purified compounds were identified using UV spectrophotometry, MS and NMR spectroscopy. Furthermore, the inhibition capacity of lipid peroxidation and DPPH radical scavenging activity of the purified compounds were also investigated.

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2. Materials and methods

2.1. Plant materials

Fresh fruits of emblica were collected from Huizhou, Guangdong province of China. The weather of the planting region was subtropical oceanic climate. The sample were picked at commercial harvest time (11 November 2005) and selected for the uniformity of shape and maturity.

2.2. Chemicals

DPPH and thiobarbituric acid (TBA) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA), while vitamin E and butylated hydroxyanisole (BHA) were obtained from Fluka Co. All other reagents used were of analytical grade.

2.3. Extraction and isolation

Fresh emblica fruit flesh were air-dried in an oven at 40 °C and then ground in a cutting mill to obtain particle size in the range of 0.2–0.4 mm. According to the method reported by Jayaprakasha, Ohnishi-Kameyama, Ono, Yoshida, and Jaganmohan (2006), the fruit powder (100 g) was extracted with 60% (v/v) methanol with a ratio of 1:10 (g/ml) at 50 °C for 3 h. After filtering of the extract through 0.45 μm filter paper, the residue was re-extracted and then filtered. Filtrates were combined and concentrated using a rotary evaporator at 40 °C, and then freeze-dried in vacuum. The methanolic extract (21.5 g) was dissolved in water (40 ml) and then partitioned with ethyl ether, ethyl acetate and butanol (each 400 ml) successively. The fractions were concentrated under reduced pressure at 35 or 65 °C to remove solvents. The yields of ethyl ether, ethyl acetate, butanol and aqueous fractions were 0.9, 5.5, 7.3 and 7.4 g, respectively. The total phenolic content (TPC) in each fracton was determined by the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). One milliliter of extract in methanol was mixed with 1 ml of 3-fold-diluted Folin–Ciocalteu phenol reagent. Two milliliters of 35% sodium carbonate solution and distilled water were added subsequently. The mixture was incubated for 30 min and the absorbance was recorded at 700 nm by a spectrophotometer (Unico, Shanghai, China). In this study, most of phenolics were detected in the ethyl acetate fraction (Table 1). Furthermore, the ethyl acetate fraction showed the lowest IC_{50} value on DPPH radical scavenging activity among four fractions (Table 1). The result suggested that the compounds with relatively high antioxidant activity might be contained in this fraction. Therefore, the ethyl acetate fraction was subjected to further purification and identification.

The ethyl acetate fraction (8.5 g) was fractionated on a Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co. Ltd., Uppsala, Sweden) column (450 × 25 mm), eluted with water/methanol (1:0–0:1) to obtain eight fractions (I–VIII). Fraction IV (130 mg) was further purified by reverse-phase HPLC (Waters 600E, Waters, Milford, USA) on a C_{18} μ-Bondapak column (300 × 7.8 mm, flow rate = 2.0 ml/min) with methanol/water (45:55) for 32 min to yield pure compounds 1–3. Fraction VI (340 mg) was also chromatographed on this C_{18} μ-Bondapak column (300 × 7.8 mm) with methanol/water (3:7) for 30 min to give pure compounds 4–6. The flow rate was also set as 2.0 ml/min.

Table 1
Total phenolic content and free-radical scavenging activity of various fractions[a]

Samples	Total phenolics (mg/g extract)	IC_{50} for DPPH radical scavenging activity (μg/ml)[b]
Ethyl ether fraction	269.3 ± 0.4	72.1 ± 3.1
Ethyl acetate fraction	439.9 ± 1.3	12.6 ± 0.2
Butanol fraction	203.5 ± 2.1	23.5 ± 3.5
Aqueous fraction	62.5 ± 0.7	142.6 ± 5.3
Fraction IV	513.5 ± 3.6	6.8 ± 0.3
Fraction VI	639.9 ± 2.9	4.2 ± 0.2
Vitamin E[c]	–	18.3 ± 0.3

[a] Each value was expressed as the mean ± SD ($n = 3$).
[b] IC_{50} value was determined to be the effective concentration at which DPPH radical was scavenged by 50%.
[c] Positive control.

2.4. UV–Vis spectrophotometric analysis

Each of compounds 1–6 (1 mg) was dissolved in 10 ml of methanol. The sample solution was scanned from 200 to 600 nm, using a UV–Vis spectrophotometer (Shimadzu UZ-2201, Shimadzu Co., Kyoto, Japan).

2.5. Molecular weight estimation

MS system (LCQ^{DECA}, Finigan company, USA), equipped with a Hewlett–Packard 9000 computer system, was used to determine the molecular weight. Sample (1 mg) was dissolved in 10 ml of methanol. One hundred microliter of sample solution was injected into the MS system. Mass spectroscopy was recorded with a heat capillary voltage of 4.5 kV, a heat capillary temperature of 280 °C, sheath gas flow rate of 70 units and auxiliary gas flow rate of 10 units. The scan range of m/z was 200–1200.

2.6. NMR spectroscopy

^{1}H and ^{13}C NMR spectra were recorded with a FT-NMR Bruker advance spectrometer (Bruker ARX 400, Bruker Biospin Co., Karlsruhe, Germany), and tetramethylsilane was used as an internal standard.

2.7. Determination of antioxidant activity

2.7.1. DPPH radical scavenging activity

The DPPH radical scavenging activity was determined by the method of Yang et al., 2006. Samples were dissolved

in methanol to prepare various solutions at concentrations of 80, 40, 20, 10, 8, 5 and 2 μg/ml. Each sample solution (2 ml) was mixed with 1 ml of 0.2 mM DPPH in methanol. The mixture was shaken vigorously and maintained for 30 min in dark. The absorbance was measured at 517 nm. The absorbance of the control was obtained by replacing the sample with methanol. BHA and vitamin E were used as positive control. The scavenging activity was calculated using the formula, Scavenging activity (%) = [(A_{517} of control A_{517} of sample)/A_{517} of control] × 100.

2.7.2. Inhibition of microsome lipid peroxidation

Lipid peroxidation of rat microsome was carried out as reported earlier (Sabu & Kuttan, 2002). Reaction mixture (0.5 ml) containing 0.1 ml (25%, w/v) of rat liver homogenate in Tris–HCl buffer (40 mM, pH 7.0), 30 mM KCl (100 μl), 0.16 mM ferrous iron (100 μl), 0.06 mM ascorbic acid (100 μl) and the sample solution (100 μl) at various concentrations were incubated for 1 h at 37 °C. The lipid peroxide formed was measured by thiobarbituric acid reactive substances (TBARS) values (Ohkawa, Ohishi, & Yagi, 1979). One milliliter of aliquot were added to 2 ml of thiobarbituric acid reagent (15% trichloroacetic acid, 0.375% thiobarbituric acid and 0.25 M hydrochloric acid). Then heated in boiling bath for 15 min. After cooling down to room temperature, it was centrifuged at 1000*g* for 10 min. The absorbance of supernatant at 532 nm was recorded. The inhibition capacity of lipid peroxidation was determined by comparing the results of the test compounds with that of control. The percent antioxidant activity using the following equation, lipid peroxidation inhibition (%) = [(A_{532} of control A_{532} of sample)/A_{532} of control] × 100. BHA and vitamin E were used as positive control.

2.8. Statistical analysis

Data were presented as mean ± standard deviation (SD) of three determinations. Statistical analyses were performed using a one-way analysis of variance. The IC_{50} values were calculated by linear-regression analysis. Results were calculated by employing the statistical software (SPSS 13.0, SPSS Inc., USA).

3. Results and discussion

3.1. Comparison of phenolic content and antioxidant activity of four fractions

Phenolic compounds have been proved to be responsible for the antioxidant activity of emblica fruit (Kumar, Nayaka, Dharmesh, & Salimath, 2006). The total phenolic contents of all fractions and their DPPH radical scavenging activities were shown in Table 1. The ethyl acetate fraction showed the highest phenolic content (439.9 mg/g) and DPPH radical scavenging activity (IC_{50} 12.6 μg/ml) as compared with other three solvent fractions. This fraction was subjected to preliminary purification on a Sephadex LH-20 column, giving eight main fractions, which were tested under the same experimental conditions. In comparison with the ethyl acetate fraction, fraction IV and VI were much stronger in the DPPH test (IC_{50} 6.8 and 4.2 μg/ml, respectively) and showed a higher level of total phenols (513.5 and 639.9 mg/g, respectively), suggesting that it contained a higher concentration of the active principles responsible for the observed free-radical scavenging activity. The activities of these two fractions were higher than that of vitamin E (IC_{50} 18.3 μg/ml) used as a positive control.

3.2. Separation of the phenolics from ethyl acetate fraction

With the aim to characterize the phytochemical profile of emblica, fractions IV and VI were purified by HPLC (Figs. 1 and 2). In this analysis, the chromatographic separation from the fraction IV yield pure compounds 1 (retention time (t_R) = 21.161 min, 18.0 mg), 2 (t_R = 22.450 min, 15.0 mg) and 3 (t_R = 23.850 min, 7.5 mg) (Fig. 1). The chromatographic separation of fraction VI afforded three major compounds 4 (t_R = 12.574 min, 13.0 mg), 5 (t_R = 16.402 min, 15.0 mg) and 6 (t_R = 21.015 min, 8.5 mg) (Fig. 2). Thus, these isolated compounds were used for further identification.

3.3. Identification of compounds 1–6

Compound 1: greenish yellow crystalline powder; UV λ_{max} (CH_3OH) nm: 218, 274; ESI-MS, *m/z* 953 $[M+H]^+$, and 975 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 4.36 (m), 4.45 (m), 4.82 (m), 4.98 (m), 5.21 (s), 5.45 (brs), 5.52 (s), 5.55 (s), 5.59 (s), 6.29 (d), 6.58 (s), 6.59 (brs), 6.69 (s), 6.71 (s), 7.10 (s), 7.16 (s), 7.22 (s), 7.24 (s), 7.26 (s) and 7.30 (s); ^{13}C NMR (CD_3OD): δ 92.6 (C-1), 74.6 (C-5), 63.9 (C-3), 71.7 (C-2), 68.2 (C-4), 65.3 (C-6), 112.1 (C-2, 6), 118.7 (C-1), 141.7 (C-4), 147.4 (C-3, 5), 166.5 (C=O) (A ring); 126.5 (C-2), 125.2 (C-2′), 147.1 (C-6), 147.1 (C-6′), 118.6 (C-1), 117.2 (C-1′), 144.3 (C-4′), 147.1 (C-4), 139.7 (C-5), 138.5 (C-5′), 109.2 (C-3), 111.4 (C-3′), 168.4 (C′=O), 170.9 (C=O) (B and C ring); 116.5 (C-1), 138.5 (C-5), 138.5(C-3), 118.1 (C-2), 146.4 (C-4), 146.2 (C-6), 166.9 (C=O) (D ring); 53.5 (C-1), 96.7 (C-5), 125.9 (C-3), 149.3 (C-2), 196.0 (C-4), 97.4 (C-6), 167.1 (C=O) (E ring). The data were in agreement with the reported literature values (Adesina et al., 2000). Thus, the structure of compound 1 was determined as geraniin.

Compound 2: yellow amorphous powder; UV λ_{max} (CH_3OH) nm: 265, 290; negative ESI-MS *m/z*: 463 $[M-H]^-$; ^1H NMR (CD_3OD): δ 6.17 (1H, d, J = 1.8 Hz, H-6), 6.36 (1H, d, J = 1.8 Hz, H-8), 7.70 (1H, d, J = 1.8 Hz, H-2′), 6.86 (1H, d, J = 8.1 Hz, H-5′), 7.56, 7.57 (1H, dd, J = 1.8, 8.1 Hz, H-6′), 5.22 (1H, d, J = 7.5 Hz, H-1″), 3.20-3.71(6H, m, H-2″, 6″); ^{13}C NMR (CD_3OD): δ 159.7 (C-2), 135.6 (C-3), 179.5 (C-4), 162.9 (C-5), 99.9 (C-6), 166.0 (C-7), 94.7 (C-8), 158.4 (C-9), 105.7 (C-10), 123.1 (C-1′), 117.6 (C-2′), 145.8 (C-3′),

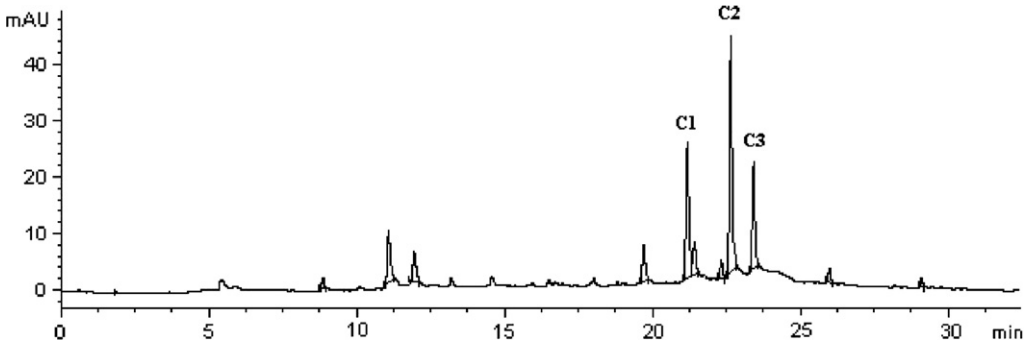

Fig. 1. Reverse-phase high-performance liquid chromatogram of fraction IV of the ethyl acetate fraction of emblica extract monitored at 275 nm.

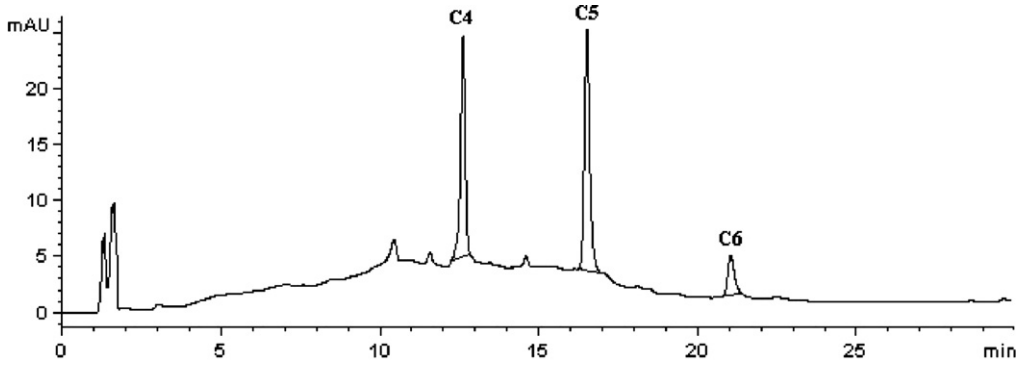

Fig. 2. Reverse-phase high-performance liquid chromatogram of fraction VI of the ethyl acetate fraction of emblica extract monitored at 275 nm.

149.8 (C-4′), 116.0 (C-5′), 123.2 (C-6′), 104.5 (C-1″), 75.7 (C-2″), 78.1 (C-3″), 71.3 (C-4″), 78.3 (C-5″), 62.6 (C-6″). The ^{1}H NMR spectrum of compound 2 showed two peaks (δ 6.17 and 6.36, $J = 1.8$ Hz) consistent with the *meta* protons H-6 and H-8 on the phloroglucinol A-ring and an ABX system (δ 6.86, $J = 8.1$ Hz; 7.56, 7.57, $J = 1.8$ and 8.1 Hz) for the catechol B-ring protons, while the ^{13}C NMR spectrum was consistent with that of quercetin 3-β-D-glucopyranoside reported by Markham and Chari (1982).

Compound 3: yellow amorphous powder; UV λ_{max} (CH_3OH) nm: 265, 340; negative ESI-MS *m/z*: 447 $[M-H]^-$; ^{1}H NMR (DMSO-d_6): δ 3.08–3.65 (m, sugar-H), 5.45 (d, $J = 7.2$ Hz, H-1″), 6.19 (d, $J = 1.9$ Hz, H-6), 6.42 (d, $J = 1.8$ Hz, H-8), 6.88 (d, $J = 8.8$ Hz, H-3′, 5′), 8.04 (d, $J = 8.9$ Hz, H-2′, 6′), 12.60 (s, OH-5); ^{13}C NMR (DMSO-d_6): δ 60.8 (C-6″), 69.8 (C-4″), 74.18 (C-2″), 76.4 (C-5″), 77.5 (C-3″), 93.7 (C-8), 98.7 (C-6), 100. 9 (C-1″), 103.8 (C-10), 115.1 (C-3′, 5′), 120.8 (C-1′), 130.8 (C-2′, 6′), 133.2 (C-3), 156.2 (C-2), 156.4 (C-9), 159.9 (C-4′), 161.2 (C-5), 164.5 (C-7), 177.4 (C-4). Compound 3 exhibited a similar ^{13}C NMR spectra to that of quercetin 3-glucoside but instead of the catechol B-ring, there was a para-substituted phenol ring (δ 115.1 × 2, 120.9, 130.8 × 2, 159.9). The latter was also confirmed by the ^{1}H NMR spectrum which contained an AB system (δ 6.86 and 8.06, $J = 8.6$ Hz). These spectral characteristics suggested that compound 3 was kaempferol 3-β-D-glucopyranoside (Lu & Foo, 1999).

Compound 4: colorless, amorphous powder; UV λ_{max} (CH_3OH) nm: 219, 270; ESI-MS: *m/z* 633 $[M-H]^-$, 657 $[M+Na]^+$; ^{1}H NMR (CD_3OD): δ 7.04 (1H, s, galloyl proton (Gal) H-2, 6), 6.67 (2H, s, 2H of hexahydroxydiphenoyl portion (HHDP)), 6.65 (1H, s, HHDP H-3′), 6.36 (1H, d, $J = 2.0$, glucose (Glc) H-1), 4.95 (1H, t, $J = 11$, Glc H_a-6), 4.79 (1H, brs, Glc H-3), 4.51 (1H, t, $J = 8$, Glc H-5), 4.45 (1H, d, $J = 2.0$, Glc H-4), 4.15 (1H, dd, $J = 11$, 8, Glc H_b-6), 3.97 (1H, d, $J = 2.0$, Glc H-2); ^{1}C NMR (CD_3OD): 95.0 (Glc C-1), 76.3 (Glc C-5), 71.7 (Glc C-3), 69.5 (Glc C-2), 65.1 (Glc C-4), 62.6 (Glc C-6), 111.1 (Gal C-2, 6), 120.8 (Gal C-1), 140.5 (Gal C-4), 146.5 (Gal C-3, 5), 166.8 (Gal C=O), 108.5 (HHDP C-2), 110.4 (HHDP C-2′), 117.3 (HHDP C-6), 116.8 (HHDP, C-6′), 125.6, 125.7 (HHDP C-1, 1′), 137.7 (HHDP C-4′), 138.2 (HHDP C-4), 145.4, 145.8 (HHDP C-3, 5), 146.2 (HHDP C-3′, 5′), 168.6 (HHDP C′=O), 170.2 (HHDP C=O). The ^{1}H and ^{13}C NMR spectra showed characteristic signals for a hydrolysable tannin, with galloyl, 1-*O*-galloyl-3, 6-(*R*)-hexa-hydroxydiphenoyl and glucose unit. Compared the ^{1}H NMR spectrum (CD_3OD) of compound 4 with corilagin (Nawwar, Hussein, & Merfort, 1994), it was almost superposable with that of corilagin, excepted that: the ^{1}H NMR spectrum of corilagin exhibited a doublet at δ 6.20 ($J = 7.2$ Hz), attributable to H-1 of the glucose moiety and indicative of the β-configuration at the anomeric centre. This finding is explicable in terms of the solvent-dependent conformational change (1C → 1B) of the glucose core (Seikel & Hillis, 1970). In contrast, the ^{1}H NMR spectra of compound 4 displayed

the signal of the corresponding proton as a doublet at δ 6.36 ($J = 2$ Hz), thus indicating an equatorial-axial arrangement of H-1 and H-2. Accordingly, compound 4 represented the unique isomer of corilagin and was named isocorilagin.

Compound 5: yellow powder; UV λ_{max} (CH_3OH) nm: 265, 344; ESI-MS: m/z 287 $[M+H]^+$; ^1H NMR (CD_3OD): δ 7.72 (1H, d, $J = 2.1$ Hz, H-2′), 7.62 (1H, dd, $J = 8.4$, 2.1 Hz, H-6′), 6.87 (1H, d, $J = 8.4$ Hz, H-5′), 6.37 (1H, d, $J = 2.0$ Hz, H-8), 6.17 (1H, d, $J = 2.0$ Hz, H-6); ^{13}C NMR (CD_3OD): δ 177.3 (C-4), 165.6 (C-7), 162.5 (C-5), 158.2 (C-9), 148.8(C-4′), 148.0 (C-2), 146.2 (C-3′), 137.2 (C-3), 124.2 (C-1′), 116.2 (C-2′, 5′), 116.0 (C-2′), 104.5 (C-10), 99.2 (C-6), 94.4 (C-8). The data were in agreement with the reported literature values of Fossen, Pedersen, and Andersen (1998). Hence, compound 5 was quercetin.

Compound 6: yellow powder; UV λ_{max} (CH_3OH) nm: 258, 365; ESI-MS: m/z 287 $[M+H]^+$; ^1H NMR (CD_3COCD_3): δ 8.10 (2H, d, $J = 9.0$ Hz, H-2′, 6′), 6.97 (2H, d, $J = 9.0$ Hz, H-3′, 5′), 6.24 (1H, d, $J = 2.0$ Hz, 6-H), 6.50 (1H, d, $J = 2.0$ Hz, 8-H), 6.28 (1H, d, $J = 2.1$, H-6); ^{13}C NMR (CD_3COCD_3): δ 175.6 (C-4), 164.1 (C-7), 156.8 (C-5), 159.3 (C-4′), 161.0 (C-9), 146.1 (C-2), 135.5 (C-3), 129.4 (C-2′, 6′), 122.1 (C-1′), 115.3 (C-3′, 5′), 103.0 (C-10), 98.1 (C-6), 93.5 (C-8). The data were in agreement with the reported literature values (Ohmura, Ohara, Hashida, Aoyama, & Doi, 2002) and compound 6 was identified as kaempferol.

Hence, compounds 1–6 were identified as geraniin (1), quercetin 3-β-D-glucopyranoside (2), kaempferol 3-β-D-glucopyranoside (3), isocorilagin (4), quercetin (5) and kaempferol (6), respectively, by detailed spectroscopic analyses and comparing with the literature data. Their chemical structures were shown in Fig. 3. Compounds 2–4 and 6 were identified from emblica fruit for the first time. Pozharitskaya, Ivanova, Shikov, and Makarov (2007) have isolated four compounds (emblicanins A, emblicanins B, gallic acid and ellagic acid) from *Emblica officinalis* extract by thin layer chromatography. Emblicanins A and B are hydrolysable tannins, while geraniin and isocorilagin identified in this work are ellagitannins which can produce gallic acid by degradation. The isolation method should be responsible for the composition differences.

3.4. Antioxidant activity

3.4.1. DPPH radical scavenging activity

Free radicals play an important role in some pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, liver cirrhosis, cardiovascular diseases, atherosclerosis, cataracts, diabetes and inflammation (Aruoma, 1998). One of the methods for measuring antioxidant potential is through determining the free radical inhibitory ability of different antioxidant by using very stable free radicals, such as DPPH in methanol solution. DPPH is a stable nitrogen-centred free radical, and its colour changes from violet to yellow when is reduced by either the process of hydrogen- or elctron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Brand-Williams, Cuvelier, & Berset, 1995).

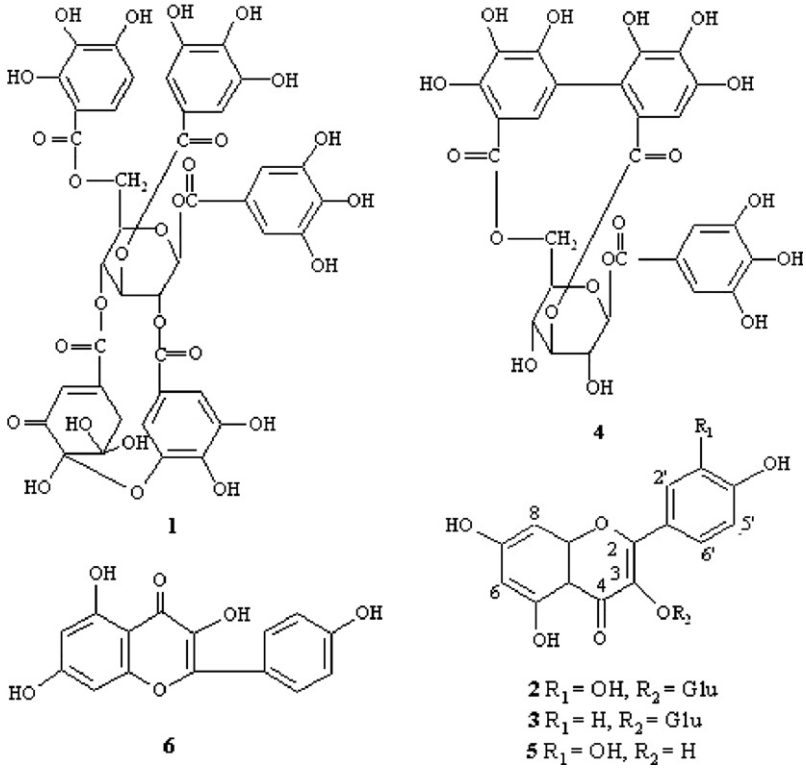

Fig. 3. Chemical structures of six phenolic compounds isolated from emblica fruit.

Table 2
Inhibition capacity of lipid peroxidation and DPPH radical scavenging activity of phenolic compounds isolated from emblica fruit[a]

Samples	IC_{50} value (μM)[b]	
	DPPH radical scavenging activity	Inhibition capacity of lipid peroxidation
Geraniin (1)	4.7 ± 0.5	65.7 ± 2.5
Qquercetin 3-β-D-glucopyranoside (2)	39.7 ± 0.9	183.6 ± 4.2
Kaempferol 3-β-D-glucopyranoside (3)	110 ± 1.1	202.1 ± 8.3
Isocorilagin (4)	5.9 ± 0.3	68.4 ± 5.1
Quercetin (5)	13.6 ± 0.7	74.8 ± 5.8
Kaempferol (6)	33.6 ± 1.5	99.0 ± 3.2
Vitamin E	201.2 ± 3.5	79.8 ± 1.7
BHA	33.3 ± 0.5	56.5 ± 0.3

[a] IC_{50} value was determined to be the effective concentration at which DPPH radical was scavenged by 50% and lipid peroxidation were inhibited by 50%, respectively. The IC_{50} value was obtained by interpolation from linear regression analysis.
[b] Each value was expressed as the mean ± SD ($n = 3$).

The antioxidant activities of compounds 1–6 and controls, BHA and vitamin E, were shown in Table 2, respectively. Most of the isolated compounds exhibited considerable scavenging activity on DPPH radical and the increasing order of their activities was vitamin E < kaempferol 3-β-D-glucopyranoside < quercetin 3-β-D-glucopyranoside < BHA < kaempferol < quercetin < isocorilagin < geraniin. This observation suggested that the antioxidant activity of phenolics increased when there were more free hydroxyl groups in the molecule, which agreed with the previous report by Kumaran and Karunakran (2006). Flavonoids (Quercetin 3-β-D-glucopyranoside, kaempferol 3-β-D-glucopyranoside, quercetin and kaempferol), isolated from emblica, displayed antioxidant activities from moderate to weak on DPPH assay and flavonoids belonging to the quercetin class (Quercetin and quercetin 3-β-D-glucopyranoside) possessed higher antioxidant activities than kaempferol and its derivative (kaempferol 3-β-D-glucopyranoside), due to their possessing an *O*-dihydroxy B-ring structure, which conferred higher stability in the radical form and participated in electron delocalisation. This conclusion was consistent with those reported in the literature (Pietta, 2000) and confirmed that *O*-glycosylation at C-3 had a negative effect on antioxidant activity, and the presence of a bigger substituent at the C-3 position will reduce the antioxidant activity (Cioffi et al., 2002). The substitution of C-3 hydroxyl group by sugar moiety is reponsible for the change of antioxidant activity.

3.4.2. Inhibition capacity of lipid peroxidation

Lipid peroxidation can inactivate cellular components and plays an important role in oxidative stress of biological systems. Furthermore, several toxic byproducts from the peroxidation can damage other bio-molecules (Box & Maccubbin, 1997). It is well established that transition of metal ions, such as iron and copper, can stimulate lipid peroxidation through various mechanisms. They may either promote the generation of hydroxyl radicals to initiate the lipid peroxidation process or propagate the chain process via decomposition of lipid hydroperoxides (Braughler, Chase, & Pregenzer, 1987).

In this study, six purified phenolics showed significant inhibition effect on the lipid peroxidation (Table 2). The highest inhibition capacity of lipid peroxidation was observed for geraniin (IC_{50} 65.7 μM), followed by iscorilagin (IC_{50} 68.4 μM), while Kaempferol 3-β-D-glucopyranoside exhibited the lowest inhibition capacity (IC_{50} 202.1 μM). The IC_{50} values of DPPH radical scavenging activity was correlated well with the inhibition capacity of lipid peroxidation (correlation coefficient = 0.8713, data not shown). This result was consistent with the report of Rekka and Kourounakis (1991).

4. Conclusion

The ethyl acetate fraction contained most of phenolics presented in emblica fruit. Six phenolic compounds were purified by reverse-phase HPLC and identified as geraniin (1), quercetin 3-β-D-glucopyranoside (2), kaempferol 3-β-D-glucopyranoside (3), isocorilagin (4), quercetin (5) and kaempferol (6), respectively, by analyses of UV–Vis spectrophotometry, MS and NMR spectroscopy, and comparison with the literatures. In addition, most of the purified compounds showed strong antioxidant and radical scavenging activities. Thus, the antioxidant activity of emblica fruit may be one of the mechanisms by which these compounds are used as foodstuff as well as traditional medicine.

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