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Comparison of phenolic content and antioxidant activity of two common fruits of Bangladesh in solvents of varying polarities

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Abstract

Phenolic content and antioxidant activity of two common fruits of Bangladesh, namely *Phyllunthus emblica* and *Elaeocarpus floribundus*, were measured in water, methanol, ethanol, acetone and hexane extracts. Several *in vitro* models including phosphomolybdenum assay, DPPH free radical scavenging assay, FRAP assay and reducing power assay were used to assess the antioxidant activity of these extracts in comparison with reference antioxidants. Between the two fruits, *P. emblica* showed higher phenolic content and antioxidant activity in all the solvents used. In the DPPH scavenging assay, the activity of *P. emblica* extracts was close to reference antioxidants, ascorbic acid and BHT. Besides, considering the solvents used, extracts of both fruits had the highest phenolic and antioxidant activity in polar solvents. The correlation coefficient between total phenolics and antioxidant activities was found statistically significant. These findings suggest that *P. emblica* could be an excellent antioxidant resource for industries like food, pharmaceutical, and cosmetics.

1. Introduction

In a biological system, reactive oxygen species and reactive nitrogen species such as superoxide, hydroxyl, and nitric oxide radicals can damage DNA and lead to the oxidation of lipid and protein in cells, causing some chronic and degenerative diseases (Nunes *et al*., 2012; Aktumsek *et al*., 2013; Xu *et al*., 2017). Recent studies indicate that because of the presence of antioxidants especially, polyphenols and carotenoids, frequent consumption of fruits and vegetables is associated with a lower risk of inflammation, stroke, cancer, diabetes and neurodegenerative diseases (Garsia-Salas *et al*., 2010; Khoddami *et al*., 2013; Zhang *et al*., 2015). Antioxidant molecules block both the initiation and propagation of oxidizing chain reactions, thereby impeding or slowing the oxidation process (Lobo *et al*., 2010; Nunes *et al*., 2012).

Fruits of *P. emblica* and *E. floribundus*, locally known as amloki and jolpai, respectively, are two well-consumed fruits of Bangladesh. The plant *P. emblica*, belonging to the family Euphorbiaceae, is indigenous to the tropical region of Southeast Asia. It is generally found throughout the forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Dinajpur, Tangail, Sylhet, and villages of Bangladesh (Uddin *et al*., 2016). It is highly nutritious and rich in vitamin C, minerals and amino acids. All parts of this plant have medicinal properties, especially the fruits that are used in Ayurveda as a potent Rosayan (rejuvenator). The plant is reported to have various pharmaceutical potentials including anti-inflammatory, analgesic and antipyretic, antioxidant, antimicrobial, hepatoprotective, antitumor, immunomodulatory, anti-atherogenic, antiulcerogenic, and adaptogenic activities (Khopde *et al*., 2001; Krishnaveni and Mirunalini, 2010; Gaire and Subedi, 2014; Uddin *et al*., 2016; Chaphalkar *et al*., 2017).

On the other hand, plant *E. floribundus* belongs to the family Elaeocarpaceae and is found in the South and East Asian region including Bangladesh (Zaman, 2016; Swargiary *et al*., 2017). The fruits of this plant are traditionally used to prepare pickles (Zaman, 2016) and the seeds are used to prepare oil in Myanmar (Shin *et al*., 2018). The plant has various biological activities like antioxidant, antibacterial, cytotoxic, anti-ageing and anticancer (Utami *et al*., 2013; Sircar and Mandal, 2017; Deivasigamani and Devi, 2018).

The phenolic composition of plants depends on many factors such as genetic, seasonal, agronomic, maturation stage and growing conditions like temperature and rainfall (Hilton and Palmer-Jones, 1973;

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Wang and Zheng, 2001; Zietz *et al*., 2010). The solubility of phenolic compounds in the extraction medium, however, affect their extraction from plant tissues (Park *et al*., 2014).

There are many reports on the phenolic contents and antioxidant activities of *P. emblica* fruits extracted with different solvents and grown in South and East Asian regions especially in India, China, Indonesia, Thailand (Khopde *et al*., 2001; Charoenteeraboon *et al*., 2010; Li *et al*., 2015; Fitriansyah *et al*., 2018; Li *et al*., 2019). However, there is a scarcity of studies, if any, showing the effect of solvent types, covering apolar to the polar range, on the phenolic content and antioxidant activities of this fruit. Although the phenolic contents and antioxidant activities of *P. emblica* fruit grown in Bangladesh has been reported (Rahman *et al*., 2016; Mondal *et al*., 2017) but the effect of the solvents on the extraction of phenolic contents and antioxidant activities are not studied. Moreover, detail antioxidant activities of this fruit by different *in vitro* methods and their comparison with reference antioxidants were not studied yet.

On the other hand, to the best of our knowledge, limited research has been done on extraction, quantification and comparison of phenolic compounds of *E. floribundus* fruit with different solvents. Sircar and Mandal (2017) reported the phenolic content and DPPH free radical scavenging activity of this fruit in ethanol and aqueous extract. However, the report on the phenolic compositions and antioxidant activities of this fruit grown in Bangladesh is seldom, if any.

In this study, the phenolic contents and antioxidative capacities of *P. emblica* and *E. floribundus* fruits grown in Bangladesh have been investigated and compared. Moreover, the effect of solvents on the extraction of phenolic compounds with the antioxidant potential of these two fruits has also been studied in order to find the appropriate solvent for extraction.

2. Materials and methods

2.1 Chemicals and reagents

Folin-ciocalteu reagent, sodium carbonate, sodium nitrate, ferric chloride, sodium acetate were purchased from BDH Chemicals Ltd. Poole, England. Ethanol, sulphuric acid, hydrochloric acid, glacial acetic acid and disodium hydrogen phosphate were purchased from Merck, Darmstadt, Germany. Aluminium chloride, sodium hydroxide and sodium dihydrogen phosphate were purchased from Merck, Mumbai, India. Gallic acid, 2,4,6-Tris (1-pyridyl)-5-triazine (TPTZ), 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT) and potassium ferricyanide were purchased from Sigma-Aldrich, St. Louis, USA. Ammonium molybdate and trichloroacetic acid (TCA) were purchased from Loba chemie, Mumbai, India. All chemicals and reagents used in this study were of analytical grade.

2.2 Plant materials

Fruits of *Phyllanthus emblica* L. and *Elaeocarpus floribundus* Blume (Figure 1) were collected from Chittagong, Bangladesh in October to November, 2018 and were properly authenticated by Dr. Sarder Nasir Uddin, Principal Scientific Officer, Bangladesh National Herbarium, Dhaka, Bangladesh where voucher specimens with accession numbers DACB 51334 and DACB 51335, respectively have been deposited. All the selected fruits were identical in size, shape, colour, ripening stages and were also of eating quality.

Figure 1. The studied fruits. A – *P. emblica*, B – *E. floribundus*.

2.3 Sample preparation

The freshly collected matured fruits were initially washed thoroughly with tap water until the attached dust particles, unicellular algae etc. were removed. Finally, they were washed with distilled water. The fruits were chopped and seeds were removed. The resulting fruit parts were dried in Economy Incubator (Size 2) at 50°C for 10 hrs. The dried fruits were grounded into powder with a grinder. The powders were stored separately in air -tight containers and kept in a refrigerator at -80°C.

For extraction of phenolic compounds, 10 g powder of each fruit was placed separately in a conical flask and soaked in 100 mL of a different solvent system. Each container was sealed and shaken intermittently for 4 days. On the 5th day, fruit extracts were filtered through 0.45 µm filter paper and the filtrate was stored in a fresh conical flask at 4°C. To the residue, 100 mL solvent was further added and left for 3 days with regular shaking. On the 7th day, the filtration process was repeated as was done previously and the resulting extract was mixed with those previously stored. Then the preparations were evaporated in a rotary evaporator at 50°C to get the solid crude extract. The extraction yield was measured and expressed as a percentage. The obtained crude extract

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was kept in a refrigerator at 4°C until further analysis. Five different pure solvent systems including water, methanol, ethanol, acetone and n-hexane (Gorinstein *et al*., 2010; Lee *et al*., 2015) with the relative polarity of 1, 0.762, 0.654, 0.355 and 0.009, respectively were used for extraction. To avoid microbial contamination while extracting with water, aseptic conditions were maintained (Aiyegoro and Okoh, 2009). Dimethyl sulfoxide (DMSO) was used to dissolve all extracts for subsequent analysis.

2.4 Determination of total polyphenol content

Total polyphenol content was estimated following the method of Ough and Amerine (1988). A 20 µL of sample extract was diluted with 2.58 mL distilled water. Then 100 µL of Folin-Ciocalteu reagent was added. After 1 min, 300 µL of 20% sodium carbonate solution was added and mixed. The mixture was then incubated for 2 hours at room temperature. The absorbance of the resulting blue colour supernatant was measured at 765 nm (UV-1601 Shimadzu, Kyoto, Japan). All samples were analyzed in triplicates. A standard calibration curve of gallic acid (0.002 - 0.01 mg/mL, r^2 = 0.997) was plotted. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry fruits weight (DW).

2.5 Determination of total flavonoid content

Total flavonoid contents of the fruit extracts were determined following the method of Zhishen *et al*. (1999). An aliquot (0.2 mL) of fruit extract was mixed with 4.8 mL of distilled water and 0.3 mL of (5% w/v) $NaNO_2$ was added. After 5 min, 0.3 mL of (10% w/v) $AlCl_3$ was added and after another minute, 2 mL of NaOH (1 M) was added. To make the final volume 10 mL, 2.4 mL of distilled water was added immediately. The absorbance of the vigorously mixed resulting solution was read at 510 nm (UV-1601 Shimadzu, Kyoto, Japan). A calibration curve was prepared using a standard solution of quercetin (0.002- 0.01 mg/mL, r^2 = 0.992). The results were presented as mg quercetin equivalents (QE)/ g DW.

2.6 Determination of antioxidant activities

2.6.1 DPPH free radical-scavenging activity (FRSA) assay

The reaction mixture (total volume, 3 mL), consisting of 0.5 mL of 0.5 M acetic acid buffer solution (pH 5.5), 1 mL of 0.2 mM DPPH in ethanol, and 1.5 mL of 50% (v/v) ethanol aqueous solution with different concentrations of extracts, was shaken vigorously (Hossain *et al*., 2008). After incubation at room temperature (25°C) for 30 mins, the amount of DPPH remaining was determined by measuring absorbance at 517 nm. Ascorbic acid and BHT were used as a reference. The percentage of inhibition of DPPH radicals was calculated using the following formula:

$$\%\ \text{inhibition} = \frac{(\text{Acontrol} - \text{Asample}) \times 100}{\text{Acontrol}}$$

Where Acontrol is the absorbance of DPPH solution without extract.

2.6.2 Evaluation of total antioxidant capacity

The total antioxidant capacities of the extracts were evaluated by phosphomolybdenum method according to Prieto *et al*. (1999). An aliquot of 400 µL of sample solution was combined in a screw cap tube with 4 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95^0C for 90 mins and cooled to room temperature. The absorbance was measured at 695 nm against a blank. The typical blank was prepared as a sample except solvent DMSO was added instead of sample and was incubated under the same conditions as that of a sample. The antioxidant capacity of the sample was expressed as equivalents of ascorbic acid (AAE), utilizing a calibration curve of ascorbic acid in the concentration range from 0.02 mg/mL to 0.32 mg/mL (r^2 = 0.994).

2.6.3 Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay was determined by the method described by Benzie and Strain (1996) with slight modifications. The FRAP reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM ferric chloride in a ratio of 10:1:1 (v/v/v). FRAP reagent was pre-warmed at 37°C for 30 mins and different volumes (10, 20, 40, 80, 160, 240 and 320 µL) of properly diluted fruit extract was mixed with 3 mL of FRAP reagent. The reaction mixture was then incubated in dark for 30 mins. and the absorbance was determined at 593 nm. The antioxidant potential of the fruit extract was determined based on a calibration curve plotted using $FeSO_4.7H_2O$ (4.29 - 85.8 µM, r^2 = 0.999). Ascorbic acid and BHT were used as a reference.

2.6.4 Reducing power assay

The ferric reducing power was assayed with slight modifications of the method described by Oyaizu (1986). Various concentrations of the extracts (40 µL) were mixed with 2.46 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 mins. After incubation, followed by cooling for a few minutes, 2.5 mL of 10% trichloroacetic acid was added. The resulting mixture was centrifuged at 650 g for 10 mins. A 2.5 mL of the

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supernatant was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of the mixture was measured at 700 nm. Ascorbic acid was used as a reference.

2.7 Statistical analysis

The SPSS package, version 19.0 (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Analysis of variance (ANOVA) and Duncan's multiple range method was used to compare the mean values. Data were expressed as mean ± standard deviation of triplicate measurements. Differences were considered significant at $P < 0.05$. The IC_{50} values were calculated by linear regression analysis. Pearson's correlation coefficient was used to do determine correlations.

3. Results and discussion

In this study, the phenolic contents and antioxidant activities of two commonly consumed fruits of Bangladesh named amloki and jolpai were first compared. To the author's knowledge, there is a single previous study investigating the efficiency of solvents of different polarities (n-hexane, ethyl acetate and hexane) on the extraction of phenolic contents of *P. emblica* grown in Indonesia (Fitriansyah *et al*., 2018). Furthermore, study on phytochemical contents and antioxidant activity of *E. floribindus* fruit is rare. In this study, we used five solvents of differing polarities in the following order: water, methanol, ethanol, acetone, hexane.

3.1 Extraction yield and antioxidant components

The influence of solvents on the extraction yield of both fruits is shown in Table 1. Among the different solvent extracts, the water extracts had the highest yield for both fruits, followed by methanol, ethanol, acetone, then n-hexane extracts. The aqueous extracts of plants are commonly found to show higher yields than other solvent extracts (Kong *et al*., 2012). Of the two fruits, the extraction yield of *P. emblica* was higher than that of the *E. floribundus* for all solvents.

Table 1. Percent yield of crude extract from two fruits in different solvent

Extraction Solvent	Yield (%)	
	P. emblica	*E. floribundus*
Water	45.7	23.9
Methanol	26	7.3
Ethanol	23.1	6.7
Acetone	6.9	1.8
n-Hexane	0.8	0.7

The total phenolic and flavonoid contents in the *P. emblica* and *E. floribundus* extracts are summarized in Table 2. It was found that the phenolics and flavonoids of *P. emblica* and *E. floribundus* were higher in a polar solvent such as water, methanol, and ethanol, implying that most polyphenols in these fruits are polar. A similar effect of the polarity of solvent on phenolic and flavonoid contents of plant materials were reported by Kong *et al*. (2012), Lee *et al*. (2015), and Belyagoubi *et al*. (2016), as well. Similar to the extraction yield, *P. emblica* had higher phenolic (0.28-103.95 mg GAE/g DW) and flavonoid contents (0.56-30.88 mg QE/g DW) than the phenolic (0.04-3.62 mg GAE/g DW) and flavonoid (0.16-1.42 mg QE/g DW) contents of *E. floribundus* fruit in all solvents. It is difficult to compare the phenolic and flavonoid content of a particular fruit determined by other studies as the method of extraction, assay and unit of expression varies. However, similar phenolic content (81.5 to 120.9 mg (GAE)/g DW) and flavonoid content (20.3 to 38.7 mg (QE)/g DW) was reported by Liu *et al*. (2008) in the methanolic extract of the fruit emblica from six regions of China. Microwave extraction of *P. emblica* fruits, collected from Fujian province of China, with 66% ethanol produced 133.58±15.61 mg GAE/g DW phenolics (Li *et al*., 2019). The Folin–Ciocalteu reagent method may overestimate TPC, because reducing agents, such as ascorbic acid, may interfere with the results. However, different phenolic compounds respond in the Folin–Ciocalteu method (Ikram *et al*. 2009). Correlation analysis was

Table 2. Phenolic and flavonoid content of two fruits extracted with different solvent

Solvent	*P. emblica*		*E. floribundus*	
	Total phenolics (mg GAE/g dw)	Total Flavonoids (mg QE/g dw)	Total phenolics (mg GAE/g dw)	Total Flavonoids (mg QE/g dw)
Water	$98.25±0.00^{d,B}$	$30.88±0.32^{e,B}$	$3.62±0.18^{e,A}$	$1.42±0. 12^{e,A}$
Methanol	$84.93±0.75^{c,B}$	$18.21 ±0. 46^{c,B}$	$0.91±0.00^{d,A}$	$0.96±0.02^{d,A}$
Ethanol	$103.95±0.00^{e,B}$	$19.4±0.24^{d,B}$	$0.67±0.07^{c,A}$	$0.79±0.02^{c,A}$
Acetone	$14.83±0.00^{b,B}$	$5.57±0.03^{b,B}$	$0.12±0.00^{b,A}$	$0.30±0.01^{b,A}$
n-Hexane	$0.28±0.0023^{a,B}$	$0.56±0.01^{a,B}$	$0.04±0.00^{a,A}$	$0.16±0.00^{a,A}$

Values are presented as mean±SD, n = 3. Values with different lowercase superscript are significantly different at $p < 0.05$ among the extraction solvents while values with different uppercase superscript are significantly different at $p < 0.05$ between fruits.

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performed between the phenolic contents and flavonoid contents of both fruits. The correlations between TP and TF assays were 0.919 for *P. emblica* and 0.897 for *E. floribundus*, respectively which were highly significant at the 0.01 level, indicating that the flavonoids are an important phenolic group that contributes to the antioxidant capacity of *P. emblica* and *E. floribundus.*

3.2 Antioxidant activities

Due to the presence of a variety of antioxidant compounds with different polarities in fruits the antioxidant capacity of fruits differs with the solvent extraction methods. Moreover, antioxidants may respond to different radical or oxidant sources in a different manner. Thus, no single assay can accurately reflect all of the radical sources and antioxidants present in a mixed or complex system as multiple reaction characteristics, mechanisms, and phase localizations are usually involved (Park *et al*., 2014). Many different methods are used to assay the antioxidant activity of plant extracts (Alam *et al*., 2013; Pisoschi *et al*., 2016; Zengin *et al*., 2018). In this study, we applied four different antioxidant assay methods that would provide a better insight into the true antioxidant potential of the extracts.

3.2.1 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical-scavenging activity

The assay of scavenging of stable organic radical DPPH has been widely used in antioxidant capacity studies of plant extracts or antioxidant compounds. The antioxidant activity of plant extracts is thought to be due to its ability to transfer hydrogen or electron to DPPH, thus neutralizing its free radical character. In this assay, the purple radical (picrylhydrazyl) is reduced by antioxidant compounds to the corresponding pale yellow hydrazine (picrylhydrazine), in a concentration-dependent manner. The discolouration indicates the FRSA of the tested sample (Rahman *et al*., 2015).

The abilities of the studied fruit extracts to scavenge DPPH were assessed based on their IC_{50}. The IC_{50} of an antioxidant is defined as the amount of the antioxidant needed to decrease the radical concentration by 50% and its values are inversely related to the antioxidant capacities. Table 3 shows the FRSA of the extracts of *P. emblica* and *E. floribundus* fruits. For both fruits, the antioxidant strength varied with the polarity of the extraction solvent. Similar to its highest total phenolic content in ethanolic extract, the same extract of *P. emblica* showed the highest FRSA, closer to the reference antioxidant BHT (0.10±0.001 mg/mL) but weaker than ascorbic acid (0.07±0.002 mg/mL), implying its potencies. The methanol and acetone extracts of *P. emblica* also showed strong FRSA which were close to BHT. Water extract showed relatively poor scavenging activity compared to BHT and n-hexane extract showed the least activity. A similar effect of solvents on the DPPH radical scavenging activity was observed by Do *et al*. (2014) while studying the solvent effect on the antioxidant activity of *L. aromatica*. In most instances, the hexane extracts were the least reactive in scavenging the DPPH radicals (Kong *et al*., 2012). A high total phenolic content is not always associated with a high FRSA. As reported by Bhebhe *et al*. (2016), ethanol (50%) extracts from *S. jambolonum* had the highest FRSA, yet they recorded lower TPC than 50% acetone and hot water. In this study, although water extract had higher phenolic content than methanol and acetone extract but the former showed lower FRSA.

The scavenging activity of *E. floribundus* varied clearly with the polarity of the solvent, i.e. strongest scavenging activity in water but the weakest in acetone, in accordance with its phenolic and flavonoid contents in different solvents. Earlier reports indicated that the FRSA of phenolic extracts varies from plant to plant such that only a suitable solvent for a particular plant may extract phenolics with the highest activity. A solvent may be efficient on one plant and less efficient on another. The phenomena may be explained by the fact that the recovery of antioxidant compounds such as polyphenols from plant materials is influenced by the

Table 3. Antioxidant activities of the fruit extracts in different solvent

Solvent	*P. emblica*			*E. floribundus*		
	DPPH· scavenging	Total antioxidant	FRAP	DPPH· scavenging	Total antioxidant	FRAP
	IC_{50} (mg/mL)	(mg AAE/g dw)	(mmol Fe^{2+}/g dw)	IC50 (mg/mL)	(mg AAE/g dw)	(mmol Fe^{2+}/g dw)
Water	$0.15±0.002^{c,A}$	$158.81±0.99^{e,B}$	$1362.15 ±33.49^{e,B}$	$3.03±0.04^{a,B}$	$53.38±0.28^{d,A}$	$29.50±0.58^{d,A}$
Methanol	$0.11±0.001^{b,A}$	$96.18±0.72^{d,B}$	$933.76±7.35^{d,B}$	$5.79±0.03^{c,B}$	$10.65±0. 64^{c,A}$	$5.67±0.37^{b,A}$
Ethanol	$0.09±0.001^{a,A}$	$82.54±1.13^{c,B}$	$730.86±17.63^{c,B}$	$4.87±0.11^{b,B}$	$10.54±0.07^{c,A}$	$6.20±0.24^{c,A}$
Acetone	$0.11±0.000^{b,A}$	$22.76±0.86^{b,B}$	$236.33±6.90^{b,B}$	$12.32±0.25^{d,B}$	$2.66±0.09^{b,A}$	$0.85±0.06^{a,A}$
n-Hexane	$1.02±0.006^{d}$	$1.48±0.02^{a,B}$	$8.66±0.29^{a}$	NA	$1.12±0.04^{a,A}$	NA

Values are presented as mean±SD, n = 3. Values with different lowercase superscript are significantly different at $p < 0.05$ among the extraction solvents while values with different uppercase superscript are significantly different at $p < 0.05$ between fruits.

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solubility of the phenolic compounds in the solvent used for the extraction. Moreover, the polarity of the solvent will play an important role in increasing the solubility of phenolic compounds (Bhebhe *et al*., 2016). All extracts of *E. floribundus* showed lower DPPH radical scavenging activities than *P. emblica* and standard antioxidants. This can be attributed mainly due to the lower phenolic content of *E. floribundus* than *P. emblica*. However, the hexane extract of *E. floribundus* did not reach the 50% inhibition of the DPPH radicals at the concentration tested.

3.2.2 Total antioxidant capacity

The total antioxidant capacity was determined by phosphomolybdenum method based on reduction of Mo (VI) to Mo(V) by antioxidant substance and subsequent formation of a green phosphate Mo(V) compounds at acidic pH with an absorbance at 695 nm (Aktumsek *et al*., 2013). The water extracts of *P. emblica* and *E. floribundus* fruits had the highest antioxidant capacity (158.81±0.99 and 53.3±0.28 mg AAE/g dw, respectively), whereas hexane extracts had the lowest (1.48±0.02 and 1.12±0.04 mg AAE/g DW, respectively). Besides, *P. emblica* showed a higher total antioxidant capacity than *E. floribundus* for all solvent extracts (Table 3).

3.2.3 Ferric reducing antioxidant activity

The assay is based on the ability of antioxidant compounds to a reducing complex, Fe(III)–TPTZ to Fe (II)–TPTZ. This reduction is associated with the colour change from light blue to dark blue with an absorbance maximum at 593 nm (Ezzati Nazhad Dolatabadi *et al*., 2014). The ferric reducing antioxidant activities of the different extracts of *P. emblica* and *E. floribundus* fruits are presented in Table 3. The ferric reducing power of *P. emblica* fruit was higher than *E. floribundus* for all solvents. Again, water extract and hexane extract of both *P. emblica* and *E. floribundus* fruits showed the highest (1362.15 ±33.49 and 29.50±0.58 mmol Fe^{2+}/g DW, respectively) and lowest reducing activity (8.66±0.29 mmol Fe^{2+}/g DW and undetectable, respectively), respectively. The ferric reducing antioxidant power of ascorbic acid and BHT were 413.96±12.35 mmol/g and 157.15±6.77 mmol/g, respectively. The highest and lowest FRAP activity was also reported in the water and hexane extract, respectively of black mustard seeds (Lee *et al*., 2015). Moreover, the FRAP and TAC values of both fruits showed a similar trend. The FRAP activity of hexane extract of *E. floribundus* could not be measured due to its low activity.

3.2.4 Reducing power activity (Iron(III) to iron(II) reduction)

Literature reports suggest that the reducing properties are generally associated with the presence of reductones. The reductones have been shown to exert antioxidant activity by breaking the free radical chain by donating a hydrogen atom. In the ferric reducing assay, the presence of antioxidants causes the conversion of the Fe^{3+}/ferricyanide complex to the ferrous form which can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Alam *et al*., 2016). In this study, the ferric reducing activity of different extracts of two fruits was evaluated. Figure 2 shows the dose-dependency of ferric reducing power of different solvent extract of both fruits and ascorbic acid. The reducing power of all extracts increased with increased concentration. Dose dependency was also shown by ascorbic acid. Ethanol and water extracts showed the highest reducing activity for *P. emblica* and *E. floribundus* fruits, respectively, in accordance with their highest phenolic content whereas, for both fruits, hexane showed the lowest activity similar to other antioxidant assays. All the solvent extracts of *P. emblica* showed higher ferric reducing activity than ascorbic acid except hexane. The ferric reducing activity of hexane extract of *E. floribundus* was not found at the concentration tested due to its poor activity.

3.3 Correlation analyses

Pearson correlation analyses were done to estimate

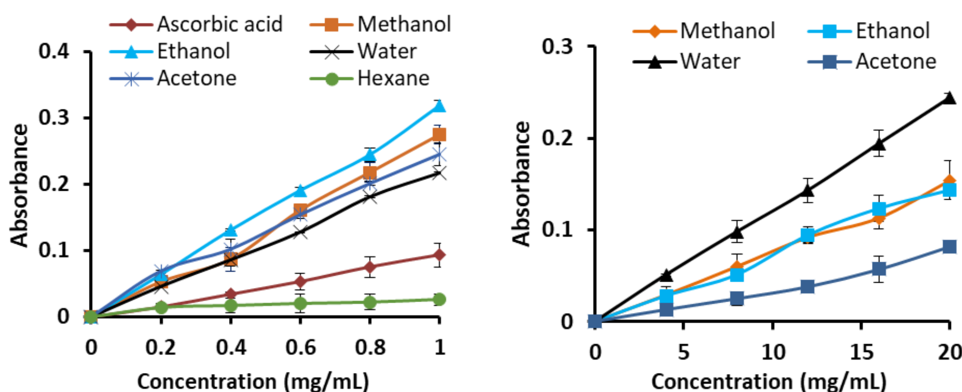

Figure 2. Dose dependency of *P. emblica* (A) and *E. floribundus* (B) fruit extracts in different solvent and comparison with reference antioxidant ascorbic acid.

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Table 4. Correlation coefficient between total phenolic content and antioxidant activity

	Correlation coefficient (r)			
	P. emblica		*E. floribundus*	
	Total phenolics	Flavonoids	Total phenolics	Flavonoid
Free radical scavenging activity (IC_{50})	-0.681[b]	-0.642[b]	-0.724	-0.918[a]
Total antioxidant capacity	0.882[a]	0.992[a]	0.997[a]	0.874[a]
Ferric reducing antioxidant power	0.891[a]	0.986[a]	0.997[a]	0.889[a]

[a]Correlation is significant at the 0.01 level, [b]Correlation is significant at the 0.05 level

the relationship between antioxidant activity and different phenolic content (Table 4). The total antioxidant capacity and ferric reducing activity power of both fruits showed a very strong correlation (r > 0.8) with both phenolic and flavonoid content. The lower values in IC_{50} of DPPH had higher antioxidant activity. The scavenging activity (IC_{50}) showed a negative correlation with the antioxidant compounds. The scavenging activity of *P. emblica* showed a strong correlation (r = 0.6-0.79) with both phenolic and flavonoid content. This implies the ability of polyphenols to act as antioxidants and neutralize free radicals. Choe *et al*. (2014) also reported a negative correlation between phenolic content and DPPH radical scavenging activity of ethanol extract of persimmon peel.

4. Conclusion

This study indicated that the *P. emblica* fruit had very high phenolic content and antioxidant activity compared to the *E. floribundus* fruit. The antioxidative activity of *P. emblica* grown in Bangladesh was very close to the reference antioxidant BHT and ascorbic acid. The antioxidant activity of both fruits showed a clear correlation with the phenolic content proving that the activity is due to the presence of bioactive compounds. Polar solvents especially water and ethanol showed higher efficiency in extracting the phenolic compounds of *P. emblica*, resulting in higher antioxidative activity. However, the effect of single or mixed organic solvent or organic solvent-water mixtures on the extraction of phenolic content and antioxidant activity of *P. emblica* remains to be studied.

Conflict of interest

The authors have no conflict of interest to declare.

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