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Total Phenolic Content, Antioxidative, Anti-amylase, Anti-glucosidase, and Antihistamine Release Activities of Bangladeshi Fruits

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To seek out a cheap source of dietary polyphenols and antioxidants along with anti-amylase and anti-glucosidase activities, ethanol extracts of eleven cheap Bangladeshi fruits were investigated. The extracts were also examined for anti-allergic activity using rat peritoneal exudate cells exposed to the calcium ionophore A23187. *Phyllanthus emblica* (emblic myrobalan) had the highest total polyphenol content (339 mg gallic acid equivalent (GAE)/g), followed by *Syzygium cumini* (Indian blackberry; 192.3 mg GAE/g), and *Aegle marmelos* (wood apple; 53.7 mg GAE/g). *P. emblica*, and *S. cumini* also exhibited the most potent DPPH radical scavenging activity, with an IC_{50} of 2.1 and 8.6 μg/mL respectively. These extracts also showed promising reducing powers with *P. emblica* having the greatest such activity (optical density (O.D.) 1.66), followed by *S. cumini* (O.D. 1.34), at a concentration of 0.2 mg/mL. The extracts of *Artocarpus heterophyllus* (jackfruit) and *S. cumini* showed promising chelating activities. At a concentration of 1 mg/mL, *Dillenia indica* (chalta) showed the highest inhibition of α-amylase activity (60%), and *A. marmelos*, *D. indica*, *P. emblica*, *Spondias dulcis* (hog-plum) & *S. cumini* completely inhibited α-glucosidase activity (100%). Apart from *A. heterophyllus*, *D. indica* and *Phyllanthus acidus* (star-gooseberry), all other extracts inhibited the release of histamine from the peritoneal exudate cells, with *S. cumini* having the strongest effect. These fruits therefore have activities beneficial to physiological health.

Keywords: antioxidant, anti-amylase, anti-glucosidase, anti-allergy, antihistamine release, fruits, polyphenol

Introduction

Fruits are major sources of health promoting agents. Of these agents, antioxidants are most important because they inhibit the initiation of lipid peroxidation, which is related to aging and the pathogenesis of diseases such as cardiovascular disorders, cancer, inflammation, and brain dysfunction (Ames, 1983; Shon *et al.*, 2004). Various epidemiological studies have suggested that consumption of fruit and vegetables is associated with reduced risk of cardiovascular diseases and cancers (Kris-Etherton *et al.*, 2002), neurodegenerative diseases such as Parkinson's and Alzheimer's diseases (Di Matteo and Esposito, 2003), as well as with inflammation and aging (Ames *et al.*, 1993).

Natural products are reportedly beneficial to physiological health. Various flavonoids and non-flavonoids have been reported as showing radical scavenging activity (Sawa *et al.*, 1999): flavone, and flavonoids inhibit α-amylase and α-glucosidase activities (Havsteen, 1983; Kim *et al.*, 2000); polyphenols have anti-hyperglycemic effects (Hossain *et al.*, 2002; Hanamura *et al.*, 2006), and inhibit the development of diabetes (Zunimo *et al.*, 2007). Since glucose release from food sources is the main factor affecting post-prandial hyperglycaemia, retardation of the digestion of starch by key gastrointestinal enzymes can be of benefit to diabetic patients (Puls and Keup, 1973). Also, various dietary components such as polyphenols in tea (Matsuo *et al.*, 2000), soybean (Takasugi *et al.*, 2002), and apple (Kanda *et al.*, 1998), fla-

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vonoids (Corvazier and Maclouf, 1985), and polyunsaturated fatty acids (Yamada *et al.*, 1996) suppress the release of mediators from basophils or mast cells, which in turn prevent the pathogenesis of type 1 allergy. A type I allergic reaction involves the production of allergen-specific IgE and release of a chemical mediator such as histamine or leukotrienes (LT) from basophils or mast cells. Inhibition of the release of mediators may alleviate the symptoms.

A plant, however, especially in its production of secondary metabolites, is largely affected by its environment. Therefore, the secondary metabolites in fruits grown in Bangladesh may not be identical to those of the same fruits grown in other countries. In Bangladesh, despite the consumption of these fruits, no scientific data is available comparing their physiological activities. This work is the first to compare the physiological role of the cheapest fruits in Bangladesh. The present research is aimed at investigating and recording the polyphenol content, antioxidative, anti-amylase, anti-glucosidase, and antihistamine release activities of eleven of the cheapest fruits in Bangladesh. People all over the country usually consume these fruits fresh, and sometimes in various preparations. The aim of this study also includes identifying cheap fruit(s) for preparing dietary supplements, functional foods, or food preservatives that could be used in the food and pharmaceutical industries.

Materials and Methods

Chemicals Folin-Ciocalteu's phenol reagent, fish gelatin, and histamine dihydrochloride were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). A-23187, bovine serum albumin (BSA), DPPH, and α-amylase from bacteria were purchased from Wako Pure Chemical Industry, Ltd., Osaka, Japan, and yeast α-glucosidase was purchased from Oriental Yeast Co., Ltd., Tokyo, Japan. Gallic acid was purchased from Nacalai Tesque, Kyoto, Japan. All of these chemicals and reagents were of analytical grade.

Plant materials Green-matured fruits of woodapple: *Aegle marmelos* (L.) Correa ex Roxb. (Rutaceae), jackfruit: *Artocarpus heterophyllus* Lam. (Moraceae), chalta: *Dillenia indica* L. (Dilleniaceae), sour woodapple: *Limonia acidissima* L. (Rutaceae), sapodilla: *Manilkara zapota* (L.) Royen (Sapotaceae), star-gooseberry: *Phyllanthus acidus* (L.) Skeels (Euphorbiaceae), emblic myrobalan: *Phyllanthus emblica* L. (Euphorbiaceae), hog-plum: *Spondias dulcis* G. Forst. (Anacardiaceae), Indian blackberry: *Syzygium cumini* (L.) Skeels (Myrtaceae), Indian jujube: *Zizyphus mauritiana* Lam. Var. deshi, and *Zizyphus mauritiana* Lam. Var. *narikeli* (Rhamnaceae), collected from a local market in Khulna city, Bangladesh, were cut into small pieces and sun-dried. The dried fruits were ground into a powder with a grinder. The powders were stored separately in air-tight containers and kept in a cool, dark, dry place.

Preparation of fruit extracts The powder of each type of fruit was placed separately in a clean, flat-bottomed glass container and soaked in 99.5% ethanol. Each container was sealed and left for a period of 12 days with regular shaking and stirring of the contents. Hereafter, the preparations were filtered and evaporated to give dried concentrates. The dried concentrates were the ethanolic extracts of the fruits. The weight of the final crude extracts was expressed as a percentage of the dry weight (% d.w.) of the powder, and dry weights for *A. marmelos*, *A. marmelos* (pods), *A. heterophyllus*, *D. indica*, *L. acidissima*, *M. zapota*, *P. acidus*, *P. emblica*, *S. dulcis*, *S. cumini*, *Z. mauritiana* var. *deshi*, and *Z. mauritiana* var. *narikeli* were 5.58, 4.27, 18.5, 8.4, 5.4, 11.9, 6.7, 8.25, 3.6, 5.2, 11.5, and 10.2 % d.w. respectively. Twenty milligrams of each extract was dissolved in 1 mL of ethanol to prepare a stock-solution for experiments.

Determination of total phenolic content The total concentration of phenolics (TPH) in the extracts was determined according to the Folin-Ciocalteu method (Ough and Amerine, 1988) with gallic acid (GA) as the standard and expressed (mg) as gallic acid equivalents (GAE)/g of extract (Aoshima and Ayabe, 2007).

DPPH radical scavenging activity The reaction mixture (total volume, 3 mL), consisting of 0.5 mL of a 0.5 M acetic acid buffer solution at pH 5.5, 1 mL of 0.2 mM DPPH in ethanol, and 1.5 mL of a 50% (v/v) ethanol aqueous solution with the extracts, was shaken vigorously (Blios, 1958; Hatano *et al.*, 1988; Aoshima *et al.*, 2004). After incubation at room temperature for 30 min, the amount of DPPH remaining was determined by measuring absorbance at 517 nm.

Reducing power activity The reducing power of the extracts was determined according to the method of Oyaizu (1986). Phosphate buffer (2.5 mL, 0.2 M, pH 6.6) containing different concentrations of the extract were prepared. Then it was added to 2.5 mL of 1% (w/w) potassium ferricyanide, and mixed. After incubation at 50 °C for 20 minutes, the mixtures were mixed with 2.5 mL of 10% (w/w) trichloroacetic acid followed by centrifugation at 650g for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. Then the absorbance of this solution was measured at 700 nm. One mM potassium ferrocyanide in the buffer solution, which is produced from potassium ferricyanide by reduction, produced the absorbance of OD 0.985 at 700 nm in a cell with 1-cm long light path. Ascorbic acid (40 μg/mL phosphate buffer) served as positive control.

Measurement of chelating activity The activity of extracts to chelate Fe^{2+} was measured according to the method

of Carter (1971). Briefly, the extract (1, 2, 3 and 4 mg) was dissolved in 0.2 mL of 1.0 mM $FeCl_2.4H_2O$. It was then added to 0.8 mL of 5.0 mM ferrozine, and the volume was adjusted to 3.0 mL with methanol. After 10 min., the absorbance of the mixture was measured at 562 nm. EDTA (0.4 mg) instead of the extract served as the positive control, and reaction mixture without the extract or EDTA served as the negative control. Metal chelation (%) = {absorbance of negative control-(absorbance of sample - absorbance of sample itself)} × 100/absorbance of negative control.

α-Amylase assays in vitro α-Amylase activity was carried out using the starch-iodine method. Briefly, 10 μL of α-amylase solution (0.025 mg/mL) was mixed with 390 μL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentrations of extract. After incubation at 37 °C for 10 min, 100 μL of the starch solution (1 or 5%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 mL of 1% iodine solution was added, and after adding 5 mL distilled water, the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were undertaken under the same conditions. Inhibition of enzyme activity was calculated as (%) = (A-C) × 100/ (B-C), where, A = absorbance of the sample, B = absorbance of blank (no α-amylase), and C = absorbance of control (no starch).

α-Glucosidase assays in vitro Phosphate buffer (80 μL, 100 mM, pH 6.8) containing different concentrations of the extract was mixed with 20 μL of α-Glucosidase (0.01 mg/ mL) and incubated at 37 °C for 10 min. Next, 50 μL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (pNPG) was added to the mixture to start the reaction. The reaction mixture was incubated at 37 °C for 60 min and stopped by adding 2.5 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the absorbance at 400 nm. Mean values were obtained from triplicate experiments. Inhibition of enzyme activity was calculated as (%) = (A-C) × 100/ (B-C), where, A = Sample, B = Blank, and C = Control reading.

Preparation of rat peritoneal exudate cells (PECs) Male SD rats (8 weeks old) were purchased from Kyudo Co., Ltd., Tosu, Japan. Twenty mL of Tyrode buffer (137 mM NaCl, 2.7 mM KCl, 0.4 mM NaH_2PO_4, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 12 mM $NaHCO_3$ and 5.6 mM glucose, pH 7.4) containing 0.1% (w/w) BSA was injected into the peritoneal cavity. After the abdomen was gently massaged for 2 min, the cavity was opened, and the fluid containing the peritoneal exudate cells (PECs) was collected with a Pasteur pipette. The cells were gently washed with Tyrode buffer and then centrifuged at 200 × *g* for 10 min at 4 °C. To remove contaminating erythrocytes by hypotonic lysis, the cell pellets were resuspended in a modified ammonium chloride buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, and 10 mM EDTA.2Na, pH 7.4) and incubated for 5 min on ice. The cell suspension was then centrifuged at 200 × *g* for 5 min at 4 °C and the cells were resuspended in the Tyrode buffer at 1 × 10^6 cells/mL. Cell viability was measured by trypan blue staining and mast cells were identified by toluidine blue staining (Takasugi *et al.*, 2002). The cell viability of this preparation was more than 95% and the proportion of mast cells was 5-10% of all the cells (Matsuo *et al.*, 2000).

Measurement of the inhibition of histamine release (%) Rat peritoneal exudate cells (PECs; 500 μL, 1 × 10^6) were suspended in 48 μL of 25 mM $CaCl_2$, 12 μL of various concentrations of samples and/or 120 μL of 5 μM A23187 solution, and then the volume was adjusted to 1.2 mL with tyrode buffer, and incubated for 20 min at 37 °C. The reaction was terminated by incubating for 5 min at 4 °C. The cell suspension was then centrifuged at 300 × *g* for 10 min, and the amount of histamine in the supernatant was measured.

The histamine content was measured by fluorometric assay (Shore *et al.*, 1959). The percent inhibition of histamine release was calculated with the following formula: inhibition of histamine release (%) = (histamine release without extract – histamine release with extract) × 100/histamine release without extract. The negative control was the histamine content without stimulation. The positive control was that after stimulation by A23187. All results were expressed as the mean ± SD of at least four determinations (n = 4).

Results

Total phenolic (TPH) content The TPH content of the different fruits ranged from 339 to 6.8 mg GAE/g of extract (Table 1). The amount was largest in the extract of *P. emblica* followed by *S. cumini* and *A. marmelos*, whereas it was smallest in the extract of *M. zapota* followed by *Z. mauritiana* and *L. acidissima*. The TPH contents of the two varieties of *Z. mauritiana* var. *deshi* and *narikeli*, were almost identical.

DPPH radical scavenging activity The DPPH radical scavenging activities of the different extracts are also shown in Table 1. At a concentration of 0.2 mg/mL of extract, the level of free radical (DPPH) scavenging activity was highest in *P. emblica* (93.7%) and lowest in *A. heterophyllus* and *Z. mauritiana* var. *Narikeli* (14.6%). At this concentration, extracts of five fruits, *P. emblica*, *S. cumini*, *D. indica*, *S. dulcis*, and *P. acidus*, showed more than 90% DPPH radical scavenging activity. These extracts dose-dependently scavenged the DPPH radical, with IC_{50} values of 2.1, 8.6, 57, 81, and 93 μg/mL respectively (Fig. 1). *P. emblica* had the lowest IC_{50}, which means that of all the fruits tested, it had the strongest radical scavenging activity.

Table 1. Total phenolic content, DPPH radical scavenging activity, reducing power, and chelating activity of the different fruit extracts[a].

Name of fruits	Total phenolic content mg GAE/g	DPPH radical scavenging activity (%) at 0.2 mg/mL	Reducing power (O. D.) at 0.2 mg/mL	Chelating activity (%) at 1.0 mg/mL
A. marmelos	53.7 ± 0.5	47.7 ± 0.9	0.246 ± 0.04	13.0 ± 0.16
A. marmelos (pods)	42.4 ± 1.2	49.4 ± 0.4	0.223 ± 0.02	9.4 ± 0.64
A. heterophyllus	34.9 ± 0.2	14.6 ± 0.3	0.112 ± 0.03	33.4 ± 0.14
D. indica	37.8 ± 0.3	92.9 ± 0.1	0.337 ± 0.05	16.4 ± 0.61
L. acidissima	14.4 ± 0.1	31.3 ± 0.6	0.241 ± 0.02	22.7 ± 1.30
M. zapota	6.8 ± 0.1	18.5 ± 0.8	0.064 ± 0.02	Nill
P. acidus	31.4 ± 0.5	90.8 ± 0.2	0.339 ± 0.07	9.7 ± 0.66
P. emblica	339.0 ± 6.0	93.7 ± 0.7	1.660 ± 0.03	2.9 ± 0.22
S. dulcis	26.1 ± 0.4	91.5 ± 0.3	0.313 ± 0.04	23.0 ± 1.28
S. cumini	192.3 ± 13.8	92.9 ± 0.5	1.337 ± 0.01	57.1 ± 3.35
Z. mauritiana var. *Deshi*	8.6 ± 0.5	18.0 ± 0.7	0.101 ± 0.04	18.5 ± 0.95
Z. mauritiana var. *Narikeli*	9.6 ± 0.2	14.6 ± 1.6	0.063 ± 0.03	0.2 ± 0.08[#]

$p < 0.01$ by Student's t test for values between the sample and the control in DPPH, and chelating experiments. [#]Not significant.
[a]Values are the mean of three replicates ± SD.

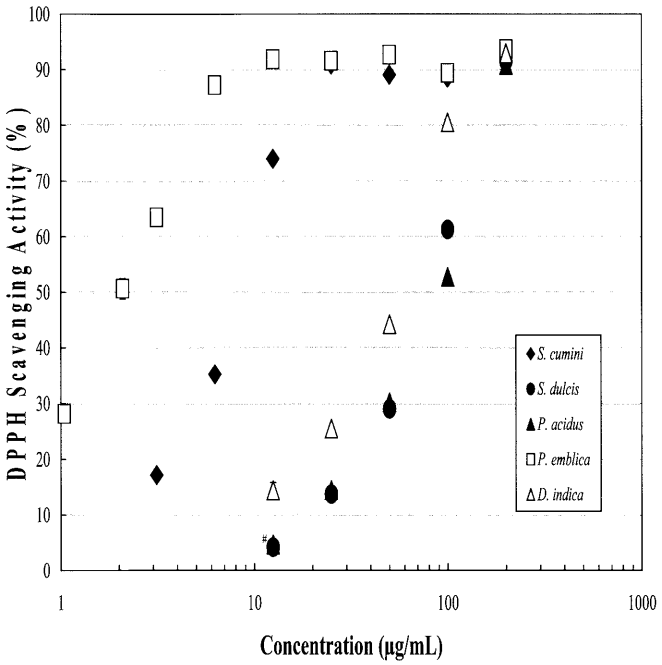

Fig. 1. Dose-dependency of the DPPH radical scavenging activities of the ethanol extracts of fruits. Data are mean ± SD (bars) values from three experiments. $P < 0.05$ by Student's t test for values between the sample and the control, except for the value marked #.

Reducing power Reportedly, the activity of antioxidants is concomitant with the development of reducing power (Duh *et al.*, 1999). Table 1 shows the reducing power of the extracts determined using the potassium ferricyanide reduction method. *P. emblica* and *S. cumini* have the highest levels of reducing activity and they showed a dose-dependent increase in their reducing power (data not shown).

Chelating activity Table 1 shows the chelating effect of the fruit extracts on ferrous ions. The extracts of *A. heterophyllus* and *S. cumini* were the most promising chelators, and their IC_{50} values are 333.3 and 934.5 μg/mL respectively. Extracts of *A. heterophyllus*, and *S. cumini* exhibited chelating effects on ferrous ions, suggesting that they minimize the concentration of metal in the Fenton reaction.

Relationship between total phenolic content (TPH) and DPPH radical scavenging activity, reducing power, or chelating activity (%) Generally, extracts with a higher phenolic content show more DPPH radical scavenging activity and vice-versa. Considering TPH content versus DPPH scavenging activity (%) of all extracts of the fruits, the correlation was just 0.54. However, we found a strong correlation (r = 0.95) between TPH content and the $1/IC_{50}$ values of different extracts (Fig. 2) that were calculated from Fig. 1. Since reducing power develops concomitant with antioxidative activity (Duh *et al.*, 1999), we examined the correlation between TPH content and the reducing power of all extracts, and obtained a correlation of 0.97. Our results, however, were unable to show a good correlation between TPH content and the chelating activity of the extracts (r = 0.14).

Inhibition of α-amylase activity in vitro In the present study, nine out of the eleven fruits tested, *A. marmelos*, *A. heterophyllus*, *D. indica*, *L. acidissima*, *M. zapota*, *P. acidus*, *S. dulcis*, and *Z. mauritiana* var. *Deshi* and *Narikeli* were found to possess significant ($p < 0.01$) inhibitory effects on starch breakdown *in vitro* as shown in Fig. 3. The highest inhibitory activity was from *D. indica* and its IC_{50} was 410 μg/mL. Increasing starch concentration (5%) did not affect the inhibitory activities of these extracts, indicating a non-competitive inhibition.

Inhibition of α-glucosidase activity in vitro Except *A. heterophyllus*, *L. acidissima*, and *Z. mauritiana* var. *Narikeli*, all other fruits had inhibitory effect on α-glucosidase activity at 1 mg/mL extract (Fig. 3). At this concentration, *A. marmelos*, *D. indica*, *M. zapota*, *P. emblica*, *S. dulcis*, and *S.*

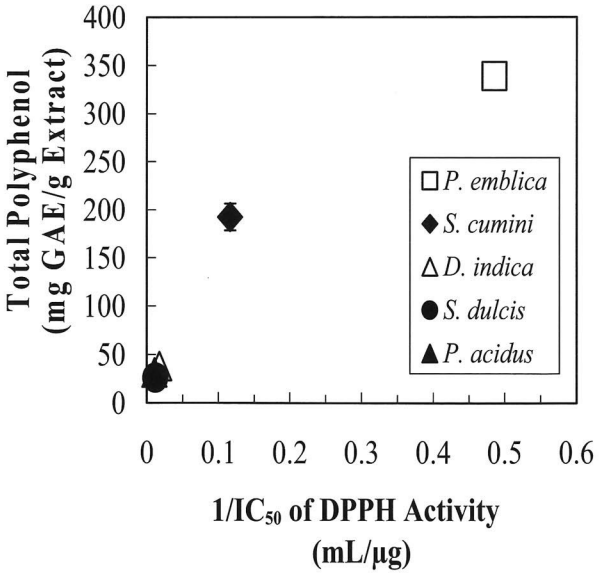

Fig. 2. Relationship between total polyphenol content and the reciprocal of IC_{50} values for DPPH radical scavenging activities of different extracts of fruit (*D. indica*, *P. acidus*, *P. emblica*, *S. dulcis*, and *S. cumini*). The values for total polyphenol content are means ± SDs (bars) from three experiments.

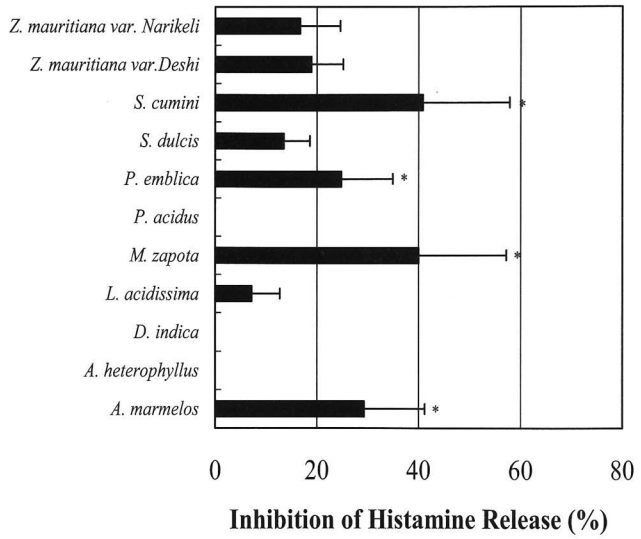

Fig. 4a. Effect of fruit extracts on the ionophore A23187-induced release of histamine from rat PECs. PECs (1 x 10^6) were incubated with the same concentration of polyphenols, 10 μM GAE of extract, at 37 °C for 20 min. Each datum represents the mean ± SD of at least four independent experiments. $P^* < 0.05$ by Student's *t* test for values between the sample and the control.

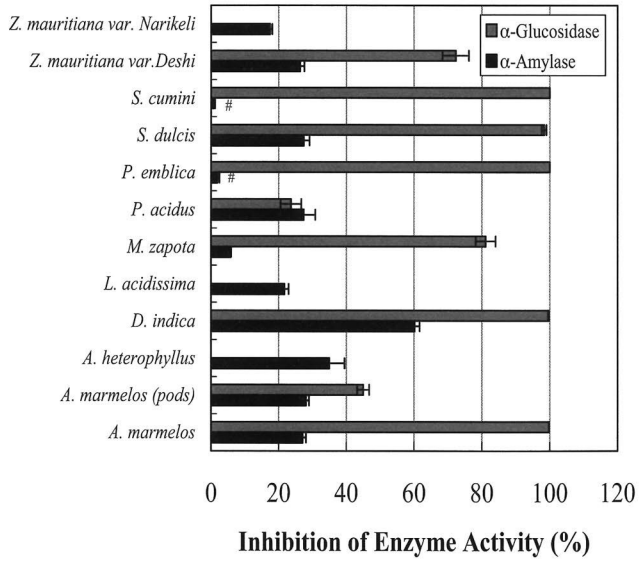

Fig. 3. Effect of fruit extracts on the inhibition of α-amylase, and α-glucosidase activities (%). α-Amylase or α-glucosidase without any extract was taken to be 100% activity (control). Inhibition (%) of the activity was studied in presence of 1 mg/mL extract. $P < 0.01$ by Student's *t* test for values between the sample and the control, except for values marked #.

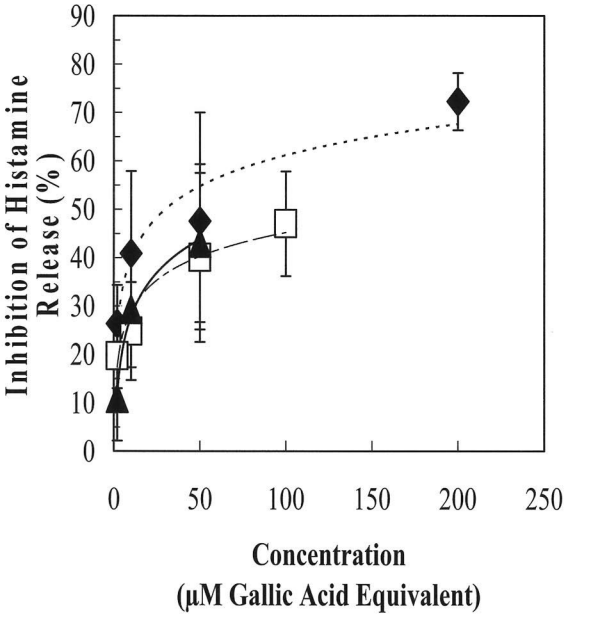

Fig. 4b. Dose-dependent inhibition of histamine-release (%) by the ethanol extracts of fruits of *A. marmelos* (▲), *P. emblica* (□), and *S. cumini* (◆) from peritoneal exudate cells (PECs). Cells (1 x 10^6) were incubated for 20 min in the presence of various concentrations of polyphenols as GAE in these extracts. Results are the mean ± SD of at least four independent experiments.

cumini had more than 80% inhibition of the enzyme activity. At a concentration of 0.1 mg/mL, *A. marmelos*, *D. indica*, *P. emblica*, *S. dulcis*, and *S. cumini* showed an inhibitory effect of 69.5 ± 15.7, 93.1 ± 2.9, 98.4 ± 0.2, 61 ± 1.4, and 99.7 ± 0.4% respectively, whereas other extracts had no effect.

Effect of fruit extracts on the inhibition of histamine release We examined the effect of fruit extracts on the inhibition of histamine release from rat PECs. The PECs (1 × 10^6/mL) were stimulated with 5 μM A23187 for 20 min in the presence of the extracts. As shown in Fig. 4a, *P. emblica*,

S. cumini, *A. marmelos*, and *M. zapota* showed significant inhibition of the release of histamine from the cells at the same concentration of polyphenols (10 μM GAE). Since the total phenolic content (GAE/g) of *M. zapota* is low, as shown in Table 1, its inhibition of the histamine release is possibly weak when *M. zapota* is ingested. Since the total phenolic contents of *P. emblica*, *S. cumini*, and *A. marmelos* (Table 1) are high and significantly inhibit histamine release (Fig. 4a), the dose-dependence of their inhibition of histamine release was examined and shown in Fig. 4b. Of these three, *S. cumini* exhibited the strongest activity.

Discussion

Fruits are a natural source of dietary fiber, trace elements, antioxidants and bio-active compounds beneficial to health (Lorgeril *et al.*, 1994; Hertog *et al.*, 1995). Two of the eleven fruits studied here, *P. emblica* and *S. cumini*, are potential sources of natural antioxidants because they exhibited extensive DPPH radical scavenging activity and reducing power, although *P. emblica* had no chelating effect on ferrous ions. A strong correlation between TPH content and the $1/IC_{50}$ values for scavenging the DPPH radical suggested that the level of scavenging activity of the fruit extracts was closely related to their phenolic groups. A very high correlation between TPH content and the reducing power of these extracts also suggested that with increase in polyphenol content, the antioxidative activities increase (Duh *et al.*, 1999). Antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from their phenolic hydroxyl groups, thereby forming a stable end-product, which does not initiate or propagate further oxidation of lipids (Sherwin, 1978). Lipid hydroperoxides are reported to cause apoptosis in PC12 cells (Aoshima *et al.*, 1997). Since phenolic compounds present in the extracts are a good source of electron donors, they show reducing power. In this study, *A. heterophyllus*, and *S. cumini* displayed a remarkable capacity to bind iron, suggesting that they may protect against peroxidation. An affinity for ferrous ions minimizes the concentration of the catalyzing transition metal needed in a lipid peroxidation reaction. Owing to the complexity of the oxidation-antioxidation process, no single testing method is capable of providing a comprehensive view of the antioxidative profile of a sample (Parejo *et al.*, 2002). Therefore, a multi-method approach is necessary to assess antioxidative activity.

Fruits that reduce post-prandial hyperglycaemia by suppressing the hydrolysis of carbohydrates may be helpful in the control of diabetes mellitus. In the present study, both α-amylase and α-glucosidase activities were most strongly inhibited by *D. indica*, whereas *P. emblica*, and *S. cumini* were strongest in the inhibition of only α-glucosidase activity. In dose-dependent effects on α-amylase, at high concentrations probably there was saturation of component(s) thereby causing no further increase in inhibition. However, these fruits may have different inhibitory effects on α-amylase and α-glucosidase of different origins. Reportedly, various natural products, such as flavone, and flavonoids, inhibit α-amylase and α-glucosidase activities (Havsteen, 1983; Kim *et al.*, 2000). Moreover, polyphenols also have anti-hyperglycemic effects (Hossain *et al.*, 2002; Hanamura *et al.*, 2006), and inhibit the development of diabetes (Zunimo *et al.*, 2007).

A type I allergy is an immediate hypersensitive reaction to, for example, food or environmental allergens (Younginger, 1992; Marks and Marks, 1993). Mast cells play a crucial role in the pathogenesis of this type of allergy through the production and release of chemical mediators such as histamine and eicosanoids (Matsuo *et al.*, 2000), which trigger various pathophysiological events in the acute phase of the reaction, including an increase in vascular permeability, the contraction of bronchial smooth muscle or production of mucus, and neutrophil chemotaxis (Kaliner *et al.*, 1982; Robinson and Holgate, 1985). Therefore, it is important to inhibit the release of mediators for the prevention and/or alleviation of allergic symptoms. Polyphenols inhibit the binding of specific radio-ligands to various receptors (Zhu *et al.*, 1997). In the immediate type of allergic reaction cascade, IgE sensitizes basophils or mast cells which have a specific Fc receptor for IgE on their surface. It is therefore necessary to elucidate whether these fruit extracts have an effect at a later stage in the sensitization by IgE in the cascade, and inhibit histamine release. Also, histamine is released from intracellular secretory granules with an increase in the intracellular calcium ion concentration and by the activation of signal transduction (Beaven *et al.*, 1984; Penner, 1988). Kee and Lim (2007) reported that polyphenols of *Rubus coreanum* inhibit both the calcium influx into the cells and the uptake of calcium into the cytoplasmic calcium store. Figs. 4a and 4b show the inhibition of calcium-ionophore-induced release of histamine from rat PECs by the extracts. These extracts might inhibit the influx of calcium and affect the transduction of signals as well as release of histamine. Further studies are needed to determine whether intake of these fruits prevents or reduces allergic symptoms in humans.

Conclusion

Many of the health-promoting activities of fruit, such as anti-cancer, anti-diabetic, anti-mutagenic, antimicrobial, and anti-allergic effects, may be related to antioxidative activity. A relationship between polyphenols and antioxidative and anti-allergic activities has already been reported (Yamada

et al., 1999). The results support the possibility that these fruits, which are commonly consumed in Bangladesh, contribute to health. However, further *in vitro* and *in vivo* studies are needed to confirm the present observations. Attention should be paid to potential cytotoxic effects when they are used for the preparation of dietary supplements, and in the enrichment of beverages or foods, since some polyphenols perturb the membrane structure (Hossain *et al.*, 2002; Aoshima *et al.*, 2005). Moreover, fractionation of potential extracts is essential for determining the phenolic or nonphenolic compound(s) responsible for the antioxidative, anti-amylase, anti-glucosidase, and anti-allergic activities.

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