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NUTRIENT CONTENT AND ANTIOXIDANT PROPERTIES OF SOME POPULAR FRUITS IN BANGLADESH

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Keywords:

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ABSTRACT: The present study discussed the nutritional content and quantification of eleven bioactive phenolic antioxidant contents in the water, methanol and acetone extracts of four different edible fruits viz. *Musa acuminate* (banana), *Ananascomosus* (pineapple), *Psidiumguajava* (guava) and *Malusdomestica* (apple) using HPLC-DAD. Nutritional analysis showed that the most moisture and fiber-containing fruit was guava where as banana had the lowest amount. On the other hand banana had the highest percentage of protein and carbohydrate whereas *guava* had the lowest percentage. The result showed that the antioxidant content determined by HPLC was varied in the water, methanol and acetone extracts of these fruits. According to the study, acetone was appeared as the most suitable extraction system of phenolic antioxidants present in these fruits, i.e. in the apple,*(+)* catechin hydrate was 486.80 ± 16.75, 28.64 ± 2.21and 0.0 ± 0.0 mg/100g of the extract; ellagic acid was 1735.14 ± 33.17, 33.44 ± 4.07 and 0.0 ± 0.0 mg/100g of the extract and *(-)* epicatechin was 138.02 ± 7.69, 6.78 ± 1.06 and 0.0 ± 0.0 mg/100g of acetone, methanol and water extract respectively. Phenolic antioxidant contents were found highest in the extract of apple where as least amount present in any extract of banana although Nutrient content analysis showed it as a good source of energy. Therefore, the results suggest that these edible fruits might be a good source of natural antioxidant.

INTRODUCTION: Fruits are nature's wonderful gift to mankind, which are packed with vitamins, minerals, antioxidants, soluble dietary fiber and many phyto-nutrients. They are absolute feast for their unique nutrition-profile that help the human body free of diseases and are essential for optimizing our health. Increased consumption of fruit and vegetables significantly reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and other aging-related pathologies.

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Fruits offer protection against free radicals that damage lipids, proteins, and nucleic acids. Polyphenols, carotenoids (pro-Vitamin A), vitamins C and E present in fruits have antioxidant and free radical scavenging activities and play a significant role in the prevention of many diseases [1]. However, food surveys show continuing low consumption of fruits and vegetables in many regions of the developing world [2]. The total fruit availability per person per day is 155 g, which is much higher than the current consumption of 34 g per person per day in Bangladesh [3]. This is partly due the shortage of proper nutritional facts of common fruits available in the market.

Antioxidants are compounds that when present at low concentration are capable of prevent or delay the oxidation of lipids, proteins and nucleic acids

by inhibiting the initiation or propagation of oxidizing chain reactions. The most abundant antioxidants in fruits are polyphenols, Vitamin C, Vitamins A, B and E while carotenoids are present to a lesser extent in some fruits. These polyphenols, most of which are flavonoids, are present mainly in ester and glycoside forms [4]. There are two basic categories of antioxidants, namely, synthetic and natural. Although some synthetic antioxidants such as butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity and side effects [5, 6]. Thus, the interest in natural antioxidants has increased considerably [7].

Information about the composition of food is important for nutrition education, training and research [8] as well as dietary recommendation and supplementation of food. Though, the presence of trace elements, heavy metal and Vitamin C of several fruits has recently been reported [9, 10]; however, information on the antioxidant activity and phenolic content of majority fruitsin Bangladesh is not available. Moreover phenolic acids, such as caffeic, chlorogenic, ferulic, sinapic, and pcoumaric acids, appear to be more active antioxidants than the hydroxy derivatives of benzoic acid such as p-hydroxybenzoic, vanillic, and syringic acids [11, 12] therefore, the objective of this study was to determine the nutrient content (proximate analysis) and phenolic antioxidant contents of banana (*Musa acuminata*), pineapple (*Ananascomosus*), guava (*Psidiumguajava*) and apple (*Malusdomestica*), which are now commonly available around the year and mostly consumed fruits in Bangladesh.

METHODS AND MATERIALS:

Sample collection:

The selected four types fresh, matured, and free from insect's bites or microbial damage and other organoleptic deterioration fruits were collected from at least three different local markets in Dhaka city. The freshly collected sample was cleaned with deionized water to remove any visible dirt present on the surface and removed the water quickly with a blotting paper.

Determination of nutritional properties:

Moisture of fruit is determined by drying a sample at elevated temperature (105^0C) and reporting the loss in weight in terms of moisture [13]. The estimation of total protein was made by Kjeldahl method [14]. The total fat and crude fiber content of samples were determined by AOAC method [13]. Ash was determined by heating sample at 600^0C for six hours until a constant weight was reached [15]. The content of total carbohydrate was determined by the following equation [16]:

Total Carbohydrate (%) =100 - {Moisture (%) + Protein (%) + Fat (%) + Ash (%)}.

Extraction of fruit materials (Methanol, Acetone and Water):

Total 15 g powdered fruit samples (each) were weighted and taken into a screw capped 500 ml bottles. 150 ml of HPLC grade methanol, acetone or distilled water was added to each sample separately. These samples were kept in anorbital shaker (VNR-480, Gemmy, Taiwan) for 3-4 days at ambient temperature with agitation. After this samples were filtered by whatman grade-1 filter papers (Bibby RE200, Sterilin Ltd., UK) with the help of vacuum pump and kept at 50°C water bath with rotation to evaporate residual solvent. The extracts were store in a cool place for further research.

High performance liquid chromatography (HPLC) system:

Chromatographic analyses were carried out on a Thermo Scientific Dionex Ulti Mate 3000 Rapid Separation LC (RSLC) systems (Thermo Fisher Scientific Inc., MA, USA), coupled to a quaternary rapid separation pump (LPG-3400RS), Ultimate 3000RS auto-sampler (WPS-3000) and rapid separation diode array detector (DAD-3000RS). Phenolic compounds were separated on a Acclaim® C18 (4.6 x 250 mm; 5µm) column (Dionix, USA) which was controlled at 30°C using a temperature controlled column compartment (TCC-3000). Data acquisition, peak integration, and calibrations were performed with Dionix Chromeleon software (Version 6.80 RS 10).

Chromatographic condition:

The phenolic composition of the extracts were determined by HPLC, as described by Islamet

al.[17]and Sarunya & Sukon[18]. For equilibration of the column, a 5min post run was set-up at initial conditions. A constant flow rate was maintained (1ml/min) and the injection volume used was 20µl. For UV detection, the wavelength program was optimized to monitor phenolic compounds at their respective maximum absorbance wavelengths as follows: λ 280 nm held for 18.0 min before switching to λ 320 nm where it was held for 6 min, and finally to λ 380 nm, which was kept constant for the rest of the analysis. The diode array detector was set at an acquisition range of 200 nm to 700 nm. The detection and quantification of GA, CH, VA, CA, and EC was done at 280 nm, PCA, RH, and EA at 320 nm, and MC, QH and KF at 380 nm, respectively.

Standard and sample preparation:

Stock solutions (100µg/ml) for each of the phenolic compounds were prepared and the calibration curves of the standards were performed according to Jahan et al.[19]. A solution at a concentration of 5 mg/ml were prepared by dissolving in methanol for acetone and methanol extract and in water for water extract by mixing with a vortex (Branson, USA) for 30 min. The samples were stored in the dark at low temperature (5°C). Spiking the sample solutions with phenolic standards was done for additional identification of individual polyphenols. Prior to HPLC analysis, all solutions (mixed standards, sample, and spiked solutions were filtered through 0.20µm nylon syringe filter (Sartorius, Germany) and then degassed in an ultrasonic bath (Hwashin, Korea) for 15 min.

RESULTS:

The study was conducted with four different fruits collected from at least three different local markets. In this study, the collected fruit samples were analyzed for nutrient content (carbohydrate content, protein content, fat content, ash content, moisture content, fiber content) and antioxidant property. Each value represents the average from at least three replications and the outcomes expressed as mean values ± standard deviations (SD).

Nutrient content:

Moisture content of a sample refers to the amount of water present in it and among the analyzed food guava is appeared as the most moisture and fiber containing fruit whereas banana is the lowest (**Table 1**). On the other hand, the banana has the highest percentage of protein and carbohydrates but guava has the lowest. In case of fat apple has the maximum fat percentage and guava has the minimum (**Table 1).** While total mineral content was measure, banana appeared as the maximum mineral containing fruit.

TABLE 1: NUTRIENT CONTENT IN SELECTED FRUITS (PROXIMATE ANALYSIS) EXPRESSED IN g/100g EDIBLE PARTOF FRUIT.

Attribute	Apple	Banana	Guava	Pineapple
Moisture (%)	85.49 ± 3.3 1.30.1	75.74 ± 3.1	91.27 ± 2.9	82.32 ± 2.6
Ash (%)	0.15± 0.06	0.63 ± 0.11	0.25 ± 0.09	0.32 ± 0.10
Fat (%)	1.03± 0.21	0.94± 0.17	0.26± 0.10	0.30± 0.07
Protein (%)	0.93± 0.19	5.42± 0.45	0.91± 0.07	1.09± 0.14
Fiber (%)	1.15± 0.23	0.68± 0.13	1.44± 0.21	0.70± 0.16
Carbohydrate (%)	11.25± 2.3	16.59± 3.4	5.87± 2.1	15.27±2.7

*The values of individual compound were with the mean ± standard deviation (n = 3).

Antioxidant property of the selected fruit extract in different solvents:

Three different types of solvent were used for the extraction of antioxidant chemicals form the fruits and analyzed by HPLC-DAD. Identification and quantification of individual phenolic compounds in acetone, methanol and water extracts of four fruits were analyzed by HPLC. The chromatographic separations of polyphenols in the acetone extracts of four fruits are shown in figure 1, 2, 3 and 4.

A total eleven phenolic antioxidant were identified and quantified (**Table 2-4**),which were gallic acid (GA), *(+)* catechin hydrate (CH), vanillic acid (VA), caffeic acid (CA), (-) epicatechin (EC), p-coumaric acid (PCA), rutin hydrate (RH), ellagic acid (EA), myricetin (MC), quercetin hydrate (QH), and kaemferol (KF). In acetone extract, majority of the standard antioxidants were present in four fruits extract with maximum level in apple except for caffeic acid and rutin hydrate, which

were found highest in pineapple and guava respectively (**Table 2**). It is notable that gallic acid, myricetin and kaenferol were undetected in acetone extract of all four fruits.

In methanol extract the amount of detected antioxidants were lower than the acetone extract (chromatographic figures are not shown).When methanol extract was analyzed although majority of the antioxidant were found in guava and pineapple but higher quantity was detected in pineapple than guava extract (**Table 3**). In methanol extract myercetin was only detected in guava and no quercetin hydrate and kaemferol were detected in any fruit extract. In acetone extract though nogallic acid was detected but in methanol extract it was found exclusively in pineapple extract.

The water extraction analysis (chromatographic figures are not presented) showed that none of the antioxidants detected in apple, banana and pineapple except for (-) epicatechin in pineapple (**Table 4**). Only few (gallic acid, catechin hydrate, vanillic acid, caffeic acid and rutin hydrate) were detected only in guava extract (**Table 4**).

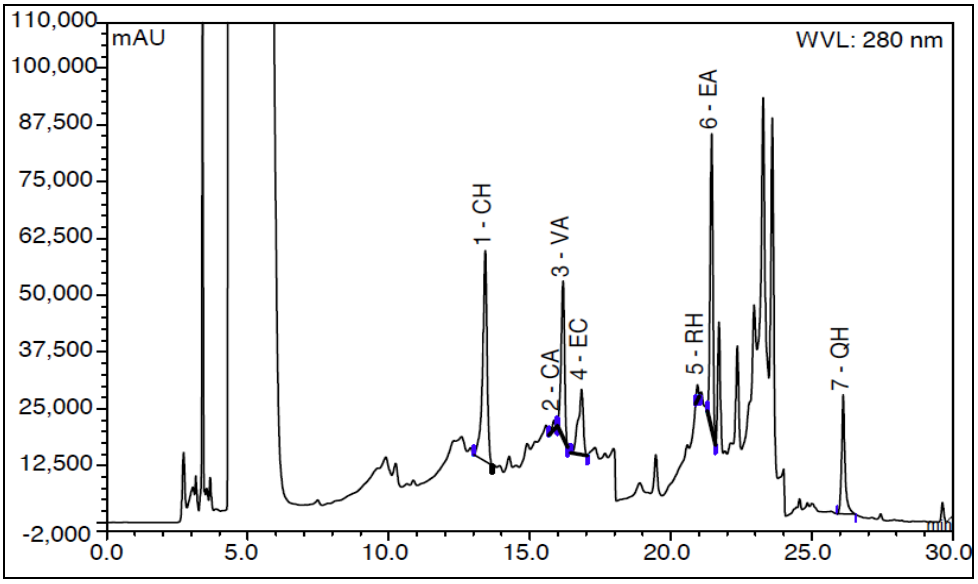

FIG. 1: HPLC CHROMATOGRAM OF ACETONE EXTRACT OF APPLE. PEAKS: 1- (+) CATECHIN HYDRATE; 2- CAFFEIC ACID; 3-VANILLIC ACID; 4- (-) EPICATECHIN; 5-RUTIN HYDRATE; 6-ELLAGIC ACID; 7-QUERCETIN HYDRATE.

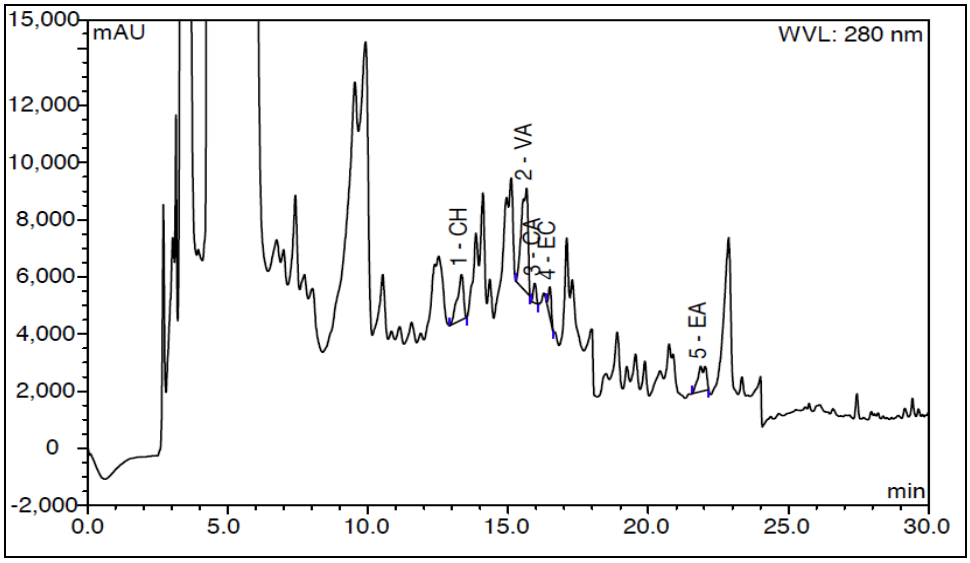

FIG. 2: HPLC CHROMATOGRAM OF ACETONE EXTRACT OF BANANA. PEAKS: 1-(+) CATECHIN HYDRATE; 2-VANILLIC ACID; 3-CAFFEIC ACID; 4- (-) EPICATECHIN; 5-ELLAGIC ACID.

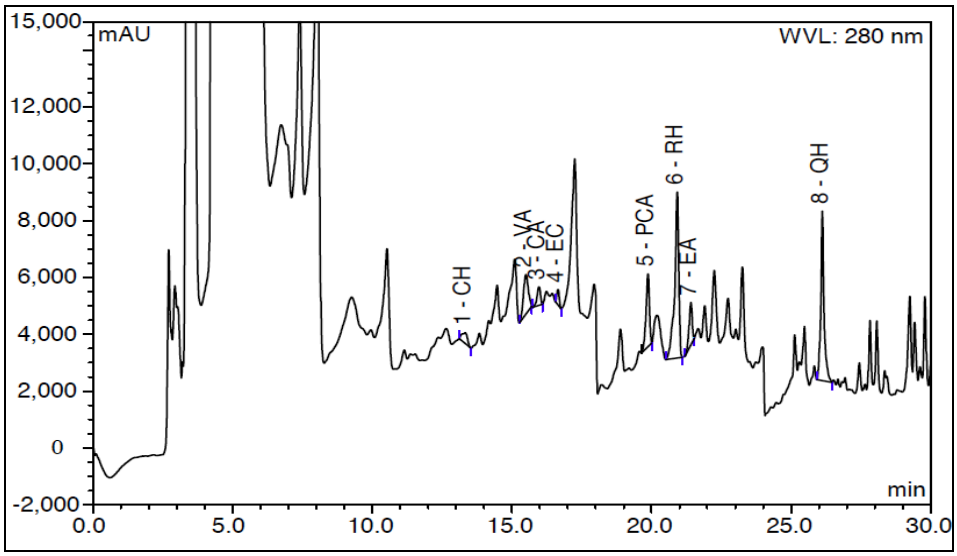

FIG.3: HPLC CHROMATOGRAM OF ACETONE EXTRACT OF GUAVA. PEAKS: 1-(+) CATECHIN HYDRATE; 2-VANILLIC ACID; 3-CAFFEIC ACID; 4- (-) EPICATECHIN; 5- P-COUMARIC ACID; 6-RUTIN HYDRATE; 7-ELLAGIC ACID; 8-QUERCETIN HYDRATE

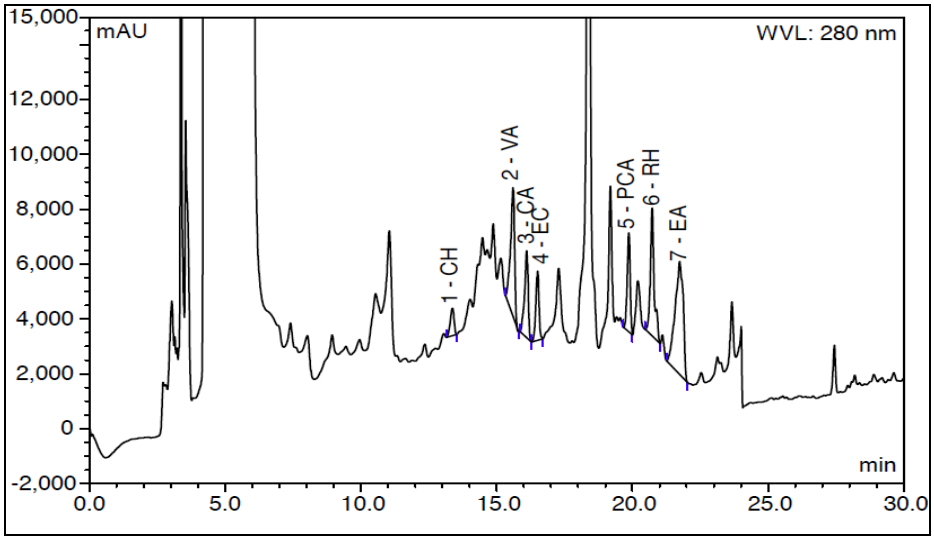

FIG. 4: HPLC CHROMATOGRAM OF ACETONE EXTRACT OF PINEAPPLE. PEAKS: 1-(+) CATECHIN HYDRATE; 2-VANILLIC ACID; 3-CAFFEIC ACID; 4- (-) EPICATECHIN; 5- *P*-COUMARIC ACID; 6-RUTIN HYDRATE; 7-ELLAGIC ACID.

TABLE 2: AMOUNT OF ANTIOXIDANT EXPRESSED IN mg/100g OF ACETONE EXTRACT.

Antioxidants	Apple	Banana	Guava	Pineapple
Gallic acid	ND	ND	ND	ND
(+) Catechin hydrate	486.80 ± 16.75	23.34 ± 4.56	4.88 ± 1.32	8.52 ± 1.19
Vanillic acid	42.07 ± 8.12	8.54 ± 2.03	2.48 ± 0.97	7.00 ± 1.09
Caffeic acid	2.78 ± 0.61	1.36 ± 0.26	1.44 ± 0.20	7.88 ± 1.33
(-) Epicatechin	138.02 ± 7.69	5.50 ± 1.01	2.44 ± 0.65	15.82 ± 4.31
p-Coumaric acid	ND	ND	1.38 ± 0.61	1.78 ± 0.76
Rutin hydrate	7.36 ± 1.36	ND	26.24 ± 2.11	20.18 ± 3.12
Ellagic acid	1735.14 ± 33.17	68.82 ± 6.10	44.18 ± 5.70	333.16 ± 9.15
Myricetin	ND	ND	ND	ND
Quercetin hydrate	33.90 ± 4.10	ND	7.56 ± 1.13	ND
Kaemferol	ND	ND	ND	ND

*The values of individual compound were with the mean ± standard deviation (n=3).ND: Not Detected.

TABLE 3: AMOUNT OF ANTIOXIDANT EXPRESSED IN mg/100g OF METHANOL EXTRACT.

Antioxidants	Apple	Banana	Guava	Pineapple
Gallic acid	ND	ND	ND	6.34 ± 1.18
(+) Catechin hydrate	28.64 ± 2.21	2.52 ± 0.51	3.02 ± 0.88	9.64 ± 2.37
Vanillic acid	2.0 ± 0.47	ND	1.30 ± 0.38	15.92 ± 3.14
Caffeic acid	ND	1.08 ± 0.14	0.92 ± 0.11	ND
(-) Epicatechin	6.78 ± 1.06	ND	2.70 ± 0.82	25.34 ± 4.32
p- Coumaric acid	ND	ND	0.34 ± 0.04	3.18 ± 1.12
Rutin hydrate	ND	ND	9.06 ± 1.76	20.56 ± 4.18
Ellagic acid	33.44 ± 4.07	ND	14.06 ± 2.12	280.28 ± 9.31
Myricetin	ND	ND	0.64 ± 0.06	ND
Quercetin hydrate	ND	ND	ND	ND
Kaemferol	ND	ND	ND	ND

*The values of individual compound were with the mean ± standard deviation (n=3). ND: Not detected.

TABLE 4: AMOUNT OF ANTIOXIDANT EXPRESSED IN mg/100g EDIBLE PART OF DRY FRUIT IN H_2O EXTRACT.

Antioxidant	Apple	Banana	Guava	Pineapple
Gallic acid	ND	ND	3.30 ± 0.88	ND
(+) Catechin hydrate	ND	ND	34.12 ± 4.19	ND
Vanillic acid	ND	ND	1.16 ± 0.07	ND
Caffeic acid	ND	ND	1.78 ± 0.66	ND
(-) Epicatechin	ND	ND	ND	4.11 ± 0.89
p-Coumaric acid	ND	ND	ND	ND
Rutin hydrate	ND	ND	7.38 ± 1.07	ND
Ellagic acid	ND	ND	ND	ND
Myricetin	ND	ND	ND	ND
Quercetin hydrate	ND	ND	ND	ND
Kaemferol	ND	ND	ND	ND

*The values of individual compound were with the mean ± standard deviation (n=3).ND: Not detected.

DISCUSSION: In this study apple, banana, guava and pineapple were investigated since they are very popular, relatively inexpensive, highly nutritious and widely available in almost all parts in Bangladesh, therefore those fruits are the part of daily dietary intake.

Among four fruits we found maximum moisture content in guava 91.27% (**Table 1**). This is very important for food's freshness and nutritional balance as well. This moisture of a fruit is very important since it helps to keep our skin hydrated, which leads to healthy, glowing skin and also help to weight lose because lesser amount of calories. When we checked the carbohydrate and fiber content interestingly we found guava had the highest fiber content (1.44%) but lowest carbohydrate content (5.87%) whereas banana had the lowest fiber and highest carbohydrate content 0.68% and 16.59 % respectively (**Table 1**). This result indicated that guava could be the better fit when we concern about the dietary intake of large number of diabetic patients in our country. Beside this guava had the lowest fat and protein content (**Table 1**) therefore it could be a worthy pick in the obesity, which has appeared as an emerging problem in Bangladesh. On the other hand, since banana had the highest carbohydrate, protein and moderate fat content among four fruits (**Table 1**) it could play an important role in malnutrition among the underprivileged people.

The ash content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific inorganic components present within a food, such as Calcium, Sodium, Potassium, Phosphorous and Chlorine. Among four samples analyzed, banana had highest ash content 0.63% and apple had the lowest 0.15% (**Table 1**). Determination of the ash and mineral content of fruits is important to asses their nutritional quality, microbiological stability and physicochemical properties especially needed during processing.

Natural antioxidants in plant materials are mainly due to their phenolic constituents and it has been established that phenolic compounds are the major plant compounds with antioxidant activity and this activity is due to their redox properties. According to our study, acetone was the most suitable for the extraction of the phenolic compounds present in the edible part of fruit. Fruits are the main source of hydroxycinnamic acid and its derivatives, including cinnamic, sinapic, *p*-coumaric and caffeic acid [20].

In this study, although we found maximum amount of *p*-coumaric acid and caffeic acid in pineapple after acetone extraction but there was no gallic acid in any of those fruits. On the other hand gallic acid was found only in methanol extract of pineapple and water extract of guava. The *p*-coumaric acid found in both pineapple and guava has antioxidant properties and is believed to reduce the risk of stomach cancer [21] by reducing the formation of carcinogenic nitrosamines [22]. Recent studies showed ellagic acid has antiproliferative and antioxidant properties in a number of *in vitro* and small-animal models [23, 24] and rutin contributes to the antibacterial [25] and antioxidant [26] properties of the plant. In the present study, ellagic acid and rutin was found maximum in the acetone extract of apple and guava respectively whereas pineapple had the highest amount of those compounds among four fruits in methanol extract (**Table 3** and **4**).

It was also found that apple has the highest amount of epicatechin, catechin and quercetin hydrate in acetone extract in compare with other three fruits but maximum epicatechin content in pineapple and guava in methanol and water extraction respectively (**Table 2-4**). In this study water has appeared as the poor extraction medium for the phenolic antioxidant extraction system. The acetone, methanol and water extracts of all of the fruits under investigation exhibited different extent of antioxidant content and thus provide a valuable source of nutraceutical supplements.

The results of his study indicated that among these four fruits if we consider for minerals we should intake banana, for fiber guava and for phenolic antioxidant apple and guava would be the best pick as daily fruits. Depending on the experimental values, some fruits are more important than others.

CONCLUSION: The study highlighted the composition of individual bioactive phenolic compounds varied among fruits analyzed, which indirectly influenced their antioxidant activity. The result of present study showed that the acetone extract of different fruits, which contain highest amount of different antioxidants that might exhibit the greatest reducing power and radical scavenging activity. As the fruits are quite safe and the use of synthetic antioxidant has been limited because of their toxicity therefore, these edible, widely available fruits could be exploited as antioxidant additives or as nutritional supplements. Moreover, no fruit serve as the sources of all nutrients, rather mixed fruits intake should be consider in maintaining healthy life.

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CONFLICT OF INTEREST: There is no conflict of interest.

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