Evaluation of Physico-Chemical and Antioxidant Properties in Different Varieties of Banana (*Musa acuminata*), Indigenous to Pakistan

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Summary: The antioxidant capacity, phenolic and flavonoid contents, and physico-chemical analysis on the pulp of three different varieties of *Musa acuminata*, were studied namely Cavendish basrai, Grand naine and Plantain, collected from Gharo, Adam Khas Kheli Road near Karachi (Pakistan). DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, reducing power (RPA) and phosphomolybdenum assays were used for determination of their antioxidant capacity. Cavendish basrai characterized the highest antioxidant capacity among three varieties, DPPH (82.46 % inhibition at 0.1 mM conc.), RPA (43.59 mg/100 g) and phosphomolybdenum (38.90 mg/100 g) in methanolic extract and DPPH (67.27 % inhibition at 0.1 mM conc.), RPA (27.03 mg/100 g) and phosphomolybdenum (24.27 mg/100 g) in water extract. The phenolic (83.04 mg/100 g, 19.50 mg/100 g) and flavonoid contents (11.66 mg/100 g, 4.77 mg/100 g) were also high in Cavendish basrai in methanolic and water extracts, respectively showed the direct relation of antioxidant capacity to the phenolic and flavonoid contents, and the DPPH assay revealed more power full assay for determination of antioxidant capacity among these assays. In correlation with antioxidant capacity, Plantain showed comparatively high physico-chemical characteristics revealed high nutritional contents such as total dry matter, total sugar contents, TSS, titratable acidity and % NaCl.

Keywords: *Musa acuminata*; Physico-chemical; Antioxidant properties; Phenolic and flavonoid contents.

Introduction

Antioxidants are substances that are capable to reduce or suppress oxidative damage of proteins, lipids and nucleic acids. Oxidative damage on cellular level caused by highly reactive oxygen species, which include reactive free radicals such as peroxide, superoxide and hypochlorous, etc. Antioxidants deactivate free radicals by breaking chain propagation, inhibiting initiation or suppressing formation of free radicals with high affinities by reducing hydrogen peroxide, binding to the metal ions and quenching superoxide and singlet oxygen [1]. Antioxidants have become an intense focus of research interest because of their superficial effects for health promoting, including anticarcinogenic, antiatherogenic, anti-ulcer, antithrombotic, anti-inflammatory, immunomodulating, antimicrobial and analgesic effects. That's why, the search for utilization of natural antioxidants, mainly from plant origin, has greatly improved in recent years [2]. Fruits show antioxidant capacities according to their polyphenolic contents, vitamin E, vitamin C, flavonoids and carotenoids [3].

The common name of *Musa acuminata* is banana. It belongs to the family "Musaceae". Banana is a well-known tropical fruit, its plant spread to India by about 600 BC and later on it reach all over the tropical world. It may be the world's oldest cultivated crop [4]. Banana is one of the most popular fruit. Several studies have indicated that both banana pulp and peel contains antibacterial and antioxidant potential [5]. Banana is a well source of potassium, starch, phenolic acid, anthocyanins, fructosans, terpenoids and sterols [6]. It has high concentration of catecholamine, dopamine, gallocatechin, norepinephrine and naringenin-7-*O*-neohesperidoside [7]. Plant parts like peels, stalks, fruits, roots and leaves of banana plants have been consumed orally or tropically for the medication of diarrhea and dysentery. It is also used in curing of intestinal lesions in colitis, inflammation, pains and snakebite [8], as well exhibited antiulcerogenic [9], hypoglycemic [10], hypolipidemic activities [11]. A constituent, hydroxyanigorufone obtained from *Musa paradisiaca*, showed to be a prospective cancer chemopreventive agent [12]. Methanolic extract of banana can be used as a potential drug for the treatment of diabetes mellitus [13].

The present research work describes physico-chemical evaluation, total phenolic and flavonoid contents, and antioxidant potential by using three different assays including DPPH radical scavenging, reducing power (RPA) and

phosphomolybdenum assays in different varieties of banana, indigenous to Pakistan and also the correlation among them.

Results and Discussion

Physico-Chemical Evaluation

The results of physico-chemical evaluation of three different varieties of *Musa acuminata* are shown in Table-1 and correlated in Figs. 1 and 2. Significant variations ($p \leq 0.05$) were observed among the selected varieties due to presences of different sources of macronutrients and micronutrients. From the present study, it was observed that the Plantain contained the maximum average fruit weight (154.40 g/fruit) in the selected varieties of *Musa acuminata* with contribution of flesh (67.37 %), maximum length (19.30 cm), maximum diameter (8.534 cm) and the lowest peel contribution (32.62 %). These results showed that Plantain has significantly different appearance from other two varieties.

Table-1: Physico-chemical evaluation of *Musa acuminata* collected from Gharo near Karachi (Pakistan).

S.No.	Properties	Unit	Varieties of *Musa acuminata*		
			Cavendish basrai Mean±S.E.M.	Grand naine Mean±S.E.M.	Plantain Mean±S.E.M.
1	Fruit weight	g/fruit	35.92 ± 3.74^{C}	54.82 ± 4.12^{B}	154.40 ± 10.72^{A}
2	Flesh contribution	%	58.09 ± 0.48^{C}	62.38 ± 1.50^{B}	67.37 ± 1.22^{A}
3	Peel contribution	%	41.90 ± 0.48^{A}	37.61 ± 1.50^{B}	32.62 ± 1.22^{C}
4	Length	cm	12.85 ± 0.05^{C}	14.30 ± 0.15^{B}	19.30 ± 0.65^{A}
5	Diameter	cm	4.216 ± 0.05^{C}	6.934 ± 0.20^{B}	8.534 ± 0.11^{A}
6	Moisture contents	%	79.18 ± 0.49^{A}	73.70 ± 0.38^{B}	73.70 ± 0.38^{B}
7	Total dry matter	%	20.81 ± 0.49^{B}	26.29 ± 0.38^{A}	26.39 ± 1.15^{A}
8	TSS	°Brix	12.20 ± 0.01^{C}	16.45 ± 0.47^{B}	20.05 ± 0.13^{A}
9	Total sugar contents	%	8.16 ± 1.09^{C}	13.19 ± 1.04^{B}	21.86 ± 2.72^{A}
10	pH	-	6.50 ± 0.30^{A}	6.59 ± 0.07^{A}	5.53 ± 0.24^{B}
11	Total organic matter	%	98.64 ± 0.10^{B}	98.96 ± 0.01^{A}	98.87 ± 0.01^{A}
12	Carotenoids	ppm	122.68 ± 2.26^{A}	68.02 ± 5.44^{B}	113.32 ± 3.44^{A}
13	Chlorophyll A	ppm	0.25 ± 0.0214^{B}	0.34 ± 0.0172^{B}	0.52 ± 0.0559^{A}
14	Chlorophyll B	ppm	0.46 ± 0.0393^{B}	0.62 ± 0.0323^{B}	0.95 ± 0.0994^{A}
15	TDS	g/1000 mL	0.464 ± 0.0268^{B}	0.775 ± 0.0242^{A}	0.758 ± 0.0375^{A}
16	Conductance	mS	0.897 ± 0.0305^{B}	1.680 ± 0.040^{A}	1.630 ± 0.0360^{A}
17	Titratable acidity	g/100 mL	0.453 ± 0.023^{B}	0.426 ± 0.023^{B}	0.506 ± 0.023^{A}
18	Total ash	%	1.35 ± 0.10^{A}	1.12 ± 0.01^{B}	1.03 ± 0.01^{B}
19	NaCl	%	0.031 ± 0.001^{C}	0.038 ± 0.002^{B}	0.05 ± 0.001^{A}

S.E.M. = Standard error of the mean of three experiments

(A-C) values in some row with different subscripts are significant differences was justified at 95% confidence level (ANOVA) by Tukey's HSD ($p \leq 0.05$)

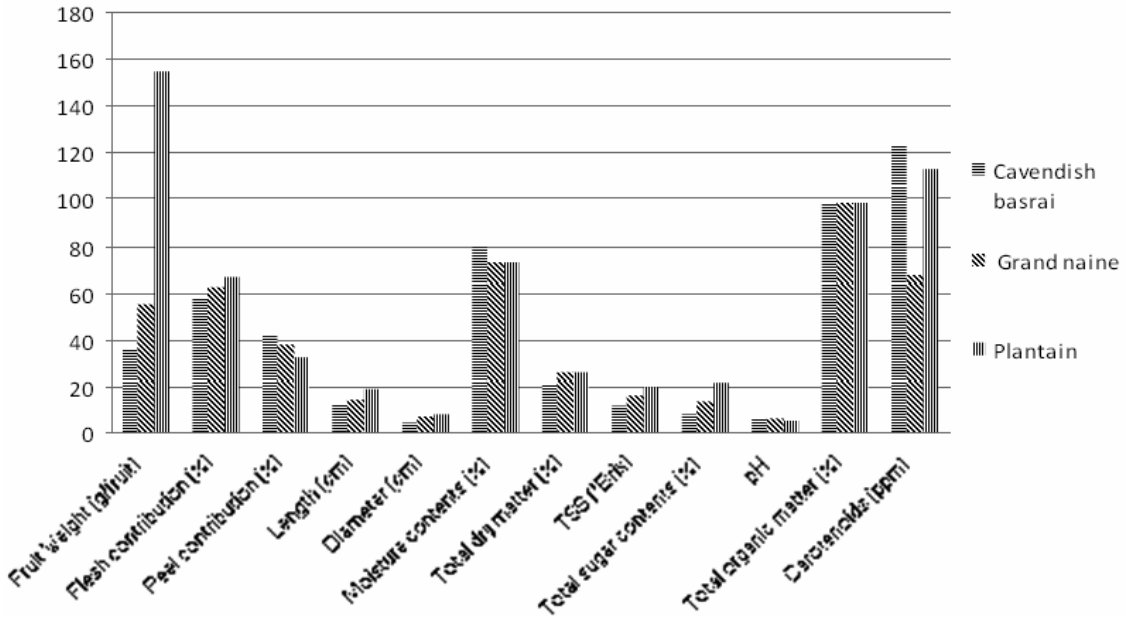

Fig. 1: Physico-chemical evaluation of *Musa acuminata* collected from Gharo near Karachi (Pakistan) at 95 % confidence level.

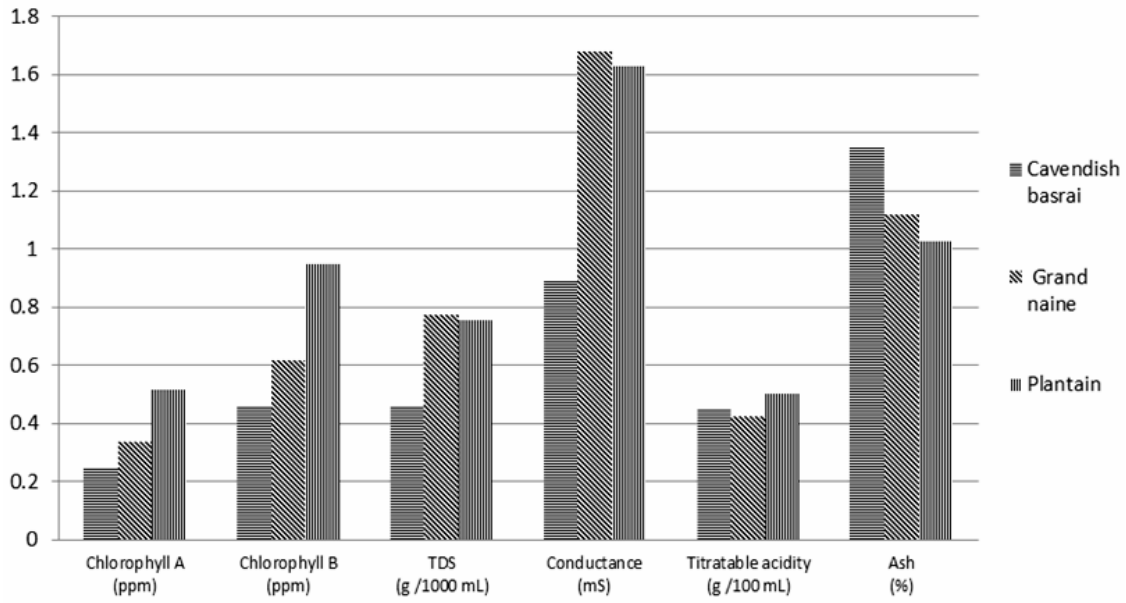

Fig. 2: Physico-chemical evaluation of *Musa acuminata* collected from Gharo near Karachi (Pakistan) at 95 % confidence level.

The highest moisture contents were observed in Cavendish basrai (79.18 %) with the lowest amount of total dry matter (20.81 %). TSS readings of Plantain (20.05 °Brix) showed the presence of high sucrose contents as compared to Grand naine and Cavendish basrai. Total soluble solids associated the sucrose contents in the flesh of banana, show the free sugar contents and used to determine the maturity, ripening and appreciation of fruits in sense of taste [14]. The significant changes ($p \leq 0.05$) in sugar contents in the selected varieties of *Musa acuminata* were observed. Plantain showed the highest sugar contents (21.86 %).

The pH and titratable acidity of different varieties of banana extracts were also investigated (Table-1). It was revealed that total titratable acidity increased in plantain (0.506 g/100 mL) as compared to Cavendish basrai (0.453 g/100 mL) and Grand naine (0.426 g/100 mL). This resulted in a decrease of pH: 5.53, 6.50and 6.59, respectively. The results exhibited direct relation between the progressive decrease in pH and increase in titratable acidity.

Total carotenoid contents results showed significant difference at 95 % confidence level ($p \leq 0.05$). Cavendish basrai contained the highest carotenoid contents (122.68 ppm). The significant changes in the different varieties of banana were also observed by Wall [15] for the analysis of carotenoid contents. Grand naine had high conductance (1.68 mS) as compared to other two varieties due to the presence of increase quantity of solvated species e.g. amount of NaCl and TDS (0.775 g/1000 mL). In case of mineral contents as ash, it was observed that Cavendish basrai contained high quantity of minerals as ash (1.35 %) as compared to Grand naine (1.12 %) and Plantain (1.03 %).

Antioxidant Analysis

Total Phenolic Contents (TPC): Methanolic extract of Cavendish basrai exhibited the highest total phenolic contents (TPC) (83.04 mg/100 g) in contrast to Grand naine (32.23 mg/100 g) and Plantain (42.06 mg/100 g). Water extract of Cavendish basrai also showed the greatest TPC (19.50 mg/100 g) as compared to the other two extracts of Grand naine (12.56 mg/100 g) and Plantain (6.35 mg/100 g). Grand naine showed higher TPC as compared to Plantain in water extract but smaller in methanolic extract (Table-2, Fig. 3).

It has been reported that yield of total phenolic contents depend upon the method and solvent used for extraction because polarity directly take part in rising phenolic solubility [16]. The highest amount of total phenolic contents in Cavendish basrai is may be due to presence of the highest content of organic acids as observed from the pH and acidity (Table-1). Organic acid reacts with Folin-Ciocalteu reagent in the TPC determination [17].

Table-2: Antioxidant analysis of *Musa acuminata* collected from Gharo near Karachi (Pakistan).

S. No.	Properties	Unit	Varities of *Musa acuminata*		
			Cavendish basrai Mean±S.E.M.	Grand naine Mean±S.E.M.	Plantain Mean±S.E.M.
1	Total phenolic contents^{m} (TPC)	mg/100 g	83.04±0.072^{A}	32.23±0.050^{C}	42.06±0.110^{B}
2	Total phenolic contents^{w} (TPC)	mg/100 g	19.50±0.30^{A}	12.56±0.11^{B}	6.35±0.06^{C}
3	Total flavonoid contents^{m} (TFC)	mg/100 g	11.66±0.23^{A}	5.48±0.02^{B}	4.29±0.03^{C}
4	Total flavonoid contents^{w} (TFC)	mg/100 g	4.77±0.020^{A}	4.56±0.010^{B}	3.06±0.060^{C}
5	DPPH Radical scavenging assay^{m}	% inhibition at 0.1 mM conc.	82.46±1.50^{A}	44.18±0.65^{B}	30.38±3.00^{C}
6	DPPH Radical scavenging assay^{w}	% inhibition at 0.1 mM conc.	67.27±0.65^{A}	33.59±0.93^{B}	22.30±0.15^{C}
7	RPA assay^{m}	mg/100 g	43.59±0.26^{A}	25.80±0.17^{B}	25.97±0.23^{B}
8	RPA assay^{w}	mg/100 g	27.03±0.06^{A}	25.18±0.16^{B}	15.13±0.11^{C}
9	Phosphomolybdenum assay^{m}	mg/100 g	38.90±0.95^{A}	20.80±0.55^{B}	19.67±0.37^{C}
10	Phosphomolybdenum assay^{w}	mg/100 g	24.27±0.49^{A}	18.34±0.53^{B}	13.57±0.89^{C}

m = methanolic extract
w = water extract
S.E.M. = Standard error of the mean of three experiments
(A-C) values in some row with different subscripts are significant differences was justified at 95 % confidence level (ANOVA) by Tukey's HSD ($p \leq 0.05$)

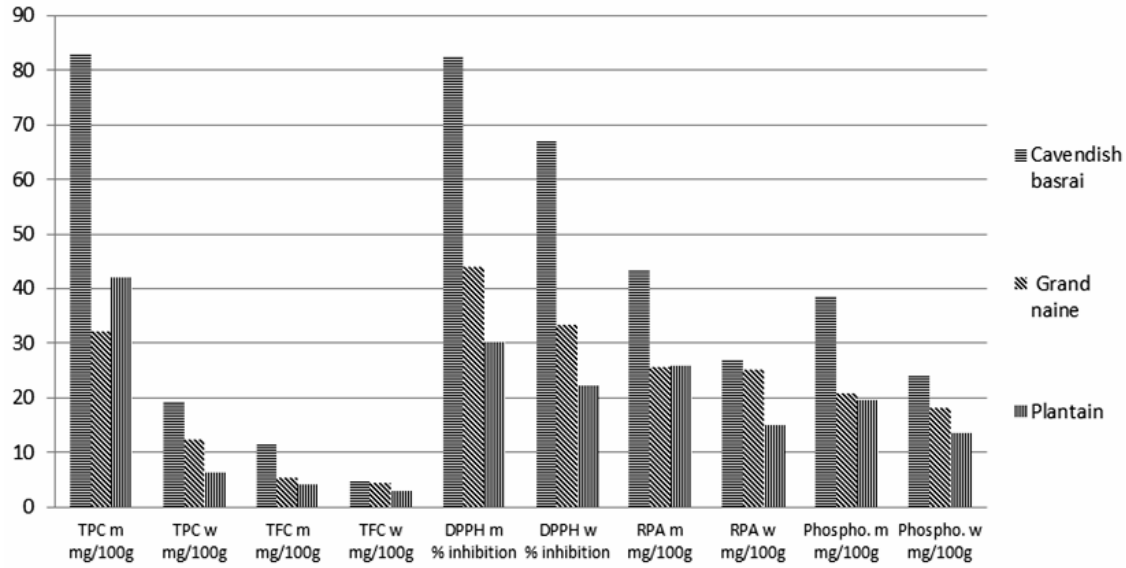

Fig. 3: Antioxidant analysis of *Musa acuminata* collected from Gharo near Karachi (Pakistan) at 95 % confidence level. m = methanolic extract and w = water extract.

Total Flavonoids Contents (TFC): The total flavonoid contents (TFC) among the three different varieties of *Musa acuminata* were also observed (Table-2, Fig. 3). Two different solvents (water and methanol) were used for the extraction of flavonoid contents. The methanolic extract of Cavendish basrai showed the highest TFC (11.66 mg /100 g) than Grand naine (5.48 mg/100 g) and Plantain (4.29 mg/100 g). Similarly water extract of Cavendish basrai was also showed the highest TFC again (4.77 mg/100 g), whereas Grand naine and Plantain showed 4.56 mg/100 g and 3.06 mg/100 g, respectively.

It has been observed that banana contained various kinds of flavonoids [9]. The high content of polyphenols (phenolics and flavonoids) was accountable for high antioxidant capacity in these varieties of *Musa acuminata*. However, the antiulcerative activity in banana is due to the presence of natural flavonoids and this activity may vary in different varieties of banana due to the different levels of these natural active components [18, 19].

DPPH Radical Scavenging Assay: The total antioxidant capacity in the selected varieties of *Musa acuminata* was determined spectrophotometrically by using DPPH radical scavenging assay. It was the most useful method for evaluating antioxidant capacity. DPPH is a radical and capable to accept electron from the donor antioxidant specie in the sample and become stable molecule. In this reaction, the DPPH radical convert into reduced form of DPPH

by decolorizing of violet color into pale yellow depends on the capacity of the antioxidant compounds in the sample, shows maximum absorbance in the range of 515 to 517 nm.

Different concentrations of DPPH radical were used in the range from 0.025 to 0.300 mM, depends upon the sensitivity of sample. As sensitivity of the sample increases the concentration of DPPH radical also increases. In this study, the concentration of DPPH radical was used 0.1 mM and as a result the highest % inhibition was observed in the methanolic extract of Cavendish basrai (82.46 %) as compared to the other two varieties, Grand naine (44.18 %) and Plantain (30.38 %), which were shown in (Table-2, Fig. 3). Water extract of Cavendish basrai also exhibited the strong % inhibition (67.27 %) than Grand naine (33.59 %) and Plantain (22.30 %) (Table-2 and Fig. 3). The results showed the significant difference in the varieties of *Musa acuminata* at 95% confidence level ($p \leq 0.05$). The highest DPPH radical scavenging activity in Cavendish basrai is may be due to the highest radical scavenging ability of total phenolic and flavonoid contents as shown in Table-2. It has been reported that antioxidant activity is proportional to the total phenolic and flavonoid contents [20].

Reducing Power Assay (RPA): The protocol of reducing power assay (RPA) is same as other antioxidant assays, based on the measurement of antioxidant reduction potential in sample solution. In this assay, potassium ferricyanide (Fe^{+3}) is converted into potassium ferrocyanide (Fe^{+2}) and finally ferric ferrous complex formed after reacting with ferric chloride, having maximum absorption at 700 nm. The change in color observes from yellow to a variety of green and blue shades depending on the reducing capability of selected samples.

The results of RPA in different varieties of *Musa acuminata* were shown in Table-2. The methanolic extract was considerably more active as compared to water extract. Methanolic extract of Cavendish basrai exhibited the highest antioxidant potential (43.59 mg/100 g) as compared to other varieties. Antioxidant potential of Grand naine (25.80 mg/100 g) and Plantain (25.97 mg/100 g) did not show any significant difference at 95% confidence level ($p \leq 0.05$). Similarly, water extract of Cavendish basrai also showed the highest antioxidant potential (27.03 mg/100 g) than Grand naine (25.18 mg/100 g) and Plantain (15.13 mg/100 g).

Phosphomolybdenum Assay: In this assay, the antioxidant ability of samples is measured by the reduction of Mo (VI) into Mo (V) in presence of antioxidants, which is observed by the green color solution of Mo (V) complex [21].

The results of phosphomolybdenum assay had similar pattern of antioxidant capacity as analyzed by DPPH radical scavenging assay and RPA (Table-2, Fig. 3). Cavendish basrai showed the greatest antioxidant capacity in both methanolic (38.90 mg/100 g) and water (24.27 mg/100 g) extracts as compared to Grand naine (20.80 mg/100 g) and Plantain (19.67 mg/100 g) in methanolic extract whereas 18.34 mg/100 g and 13.57 mg/100 g in water extract, respectively.

Experimental

Sample Collection

Three varieties of fresh *Musa acuminata*, namely Cavendish basrai, Grand naine and Plantain with no appreciable physical or microbial damage were collected from the farm of Gharo, Adam Khas Kheli Road near Karachi (Pakistan) in the stage of inflorescences (fruit is above the bud). All the fruits were of eating quality.

Sample Preparation

The flesh of banana was scalped from peel and cut down into small pieces. 10 g Flesh sample of each fruit was crushed in a motor and pastel. Crushed sample was soaked in 100 mL of water or methanol and stirred for 60 min on magnetic stirrer (10 % w/v sample solution). The extracts were filtered by using whatman filter paper (Grade 41).

Physico-chemical Evaluation

Total weight, weight of flesh, weight of peel, length and diameter: The length of the sample was determined by using inch tape. The diameter was determined by using vernier calliper. Total weight was determined by weighing the sample on electronic balance (Denver TP-214, Germany).

Moisture Contents and Total Dry Matter: Moisture contents and total dry matter were determined according to the method of I.S.I. [22]. Sample (5 g) was weighted in a dried petri dish. The petri dish was placed in a hot air oven (Binder E28#05-86486, Germany), temperature maintained at 105±2 °C at least for 2 hours until dryness. Cooled in a desiccator and weighed. The process of heating, cooling and weighing was repeated until the difference between two successive weighing was less

than 1 mg. The lowest weight was recorded. Moisture contents and total dry matter were determined according to the following formula:

Moisture (% by weight) = 100 $(M_1 - M_2)/M_1 - M$

Total dry matter (% by weight) =100 $(M_2-M)/M_1 -M$

where, M_1 = weight (g) of petri dish with material before drying
M_2 = weight (g) of petri dish with the dried material
M = weight (g) of empty petri dish

Total Soluble Solids (TSS): Total soluble solids (TSS) in the thick products of *Musa acuminata,* was determined according to the method of BIS I.S. [23]. Briefly into the beaker, suitable quantity (40-50 g) of the sample was added in 100-150 mL of distilled water. The contents of beaker were allowed to boil with continuous stirring for 5 min. After cooling, the contents were filtered. The TSS of the test solution was determined on the refractometer (KRÜSS DR 6200, Germany), expressed as sucrose content.

Total Sugar Contents: 1 g of the sample was weighed into a beaker and 10 mL of methanol was added. Solution was stirred with magnetic stirrer for 30 min. The mixture was filtered through whatman filter paper (Grade 41). 1 mL of aliquot was pipetted into a test tube and 1 mL of 5 % phenol was added into it, the solution was shaken then 5 mL of 96 % H_2SO_4 was further added. The test tube was allowed to cool in a water bath (Witeg WIG-32, Germany) at 30 °C for 10 min. The absorbance of the test solution was measured at 490 nm against the blank [24], with the help of spectrophotometer (Jenway 6300, England). Total sugar contents were measured by using the following formula.

Total sugar contents =

$$\frac{\text{absorbance of sample} \times \text{dilution factor} \times \text{gradient factor}}{10{,}000 \times \text{weight of sample (g)}}$$

pH: The pH of the test solution (water extract) was determined by using the pH meter (Jenway 3510, England).

Total Organic Matter: Total organic matter was calculated by using the formula as follows:

Total organic matter (%) = 100 - total ash (%)

Total Carotenoids: The amount of total carotenoids in the test solution (methanolic extract) was also analyzed according to the method of Dere *et al*., [25] by using the following formula.

Total carotenoids = 1000 A_{470}-2.860 C_a-129.2 C_b/245

where, A_{470} = Absorbance at 470 nm
C_a = Chlorophyll a
C_b = Chlorophyll b

Chlorophyll a (C_a) and Chlorophyll b (C_b): Chlorophyll a (C_a) and chlorophyll b (C_b) in a test solution of methanol was determined by using the method of Dere *et al*., [25]. The amount of these pigments was calculated according the formula as follows:

$C_a = 15.65\ A_{666} - 7.340\ A_{653}$

$C_b = 27.05\ A_{653} - 11.21\ A_{666}$

Where, A_{666}=Absorbance at 666 nm
A_{653}=Absorbance at 653 nm

Total Dissolved Solids (TDS) and Conductance: The TDS of the sample solution (water extract) was determined by using TDS meter (S 518860, Korea). Conductance was analyzed by using conductivity meter (Jenway 4510, England).

Titratable Acidity: Titratable acidity was determined briefly according to the method of A.O.A.C. [26]. It is expressed in g acid per 100 g, by using the factor appropriate to the acid as, 1 mL of 0.1 N NaOH equals to 0.0060 g acetic acid. In which simply 5 mL of the test solution (water extract) was titrated with 0.1 N NaOH using phenolphthalein as indicator. Acidity reported as mL of 0.1 N NaOH per 100 g or 100 mL as required.

Total Ash: Total ash was determined according to the method of A.O.A.C. [24]. Briefly 5 g of the sample was weighed in silica crucible, dried in the hot air oven (Binder E28#05-86486, Germany) and then kept in the muffle furnace (Nabertherm GmbH Bahnhofstr. 20, 28865 Lilienthal, Germany) at 550 °C for 6-8 hours until white ash was obtained. The dish was cooled, weighed and kept in the muffle furnace again for 1 hour to obtain the lowest weight of total ash. % Ash and organic matter was calculated by using the formula as follows:

$$\text{Total ash (\% by weight)} = \frac{\text{weigh of ash (g)} \times 100}{\text{original weight of sample (g)}}$$

% of NaCl: % of NaCl was determined according to the method of I.S.I. [27]. Simply 5 mL of the sample solution (water extract) was neutralized with standard NaOH solution using phenolphthalein as indicator, then 1 mL of 5 % aqueous K_2CrO_4 solution was added into it and titrated with 0.1 N $AgNO_3$ solution to produce red-brown end point. The % of NaCl was determined by the following formula:

% of NaCl =

$$\frac{\text{titre value} \times \text{Normality of } AgNO_3 \times 58.4 \times 100}{\text{weight of the sample (g)} \times 1000}$$

Antioxidant Analysis

Total Phenolic Contents (TPC): Total phenolic contents (TPC) were determined [28]. Briefly, 40 µL of sample solution (water or methanolic extract) were mixed with 1.8 mL of pre-diluted Folin-Ciocalteu reagent (10 times with distilled water). 1.2 mL of (7.5 % w/v) sodium carbonate solution was added after 5 min at room temperature. Then the solution was mixed and allowed to stand at room temperature for 1 hour. The absorbance was recorded at 765 nm by using spectrophotometer (Jenway 6300, England). Calibration curve was plotted using a standard solution of gallic acid (20, 40, 60, 80 and 100 mg/L, r^2 = 0.997). Results were expressed as mg of gallic acid equivalents/100 g of sample.

Total Flavonoid Contents (TFC): Total flavonoid contents (TFC) were also determined [29]. 1 mL of sample solution (water or methanolic extract) was mixed with distilled water (4 mL) and 0.3 mL of (5 % w/v) $NaNO_2$ was added. 0.3 mL of (10 % w/v) $Al(NO_3)_3$ was also added after 5 min. Then volume was made up to 10 mL by the addition of distilled water (2.4 mL). After shaking the mixture, absorbance was measured at 510 nm. A calibration curve was plotted using a standard solution of catechin (20, 40, 60, 80 and 100 mg/L, r^2 = 0.996). Results were expressed as mg of catechin equivalents (CEQ) /100 g of sample.

DPPH Free Radical Scavenging Assay: Antioxidant capacity was obtained through DPPH (2,2-diphenyl-1-picrylhydrazyl) assay according to the method proposed by De Ancos *et al*. [30]. 5 µL of sample solution (water or methanolic extract) was mixed with 0.1 mM methanolic solution of DPPH (95 µL). This mixture was then mixed on vortex mixer (VM-300, Gemmy Industrial Corp., Taiwan) and kept in dark for 30 min. Its absorbance was recorded at 515 nm against a blank (methanol without DPPH). The results were expressed as % inhibition of the DPPH radical.

% Inhibition of DPPH radical =

$$\frac{(\text{absorbance of control}^* - \text{absorbance of sample}) \times 100}{\text{absorbance of control}^*}$$

where, *Control = DPPH solution without sample solution

Reducing Power Assay (RPA): Antioxidant capacity through reducing power assay was also determined [31]. 0.1 mL Sample solution (water or methanolic extract) was mixed with potassium ferricyanide (2.5 mL) and phosphate buffer (2.5 mL). This mixture was kept on water bath at 50 °C for 20 min. After cooling, 10 % trichloro acetic acid (2.5 mL) was added and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with a freshly prepared ferric chloride solution (0.5 mL) and distilled water (2.5 mL). The absorbance was measured at 700 nm. A calibration curve was obtained using a standard solution of ascorbic acid at various concentrations (20, 40, 60, 80 and 100 mg/L, r^2 = 0.993). The results were expressed as mg of ascorbic acid equivalents/100 g of sample.

Phosphomolybdenum Assay: Antioxidant capacity of samples was also determined through phosphomolybdenum assay [21]. 0.1 mL Sample solution (water or methanolic extract) was mixed with 4 mL reagent solution (28 mM sodium phosphate, 0.6 M sulphuric acid and 4 mM ammonium molybdate) and heated on water bath at 95 °C for 90 min. Samples were cooled at room temperature and absorbance were measured at 695 nm. A calibration curve was obtained using a standard solution of ascorbic acid at various concentrations (20, 40, 60, 80 and 100 mg/L, r^2 = 0.994).The results were expressed as mg of ascorbic acid equivalents/100 g of sample.

Statistical Analysis

All the results were expressed as a mean of triplicate ± SEM (Standard error mean). Data were statistically analyzed by one-way ANOVA Tukey's HSD with $\alpha = 0.05$ test by using Minitab 16 software, significant difference was justified at 95% confidence level.

Conclusion

The physico-chemical evaluation showed that Plantain contained extensive nutritional components whereas Cavendish basrai contained the

highest total phenolic and flavonoid contents as well as the potent antioxidant activity among three varieties of *Musa acuminata* regarding DPPH radical scavenging, reducing power and phosphomolybdenum assays. Grand naine has significant antioxidant potential than Plantain. It was also observed that methanol is better solvent than water because it dissolved more antioxidants (phenolic and flavonoids). It was also assumed that DPPH radical scavenging assay is the best among three assays for determination of antioxidant potential in the three varieties of *Musa acuminata*, whereas reducing power assay was better than phosphomolybdenum assay.

The aim of this research was to explore the importance of plant based antioxidants in *Musa acuminata* of Pakistani origin and to identify the antioxidant capacity in different varieties of *Musa acuminata* from the same origin and stage.

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