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Phytochemical and antioxidant comparison of different varieties of banana

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Abstract
Banana is widely cultivated fruit around the world. The edible portion (flesh) of banana has been used for making various foods as well as eaten directly. But the outer portion (peel) is not used so much and thrown directly into the environment in most of the cases. But bioactive compounds are mostly present into the peel and in this study the comparative phytochemical, antioxidant and antibacterial property of banana peel and fleshes are evaluated. Three different varieties of unripe raw banana were selected for this study. The peel and flesh of the banana was separated, dried, crushed into coarse powder and extracted by methanol and ethyl acetate. The total phenolic and flavonoid content, total antioxidant capacity, ferrous reducing power assay and DPPH radical scavenging activity was analyzed. All the extracts have considerable amount of phenolic and flavonoid content. The extracts also exhibit very good activity in total antioxidant assay, iron reducing power activity and DPPH radical scavenging assay. The methanolic extract of Dimkumari Peel (MDP) is the most effective among all the extracts. It has phenolic content equivalent to 10.215±0.873 mg/g of GAE and flavonoid content equivalent to 9.285±0.452 mg/g of CAE. The MDP has scavenging power 69.44% at 100 µl/ml concentration which is very reasonable compared to the standard BHT 77.48%. From this study it was found that banana peel and flesh both are rich source of antioxidants.

Keywords: Banana, total phenolic, total flavonoid, DPPH, iron reducing, antioxidant

Introduction
Plants are great source of new classes of drugs and thus, research is persistently progressing on novel drugs from plant-based products and curing diseases. Approximately, 80% of the global population, particularly in the emerging countries, use medicinal plants as their first choice of medications [1]. Traditional medicines are potential reservoir of new therapeutics along with more than 2000 plant species [2]. A number of antimicrobial drugs are discovered and some of them yet to be established which are hidden in nature. As several pathogenic organisms has already developed resistant to the current antimicrobial agents so it is an urgency to find out newer classes of antimicrobial [3].

Banana is elongated fruit botanically a berry [4] and produced from several kinds of large herbaceous flowering plants. Banana fruit is variable size, color and firmness and usually curves with green, yellow, red, purple or brown rind. At present most of the edible bananas are seedless (parthenocarpy) come mainly from two wild species namely *Musa acuminata* and *Musa balbisiana*. Banana is cultivated all over the world and is extremely popular fruit in Bangladesh. It is a tropical fruit grown in over 122 countries worldwide [5]. In 2013 the worldwide production of banana was 106.71 million metric tons [6]. In 2016 the production of banana was increased to 148.4 million metric tons [7] which raised to 153.1 million metric tons in 2017 [8].

There are various types of compounds are present in banana. The most common enzymes are polyphenol oxidase, pectin and amylases and glucosidase like enzyme [9]. Beside this flavonoid, alkaloids, tannins, terpenoids and glycosides are present. In addition, banana contains vitamin A, vitamin C, gallocatechine, dopamine, vitamin E, vitamin B_6, malic acid, succinic acid and palmitic acids. Minerals mainly magnesium, potassium, phosphorus and iron are also present in banana [10].

Banana contains high proportion of carbohydrates, fibers and low percentages of protein. Banana peels is a major agricultural waste which have been used as medicine, animal feeds, blacking of leathers, soap making, fillers in rubber and many others [11]. This fruit wastes are highly perishable and are a problem to the processing industries and pollution monitoring agencies. Banana fruit peel, is one of the major underutilized sources of phenolic compounds which is considered as a good source of antioxidants for foods and functional foods against cancer and heart disease [12].

Materials and Methods

Collection of the plant sample

Three different varieties of unripe raw banana were collected from Pabna. The banana was collected pure state as it does not contain foreign chemicals.

Dimkumari Sagor Madna

Fig 1: Studied varieties of banana peel and flesh.

Preparation and extraction of the banana

After collection, the banana was washed thoroughly in distilled water and sun dried. The banana was peeled off by scrapper and knife and the peels and fleshes are oven dried at 55˚C. Then they (peels and fleshes) are crushed into coarse powder by blender (Model: Miyako Electric Grinder MC-07, Origin: India) and stored at room temperature for further use.

The samples are extracted by both polar and non-polar solvent. For polar fraction methanol (relative polarity 0.762) and for non-polar ethyl acetate (relative polarity 0.228) was used (Solvent Polarity Table, 2022). The liquid extract was filtered by Whatman No.1 filter and then concentrated with Rotary evaporator (Model: RE-200A, Manufacturer: USA Lab Equipment, Origin: USA).

For smooth working, the samples are coded as follows:

Table 1: Banana varieties with designated code name.

Banana Varieties with extracted solvent	Sample Code
Methanolic Extract of Dimkumari Flesh	MDF
Methanolic Extract of Dimkumari Flesh	MDP
Ethyl Acetate Extract of Dimkumari Flesh	EADF
Ethyl Acetate Extract of Dimkumari Peel	EADP
Methanolic Extract of Sagor Flesh	MSF
Methanolic Extract of Sagor Flesh	MSP
Ethyl Acetate Extract of Sagor Flesh	EASF
Ethyl Acetate Extract of Sagor Peel	EASP
Methanolic Extract of Madna Flesh	MMF
Methanolic Extract of Madna Flesh	MMP
Ethyl Acetate Extract of Madna Flesh	EAMF
Ethyl Acetate Extract of Madna Peel	EAMP

Total phenolic content

The total phenolic content of the samples was determined by Folin-Ciocalteu method [13]. Briefly 2.0 ml of 10% (v/v) Folin-Ciocalteu reagent (FCR) was added in 0.4 ml extract solution followed by addition of 2.0 ml sodium bicarbonate (7.5%) and then incubation of 20 min at 25 °C under dark condition. The absorbance of the complex was taken at 760 nm using a UV spectrometer (Model number: SE6300, Manufacturer: Azzota, Origin: USA). Result was expressed as gallic acid equivalent (GAE) in milligrams per gm of dry weight of peel.

Total flavonoid content

Total flavonoid content of different fruit peal extracts was measured by aluminium chloride colorimetric method where quercetin (QE) was used as standard [14]. Briefly 0.5 ml extract was mixed with 0.1 ml of 10% aluminium chloride. After that 0.1 ml potassium acetate (1M) was added and incubated for 30 minutes at room temperature under dark. Finally, the absorbance was taken at 420 nm using the UV spectrometer. The total flavonoids content was expressed as mg quercetin equivalent (QE) per gram of dried sample peel extract.

Total Antioxidant Capacity

The total antioxidant capacity of citrus fruit peal was determined by phosphomolybdenum method [15]. In this process 0.5 ml extract was mixed with 3 ml reagent solution (0.6 M sulfuric acid, 28 ml sodium phosphate and 1% ammonium molybdate). Then the mixture was incubated in the oven for 90 minutes at 95° C. After cooling at room temperature, the absorbance was taken at 695 nm using UV spectrometer.

DPPH radical scavenging activity

DPPH (1, 1-diphenyl 2-picrylhydrazyl) radical scavenging activity is used to evaluate scavenging activity of antioxidants on free radicals [16]. Here the absorbance of the sample is measured and the absorbance is decreased due to the scavenging of DPPH radical by donation of proton from the antioxidant compounds present in the sample. With 1 ml of fruit peel extract, 2.4 ml DPPH (0.004% w/v) was added. After incubation at dark for 30 minutes absorbance was taken at 517 nm against blank using the UV spectrometer.

Ferric reducing power assay

The reducing power capacity of sample was determined by the method described by Do QD [17]. Here 0.625 ml phosphate buffer (0.2 M) and 0.625 ml potassium ferricyanide [K_3 Fe $(CN)_6$] (1% w/v) was added in 0.25 ml fruit peel extract. Then 0.625 ml 10% trichloro-acetic acid (TCA) was added after incubation of 20 minutes at 50°C. Then the total mixture was centrifuged at 3000 rpm for 10 minutes (Model Number: UNICO Powerspin Lx C858, Manufacturer: Unico, Origin: USA). After centrifugation, 1.8 ml supernatant was withdrawn and diluted with 1.8 ml distilled water, then it is mixed with 0.36 ml 0.1% ferric chloride ($FeCl_3$) and finally absorbance was taken at 700 nm against blank solution using the UV spectrometer.

Result

Total phenolic content

Phenolic compounds are plant secondary metabolites, originated from shikimic acid and pentose phosphate via phenylpropanoid metabolization [18]. This phenolic compound has hydroxyl substituents in the benzene ring [19]. The plant phenolic compounds are flavonoids, phenolic acids, complex flavonoids and anthocyanins [20]. The phenolic compounds have beneficial role mainly anti-aging, anti-inflammatory, antioxidant and anticancer effects [21].

The total phenolic content of banana extracts (peels and fleshes) was determined from the calibration curve of gallic acid (standard compound).

Table 2: Calibration curve of standards

Test	Standard	Conc. range	Calibration Curve	R^2
Total phenolic content	Gallic acid (GA)	2.5-30µg/ml	y =0.0099x+0.0003	0.9891
Total flavonoid content	Catechin (CA)	2.5-30µg/ml	y=0.0071x+0.0074	0.9834
Total Antioxidant Assay	Ascorbic acid (AA)	12.5-200µg/ml	y=0.0015x+0.0135	0.9808

The three studied banana varieties have considerable amount of phenolic content. Among all the extract, the methanolic extract of Dimkumari Peel (MDP) has highest amount of phenolic content which is equivalent to 10.215±0.873 mg/g of GAE. The methanolic extract of Sagor Flesh (MSP) also contain very high amount of phenolic content 9.719±0.147 mg/g of GAE. The ethyl acetate extract of Sagor Flesh has lowest amount of phenolic content 2.889±0.057 mg/g of GAE.

Table 3: Total phenolic content of banana varieties.

Sample	Average Phenolic Content (mg/g of GAE) ± Standard Deviation (SD)
MDP	10.215±0.873
MDF	4.644±0.311
EADP	5.571±0.151
EADF	3.376±0.144
MSP	9.719±0.147
MSF	3.171±0.028
EASP	4.047±0.374
EASF	2.889±0.057
MMP	5.179±0.182
MMF	4.757±0.051
EAMP	5.059±0.059
EAMF	4.613±0.143

Total flavonoid content

Flavonoids are polyphenolic compounds with benzo-γ-pyrone structure and synthesized by phenylpropanoid pathways. They are part of dietary component and has health promoting activities mainly *in vivo* and *in vitro* antioxidants. The flavonoids have antibacterial, antiviral, cardioprotective, cancers and other diseases [22].

The total flavonoid content was measured as catechin equivalent (CAE). Generally, the peels contain higher amount of flavonoid content compared to the flesh. The extract MDP (Methanolic Extract of Dimkumari Peel) has flavonoid content of 9.285±0.452 mg/g of CAE followed by MSP (Methanolic Extract of Sagor Flesh) 7.811±0.379 mg/g of CAE and MMP (Methanolic Extract of Madna Flesh) 7.060±0.768 mg/g of CAE.

Table 4: Total flavonoid content of banana varieties.

Sample	Average Flavonoids Content (mg/g of CAE) ± Standard Deviation (SD)
MDP	9.285±0.452
MDF	4.393±0.156
EADP	4.375±0.814
EADF	6.210±1.464
MSP	7.811±0.379
MSF	2.981±0.136
EASP	6.178±0.539
EASF	3.154±0.155
MMP	7.060±0.768
MMF	5.656±0.134
EAMP	6.863±1.396
EAMF	4.868±1.614

Total antioxidant assay: Total antioxidant capacity (TAC) is an analytical method widely used to assess antioxidant status of plant samples. It was measured with standard antioxidant, ascorbic acid equivalent (AAE). All the extracts have significant amount of antioxidant activity. The extract MMP has highest amount of phenolic content 39.044±0.656 mg/g of AAE whereas the extract MMF has lowest amount of antioxidant content 16.644±3.533 mg/g of AAE among all the extracts. The antioxidant activity of EADP 30.467±1.638 mg/g of AAE, MSP 30.644±0.804 mg/g of AAE and EAMF 30.644 ±2.579 mg/g of AAE are also highly significant.

Table 5: Total antioxidant assay of banana peel and fleshes.

Sample	Total Antioxidant Assay (mg/g of AAE) ± Standard Deviation (SD)
MDP	29.00±0.481
MDF	18.20±0.581
EADP	30.467±1.638
EADF	29.489±3.593
MSP	30.644±0.804
MSF	17.222±0.154
EASP	25.622±2.006
EASF	25.533±1.313
MMP	39.044±0.656
MMF	16.644±3.533
EAMP	22.111±0.965
EAMF	30.644±2.579

Iron reducing power assay

The ferrous reducing power assay of the banana peel and pulp extracts was measured compared to the standard antioxidant ascorbic acid. Almost all the extracts have good reducing power activity against the standard ascorbic acid except EADP.

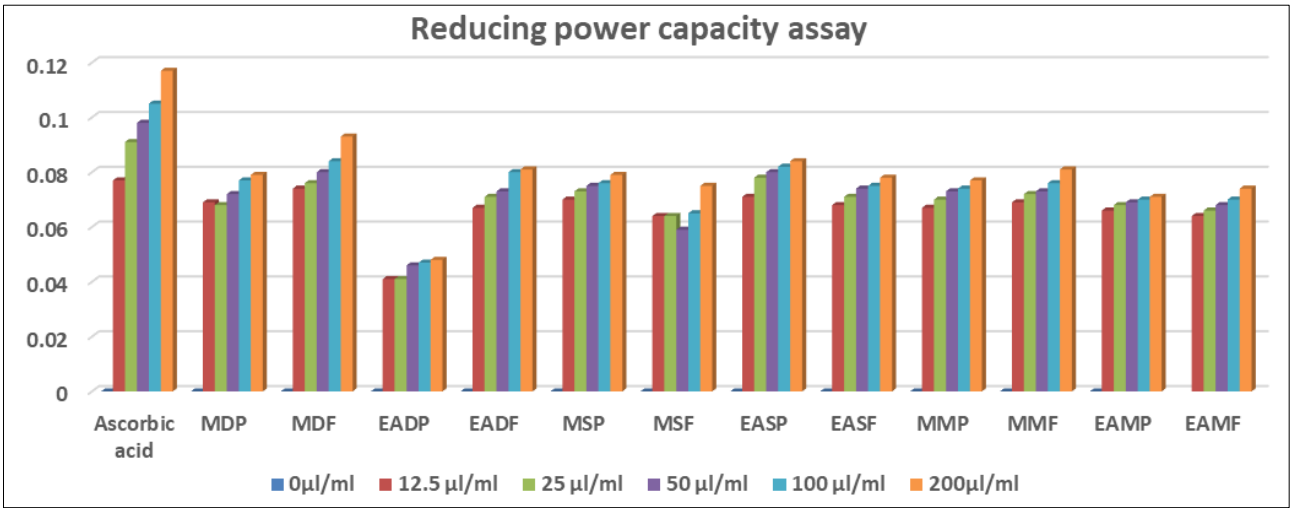

Fig 2: Reducing power capacity assay of banana varieties.

DPPH radical scavenging assay

This assay is used to measure the scavenging power of the bioactive compounds present in the plant sample. In this study the radical scavenging activity was measured compared to the standard antioxidant BHT. At 100 µl/ml concentration the BHT scavenges 77.48% whereas the extract MDP scavenges 69.44%, followed by MSP 58.81%. The extract EADF and MMF has comparatively lower scavenging activity which was 30.49% and 31.54% respectively.

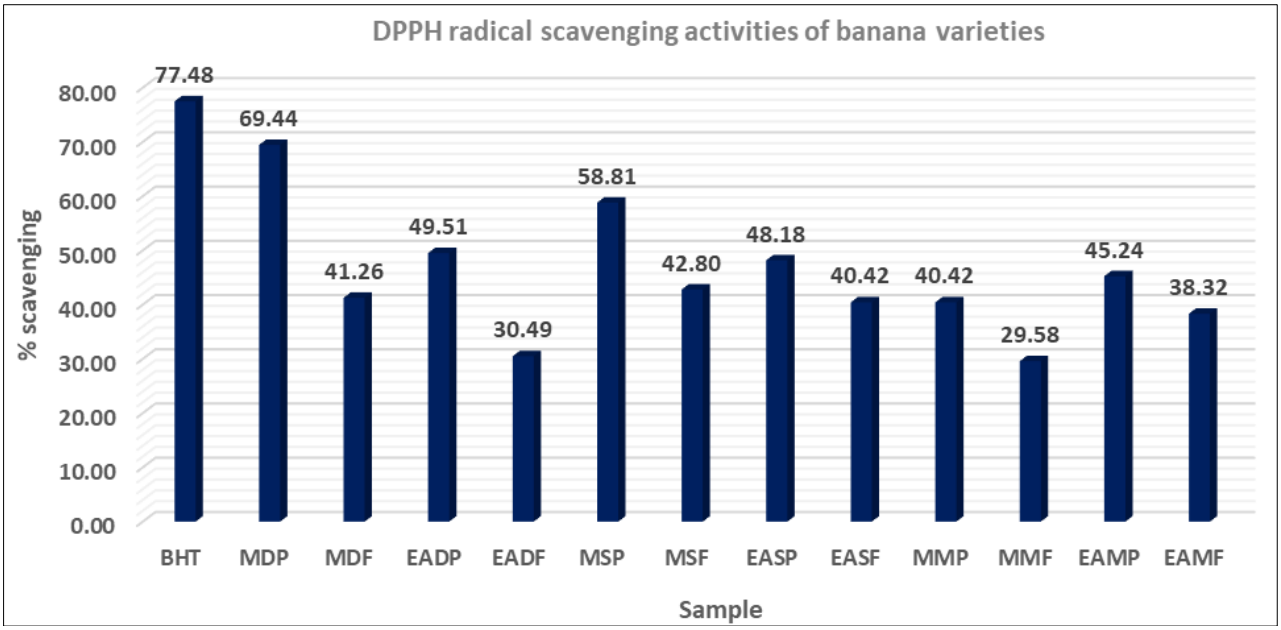

Fig 3: DPPH radical scavenging assay of banana varieties.

Discussion

Free radicals are continuously producing inside the living cell for normal metabolic process. This free radical can generate many types of disease by oxidizing vital cellular constituents mainly lipids, proteins and DNA. Antioxidants both in vivo and in vitro neutralizes those free radicals. Antioxidant enzymes like superoxide dismutase, glutathione peroxidase and catalase continuously work inside the cell to inhibit generation of free radicals [23]. Phytochemicals mainly phenolics, flavonoids, alkaloids and glycosides present in a plant sample is a good indication of bioactivity [24].

Previously it was established that peel and fleshes of edible fruits that are cultivated in Bangladesh are rich of phenolic and flavonoid content [25] and they exert antioxidant activity [26]. This studied fruit banana also exhibits similar type of activity. In total phenolic content test, the extract MDP showed highest amount phenolic content, 10.215±0.873 GAE/g of extract closely followed by MSP 9.719±0.147 mg GAE/g of extract. In previous study, total phenolic content of banana was found between 6.5±0.1 to 18.9±1.4 mg/g GAE equivalents of dry weight [27]. In another study, total phenolic content of banana (extracted by acetone 70%+ water 28%+acetic acid 2%) was found 4.75 mg of CAE (catechine equivalent) [28]. The phenolic content of banana blossom was found 4.33 mg GAE/g for acetone extract [29]. In total flavonoid test, MDP extract possess highest amount of

flavonoid content which is equivalent to 9.285±0.452 mg CAE/g of dry extract. The other extract particularly MSP, EADF, EASP, MMP and EAMP has considerable amount flavonoid content. The total flavonoid content of green banana varieties was found between 2.9-4.08 mg/g Quercetin equivalent in other studies [30]. The extract MMP showed highest value in total antioxidant assay 39.044±0.656 mg/g of AAE while the extract EADP 30.467±1.638 mg/g of AAE, MSP 30.644±0.804 mg/g of AAE and EAMF 30.644±2.579 mg/g of AAE has very high antioxidant activity. In DPPH radical scavenging activity, the extract MDP 69.44% and MSP 58.81% has significant scavenging activity compared to the standard antioxidant BHT 77.48% at 100 µl/ml concentration.

Conclusion

From this study, it can be concluded that the studied banana varieties are full of bioactive compounds and nutrients. Further work is needed to assess pharmacological activities like antimicrobial, anticancer, anti-arthritis, antidiarrheal, antiulcer and hepatoprotective activities. In the past it was found that phytochemical and bioactive compounds of Bangladeshi fruits are responsible for various types of pharmacological activities [31-34]. In addition, food supplements can be prepared from banana peel that are rich of beneficial phytochemicals. At the same time effective utilization of banana peel could prevent environmental pollution.

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