

Antioxidant Potential of Peel Extracts of Banana Varieties (*Musa sapientum*)

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Received August 28th, 2011; revised October 28th, 2011; accepted November 6th, 2011.

ABSTRACT

The study has been aimed to evaluate and compare phytochemical content and the antioxidant activity in peel extracts of nine local varieties of banana, i.e. *Musa sapientum* species. Ethanol extract of peels of these varieties were subjected to in vitro free radical scavenging assays like DPPH, ABTS and lipid peroxidation inhibition assay. Total antioxidant capacity assay to confirm the antioxidant potential and phytochemical content such as total phenols, flavonoids were also determined. The results obtained were analyzed statistically by ANOVA and DMRT analysis. The peel extracts of all the nine varieties of banana exhibited significant antioxidant and phytochemical activities with *Musa* spp. - Blueggoe (Monthan) - AAB and *Musa* spp. - Rasthali - AAB showing highest free radical scavenging activity and *Musa* spp. - Karpooravalli - ABB, *Musa* spp. - Rasthali - AAB, *Musa* spp. - Ney Poovan (Kadali) - AB and *Musa* spp. - Mysore (Poovan) - AAB having highest phytochemical content. The study suggests that peel extracts of these banana varieties could be useful to combat free radical mediated diseases.

Keywords: Antioxidants, Banana Peel, Lipid Peroxidation, Oxygen Radicals, Phytochemicals

1. Introduction

Free radicals are continuously produced in our body either naturally or on exposure to environmental stress as well as other factors and can be implicated in many diseases like cancer, atherosclerosis, arthritis, Parkinson's disease, Alzheimer's disease, aging and other age related problems [1]. Mammalian cells possess elaborate defense mechanisms for radical detoxification. Antioxidants are agents, which scavenge the free radicals and prevent the damage caused by them. In spite of these in-built defense mechanisms, it seems more meaningful to utilize extra antioxidants available in diets, especially from fruits, vegetables and whole grains [2]. Due to their minimal side effects, there are growing interests in using natural products for preventive and therapeutic medicine [3].

Musa spp., comprising banana and plantain are among the world's leading fruit crops and in terms of economical value, it is the number five agricultural crop in world trade. The edible fruit cultivars are a man-made complex based on two wild diploid species originating from South-East Asia: *Musa acuminata* Colla (AA), which is highly polymorphous, with spindly plants that grow in clumps, and *Musa balbisiana* Colla (BB), a homogeneous hardy

plant with a massive pseudo-trunk. There are diploid, triploid or tetraploid genome groups. The main genome groups are AA, AB, AAA, AAB and ABB [4].

The peels of a variety of fruits have gained attention as a natural source of antioxidants and phytochemical content which are rich in compounds with free radical scavenging activity. Banana and Plantain peels are major agricultural wastes which have been used as medicine, animal feeds, blacking of leathers, soap making, fillers in rubber and so on [5]. Fruit wastes are highly perishable and seasonal and are a problem to the processing industries and pollution monitoring agencies. This problem can be recovered by utilizing its high value compounds, including the dietary fibre fraction that has a great potential in the preparation of functional foods [6]. Banana peel, an underutilized source of phenolic compounds is considered as a good source of antioxidants for foods and functional foods against cancer and heart disease [7]. The peel of the fruit contains various antioxidant compounds such as gallicocatechin [7] and dopamine [6].

Recent trends focus on the isolation, characterization and utilization of natural antioxidants, especially growing interest in polyphenols as potential disease preventing

agents. As these compounds are predominantly found in most of fruit tissues, it would be worthwhile investigating the nature of polyphenols that are present in banana peel, a potential source of antioxidant and antimicrobial activities. Hence, the present study has been aimed to evaluate and compare the phytochemical contents and their *in vitro* antioxidant activities in peel extracts of nine local varieties of banana to assess its protective role against free radical induced cell damage.

2. Materials and Methods

2.1. Materials, Chemicals and Reagents

The banana varieties belonging to *Musa balbisiana* viz., Monthan (*Musa spp.* - Blueggoe - AAB), Karpooravalli (*Musa spp.* - Karpooravalli - ABB) Nendran (*Musa spp.* - French Plantain - AAB), Kadali (*Musa spp.* - Ney Poovan - AB), and those belonging to *Musa acuminata* viz., Pachainadan (*Musa spp.* - Pachainadan - AABS), Poovan (*Musa spp.* - Mysore - AAB), Rasthali (*Musa spp.* - Rasthali - AAB), Robusta - Cavendish sub group (*Musa spp.* - Robusta - AAB) and Sevvazhai (*Musa spp.* - Red banana - AAA) were collected locally from different farms in the same locality in Coimbatore (Tamil Nadu, India) and authenticated by Dr. T. S. Balamohan, Professor and Head, Faculty of Horticulture, Tamil Nadu Agricultural University, Coimbatore. A voucher specimen of the samples has been deposited in the herbarium of the department.

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), sodium nitroprusside, sulphanilamide, naphthyl ethylenediamine dihydrochloride, 2-deoxyribose, thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium dodecyl sulphate (SDS) and ammonium molybdate were all of analytical grade.

2.2. Preparation of Peel Extracts

The peel of fresh naturally ripened yellow un-pigmented bananas were shade-dried for about a week and then crushed to make a coarse powder. The dried powder (10 g) was weighed and solvent extraction using ethanol was performed at a 10% concentration. Exhaustive extraction was carried out in triplicates for about 36 h in a shaker at 37°C with a gentle shaking. The extracts were then evaporated at room temperature. The residues obtained were re-evaporated to remove impurities and stored at 4°C to carry out radical scavenging assays. The remaining residue was stored in desiccators for further use.

2.3. Free Radical Scavenging Assays

2.3.1. Total Antioxidant Capacity Assay

Aliquots of suitable working solutions (1 - 10 mg/ml) of

the samples were mixed with 1 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min [8]. The tubes were cooled to room temperature and the absorbance was measured at 695 nm against a blank. Ascorbic acid was used as a standard. Total antioxidant capacity was expressed as equivalents of ascorbic acid [9].

2.3.2. DPPH Radical-Scavenging Assay

DPPH scavenging activity was measured by the slightly modified spectrophotometric method of Brand-Williams *et al.* [10]. The absorbance of DPPH diluted in methanol was considered as control. The decrease in absorbance was measured at 517 nm. The antioxidant capacity to scavenge the DPPH radical was calculated by the following equation: Scavenging effect (%): [(1-absorbance of sample/absorbance of control) × 100]. Results were expressed as Mean ± SD of three experiments made by triplicate.

2.3.3. ABTS Radical Cation-Scavenging Assay

The assay was performed by a slightly modified protocol of Re *et al.* [11]. ABTS solution (7 mM) was reacted with ammonium persulphate (2.45 mM) solution to produce a dark coloured solution containing ABTS radical cations. The initial absorbance was measured at 745 nm. This stock solution was diluted with methanol to give a final absorbance value and equilibrated at 30°C. The decrease in absorbance was measured exactly one minute after mixing the solution, up to six minutes. The final absorbance was noted. The percentage inhibition was calculated according to the formula:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100\%$$

2.3.4. Lipid Peroxidation Inhibition Assay

The lipid peroxidation assay was carried out by a modified procedure of Ohkawa *et al.* [12]. Different concentration of the sample (1 - 10 mg/ml) in water was added to 0.5 ml of the 10% goat liver homogenate. Lipid peroxidation was initiated by adding 0.05 ml of 0.07 mol/m³ ferrous sulphate to the reaction mixture. After 30 min, 1.5 ml of 20% acetic acid (pH 3.5), 1.5 ml of 0.8% TBA (in 1.1% SDS) and 0.05 ml of 20 × 10⁻² TCA was added to the incubation solution, vortexed and boiled at 100°C for 1 h, cooled to room temperature and read the absorbance at 532 nm. The percentage inhibition was then calculated.

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100\%$$

2.4. Determination of Phytochemicals

2.4.1. Determination of Total Polyphenol Content

Total phenolic contents were determined according to the

spectrophotometric methods of Tanner and Brunner [13] and Kaur and Kapoor [14]. To 0.5 ml of a methanolic solution of the extracts, 7 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent were added and mixed well. After 3 min, 2 ml of 20% sodium carbonate was added and mixed well again. Absorbance of the resultant solution was read at 720 nm, after 1 h in a water bath at 25°C [15]. The total polyphenol content was calculated from the standard calibration curve obtained from catechol.

2.4.2. Determination of Flavonoids

A slightly modified version of the spectrophotometric method was used to determine the flavonoid contents of samples [15]. A 0.5 ml aliquot of the sample in aqueous methanol was diluted with 3 ml of distilled water to which 0.3 ml of 5% sodium nitrite was added and mixed well. After 5 min at room temperature, 0.6 ml of 10% aluminium chloride was added. After 6 min, 2 ml of 1 M sodium hydroxide was added and the absorbance was read at 510 nm. Flavonoid contents were then calculated using a standard calibration curve, prepared from rutin.

2.5. Statistical Analysis

The experimental results were expressed as mean \pm SD of three replicates. The data were subjected to two-way ANOVA (Analysis of Variance) and significance of difference between sample means were calculated by DMRT analysis using IRRISTAT software version 3.1. Difference in mean values were considered significant when $P < 0.05$.

3. Results and Discussion

3.1. Total Antioxidant Activity

The total antioxidant activities of various banana peel extracts are depicted in **Table 1**. The values represent the total antioxidant capacity of banana peel extracts expressed in terms of equivalents of ascorbic acid. This assay gives an estimate of the overall antioxidant potential of the banana peel [16]. In the presence of the extracts, Mo(VI) is reduced to Mo(V) and forms a green-coloured phosphomolybdenum V complex, which shows maximum absorbance at 700 nm. According to the results, the ethanolic extract of Pachainadan showed higher activity in the range of 5.85 mM·g⁻¹ in comparison to other varieties of banana peel, whereas the ethanolic extract of Nendran showed least activity. Similar studies by Gonzalez-Montelongo *et al.* [17] showed the total antioxidant activity of banana peel extract under different solvent and incubation conditions.

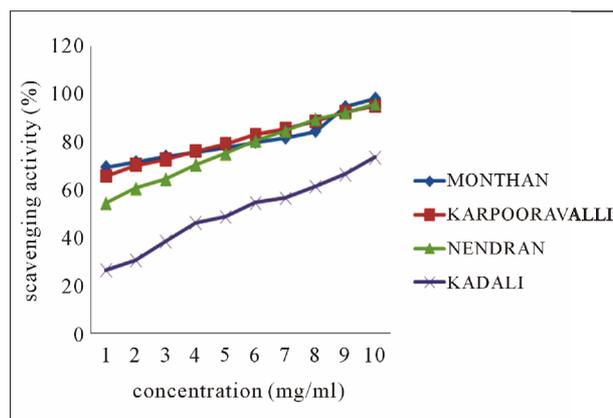
3.2. DPPH Radical Scavenging Activity

The line chart shown in **Figures 1(a)-(b)** for DPPH radical scavenging potential of the ethanolic extracts of

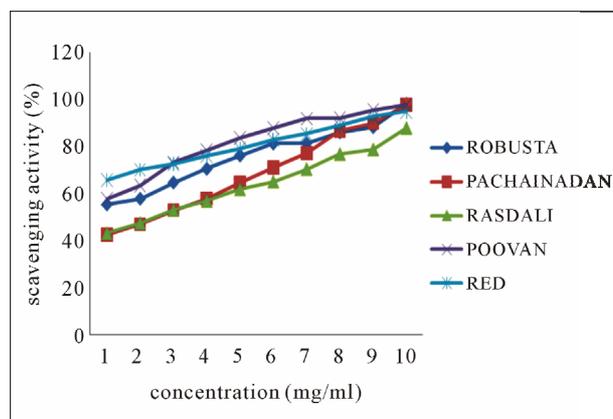
Table 1. Total Antioxidant activity in peels of banana varieties.

Banana Varieties	Total Antioxidant Activity mM AAE g ⁻¹
Kadali	4.89 ^d \pm 0.33
Karpooravalli	3.49 ^b \pm 0.02
Monthan	4.73 ^d \pm 0.06
Nendran	2.64 ^a \pm 0.06
Poovan	3.59 ^b \pm 0.03
Pachainadan	5.85 ^e \pm 0.11
Rasthali	3.39 ^b \pm 0.09
Robusta	4.79 ^d \pm 0.11
Sevvazhai	4.04 ^c \pm 0.09

Values represent mean \pm SD of 3 replicates; Total antioxidant activity was expressed as mM ascorbic acid equivalents g⁻¹ peel extract; Mean followed by a common letter are not significantly different at the 5% level by DMRT.



(a)



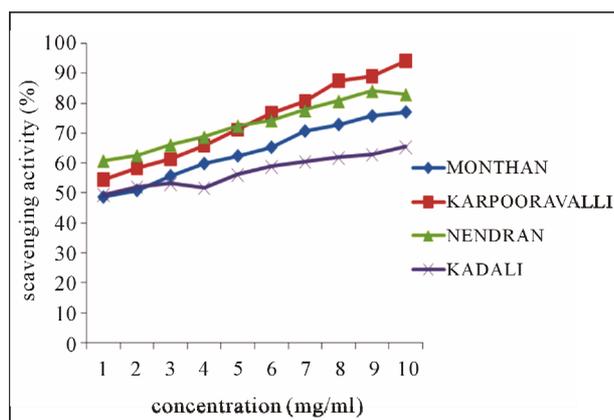
(b)

Figure 1. Scavenging activity (%) on DPPH radical by ethanolic extracts of (a) *balbisiana* (b) *acuminata* type of banana varieties.

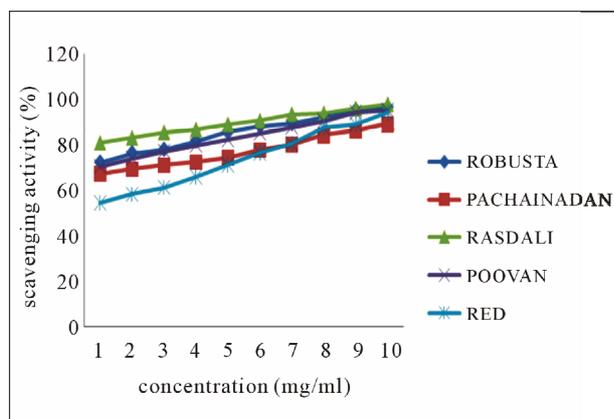
balbiana and *acuminata* type of local varieties of banana peel respectively, elucidating the mean values across the concentration range, clearly indicates the potential of Mondhan peel extract in scavenging free radicals as high percentage inhibition (98.19%) was noticed at 10 mg·ml⁻¹ in comparison to the peels of other varieties from both groups. DPPH radicals react with suitable reducing agents, during which the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up [18]. In the experiment, the solution progressively reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the extracts in a concentration-dependent manner.

3.3. ABTS Radical Scavenging Activity

The line charts 2(a) and 2(b) of Figure 2 indicates the ABTS radical scavenging potential of the ethanolic extracts of the various peel extracts of both groups of bananas at various concentrations.



(a)



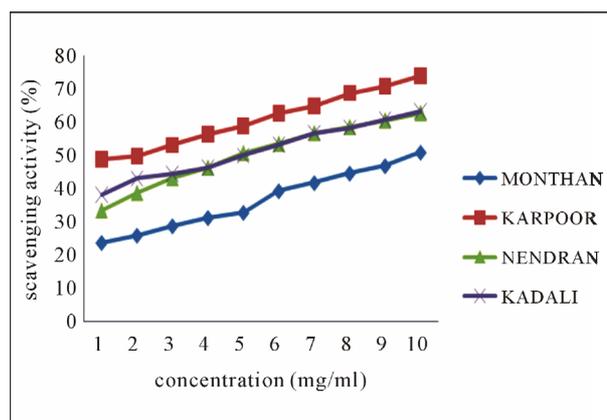
(b)

Figure 2. Scavenging activity (%) on ABTS radical by ethanolic extracts of (a) *balbiana* (b) *acuminata* type of banana varieties.

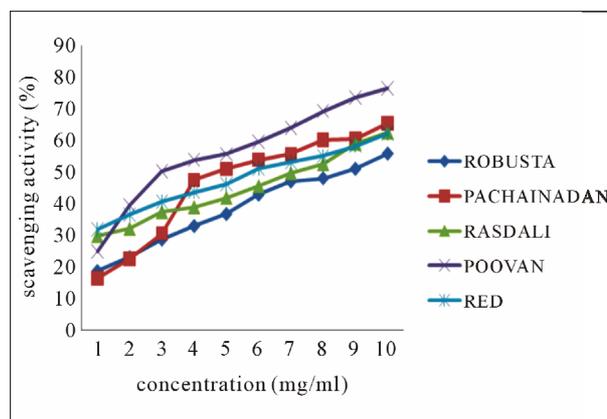
From the results, it may be postulated that ethanolic extract of Rasthali, among all species, inhibit the radical or scavenge the radical in a concentration dependent manner with highest percentage inhibition (97.66%) noticed at 10 mg·ml⁻¹. Studies by Okonogi *et al.* [19] determined the scavenging activity of banana peel extract on ABTS radical.

3.4. Lipid Peroxidation Inhibition Activity

Inhibition of lipid peroxidation by the ethanolic extracts of various banana peels is represented in Figures 3(a)-(b). Lipid peroxides are unstable and decompose to form reactive carbonyl compounds responsible for DNA damage, generation of cancer and age related diseases [20]. Most abundant among them is malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA) to form a pink chromogen [21]. The decrease in the MDA levels in the presence of increased concentration of each extract indicates the role of extracts as antioxidants. TBARS assay was used to determine the anti-lipid per-



(a)



(b)

Figure 3. Scavenging activity (%) on lipid peroxidation by ethanolic extracts of (a) *balbiana* (b) *acuminata* type of banana varieties.

oxidation properties of the banana peel extracts. The result values obtained indicate a moderate percentage inhibition with Poovan peel extract exhibiting highest inhibition among other varieties at 10 mg·ml⁻¹.

3.5. Total Phenol and Flavonoid Content

The total phenol and flavonoid content of the different banana peel extracts is shown in **Table 2**.

The antioxidant activity of the plant products is associated to their bioactive compounds, mainly antioxidant phenolics, because of their ability to scavenge free radicals [22,23]. Phenols are secondary metabolites in plants and are known to possess a wide range of therapeutic uses, such as antioxidant, antimutagenic, anticarcinogenic, free radical-scavenging activities and also decrease cardiovascular complications [24]. From the results, it is inferred that Rasthali extract showed higher phenol content compared to other varieties, which could be related to its antioxidant potential.

Many flavonoids are found to be strong antioxidants capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups [25]. In our study, from the table values, Poovan peel extract exhibited higher flavonoid content which might be correlated with its anti-lipid peroxidation activity.

Several mechanisms have been proposed to be involved in the antioxidant activity such as hydrogen donation, termination of free radical mediated chain reaction, prevention of hydrogen abstraction, chelation of catalytic ions, and elimination of peroxides [26].

Antioxidant activity is a dependent system and the characteristic of a particular system can influence the outcome of the analysis. Hence, a single assay would not be representative of the antioxidant potential of plant extracts. In the present study, we employed different models of antioxidant assays which could provide a more reliable approach to assess the antioxidant and radical scavenging potential of peel extracts of various banana varieties.

4. Conclusions

The investigation of the antioxidant potential and phytochemical content of banana peel of nine different local varieties showed that the content of total phenols were higher in Rasthali compared to other species which might be correlated with its high ABTS scavenging activity. In contrast, the content of flavonoids was higher in Poovan banana and is highly correlated with its lipid peroxidation inhibition activity. Hence the relationship between phytochemical content and free radical scavenging activity of banana peel indicates that peel extracts from these varieties may be useful to combat free radical mediated diseases. These observations may be used to substantiate

Table 2. Phytochemical contents in peels of banana varieties.

Banana varieties	Total Phenols	Flavanoids
	mg CE g ⁻¹	mg rutin g ⁻¹
Kadali	0.15 ^a ± 0.01	13.80 ^b ± 0.50
Karpooravalli	0.19 ^a ± 0.01	16.91 ^c ± 0.23
Monthan	0.32 ^b ± 0.03	11.91 ^a ± 0.23
Nendran	0.49 ^d ± 0.06	21.72 ^e ± 0.71
Poovan	0.39 ^e ± 0.09	22.83 ^e ± 1.54
Pachainadan	0.30 ^b ± 0.02	18.79 ^d ± 1.35
Rasthali	0.60 ^c ± 0.01	21.33 ^e ± 1.44
Robusta	0.20 ^a ± 0.02	17.93 ^{cd} ± 0.52
Sevvazhai	0.46 ^{cd} ± 0.07	12.96 ^{ab} ± 1.04

Values represent mean ± SD of 3 replicates; Total phenols was expressed as mg catechol equivalents g⁻¹ fresh tissue; Flavonoids was expressed as mg rutin equivalents g⁻¹ fresh tissue; Mean followed by a common letter are not significantly different at the 5% level by DMRT.

the scientific reasoning that free radical-scavenging is indeed the mode of operation of these peel varieties in the treatment or prevention of the onset of diseases that evidence free radical activity.

5. Acknowledgements

The authors gratefully acknowledge the Management of Kumaraguru College of Technology, Coimbatore for financial assistance to carry out this work.

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