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# Screening of different banana peels extracts for antioxidant capacity and total phenols

**P.Sathish Kumar**
**S.T.Joseph's College of Engineering, Sholinganallur, 600119, Chennai, (INDIA)**

## ABSTRACT

The total phenolic content and related total antioxidant capacity of 4 types of banana peels were analyzed. Banana (Musa acuminata Colla AAA) peel extracts obtained in this work had a high capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,22 -azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS+) free radicals NBR (Nitro blue tetrazolium), and they were also good lipid peroxidation inhibitorsAcetone:water extracts were considerably more effective (compared with methanol, ethanol, acetone, water, methanol:water or ethanol:water) at inhibiting the peroxidation of lipids in the β-carotene/linoleic acid system or scavenging free radicals. However, aqueous extracts had a high capacity to protect lipids from oxidation in the thiobarbituric acid reactive substances (TBARS) test, as well as in the β-carotene bleaching assay. To make practical comparison of relative antioxidant potential of phenolics extracted from selected banana peels, the phenol antioxidant coefficient (PAC) was calculated for each infusion. In addition, acetone:water most efficiently extracted all extractable components (54 ± 4%), phenolic compounds (3.3 ± 0.8%), and anthocyanin compounds (434 ± 97 µg cyanidin 3-glucoside equivalents/100 g freeze-dried banana peel). Banana peel contained large amounts of dopamine and L-dopa, catecholamines with a significant antioxidant activity. However, ascorbic acid, tocopherols or phytosterols were not detected in the different extracts. The antioxidant activity of banana peel extracts from different cultivars was similar. However, the impact of extraction time or temperature should be studied in greater depth. © 2015 Trade Science Inc. - INDIA

## KEYWORDS

FRAP;
DPPH;
ABTS;
NBR.

## INTRODUCTION

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Diets high in vegetables and fruits, which are good sources of antioxidants, have been found to be healthy; however, research has not shown antioxidant supplements to be beneficial in preventing diseases. Examples of antioxidants include vitamins C and E, selenium, and carotenoids, such as beta-carotene, lycopene, lutein, and zeaxanthin. This fact sheet provides basic information about antioxidants, summarizes what the science says about antioxidants and health, and suggests sources for additional information. The oxidative stress, defined

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as “the imbalance between oxidants and antioxidants in favor of the oxidants potentially leading to damage” has been suggested to be the cause of aging and various disease in humans. In modern western medicine, the balance between antioxidation and oxidation is believed to be a critical concept maintaining a healthy biological system[1,8,9,10,34,36]. The similar concept of balance called yinyang has existed in traditional Chinese medicine for more than 2000 years. Ou, Huang, Hampsch-Woodili, and Flanagan (2003) and Prior and Cao (2000) have shown that the effective compositions of the yin-tonic herbs are mainly flavonoids which are phenolic compounds with strong antioxidant activity. According to them the clear trend of antioxidant activity supported the hypothesis that yin in traditional chines medicine refers to antioxidant process, whereas yang relates to oxidation process. A general recommendation to the consumer is to increase the intake of foods rich in antioxidant compounds (e.g. polyphenols, carotenoids) due to their well-known healthy effects. As a consequence these evidences accelerated the search for antioxidants principles, which led to the identification of natural resources and isolation of active antioxidant molecules. Many plants have been identified as having potential antioxidant activities and their consumption recommended[19,23,24,26,36,38,40]. Bioactivephenols, especially bioflavonoids, are very interesting as antioxidants because of their natural origin and the ability to act as efficient free radical scavengers[16,22]. In last two decades the number of publications on the potential health benefits of polyphenols, has increased enormously[1,9,12,23,28,30,34,36]. Tea (black and greentea) is one of the most commonly consumed beveragesin the world and is rich in polyphenolic compounds collectivelyknown as the tea flavonoids[16,22,25]. The currentfocus is toward natural antioxidants, especiallyplant polyphenolics. It is of interest to investigate theantioxidant properties of herbal infusions especially those traditionally used in folk medicine. The aim of the present study was to examine the total phenolic content and related total antioxidant potentialIn four banana peel extract prepared in common way in which teas are prepared for human consumption. Total antioxidant potential has been determined using ferric reducing ability of plasma assay (FRAP) of Benzieand Strain (1996). The efficiency of extracted phenolics was evaluated using the phenol antioxidant coefficient (PAC). The effect of infusion temperatures and infusion time has been considered over a range similar to that encountered in a domestic environment.

## MATERIALS AND METHODS

### Materials, chemicals and reagents

The banana varieties belonging to Karpooravalli (*Musa spp.* - Karpooravalli - ABB) and those belong-

ing to *Musa acuminata viz.*, Pach- ainadan (Musa *spp.* - Pachanadan - AABS), Poovan (*Musa spp.* - Mysore - AAB), Rasthali (*Musa spp.* - Rast-hali - AAB) were collected locally from different farms in the same locality in Tiruchirapalli (Tamil Nadu, India).1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), nitro blue tetrazolium (NBR), free radical, sodium dodecyl sulphate (SDS) and ammonium molybdate were all of analytical grade.

**Preparation of peel extracts (10g)**

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 methanol 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.

**Free radical scavenging assays**

**(a) 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 560 nm against a blank. Ascorbic acid was used as a standard. Total antioxidant capacity was expressed as equivalents of ascorbic acid.

**(b) Total phenol concentration**

Total phenol concentration in selected 4 banana peel extract were determined spectrophotometrically according to the Folin–Ciocalteu colorimetric method[35], using (+)-catechin as the standard and expressing the results as catechin equivalents (CE). The levels of total phenols in infusions determined according to the Folin – Ciocalteu method are not absolute measurements of the amounts of phenolic materials but are in fact based on their chemical reducing capacity relative to an equivalent reducing capacity of (+) - catechin. Data presented are average of four measurements.

**DPPH (1,1-diphenyl-2-picrilhydrazyl) 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 560 nm. The antioxidant capacity to scavenge the DPPH radical was calculated by the fol-lowing equation: Scavenging effect (%): [(1-absorbance of sample/absorbance of control) × 100]. Results were expressed as Mean ± SD of three experiments made by triplicate. Free radical scavenging ability by the use of a stable DPPH radical (1,1-diphenyl-2-picrilhydrazyl). The DPPH radical scavenging activity of samples were determined using the method proposed by Von Gadow, Joubert, and Hansmann (1997). Aliquot (50 lL) of the tested sample was placed in a cuvette, and 2 mL of 6 · 10_5 M methanolic solution of DPPH radical was added. Absorbance measurements commenced immediately. The decrease in absorbance at 560 nm was determined after 16 min for all samples. Methanol was used to zero spectrophotometer. The absorbance of the DPPH radical without antioxidant, i.e. the control was measured daily. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution[4]. Methanolic solutions of pure compounds ((+)-catechin, vitamin C and quercetin) were tested too at different concentrations (· mol of antioxidant/1 mol DPPH radical). All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yenand Duh (1994) inhibition.

**% INHIBITION = $\{(A_C(0)_-A_A(t)/A_C(0)\}*100$**

where $A_C(0)$ is the absorbance of the control at t = 0 min;and $A_A(t)$ is the absorbance of the antioxidant a.

**NBT (nitro blue tetrazolium)(2oμg)**

Superoxide radical scavenging assay was carried out according to the method of zhishen et al.(1999). All solutions were prepared in 0.05M phosphate buffer (ph7.8). The photo induced reactions were performed

| SAMPLES | OD AT 560nm | PERCENTAGE% |
|---|---|---|
| 1. Karpooravalli | 0.411 | 32.95 |
| 2. Rasthali | 0.127 | 79.28 |
| 3. Pachainadam | 0.182 | 0.182 |
| 4. Poovan | 0.209 | 0.209 |

in aluminium foil lined with two 20w fluorescent lamps. The distance between reactant and the lamp was adjusted until reach the 4000 lux intensity of illumination. The total volume of the reactant mixture was 5ml and the concentration of the riboflavin methionine and NBT were $3*10^{-6}$,$1*10^{-2}$. The reactant was illuminated at 25c for 25min. The photo chemically reduced riboflavins generated oxygen which reduced NBT from blue formazon. The unilluminated reaction mixture was used as a blank and the absorbance (A) was measured at 560nm. The rhizome extract were added to the reaction mixture in which oxygen was scaveged then by inhibiting the NBT reduction absorbance was measured and decrease in oxygen was represented by A-A1. The degree of scavenging was calculated by the following equation,

**SCAVENGING (%) =$(A-A_1/A)*100$**

## ABTS (2,20azinobis-(3-ethylbenzthiazoline -6-sulphonic acid)

The free radical scavenging activity of extracted samples was determined by ABTS radical cation decolorization assay[32]. ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS*+) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Because ABTS and potassium persulfate react stoichiometrically at a ratio of 1:0.5, this will result in incomplete oxidation of the ABTS. Oxidation of the ABTS commenced immediately, but the absorbance was not maximal and stable

| SAMPLES | OD AT 560nm |
|---|---|
| 1. Karpooravalli | 0.724 |
| 2. Rasthali | 0.668 |
| 3. Pachainadam | 0.708 |
| 4. Poovan | 0.711 |

until more than 6 h had elapsed. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of infusion samples the ABTS*+ solution was diluted with Methanol to an absorbance of 0.70(Å}0.02) at 734 nm and equilibrated at 30 _C. Reagent blank reading was taken (A0). After addition of 2.0 mL of diluted ABTS*+ solution (A734 nm = 0.700 Å} 0.020) to 20 lL of antioxidant compounds (final concentration 0–15 lM) the absorbance reading was taken at 30 _C exactly 6 min after initial mixing (At). Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times. The percentage inhibition of absorbance at 560 nmwas calculated using BELOW formula and decrease of the absorbance between A0 and At

**% inhibition = [(Acontrol – Asample)/Acontrol] × 100%**

## PHENOLIC TEST

Total phenol concentration in selected medicinalplant infusions were determined spectrophotometrically according to the Folin–Ciocalteu colori-

| SAMPLES | OD AT 560nm |
|---|---|
| 1. Karpooravalli | 0.558 |
| 2. Rasthali | 0.245 |
| 3. Pachainadam | 0.753 |
| 4. Poovan | 0.615 |

metric method[35], using (+)-catechin as thestandard and expressing the results as catechin equivalents (CE). The levels of total phenols in infusions determinedaccording to the Folin–Ciocalteu method are notabsolute measurements of the amounts of phenolicmaterials but are in fact based on their chemical reducingcapacity relative to an equivalent reducing capacityof (+)-catechin. Data presented are average of fourmeasurements.

| SAMPLES | OD AT 560nm | mg GALLIC ACID EQUIVALENT |
|---|---|---|
| 1. Karpooravalli | 0.138 | 0.90 |
| 2. Rasthali | 0.920 | 0.23 |
| 3. Pachainadam | 0.660 | 0.132 |
| 4. Poovan | 0.239 | 0.65 |

## TOTAL ANTIOXIDANT ACTIVITY

### Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MQ, USA), Aldrich Chemical Co. (Steineheim, Germany), Merck (Darmstadt, Germany) and Kemika (Zagreb, Croatia).

### Spectrophotometric measurements

Spectrophotometric measurements were performed by UV–VIS spectrophotometer 2.2. (double-beam) Specord 200 Analytik Jena GmbH, Germany.

### Statistical analysis

| Total Antioxidant activity in peels of banana varie-ties. Banana Varieties | Total Antioxidant Activity mM AAE g–1 |
|---|---|
| Karpooravalli | 3.49b ± 0.02 |
| Poovan | 3.59b ± 0.03 |
| Pachainadan | 5.85e ± 0.11 |
| Rasthali | 3.39b ± 0.09 |

The direction and magnitude of correlation between variables was done using analysis of variance (ANOVA) and quantified by the correlation factor "r". The P-values less than 0.05 were considered statistically significant.

### Comparison of DPPH and ABTS

Comparison of DPPH radical and ABTS radical cation scavenging properties. Because of their high reactivity, most free radicals react rapidly with oxidizablesubstrates. Methods used for evaluation of radical-trappingproperties often utilize stable model free radicals asindicators for radical-scavenging abilities, among which1,1-diphenyl-2-picrilhydrazyl radical (DPPH*) and2,20azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation (ABTS*+), have gained the highestpopularity. From the methodological point of view the DPPH* method is recommended as easy and accurate with regard to measuring the antioxidant activity of fruit and vegetable juices or extracts. The results are highly reproducible and comparable to other free radical scavenging methods such as ABTS[14]. Reaction kinetics between phenols and ABTS* + have been found to differfrom that between phenols and DPPH* over a similar range of concentrations. Campos and Lissi (1996) have suggested that this differences can be partly result of differentequilibrium displacements in reaction (1) as a result of the fact that the reactions of DPPH* are carried out in the absence of added DPPH-H (i.e. the reduced form), but the reduced form ABTS is always present in the systems containing ABTS*[20]

## CONCLUSION

In conclusion, we might say that our results further support the view that different banana peels extracts are promising sources of natural antioxidants. Total phenol contentand total antioxidant capacity differs signifi-

cantly among different types of banana peels infusions. There was significant linear correlation between phenolics concentration and FRAP in infusions. The strongest antioxidant properties when measured with the FRAP assay had pachainadam, poovan, rasthali and karpooravalli. The best results were obtained from this samples: high phenolic concentration, very high FRAP (>20 mM/L) and PAC > 3. This study showed that they possess a significant reducing power and free radical scavenging ability to comparable.

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