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Polyphenols of Pseudostem of Different Banana Cultivars and Their Antioxidant Activities

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ABSTRACT: The present investigation is focused on potential use of banana pseudostem (BPS), which otherwise is disposed off as a waste or incinerated, as a source of polyphenols or antioxidants. The total phenolics (TP) and total flavonoids (TF) in various solvent extracts of pseudostem (PS) of different banana cultivars varied from 7.58 to 291 mg gallic acid equivalent (GAE/g of extract) and from 4 to 80 mg catechin equivalent (CE/g of extract), respectively. Acetone extract showed high antioxidant activity (AOA) in all of the in vitro models tested, whereas methanol extract exhibited high metal chelating activity. Among the banana cultivars, Nanjanagudu Rasabale (NR) showed the highest TP (291 mg GAE/g of extract), TF (80 mg CE/g of extract), and AOA. A detailed study on phenolic acids by reverse phase HPLC and ESI-MS revealed the presence of phenolic acids such as gentisic acid, (+)-catechin, protocatechuic acid, caffeic acid, ferulic acid, and cinnamic acid in cultivar NR. The differences in AOA of banana cultivars are in accordance with their phenolic and flavonoid concentrations.

KEYWORDS: banana pseudostem, total phenolics, total flavonoids, Nanjanagudu Rasabale

INTRODUCTION

Antioxidants, present in food or in the body at low concentrations, are substances that markedly delay or prevent the oxidation of that substrate.[1] Antioxidants may act by decreasing oxygen concentration, intercepting singlet oxygen, preventing first-chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products to nonradical compounds, and chain-breaking to prevent continued hydrogen abstraction from substrates.[2] Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG), are used widely in the food industry.[3−5] Uses of these antioxidants are restricted due to their carcinogenicity.[6] Hence, interest has increased in finding naturally occurring antioxidants to replace synthetic antioxidants.[7] Natural antioxidants have the capacity to improve food quality and can also act as nutraceuticals to terminate free radical chain reactions in biological systems. These benefits have been attributed to some of the phytochemical constituents and, in particular, to polyphenols.[8] Hence, in the present investigation attempts were made to study the total phenolics content (TP), total flavonoids content (TF), and the antioxidant activity (AOA) of different solvent extracts of banana pseudostem (PS), which is also called the scape, fleshy stalk, or floral stalk.

Banana is one of the most important commercial fruit crops throughout the tropical regions of the world. India is the major producer of bananas, with a total production of 26.2 million tons per year. Banana PS (BPS) is an actively growing aerial stem with closely packed leaf sheaths. It functions as a vascular bridge for the flow of water and nutrients from roots to leaves and finally to the banana fruit bunch. It is often used as a vegetable for culinary purposes in India. Juice from BPS is a well-known remedy for urinary disorders. It helps in the treatment for removal of stones in the kidney, gall bladder, and prostate and is also used as an antidote for snake bite. The K, Ca, Mg, Si, and P contents of ashes of BPS were 33.4, 7.5, 4.34, 2.7, and 2.2%, respectively.[9] Currently, <2% of PS production is used for human consumption and for production of fiber.[10]

Extensive literature review on BPS revealed that there was no report on its polyphenolic content and AOA. Therefore, TP and TF in PS of different commercial Indian banana cultivars, namely, Ney Mannan (NM) (ABB), Safed Velchi (SV) (AB), Red banana (RB) (AAA), Giant Cavendish (GC) (AAA), Monthan (MN) (ABB), Poovan (PN) (AAB), Nendran (NN) (AAB), and Nanjanagudu Rasabale (NR) (AAB) were determined. AOA of polyphenols was investigated by using various in vitro assay models such as the DPPH, superoxide, nitric oxide radical scavenging, β-carotene−linoleate model, hydrogen peroxide scavenging, metal chelation, and lipid peroxidation inhibition.

MATERIALS AND METHODS

Chemicals. All of the organic solvents used for extraction (AR grade), methanol used for HPLC (HPLC grade), and aluminum chloride were purchased from E. Merck (Mumbai, India). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), hydrogen peroxide (H_2O_2), β-carotene, Tris-HCl, Tween 20 and 40 (polyoxyethylene sorbitan monopalmitate), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), curcumin, catechin, ethylenediaminetetraacetic acid (EDTA), sodium nitrite ($NaNO_2$), and phenolic standards, namely, gallic acid, synergic acid, tannic acid, caffeic acid, gentisic acid, vanillic acid, *trans*-cinnamic acid, ferulic acid, protocatechuic acid, *p*-coumaric acid, catchol acid, chlorogenic acid, pyrocatechol, and epicatechin, were purchased from Sigma Chemicals Co. (St. Louis, MO). 2-Thiobarbituric acid was from ICN

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Biomedicals Inc. (Aurora, OH). 3-(2-Pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine (ferrozine), nicotinamide adenine dinucleotide-reduced (NADH), trichloroacetic acid (TCA), potassium ferricyanide, linoleic acid, L-ascorbic acid, and ferric chloride ($FeCl_2 \cdot 4H_2O$) were purchased from M/s Sisco Research Laboratories (Mumbai, India). Sodium nitroprusside, sulfanilamide, orthophosphoric acid (H_3PO_4), *N*-(1-naphthyl)ethylenediamine dihydrochloride, ferrous chloride, and Folin−Ciocalteu phenol reagent were from Loba Chemie Pvt. Ltd. (Mumbai, India). All of the reagents used in this study were of analytical grades of highest purity.

Plant Material. The eight different cultivars of banana plant were harvested fresh from commercial banana plantations in Mysore district, Karnataka state, India. After fruit bunch harvesting, the PS was separated from the plant at ground level and transferred to the laboratory, and the central core was obtained after removal of leaf sheaths and used for study.

Preparation of Extracts. The central core of PS was made into slices, dried in a hot air oven at 45 °C for 24 h, and powdered to 60 mesh in an apex grinder. Powdered sample was then extracted serially using solvents with increasing polarity, namely, hexane, chloroform, ethyl acetate, acetone, and methanol at room temperature (20 ± 5 °C), and the extraction process was repeated until the solvent became colorless. The extracts were then concentrated in vacuum at 40 ± 5 °C in a rotavapor (Buchi, model R-205, Germany), followed by lyophilization (Hetosicc, model CD 2.5), and the solid mass obtained was stored at 0−4 °C and resuspended in methanol solvent before use.

Determination of TP. In brief, 100 μL of sample (1 mg/mL stock) was mixed with 2 mL of 2% aqueous sodium carbonate solution. After 3 min, 100 μL of 50% Folin−Ciocalteu's phenol reagent was added to the mixture. After 30 min of incubation at room temperature, absorbance was measured at 750 nm against a blank. TP was calculated on the basis of a standard curve of gallic acid.[11]

Determination of TF. TF was determined by an aluminum chloride colorimetric method.[12] One hundred microliters of sample (1 mg/mL stock) was placed in a 10 mL volumetric flask, and then 5 mL of distilled water was added followed by 0.3 mL of 5% $NaNO_2$. After 5 min, 0.6 mL of 10% $AlCl_3$ was added. After 5 min, 2 mL of 1 M NaOH was added and the volume made up with distilled water. The solution was mixed and absorbance measured at 510 nm. TF was expressed as catechin equivalent per gram of extract using a standard curve.

Separation of Phenolics. The phenolic constituents of ethyl acetate, acetone, and methanol extracts from the PS of banana cv. NR were separated by reverse phase HPLC, on a Shimpak C-18 column (model LC-10A, Shimadzu Corp., Japan), using a diode array detector operating at 220, 280, and 320 nm. An isocratic solvent system, consisting of methanol/water/trifluoroacetic acid (89.5:10:0.5), was used as a mobile phase at a flow rate of 1 mL/min. Standards of phenolic acids, namely, gallic acid, synergic acid, tannic acid, caffeic acid, gentisic acid, vanillic acid, *trans*-cinnamic acid, ferulic acid, protocatechuic acid, *p*-coumaric acid, catchol acid, chlorogenic acid, pyrocatechol, and epicatechin, were used for identification and quantification.

Direct Infusion Electrospray Insertion Mass Spectrometry (ESI-MS). The ESI-MS fingerprints of polyphenols of extracts, namely, ethyl acetate, acetone, and methanol, from PS of cv. NR were obtained with an Alliance Waters 2695 mass spectrometer (Waters Corp., Micromass Ltd., U.K.) operating at ESI (negative mode). The capillary voltage was 3.0 kV; source and desolvation temperatures were 120 and 300 °C, respectively; cone gas (argon) and desolvation gas (nitrogen) flow rates were 50 and 500 L h^{-1}, respectively. The m/z values obtained for different standard phenolic acids and m/z values of different polyphenolic compounds available in the literature were used to match m/z values in the spectra obtained for PS polyphenols.[13]

AOA Measurement

DPPH Radical Scavenging Activity. The AOA of different extracts of PS on DPPH radical was measured.[14,15] About 100 μL of (1 mg/mL stock) PS extracts, at different concentrations, was mixed with 900 μL of Tris-HCl buffer (pH 7.4) to which 1 mL of DPPH (500 μM in methanol) was added. The mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm in a UV−visible spectrophotometer (UV-160A, Shimadzu Co. Japan). All measurements were made in triplicate with BHT as a positive control. Radical scavenging potential was expressed as EC_{50} value, which represents the sample concentration at which 50% of the DPPH radicals are scavenged.

Superoxide Radical Scavenging Activity. Superoxide radicals were generated in 1 mL of Tris-HCl buffer (0.02 M, pH 8.3) containing 0.1 mM NADH, 0.1 mM NBT, 10 μM PMS, and 100 μL (1 mg/mL stock) of different solvent extracts of PS/catechin (standard). The color reaction of superoxide radicals and NBT was detected at 560 nm, using a UV−vis spectrophotometer (UV-160A, Shimadzu Co. Japan). The EC_{50} value represents 50% of the superoxide radicals scavenged by the test sample.[16]

β-Carotene−Linoleate Model System. To assess the ability of PS extracts/BHT (standard) to prevent the bleaching of β-carotene, 0.2 mg of β-carotene in 1 mL of chloroform, 20 mg of linoleic acid, and 200 mg of Tween 20 were transferred into a round-bottom flask. Once the chloroform had been removed, 50 mL of distilled H_2O was added, and the resulting mixture was stirred vigorously. Six milliliter aliquots of the emulsion were transferred to tubes containing either 100 μL (1 mg/mL stock) of PS extracts or 100 μL (1 mg/mL stock) BHA. After mixing, an aliquot was transferred into a cuvette, and the absorbance (Abs^0) at 470 nm was recorded. The remaining samples were placed in a water bath at 50 °C for a period of 2 h. Thereafter, the absorbance of each sample was measured at 470 nm (Abs^{120}). The data (n = 3) are presented as AOA % values, calculated using equation I:

$$\text{AOA \%} = [1 - (Abs^0{}_{\text{sample}} - Abs^{120}{}_{\text{sample}})/(Abs^0{}_{\text{control}} - Abs^{120}{}_{\text{control}})] \times 100 \quad \text{(I)}$$

Inhibitory activity was expressed as EC_{50} value, which is the sample concentration that inhibited 50% of β-carotene bleaching.[17]

Lipid Peroxidation Inhibitory Activity. In brief, egg lecithin (3 mg/mL phosphate buffer, pH 7.4) was sonicated in a Dr. Hielscher GmbH, UP 50H ultraschallprozessor (Dr. Hielscher GmbH, Teltow, Berlin, Germany). One hundred microliters (1 mg/mL stock) of PS extracts was added to 1 mL of liposome mixture; control was without test sample. Lipid peroxidation was induced by adding 10 μL of $FeCl_3$ (400 mM) and 10 μL of L-ascorbic acid (400 mM). After incubation for 1 h at 37 °C, the reaction was stopped by adding 2 mL of 0.25 N HCl containing 15% TCA and 0.375% TBA; the reaction mixture was boiled for 15 min and then cooled and centrifuged. Absorbance of the supernatant was measured at 532 nm. Inhibitory activity was expressed as EC_{50} value, which is the sample concentration that inhibited 50% of lipid peroxidation.[18]

Metal Chelating Activity. PS extracts/EDTA (standard) was mixed with 2 mM $FeCl_2 \cdot 4H_2O$ and 5 mM ferrozine at a ratio of 10:1:2 and the mixture were shaken. After 10 min, the Fe^{2+} was monitored by measuring the formation of ferrous ion−ferrozine complex at 562 nm. Results were expressed as EC_{50} value, which represents the sample concentration at which 50% of the metal chelation occurred.[19]

Nitric Oxide Scavenging Activity. The procedure is based on the mechanism by which sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions.

For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with 100 μL (1 mg/mL stock) of PS extracts

Table 1. Yield, Total Phenolics Content, and Total Flavonoids Content of Different Extracts of Pseudostem of Banana Cultivars[a]

cultivar (genome)[b]	crude extract	extract yield (% w/w)	TP (mg/g of PS extract, in GAE)	TF (mg/g of PS extract, in CE)
NM (ABB)	hexane	1.76 ± 0.04 b		
	chloroform	0.095 ± 0.01 d		
	ethyl acetate	0.078 ± 0.01 d	40.3 ± 3.4 c	12.0 ± 0.8 c
	acetone	0.76 ± 0.02 c	189.2 ± 14.8 a	58.4 ± 3.7 a
	methanol	2.46 ± 0.05 a	62.9 ± 4.4 b	23.9 ± 1.6 b
SV (AB)	hexane	1.70 ± 0.05 b		
	chloroform	0.101 ± 0.01 d		
	ethyl acetate	0.081 ± 0.02 d	22.4 ± 1.6 c	8.0 ± 0.4 c
	acetone	0.79 ± 0.02 c	156.1 ± 12.58 a	37.6 ± 2.1 a
	methanol	2.51 ± 0.04 a	76.1 ± 3.7 b	16.6 ± 1.3 b
RB (AAA)	hexane	1.74 ± 0.04 b		
	chloroform	0.089 ± 0.02 d		
	ethyl acetate	0.088 ± 0.02 d	18.0 ± 1.6 c	6.0 ± 1.1 c
	acetone	0.84 ± 0.03 c	122.4 ± 11.68 a	49.7 ± 3.9 a
	methanol	2.54 ± 0.06 a	47.5 ± 3.1 b	14.3 ± 1.1 b
GC (AAA)	hexane	1.79 ± 0.04 b		
	chloroform	0.091 ± 0.01 d		
	ethyl acetate	0.070 ± 0.01 d	11.4 ± 1.1 c	5.0 ± 0.9c
	acetone	0.81 ± 0.02 c	103.5 ± 10.7 a	34.4 ± 2.3a
	methanol	2.59 ± 0.06 a	60.0 ± 4.7 b	10.7 ± 1.7b
PN (AAB)	hexane	1.83 ± 0.06 b		
	chloroform	0.095 ± 0.01 d		
	ethyl acetate	0.072 ± 0.01 d	13.5 ± 1.8 c	6.0 ± 1.3 c
	acetone	0.80 ± 0.03 c	83.4 ± 3.4 a	22.3 ± 1.4 a
	methanol	2.63 ± 0.05 a	34.57 ± 2.0 b	10.0 ± 1.9 b
NN (AAB)	hexane	1.83 ± 0.03 b		
	chloroform	0.111 ± 0.01 d		
	ethyl acetate	0.077 ± 0.02 d	7.5 ± 0.8 c	4.0 ± 0.4 c
	acetone	0.75 ± 0.01 c	81.5 ± 4.2 a	20.0 ± 1.6 a
	methanol	2.61 ± 0.06 a	27.5 ± 1.6 b	8.6 ± 0.8 b
MN (ABB)	hexane	1.62 ± 0.04 b		
	chloroform	0.109 ± 0.02 d		
	ethyl acetate	0.091 ± 0.02 d	15.5 ± 1.0 c	7.0 ± 1.0 c
	acetone	0.78 ± 0.03 c	82.2 ± 9.7 a	40.0 ± 3.3 a
	methanol	2.59 ± 0.05 a	35.5 ± 2.5 b	12.2 ± 1.3 b
NR (AAB)	hexane	1.53 ± 0.03 b		
	chloroform	0.117 ± 0.02 d		
	ethyl acetate	0.088 ± 0.01 d	49.0 ± 3.9 c	16.0 ± 0.6 c
	acetone	0.77 ± 0.02 c	291.0 ± 22.47 a	80.0 ± 4.0 a
	methanol	2.33 ± 0.04 a	92.0 ± 4.1 b	32.0 ± 2.8 b

[a] Yield (% w/w), values expressed as percent of dry raw material; TP (total phenolics), values expressed as mg GAE (gallic acid equivalents) g^{-1} dry extract; TF (total flavonoids), values expressed as mg CE (catechin equivalents) g^{-1} dry extract. Data are expressed as the mean ± standard deviation of triplicate measurements. Mean values with different letters differ significantly at $P < 0.05$. [b] NM, Ney Mannan (ABB); SV, Safed Velchi (AB); RB, Red banana (AAA); GC, Giant Cavendish (AAA); PN, Poovan (AAB); NN, Nendran (AAB); MN, Monthan (ABB); NR, Nanjanagudu Rasabale (AAB).

and incubated at room temperature for 150 min. The same reaction mixture, without the sample, but with an equivalent amount of water, served as control. After the incubation period, 0.5 mL of Greiss reagent (1% sulfanilamide, 2% H_3PO_4, and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. Curcumin was used as positive control.[20]

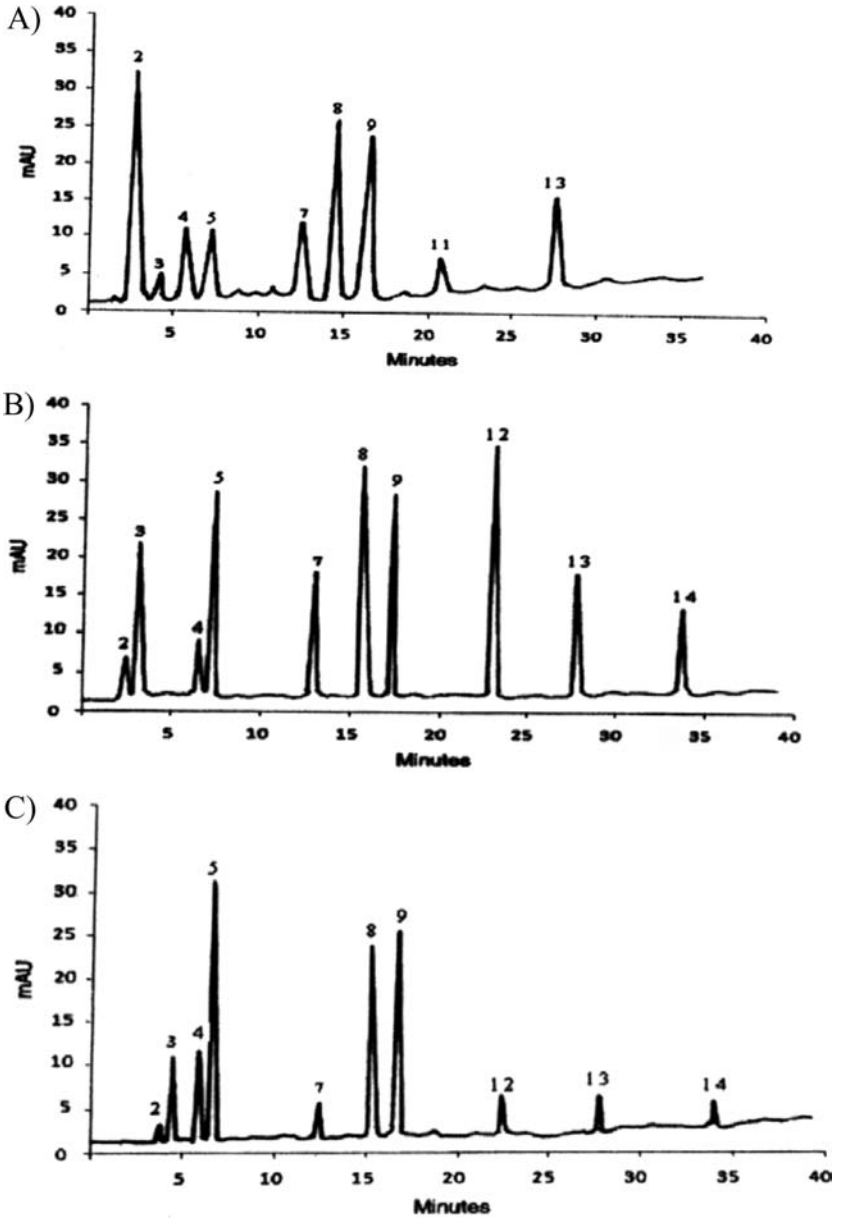

Figure 1. Phenolic acids of PS of cv. NR extracts. HPLC chromatograms at 280 nm: (A) ethyl acetate extract; (B) acetone; (C) methanol. Peaks: 2, synergic acid; 3, pyrocatechol; 4, catechol acid; 5, gentisic acid; 7, catechin; 8, protocatechuic acid; 9, gallic acid; 11, caffeic acid; 12, chlorogenic acid; 13, ferulic acid; 14, cinnamic acid.

The EC_{50} value represents 50% of the nitric oxide scavenged by the test sample.

Hydrogen Peroxide Scavenging Activity. A solution of hydrogen peroxide (2 mmol/L) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm with molar absorptivity 81 $(mol/L)^{-1}$ cm^{-1}. One hundred microliters (1 mg/mL stock) of PS extracts/ascorbic acid (standard) was added to hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage inhibition was calculated from $[(A_0 - A_1)/A_0]$ 100, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard (ascorbic acid). The EC_{50} value represents 50% of the hydrogen peroxide scavenged by the test sample.[21]

Statistical Analysis. Data are analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel XP (Microsoft Corp.), and post hoc mean separations were performed by Duncan's multiple-range test (DMRT) at $P < 0.05$.[22]

RESULTS AND DISCUSSION

Yield of Different Solvent Extracts. The yield of PS crude extracts varied widely among the banana cultivars and with different solvents (Table 1). The extract yield was higher in methanol (2.33−2.63% w/w) followed by hexane (1.53−1.83% w/w), acetone (0.75−0.84% w/w), chloroform (0.089−0.117% w/w), and ethyl acetate (0.070−0.091% w/w). Differences in the yield of extracts may be attributed to the availability and solubility of different extractable components in solvents used for extraction process.[23]

Table 2. Identification and Quantification of Phenolic Acids from Pseudostem Extracts of Cultivar NR by HPLC Analysis

			μg/mg of extract		
peak	phenolic acid	retention time	ethyl acetate	acetone	methanol
1	synergic acid	2.016			
2	tannic acid	2.955	38.14	6.67	2.23
3	pyrocatechol	3.542	3.16	23.11	10.27
4	catechol acid	5.618	8.21	8.44	12.99
5	gentisic acid	6.848	6.71	37.93	51.36
6	vanillic acid	11.701			
7	(+)-catechin	12.601	12.01	17.53	5.46
8	protocatechuic acid	14.517	32.60	42.17	36.64
9	gallic acid	16.971	29.97	38.62	40.57
10	*p*-coumaric acid	20.501			
11	chlorogenic acid	21.077	4.51		
12	caffeic acid	23.910		50.07	6.72
13	ferulic acid	26.605	14.22	16.88	6.51
14	cinnamic acid	30.872		12.97	7.03

Total Phenolics and Flavonoids of Pseudostem of Different Banana Cultivars. The TP and TF of different cultivars ranged from 7.58 to 291 mg GAE and from 4 to 80 mg CE/g of crude extract, respectively (Table 1). In all cultivars the acetone extract showed higher TP and TF, ranging from 81.55 to 291 mg GAE and from 20.09 to 80 mg CE/g of crude extract, respectively. Hexane, chloroform, and ethyl acetate showed very negligible TP and TF contents. Interestingly, acetone extract from PS of cv. NR exhibit higher amounts of TP and TF (291 GAE and 80 CE/g of crude extract) when compared to other cultivars of banana. Antioxidant activities of polyphenols from these banana cultivars were determined by different in vitro models. The acetone extract of PS from all of the cultivars showed the highest phenolic and flavonoid contents (Table 1) and, concomitantly, the highest AOA (Figures 4−10). Difference in the amounts of TP and TF in different PS could be explained by the fact that the presence of phenolics is affected by plant species and agro-climatic and soil conditions as reported earlier in bark of different plant systems.[24] Furthermore, amount and composition of phenolic compounds were observed to be diversified at subcellular level and within the tissues.[25,26] The recovery of polyphenols from plant materials was reported to be influenced by the solubility of the phenolic compounds in the solvent used for the extraction process.[27] The cv. NR, an endangered variety, is known for its exotic flavor and texture and long storage period and showed higher concentrations of TP and TF and AOA. Hence, a detailed characterization of polyphenols in cv. NR was undertaken.

Characterization of Polyphenols. Reverse phase HPLC analysis of phenolic compounds from different extracts, namely, ethyl acetate, acetone, and methanol, of PS of cv. NR indicated the presence phenolic acids, such as tannic, pyrocatechol, catechol, gentisic, (+)-catechin, protocatechuic, gallic, caffeic, chlorogenic, ferulic, and cinnamic acids along with some unidentified peaks (Figure 1). The highest concentrations of phenolic acids were observed in acetone extract (Table 2). Caffeic acid (50.07 μg/mg) was the most predominant phenolic acid recorded in acetone extract, followed by protocatechuic acid (42.17 μg/mg), gallic acid (38.62 μg/mg), gentisic acid (37.93 μg/mg), and pyrocatechol (23.11 μg/mg). In the methanol extract high concentrations of gentisic acid (51.36 μg/mg), gallic acid (40.57 μg/mg), and protocatechuic acid (36.64 μg/mg) were recorded, whereas the ethyl acetate extract showed high tannic acid (38.14 μg/mg), protocatechuic acid (32.60 μg/mg), and gallic acid (29.97 μg/mg).

Direct infusion electrospray ionization mass spectrometry (ESI-MS) was used to identify the polyphenolic compounds present in the ethyl acetate, acetone, and methanol extracts of PS of cv. NR. The ESI-MS fingerprints (Figure 2) indicate the presence of several polyphenolic compounds with different molecular weights ranging from 100 to 500 Da. Polyphenolic compounds were identified by their m/z values from ESI-MS fingerprints and are listed in Table 3. Mass spectra of the ethyl acetate, acetone, and methanol extracts from PS of NR showed the presence of 4-vinylphenol, *p*-anisaldehyde, sesamol, 4-ethylcatechol, protocatechuic aldehyde, tyrosol, 2-hydroxybenzoic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, anethole, estragole, cinnamic acid, gallic aldehyde, hydroxytyrosol, 2,3-dihydroxybenzoic acid, 2,4-dihydroxybenzoic acid, 2,6-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, gentisic acid, protocatechuic acid, 4-vinylsyringol, caffeic acid, ferulic acid, isoferulic acid, 2,3-dihydroxy-1-guaiacylpropanone, pinosylvin, sinapic acid, pinocembrin, geraldone, methylgalangin, biochanin A, glycitein, (+)-catechin/(−)-epicatechin, [6]-gingerol, 5-nonadecylresorcinol, 7-hydroxysecoisolariciresinol, and pelargonidin 3-*O*-arabinoside. The presence of phenolics acids such as gentisic acid, (+)-catechin, protocatechuic acid, caffeic acid, ferulic acid, and cinnamic acid in reverse phase HPLC analysis was also confirmed by ESI-MS. The identified phenolic acids are presented in Figure 3. Many of these phenolic compounds are also identified in both edible and nonedible parts of various fruits and vegetables.[11,24]

Antioxidant Activities of PS of Different Banana Cultivars. *DPPH Radical Scavenging Activity.* DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.[28] In the present study acetone and methanol extracts of PS from all of the banana cultivars were capable of inhibiting the DPPH free radicals. DPPH reducing activities of acetone, methanol, and ethyl acetate extract ranged from 70.47 to 23.64%, from 40.02 to 12.73%, and from 28.55 to 5.3%, respectively. The scavenging effect of acetone extract and standard BHT with the DPPH radical is in the following order: BHT (92.61%) > NR (70.47%) > NM (61.98%) > SV (55.28%) > RB (47.59%) > GC (41.36%) at the concentration of 100 μg/mL (Figure 4). The EC_{50} values for acetone extracts of cv. NR, NM, SV, RB, and GC are 76, 86, 94, 122, and 141 μg mL^{-1}, respectively (Table 4). The high polyphenols found in the acetone and methanol extracts of different cultivars of PS may be responsible for free radical scavenging activity by virtue of their hydrogen-donating ability.[29]

Superoxide Radical Scavenging Activity. Figure 5 shows the in vitro superoxide radical scavenging activity of the PS extracts. Superoxide anions derived from dissolved oxygen by PMS/NADH coupling reaction reduce NBT. The decrease of absorbance at 560 nm with the presence of antioxidants indicates the consumption of superoxide anions in the reaction mixture. Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species.[30] This radical is a powerful oxidizing agent that can react with biological membranes and induce tissue damage.[31] It may also decompose to singlet oxygen, hydroxyl radical, or hydrogen peroxide.[32]

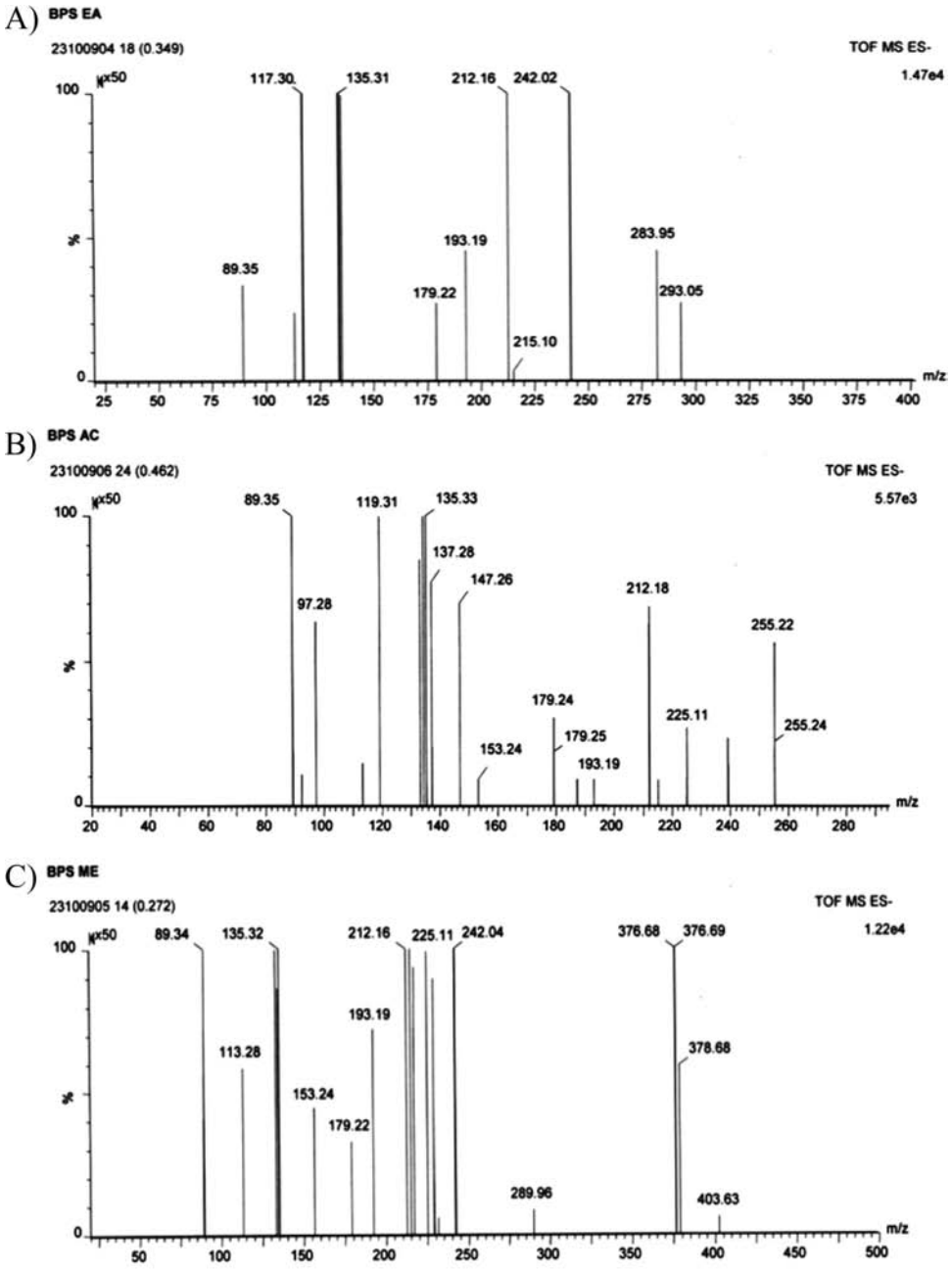

Figure 2. ESI-MS fingerprints of PS extracts of cv. NR. Zoomed spectra: (A) ethyl acetate; (B) acetone; (C) methanol.

The percentage and EC_{50} values of the superoxide radical scavenging activity of acetone extract of different banana cultivars, namely, NR, NM, SV, GC, and RB, were 65.53, 52.37, 47.98, 46.31, and 41.47% at 100 μg/mL (Figure 5) and 85, 96, 110, 115, and 130 μg mL^{-1} (Table 4), respectively. These results indicated that the tested extract had a notable effect on scavenging of superoxide when compared with standard catechin.

β-Carotene–Linoleate Model System. The β-carotene bleaching inhibitory activity of various extracts of PS is presented in Figure 6. In this system β-carotene undergoes rapid discoloration because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. The linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated β-carotene molecules. As a result, β-carotene will be oxidized and broken down in part; subsequently, the system loses its chromophore and characteristic orange color, which can be monitored spectrophotometrically at absorbance 470 nm. The percent of β-carotene bleaching inhibitory activity of various extracts of PS ranged from 5.20 to 46.37%. The decreasing order of activity in acetone extracts of cv. NR, NM, SV, RB, and GC was 46.37, 40.08, 34.87, 30.07, and 27.77% at the dose of 100 μg/mL, respectively (Figure 6). The EC_{50} values for cv. NR, NM, and SV were 119, 120, and 160 μg mL^{-1}, respectively (Table 4). An extract capable of inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant.[33] The present study indicated that acetone and methanol extracts are capable of scavenging free radicals in a complex heterogeneous medium. Moderate radical

Table 3. Polyphenol Compounds Identified from the ESI-MS Fingerprint of Different Extracts of Cultivar NR

				$[M - H]^-$ (m/z), PS extracts		
sl	polyphenolic compound	mol wt	mol formula	ethyl acetate	acetone	methanol
1	4-vinylphenol	120.0	C_8H_8O		119.31	
2	*p*-anisaldehyde	136.0	$C_8H_8O_2$	135.31	135.33	135.32
3	sesamol	138.0	$C_7H_6O_3$		137.28	
4	4-ethylcatechol	138.0	$C_8H_{10}O_2$		137.28	
5	protocatechuic aldehyde	138.0	$C_7H_6O_3$		137.28	
6	tyrosol	138.0	$C_8H_{10}O_2$		137.28	
7	2-Hydroxybenzoic acid	138.0	$C_7H_6O_3$		137.28	
8	3-hydroxybenzoic acid	138.0	$C_7H_6O_3$		137.28	
9	4-hydroxybenzoic acid	138.0	$C_7H_6O_3$		137.28	
10	anethole	148.0	$C_{10}H_{12}O$		147.26	
11	estragole	148.0	$C_{10}H_{12}O$		147.26	
12	cinnamic acid	148.0	$C_9H_8O_2$		147.26	
13	gallic aldehyde	154.0	$C_7H_6O_4$		153.24	153.24
14	hydroxytyrosol	154.0	$C_8H_{10}O_3$		153.24	153.24
15	2,3-dihydroxybenzoic acid	154.0	$C_7H_6O_4$		153.24	153.24
16	2,4-dihydroxybenzoic acid	154.0	$C_7H_6O_4$		153.24	153.24
17	2,6-dihydroxybenzoic acid	154.0	$C_7H_6O_4$		153.24	153.24
18	3,5-dihydroxybenzoic acid	154.0	$C_7H_6O_4$		153.24	153.24
19	gentisic acid	154.0	$C_7H_6O_4$		153.24	153.24
20	protocatechuic acid	154.0	$C_7H_6O_4$		153.24	153.24
21	4-vinylsyringol	180.0	$C_{10}H_{12}O_3$	179.22	179.24	179.22
22	caffeic acid	180.0	$C_9H_8O_4$	179.22	179.24	179.22
23	ferulic acid	194.0	$C_{10}H_{10}O_4$	193.19	193.19	193.19
24	isoferulic acid	194.0	$C_{10}H_{10}O_4$	193.19	193.19	193.19
25	2,3-dihydroxy-1-guaiacylpropanone	212.0	$C_{10}H_{12}O_5$	212.16	212.18	212.16
26	pinosylvin	212.0	$C_{14}H_{12}O_2$	212.16	212.18	212.16
27	sinapic acid	224.0	$C_{11}H_{12}O_5$		225.11	225.11
28	pinocembrin	256.0	$C_{15}H_{12}O_4$		255.22	
29	geraldone	284.0	$C_{16}H_{12}O_5$	283.95		
30	methylgalangin	284.0	$C_{16}H_{12}O_5$	283.95		
31	biochanin A	284.0	$C_{16}H_{12}O_5$	283.95		
32	glycitein	284.0	$C_{16}H_{12}O_5$	283.95		
33	(+)-catechin	290.0	$C_{15}H_{14}O_6$			289.96
34	(-)-epicatechin	290.0	$C_{15}H_{14}O_6$			289.96
35	[6]-gingerol	294.0	$C_{17}H_{26}O_4$	293.05		
36	5-nonadecylresorcinol	376.0	$C_{25}H_{44}O_2$			376.68
37	7-hydroxysecoisolariciresinol	378.0	$C_{20}H_{26}O_7$			378.68
38	pelargonidin 3-*O*-arabinoside	403.0	$[C_{20}H_{19}O_9]^+$			403.63

scavenging activities of PS extract may be attributed to the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system.[34] This suggests that the extracts may have potential use as antioxidative preservatives in emulsion-type systems.

Lipid Peroxidation Inhibition Activity. Lipid peroxidation can inactivate cellular components and play a major role in oxidative stress in biological systems. Furthermore, several toxic byproducts of the peroxidation can damage other biomolecules including DNA away from the site of their generation.[35,36] Lipid peroxidation causes destabilization and disintegration of the cell membrane, leading to liver injury, atherosclerosis, kidney damage, aging, and susceptibility to cancer.[37] It is well-established that transition metal ions such as iron and copper stimulate lipid peroxidation through various mechanisms,[38] and these may either generate hydroxyl radicals to initiate the lipid peroxidation process or propagate the chain process via decomposition of lipid hydroperoxides.[39] In the present study acetone extracts of all banana cultivars showed highest lipid peroxidation activity that ranged from 17.33 to 48.43% followed by methanol extracts from 6.17 to 31.11% (Figure7). The EC_{50} values for acetone extracts of cv. NR and NM were 141 and 162 μg mL $^{-1}$, respectively (Table 4).

Nitric Oxide Scavenging Activity. Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with superoxides, such as NO_2, N_2O_4, N_3O_4, NO_3, and NO_2, are very reactive. These compounds are responsible for altering the structural and functional behavior of many cellular components. NO is a defense molecule with cytotoxic, microbiocidal, and microbiostatic activities; however, large amounts of NO,

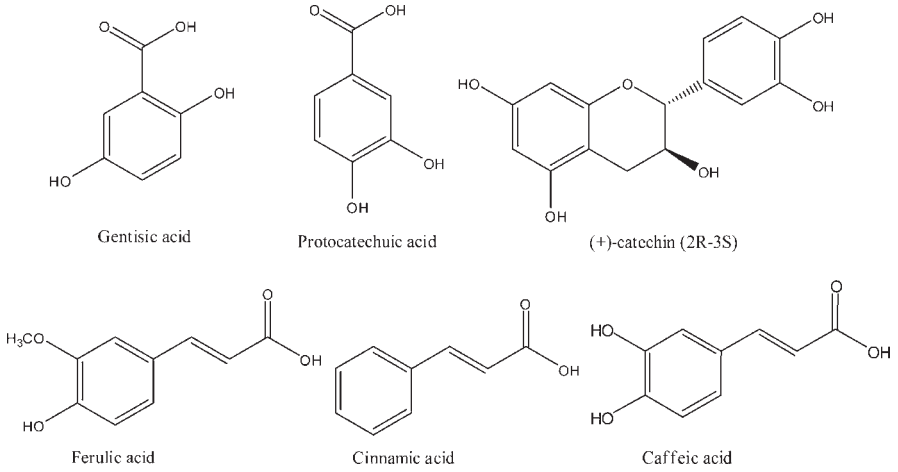

Figure 3. Phenolic acids identified from PS of banana cv. NR.

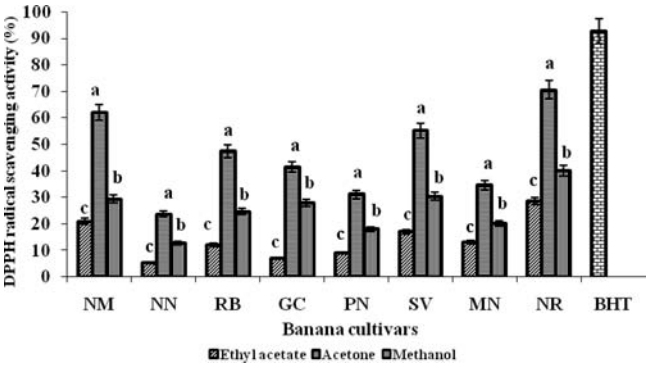

Figure 4. Free radical scavenging activity of extracts from PS of different banana cultivars and BHT (standard) as determined by the DPPH (1, 1-diphenyl-2-picrylhydrazyl) method. Data are the mean ± SD of three parallel measurements. *P* value ≤ 0.05. NM, Ney Mannan (ABB); SV, Safed Velchi (AB); RB, red banana (AAA); GC, Giant Cavendish (AAA); MN, Monthan (ABB); PN, Poovan (AAB); NN, Nendran (AAB); NR, Nanjanagudu Rasabale (AAB).

peroxynitrite, and other reactive nitrogen oxide species are considered to be potentially cytotoxic and capable of injuring the surrounding cells. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer, and other pathological conditions.[40] In the present study, the scavenging effect of PS extracts on NO was investigated. Sodium nitroprusside (SNP) is known to produce nitric oxide in phosphate-buffered saline. NO, under aerobic conditions, reacts with oxygen to form nitrate and nitrite, which can be determined using Griess reagent.[41] Incubation of sodium nitroprusside with PS extracts revealed that the inhibition of nitrite production was highest in acetone extracts. The percent inhibition activities and EC_{50} values for cv. NM, NR, SV, and RB were 53.67, 39.69, 35.57, and 31.61% (Figure 8) and 95, 120, 190, and 222 μg mL $^{-1}$, respectively (Table 4).

Metal Chelating Activity. The chelating properties of PS extracts were examined against Fe^{2+}. Ferrozine can quantitatively form complexes with Fe^{2+} ions.[19] The formation of a complex is probably disturbed by the chelating property in PS extracts, which would result in the reduction of the formation of a red-colored complex. Measurement of the rate of reduction of the color, therefore, allows estimation of the chelating activity of the PS extracts. The present data reveal that both PS extracts and standard EDTA interfered with the formation of ferrous complex with the reagent ferrozine, suggesting that it has chelating activity and captures the ferrous ion before ferrozine. The decreasing order of percent metal chelating activity of extracts from PS of different cultivars is as follows: methanol extract of PS of NR (61.28%) > GC (52.47%) > NM (48.64%) > SV (40.57%) > acetone extract of NR (35.61%) at the dose of 100 μg/mL (Figure 9). EC_{50} values are 85, 95, 110, and 140 μg mL $^{-1}$, respectively (Table 4). Its action as antioxidant may be related to its iron-binding capacity. It was reported that chelating agents that form σ-bonds with metals are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion.[42]

Hydrogen Peroxide Scavenging Activity. The scavenging ability of various PS extracts with hydrogen peroxide is shown in Figure 10. The result indicated that considerable hydrogen peroxide scavenging activity was observed in the acetone extract of PS of cv. NM (44.41%) and NR (39.09%) with EC_{50} values of 120 and 155 μg mL $^{-1}$, respectively (Table 4), followed by the methanol extract of cv. SV (37.27%) at 100 μg/mL. In general, hydrogen peroxide may act directly or indirectly as a messenger molecule, causing synthesis and activation of several anti-inflammatory mediators.[43] Hydrogen peroxide is mildly reactive by itself, but it may be converted to HO by transition metal ions, especially iron and copper.[44] Sometimes it is toxic to cells because it may give rise to hydroxyl radicals.[45] Therefore, removal of H_2O_2 is very important for antioxidant defense in cell or food systems. In the present study the polyphenolic compounds in the PS extracts may probably be involved in removing the H_2O_2. Polyphenols have been shown to protect mammalian and bacterial cells from cytotoxicity induced by hydrogen peroxide, especially compounds with the orthodihydroxy phenolic structure quercetin, catechin, gallic acid ester, and caffeic acid ester.[46,47]

Pseudostem Polyphenols and Their Multiple Antioxidant Activities. Polyphenols in acetone and methanol extracts in PS of different banana cultivars exhibited multiple AOA, whereas chloroform and hexane extracts showed negligible AOA. The differences in AOA of different banana cultivars appear to be in accordance with their concentration of phenolics and flavonoids. High concentrations of phenolics and flavonoids recorded in banana cv. NR are associated with high AOA. Several papers have

Table 4. Antioxidant Activity (EC_{50} Value, $\mu g\ mL^{-1}$) of Acetone and Methanol Extracts of Pseudostem from Different Banana Cultivars[a]

in vitro antioxidant assay model	NM	GC	SV	RB	MN	NR
DPPH radical scavenging activity	86 ± 1.2 d	141 ± 2.3 a	94 ± 1.3 d	122 ± 3.6 b	198 ± 6.2	76 ± 3.2 c
superoxide radical scavenging activity	96 ± 1.9 cd	115 ± 2.2 b	110 ± 2.0 d	130 ± 2.8 b		85 ± 2.5 c
β-carotene–linoleate model	120 ± 4.1 b		160 ± 3.6 b			119 ± 1.9 b
lipid peroxidation inhibition activity	162 ± 4.0 a					141 ± 2.6 a
metal chelating activity	110 ± 2.3 bc[b]	95 ± 2.9 c[b]	140 ± 2.3 c[b]			85 ± 3.7 c[b]
hydrogen peroxide scavenging activity	120 ± 2.7 b					155 ± 3.9 a
nitric oxide-scavenging activity	95 ± 3.1 cd		190 ± 3.7 a	222 ± 3.0 a		120 ± 3.6 b

[a] Data expressed as the mean ± SD of triplicate measurements. Mean values with different letters differ significantly at $P < 0.05$. EC_{50}, effective concentration of the sample to show 50% of antioxidant activity. [b] Methanol extract.

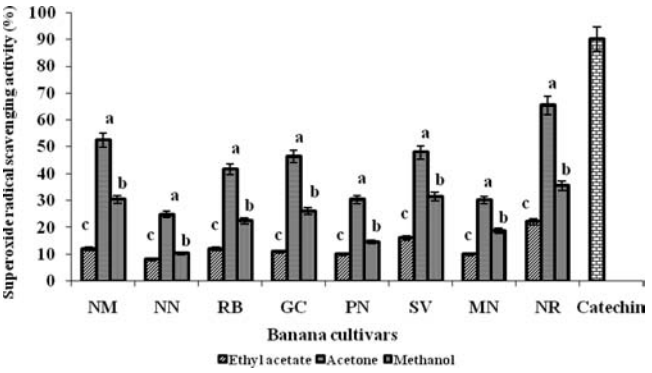

Figure 5. Superoxide radical scavenging activity of extracts from PS of different banana cultivars and catechin (standard). Data are the mean ± SD of three parallel measurements. P value ≤ 0.05. NM, Ney Mannan (ABB); SV, Safed Velchi (AB); RB, red banana (AAA); GC, Giant Cavendish (AAA); MN, Monthan (ABB); PN, Poovan (AAB); NN, Nendran (AAB); NR, Nanjanagudu Rasabale (AAB).

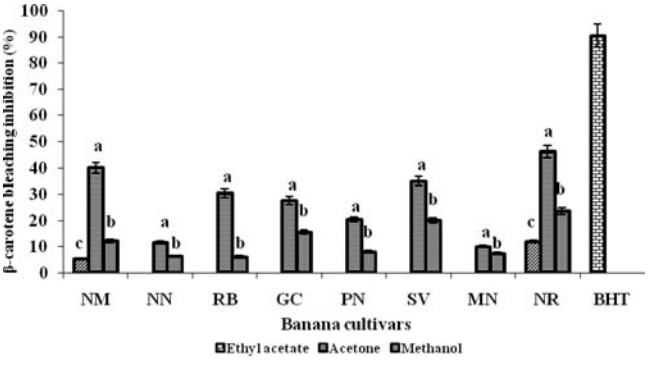

Figure 6. β-Carotene–linoleic acid bleaching activity of extracts from PS of different banana cultivars and BHT (standard). Each value is expressed as the mean ± SD of triplicate measurements. P value ≤ 0.05. NM, Ney Mannan (ABB); SV, Safed Velchi (AB); RB, red banana (AAA); GC, Giant Cavendish (AAA); MN, Monthan (ABB); PN, Poovan (AAB); NN, Nendran (AAB); NR, Nanjanagudu Rasabale (AAB).

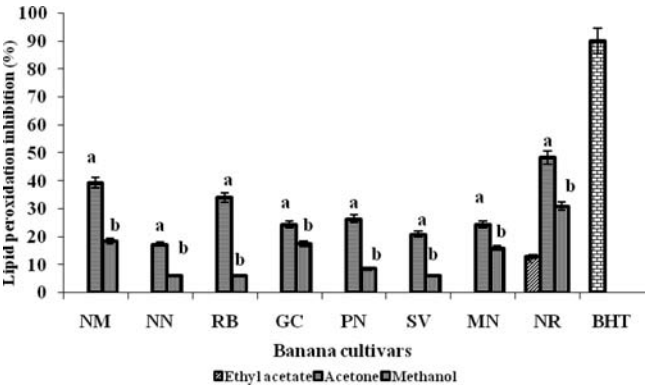

Figure 7. Lipid peroxidation inhibition activity of extracts from PS of different banana cultivars and BHT (standard). Data are the mean ± SD of three parallel measurements. P value ≤ 0.05. NM, Ney Mannan (ABB); SV, Safed Velchi (AB); RB, red banana (AAA); GC, Giant Cavendish (AAA); MN, Monthan (ABB); PN, Poovan (AAB); NN, Nendran (AAB); NR, Nanjanagudu Rasabale (AAB).

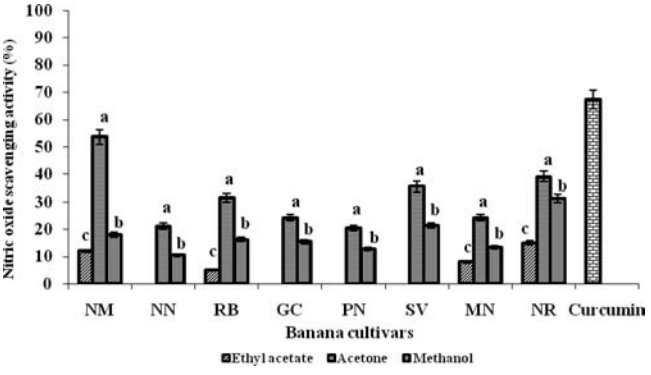

Figure 8. Nitric oxide scavenging activity of extracts from PS of different banana cultivars and curcumin (standard) using Griess reagent method. Data are expressed as the mean ± SD of triplicate measurements. P value ≤0 .05. NM, Ney Mannan (ABB); SV, Safed Velchi (AB); RB, red banana (AAA); GC, Giant Cavendish (AAA); MN, Monthan (ABB); PN, Poovan (AAB); NN, Nendran (AAB); NR, Nanjanagudu Rasabale (AAB).

conclusively shown a close relationship between TP and AOA in fruits and vegetables.[7,48,49] Furthermore, chemical composition and structures of active extract components were reported as important factors that govern the potency of natural antioxidants.[50−52] The recovery of polyphenols from plant materials is influenced by the solubility of the polyphenolic compounds in the solvent used for extraction process. In addition, solvent polarity will play a key role in increasing phenolic acid solubility.[26]

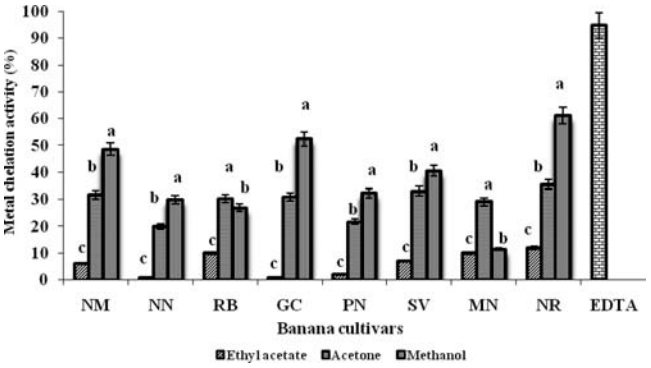

Figure 9. Metal chelating activity of extracts from PS of different banana cultivars and EDTA (standard). Each value is expressed as the mean ± SD of triplicate measurements. *P* value ≤ 0.05. NM, Ney Mannan (ABB), SV, Safed Velchi (AB); RB, red banana (AAA); GC, Giant Cavendish (AAA); MN, Monthan (ABB); PN, Poovan (AAB); NN, Nendran (AAB); NR, Nanjanagudu Rasabale (AAB).

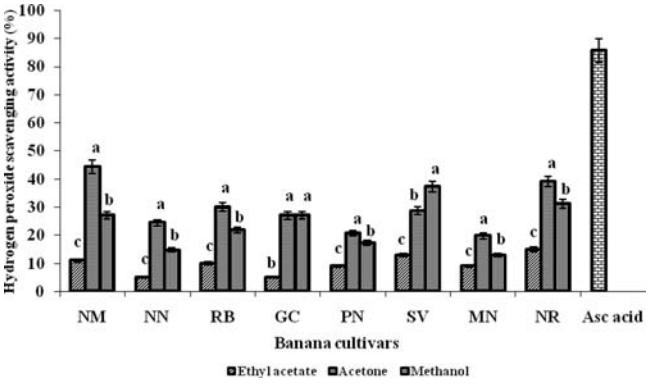

Figure 10. Hydrogen peroxide scavenging activity of extracts from PS of different banana cultivars and ascorbic acid (standard). Data are the mean ± SD of three parallel measurements. *P* value ≤ 0.05. NM, Ney Mannan (ABB); SV, Safed Velchi (AB); RB, red banana (AAA); GC, Giant Cavendish (AAA); MN, Monthan (ABB); PN, Poovan (AAB); NN, Nendran (AAB); NR, Nanjanagudu Rasabale (AAB).

The present investigation manifests clearly that BPS was found to be a potential source of polyphenols or natural antioxidants, which can be used as natural antioxidants in the food, nutraceutical, and pharmaceutical industries. The multiple antioxidant property may be an impetus to increase the consumption of BPS either in fresh or in processed form. However, the teleological role of polyphenols as biomarkers for taxonomic distinctions, for imbibing distinct quality and taste in banana fruits, is yet to be deciphered.

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