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Antioxidant Activity and Protective effect of Banana Peel against Oxidative Hemolysis of Human Erythrocyte at Different Stages of Ripening

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Abstract Phytochemicals such as polyphenols and carotenoids are gaining importance because of their contribution to human health and their multiple biological effects such as antioxidant, antimutagenic, anticarcinogenic, and cytoprotective activities and their therapeutic properties. Banana peel is a major by-product in pulp industry and it contains various bioactive compounds like polyphenols, carotenoids, and others. In the present study, effect of ripening, solvent polarity on the content of bioactive compounds of crude banana peel and the protective effect of peel extracts of unripe, ripe, and leaky ripe banana fruit on hydrogen peroxide-induced hemolysis and their antioxidant capacity were investigated. Banana (*Musa paradisica*) peel at different stages of ripening (unripe, ripe, leaky ripe) were treated with 70% acetone, which were partitioned in order of polarity with water, ethyl acetate, chloroform ($CHCl_3$), and hexane sequentially. The antioxidant activity of the samples was evaluated by the red cell hemolysis assay, free radical scavenging (1,1-diphenyl-2-picrylhydrazyl free radical elimination) and superoxide dismutase activities. The Folin–Ciocalteu's reagent assay was used to estimate the phenolic content of extracts. The findings of this investigation suggest that the unripe banana peel sample had higher antioxidant potency than ripe and leaky ripe. Further on fractionation, ethyl acetate and water soluble fractions of unripe peel displayed high antioxidant activity than $CHCl_3$ and hexane fraction, respectively. A positive correlation between free radical scavenging capacity and the content of phenolic compound were found in unripe, ripe, and leaky ripe stages of banana peel.

Keywords *Musa paradisica* · Erythrocyte lysis · Superoxide dismutase · Radical scavenging activity · Phenolic content · Ripening

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Introduction

Banana is the second largest producer after citrus fruit account for only around 16% of global world product. India is contributing 27% of the world for banana production [1]. From an environmental perspective, it is vital that plant byproducts produced by the agro-food industry be reused. Peels of a variety of fruits and plants are gaining attention as a natural source of polyphenols and bioactive compounds, which possesses various beneficial effects on human health. Apple pomace has been shown to be a good source of polyphenols and exhibits strong antioxidant and anti-proliferative activity [2]. Mango peels contain a good content of polyphenols and dietary fiber [3]. Grape pomace, a by-product of wine industry, is a good source of anthocyanins, catechins, flavanoids, phenolic acids, and dietary fiber. Tomato peels are reported to be a good source of carotenoids .There is very little or negligible work on the physiological and biochemical evaluation of edible (pulp) and non-edible portion (peel) of banana fruit [4].The main by-product of the banana processing industry is the peel, accounting for 30% of the fruit, which constitutes environmental hazard [5].High dietary fiber content of banana peels makes them promising for a variety of applications in nutraceutical and medicinal application. By doing this, the aim is to prevent oxidative stress related disorder in human. The exact mechanism behind the aging is not clearly known but there is sufficient evidence suggesting that aging could be caused by accumulation of reactive oxygen species [6]. Free radical damages the structural and functional components of the cell such as lipid, protein, carbohydrates, DNA, and RNA. Banana peel contains high content of micronutrient compared to fruit pulp [7]. It attracts great attention because of their nutritional and antioxidant properties, especially the compounds, ascorbate, catechin, gallocatechin, and dopamine. Due to the importance of these compounds, it is necessary to understand its initial production and losses during fruit development, ripening, and maturation. The changes in enzymatic and nonenzymatic components of the plant antioxidant defenses have been previously described during fruit ripening in several plants. For example, Jime'nez et al. [8] reported that the levels of glutathione (GSH) and ascorbate (AsA) in tomato increased during the ripening process. However, a different result has been shown by Wang and Jiao [9], who found that GSH, AsA, and related enzyme should decline with ripening of blackberry fruit.

The present investigation was undertaken to evaluate the antioxidant activity of banana fruit peel with the aim of exploiting the potential value of the waste banana peels and the role of these antioxidant systems in the degradation of membranes during maturation, ripening, and senescence of banana fruit peel. In the present studies, we have attempted to determine the changes in the antioxidant systems at different stages of ripening of the banana peel (*Musa paradisica*) and special emphasis was laid on maximum extraction with solvents of varying polarity. In addition, superoxide dismutase (SOD) and 2,2′-diphenyl-1-picryl hydrazyl (DPPH) activities were examined after in vitro incubation of the extract with fresh human erythrocyte.

Materials and Methods

Chemicals and Reagents

DPPH, gallic acid, methionine, nitroblue tetrazolium (NBT), riboflavin, ascorbic acid (vitamin C) were purchased from Sigma-Aldrich. All other reagents and chemicals of analytical grade were procured from local sources and milli-Q quality water was used.

Sample Collection and Extraction

Banana (*M. paradisica*, local name Bhusawal keli) fruits were purchased from local market of Kaushambi, Uttar Pradesh, India, at different stages of ripening without any ethylene and stored at 20 °C for 24 h before being extracted. Harvesting and determination of maturity was carried out by skilled workers, combining the following techniques: day count, chromacity, firmness, pH, and flavor of fruits; the mature young fruits were harvested carefully when peel tightly intact with edible part of fruit is in fully green stages and left for 1 day at room temperature .The banana fruits were peeled to get peel with firm texture and no smell. Some of the fruits were left for another 8 days at room temperature to ripen and their flesh became a soft and developed a typical banana flavor. Peel separated from the flesh part of banana fruit with yellow stages without brown spotting. Whereas overripe stages have a strong smell with leaky edible portion when fruits are left for another 3 days (in this stages peel were yellow brown). The whole fruit of banana was washed thoroughly under running tap water, dried on paper towel. The peel tissues of fresh unripe green, ripe yellow, and leaky ripe brown with traces of yellow banana fruit (200 g) were heated in 750 ml of distilled water for 5 min. Banana peel was homogenized with 70% acetone twice at room temperature using pre-chilled pestle and mortar for 48 h under shaking conditions using an electrical shaker. The combined extracts of 70% acetone extracts were then filtered and centrifuged at 4 °C in Beckman refrigerated centrifuge machine for 15 min at 15,000×*g*. The supernatant was concentrated to 150 ml. This aqueous extract was partitioned into a chloroform ($CHCl_3$) and water, using a separator and then extracted with aqueous saturated ethyl acetate. Fractionate and 70% acetone extract were concentrated using a rotary evaporator with the water bath set at 40 °C. The percentage yield of extracts ranged from 7% to 19% *w*/*w*. The antioxidant activities of hexane, $CHCl_3$, ethyl acetate (EtOAc), and water fractions were measured at different stages of peel.

Preparation of Test and Standard Solution

The extract and standard antioxidant ascorbic acid were dissolved in freshly distilled DMSO (Dimethyl Sulfoxide) separately and used for in vitro antioxidant activity using different method. For total phenol content a solution of extract in methanol was used. Gallic acid was dissolved in distilled water and used as a standard for total phenol estimation [10].

Determination of Total Phenolic Content

The amount of total soluble phenolics was determined according to the Folin–Ciocalteu's method Anagnostopoulou et al. with slight modification [11]. The reaction mixture consisted of 0.5 ml of the extract (10 mg/ml), 5 ml of distilled water, and 0.5 ml of the Folin–Ciocalteu's reagent. After a period of 3 min, 1 ml of saturated 5% sodium carbonate solution was added. The 10-ml volumetric flasks were shaken and allowed to stand for 1 h. The absorbance was measured at 725 nm (each measurement repeated three times) in a UV-visible spectrophotometer (the same equipment was used in the DPPH test). The total phenolic content was expressed as milligrams gallic acid per gram dry extract, milligram gallic acid/100 g dry fruit peel. Total content of phenolic compounds of extracts in gallic acid equivalents (GAE) was calculated by the following formula [12].

$$C = c.V/m$$

Where

- C Total content of phenolic compounds, milligrams per gram plant extract, in GAE
- c The concentration of gallic acid established from the calibration curve, milligrams per milliliter
- V The volume of extract, milliliters
- M The weight of pure plant methanolic extract (grams).

Determination of Antioxidant Activity

Scavenging Activity on 1, 1-Diphenyl-2-picrylhydrazyl Radicals

Inhibiting action of dry matter of banana peel tissue against stable free radical was measured by the following method: the radical scavenging activities of the plant extracts against 2, 2-diphenyl-1-picryl hydroxyl radical (Sigma-Aldrich) were determined by UV spectrophotometer at 517 nm. Radical scavenging activity was measured by a slightly modified method previously described Ayoola et al. [13]; Leong and Shui [14]. The following concentrations of the extracts were prepared 10, 50, 100 mg/ml. (Analar grade). Vitamin C was used as the antioxidant standard at a stock concentration of 10 mg/ml. One milligram of the extract was placed in a test tube and 3 ml of methanol was added followed by 0.5 ml of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. The mixture was shaken immediately after adding DPPH and allowed to stand at room temperature in dark and the decrease in absorbance at 517 nm was measured after 30 min until the reaction reached a plateau. These experiments were run in duplicate. The radical scavenging activity was calculated using the following formula:

$$\%\,\text{inhibition} = \{[A\text{o} - A]/A\text{o}\} \times 100$$

Where Ao is the absorbance of DPPH without sample (control) at 517 wavelengths; A is the A_{517} of sample and DPPH (test sample).

Scavenging Effect on Superoxide Anions—NBT Reduction System

SOD activity was assayed using the NBT method [15]. SOD activity of banana peel tissue extract in 70% acetone, water, ethyl acetate, chloroform, and hexane were determined by photochemical reduction of NBT, according to Giannopolitis and Ries [16] with slight modifications, using the assay system consisting of methionine, riboflavin, and NBT. NBT was reduced to blue formazan by O_2^-, which has a strong absorbance at 560 nm. However, the presence of SOD inhibits this reaction. Two sets of each sample were used. The reaction mixture consists of 1.3 μM riboflavin, 13 mm methionine, 63 μM NBT, 0.05 M sodium carbonate (pH 10.2) and the appropriate volume of extract. Distilled H_2O was added to bring to the final volume of 3 ml. The test tubes were inverted twice. One set of the reaction tube was covered with a black cloth as control. The other set was placed approximately 30 cm below a blank of two 15-W fluorescent lamps. The reaction was initiated by turning the light on for 10 min. Following light exposure, the tubes were covered with a black cloth to prevent further reaction. Illuminated mixtures lacking enzyme developed maximum color, while non-illuminated

mixtures did not develop color and were used as control. The absorbances of the illuminated mixtures were compared to non-illuminated mixtures using a spectrophotometer at 560 nm. From the graph, the volume of enzyme extract corresponding to 50% inhibition of the reaction was calculated and considered as one enzyme unit. Protein concentration was determined according to Bradford using bovine serum albumin as a standard. [17]. SOD activity was expressed as enzyme units per gram fresh weight units per gram fresh weight of banana peel.

Scavenging Effect on H_2O_2-Induced Human Blood Hemolysis (Antihemolytic Activity)

Preparation of Erythrocytes Human blood (5–10 ml) sample was withdrawn from group of healthy female (20–25 year). Samples were collected into centrifuged tubes coated with anticoagulant EDTA and the Red Blood Cells were collected by centrifugation of blood at 1,000×*g* at 4 °C for 20 min. The buffy coat and plasma were removed using a pipette. The crude RBCs were washed with the same volume of phosphate-buffered saline (PBS) pH 7.4 followed by centrifugation twice. The packed RBCs were then suspended in four volumes of PBS solution [18].

Assay for Free Radical-Mediated Hemolysis The inhibition of human erythrocyte hemolysis was done according to Rafat et al. [18] with slight modification. Here, the erythrocyte hemolysis was performed with H_2O_2 as free radical initiator, on human blood. To 500 μl of suspension of erythrocyte in IPB (isotonic phosphate buffer solution at pH 7.4), 1 ml of dry extract of banana peel extracted in solvent water, chloroform, 70% acetone, ethyl acetate, hexane was added. Concentration of sample 100 mg/ml prepared in 5% DMSO dissolved in isotonic phosphate buffer pH 7.4 and kept at 4 °C until required. The working solution 10 mg/ml for assay was made by diluting the stock solution with IPB. The reaction mixture was shaken gently while being incubated at 37 °C for 3 h. A positive control was prepared by pretreating the erythrocyte suspension with 1 ml of 10 mg/ml concentration of vitamin C dissolved in IPB. The inhibitory effect of the extract was compared with standard antioxidant vitamin C. The non-pretreated erythrocyte suspension was used as the negative control. Oxidative stress was then induced by adding 1 ml of 10 mM hydrogen peroxide (H_2O_2) and incubated at 37 °C for 150 min. After incubation, the volume of all pretreated and non-pretreated erythrocyte suspensions were adjusted to 9 ml by adding IPB. The released hemoglobin in the supernatant of the mixtures was measured using spectrophotometer at 540 nm. Erythrocyte hemolysis in pure water was based on complete erythrocytes hemolysis (100%) while hemolysis of the pretreated and non-pretreated erythrocytes was expressed as a percentage of this value.

Percentage inhibition was calculated as described by the equation $(1-A_{\text{antioxidant}}/A_{\text{H2O2}})\times 100$, where A_{H2O2} is the absorbance of sample containing no extract and $A_{\text{antioxidant}}$ is the absorbance of sample containing extract [19].

Statistical Analysis

All measurements were carried out in triplicate and the results are statistically analyzed using the statistical program to determine the average value. Analysis of variance was performed using two-way ANOVA and the significant differences ($p<0.05$) between the means were performed to determine the effect of solvent polarity and ripening stages on the content of bioactive compounds and antioxidant capacity of banana peel.

Results and Discussion

Different extracts of banana peel in different stages of ripening possess potent antioxidant activity, using the DPPH assay and the SOD assay. Fruit ripening has been described as oxidative phenomenons [20], which require a turnover of active species such as H_2O_2 and superoxide anion. It has been reported that tolerance of plant to condition-causing damage may be associated with their higher ability to remove free radical and active oxygen species through active oxygen species (AOS) detoxifying enzyme, SOD, and peroxidases implying that they may play a protecting role from oxidative damage [21].

Banana peel is rich in photochemical compound mainly antioxidant. Our results show the total phenolic content estimated to be in the range of 1.74±0.09 GAE g/100 g in unripe stages which is reported by Someya et al. [22] and Nguyen et al. [23].

Table 1 shows the results of the extraction of the unripe, ripe, and leaky ripe banana peel sample with 70% acetone (acetone/water). The highest yield (5.2%) was obtained from unripe fruit peels while the lowest yield (2.98%) was from leaky ripe fruit peels. The total phenolic contents measured in unripe to leaky ripe peel was GAE gram per 100 g dry extract using Folin–Ciocalteu's assay ranged from 1.74 to 0.844, it is noted that unripe banana peel demonstrated significantly higher total phenolic content than that of ripe and leaky ripe peels. Ethyl acetate seems to be the solvent that concentrates best phenolic substances of intermediate polarity. This is in accordance with the findings by Chung et al. [24] and Parejo et al. [25].

Kiyoshi and Wahachiro [26] reported that in immature stages 60% polyphenol compounds with molecular weight (MW) above 2×10^5 and other 40% were those with a MW below 2×10^5. During ripening polyphenol compounds with MW 2×10^5 and astringency disappeared. As ripening proceeds only low MW (below 2×10^5) polyphenolic compounds remain. Our result is in line with this, as the ripening proceeds, polyphenol compound were degraded.

Choosing the appropriate extraction conditions and solvent is one of the most important factors in obtaining extracts with a high content of bioactive compounds. The results obtained coincide with reports that the mixture of acetone/water (70% acetone extract) is an effective solvent for extracting phenolic compounds (most of the polar and non polar antioxidants were extracted in this solvent) [2, 27]. Our results reinstate the view of earlier studies that extracting banana peel with 70% was not only very efficient but also produced extracts with high antioxidant capacity, as confirmed by various model systems. This may be due to variation in the quality and quantity of phenolic compounds and other bioactive compounds present in the different extracts, which on fractionation given higher concentration in ethyl acetate solvent. [5].

Banana peel powders were consecutively fractionated with organic solvents with increasing polarity. The yields of these extractions and the total phenolic contents are reported in Table 2. It was found that pooled fraction of extract with polar solvent, gave the

Table 1 Yield of acetone extract (percentage of *w*/*w*) and total phenolic content from the banana peel tissue at the different stage of ripening

Sample (ripening stage)	Sample code	Percentage of yield (*w*/*w*)	GAE mg/100 g
Unripe banana peel	UP	5.23±0.585	1743.93±90.12
Ripe banana peel	RP	3.56±0.128	1091.41±47.8
Leaky ripe banana peel	LRP	2.63±0.124	844.61±10.7

Table 2 Yield and total phenolic content of fractionated dry extracts per 100 g of banana peel tissue at different stage of ripening

Ripening stage	Solvent	Sample code	Percentage of yield (*w*/*w*)	GA mg/100 g
Unripe	Water	WUP	48.76±1.50	741.5±4
	Chloroform	CUP	1.92±0.223	35.12±3.0
	Ethyl acetate HUP	EUP	37.09±2.77	992.46±17.9
	Hexane	HUP	0.41±0.040	87.63±3.65
Ripe	Water	WRP	59.1±2.9	371.74±3.82
	Chloroform	CRP	0.620±0.055	17.85±2.45
	Ethyl acetate	ERP	18.71±1.73	630.14±2.85
	Hexane	HRP	0.085±0.007	68.83±3.32
Leaky ripe	Water	WLP	74.03±1.45	155.58±3.20
	Chloroform	CLP	0.446±0.096	23.81±2.42
	Ethyl acetate	ELP	15.16±0.642	583.64±2.62
	Hexane	HLP	0.171±0.027	71.26±3.26

highest yields (16–26%) from the 70% acetone extract of banana peel at different stages of ripening, whereas the yields obtained after fractionation with hexane were the lowest (0.6–0.9%) [27]. The hexane extracts not only gave low yields, but also contained the lowest levels of phenolic compounds (0.1–0.3 mg/ml). The extracts of the intermediate polar solvent, EtOAc contained substantially higher levels of phenolic compounds (ranging from 0.071 to 0.996 GAE g/100 g) than polar water fraction and comparable with those found in the 70% acetone extracts (Table 1). Similar results were obtained by Mokbel and Fumio Hashinaga [28] in Cavendish variety of banana peel. Low recovery of phenols in water fraction could be caused by the oxidation of phenolic compound by polyphenol oxidase. While, in acetone, ethyl acetate, and chloroform the enzyme is inactivated (Table 3).

Figure 4 shows that the antioxidant activity (AEAC) values of the 70% extracts of the banana peel at the different stages of ripening were between 1.21+0.10 g/100 g and 0.485+0.07 g/100 g. The antioxidant activity of the extracts was significantly lower than that of

Table 3 Correlation between phenolic content and AOA of 70% acetone extract of fresh banana peel at different stages of ripening

Variation in ripening stage (70% acetone extract)		
Correlation	*R*	R^2 (%)
TPC vs. DPPH	0.998	99.6
TPC vs. SOD	0.049	0.24
TPC vs. hemolysis	0.702	49.2
DPPH vs. SOD	0.101	96.4
DPPH vs. hemolysis	0.663	57.4
SOD vs. hemolysis	0.676[a]	45.6

Ripening stages in 70% acetone extract of banana peel in the antioxidant activity, scavenging effects on superoxide anion, DPPH radicals, and H_2O_2-induced heamolysis used for correlation analysis were unripe, ripe, and leaky ripe, respectively

[a] Indicate negative correlation

vitamin C at the same concentration (Fig. 1). The extracts of the unripe peels have both excellent DPPH* radical scavenging (Figs. 2 and 3) and superoxide dismuting capacities (Figs. 6 and 7). The hexane fraction possess the lowest antioxidant activity, as determined by the DPPH assay (AEAC values ranging from 0.097 to 0.008 AEAC g/100 g) and SOD assay (ranging from 0 to 0.48 unit/g fruit weight from unripe to leaky ripe) which can be ascribed to their very low polyphenolic contents (Table 2, Figs. 4, 5, and 7). From the result we can conclude that unripe stages of banana has inherent compensatory mechanism against oxidative stress (tolerance of plant to condition causing damage may be associated with their ability to remove AOS through AOS detoxifying enzyme, SOD, CAT, etc. implying that they play a role protecting fruit from oxidative damage) though have less protein whereas in leaky ripe sample comparatively high protein causes the higher SOD activity. On fractionation, higher activity reported in ethyl acetate fraction due to the peculiar behavior of SOD isoenzyme, whereas in water, enzyme activity degrades at water pH (Figs. 6 and 7).

The extracts of the intermediate polar solvents (ethyl acetate) had substantially higher activity for DPPH* scavenging (ranging from 0.22±0.025 to 0.70±0.018 AEAC g/100 g) and SOD activity (ranging from 2.19 to 1.67 unit/g fruit weight unripe to leaky ripe) which is corresponding to their high polyphenolic levels (Table 2).

Similarly, unripe sample shows better SOD activity in water and chloroform fractions. This is because polyphenol oxidase exhibits lesser activity in immature stages of fruit and the enzyme extracts in water is not degraded at that particular pH.

Antioxidant potency of the crude extract was further investigated for their capacity to protect human erythrocytes against damage in vitro (Fig. 8). It is known that polyphenolics enhance red blood cell resistance to oxidative stress both in vitro and in vivo [29]. Figure 9 shows the inhibitory effect of different concentrations of unripe crude 70% acetone fraction (5–50 mg/ml) on an H_2O_2-induced hemolysis of human erythrocytes.

Unripe peel extracts showed higher inhibition on erythrocyte hemolysis than that of ripe peel, while, not much difference in inhibition was noticed between ripe and leaky ripe peel extract of banana peel. Ajila and Rao [30] reported acetone extract of mango peel in unripe stages inhibited the H_2O_2-induced hemolysis of rat erythrocytes. The results indicated that

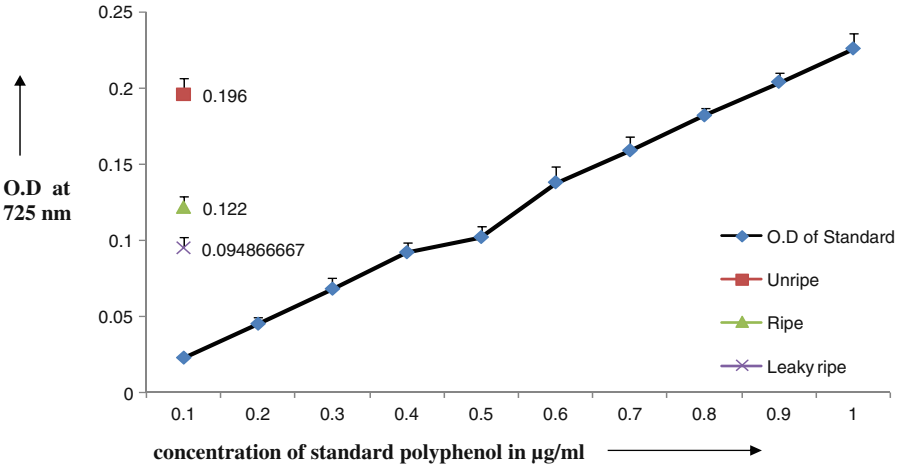

Fig. 1 Total phenolic content in GAE/10 mg of banana peel extract in 70% acetone at different stages of ripening. The data are displayed with mean+standard deviation (*bars*) of three replications

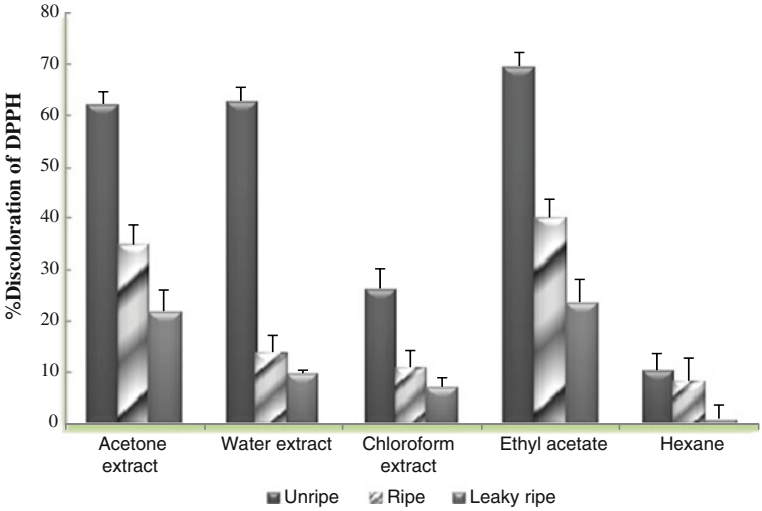

Fig. 2 DPPH radical scavenging effect of the unripe, ripe, and leaky ripe banana peels extract in solvent 70% acetone, water,chloroform, ethyl acetate, hexane. The data are displayed with mean+standard deviation (*bars*) of three replications

the differences in hemolysis of peel extracts of unripe and ripe mango fruits might be due to the synergistic actions of bioactive compounds present, Since, unripe peel extracts were rich in polyphenols than the corresponding ripe peel extracts, the increased inhibitory effect of unripe peel extracts on erythrocyte hemolysis might be due to high content of polyphenols. Furthermore, with the aim of characterizing the compound, we fractionated the peel extract using solvents of increasing polarity (hexane, ethyl acetate, chloroform, and water). This was done to investigate the fraction(s) with higher antioxidant potency

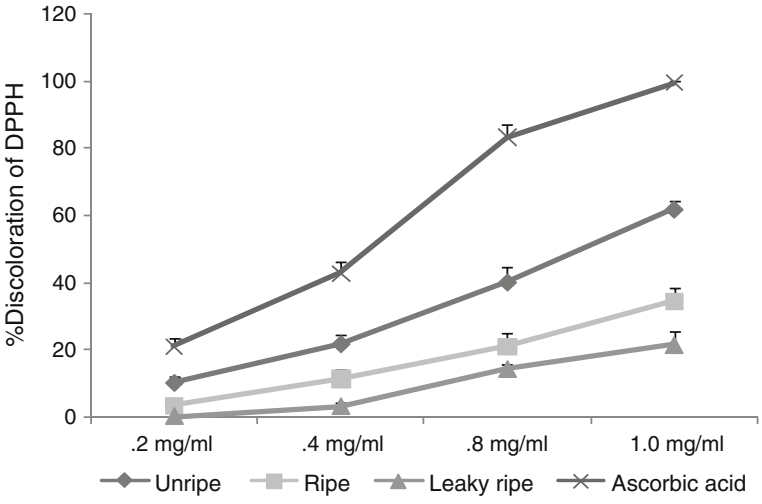

Fig. 3 Antioxidant activity of 70% acetone extract (acetone/water) of unripe ripe, leaky ripe banana peel extract at different concentration to scavenge free radical of DPPH. The data are displayed with mean+ standard deviation (*bars*) of three replication

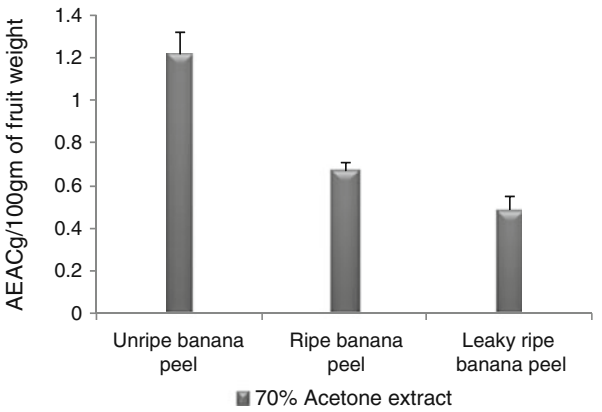

Fig. 4 Ascorbic acid equivalent Antioxidant activity of the 70% acetone extracts from the banana fruit peels at different stage of ripening using DPPH assays. Calibration was done with ascorbic acid standard (DPPH assay)

showing best inhibiting activity of erythrocyte hemolysis. The extracts of the polar solvents had substantially higher inhibitory effect on erythrocyte hemolysis (ranging from 7.3± 0.025% to 34.3±0.018% inhibition) which is corresponding to their high polyphenolic levels (Tables 2, 3). The ethyl acetate fraction of unripe had about three times higher activity than that of ripe and leaky ripe, respectively. Thus, it is possible that the compound with antioxidant capacity may have a polar nature.

Correlation Analysis

Calculated coefficients of correlations between antioxidant activity, scavenging effects on radicals and contents of phenolic compounds of banana peel fractionate in different solvents are shown in Tables 4 and 5. The antioxidant activity of banana peel fractionate was significantly correlated with their scavenging effects on superoxide anion ($P<0.01$), DPPH* radicals ($P<0.05$) and H_2O_2-induced hemolysis ($P<0.01$) on varying extractions in different solvents, whereas no significant difference was observed on varying the ripening

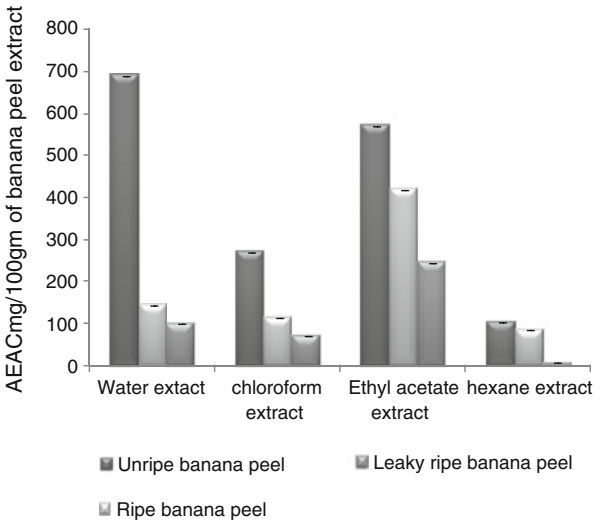

Fig. 5 Ascorbic acid equivalent antioxidant activity milligrams per 100 g of fractionated extracts (Table 2) using DPPH assay. Ascorbic were used as standards

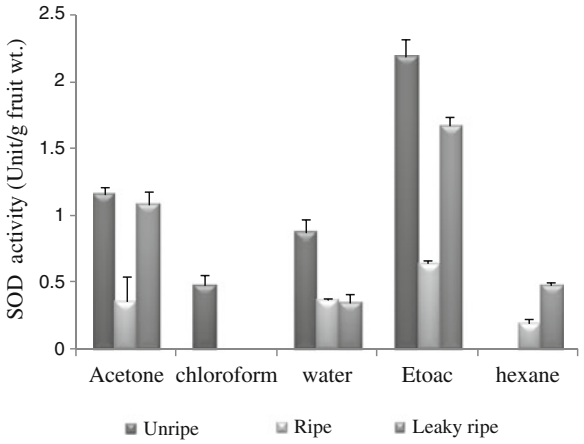

Fig. 6 Activities of superoxide dismutase (SOD) in unit per gram dry extract of banana peel tissue extracted in different solvent. Different stage of growth stands for unripe, ripe, leaky ripe banana peel sample. The data are displayed with mean±standard deviation (*bars*) of three replications

stages. Therefore, the antioxidant activities of banana peel extracts may be due to their scavenging effects on radicals and inhibiting the human erythrocyte hemolysis induced by H_2O_2.

For scavenging effects on radicals, high correlations (R^2=91.39–31.02) were observed between various radicals, on varying the extraction in different solvent in order of polarity in unripe, ripe, and leaky ripe samples. Results indicate that in unripe sample highest correlation observed between TPC vs. DPPH (91.39%).Whereas in ripe and leaky ripe peel, good correlation was observed between TPC vs. SOD (92.7% and 78.85%). The antioxidant activity (P<0.05) and scavenging effects on superoxide anion, percentage of inhibition of H_2O_2-induced hemolysis (P<0.01) of banana peel extracts was also well correlated with their contents of total phenolic compounds. The same trends were observed in the correlation of the content of gallic acid and the antioxidant activity and scavenging effects on superoxide anion. According to recent reports, a highly positive relationship existed between total phenolics and antioxidant activity in many plant species [31].

Table 5 indicates that in each stages, on varying the solvent polarity the lower correlation between DPPH and SOD observed in leaky ripe. In ripe and leaky ripe peel samples, SOD activity is not only the cause of inhibition of erythrocyte hemolysis but other phenolic compounds are also responsible for it. From Table 4, we can conclude that on varying the ripening stages (variation factor), highest correlation was observed between TPC and DPPH

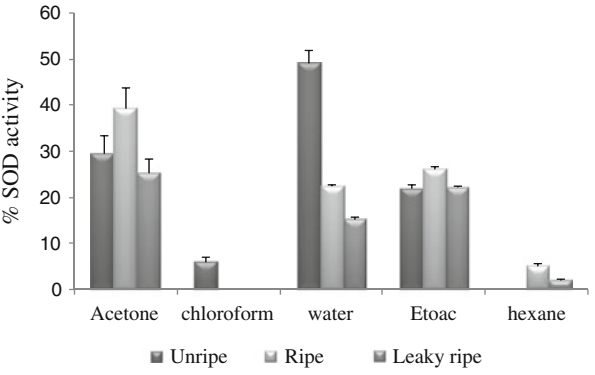

Fig. 7 Percentage of activity of superoxide dismutase in banana peel extract at different stage of growth to scavenge free radical O_2^- generated from NBT reduction.The data are displayed with the mean±standard deviation (*bars*) of three replications

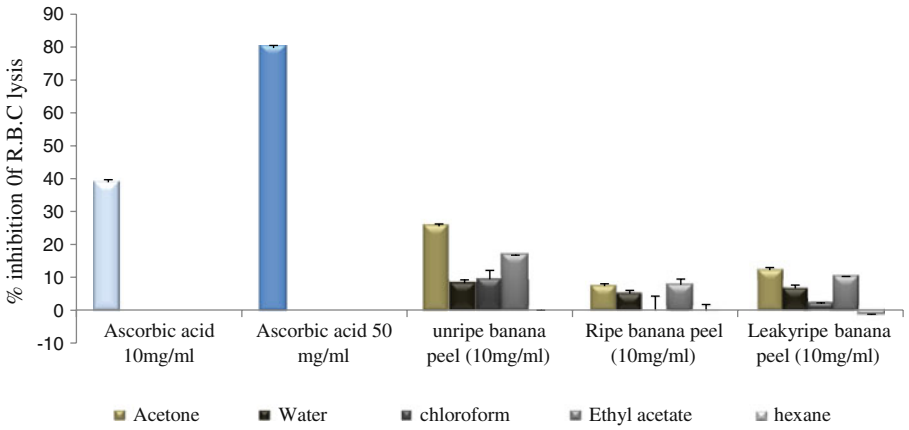

Fig. 8 Antioxidant activity of unripe, ripe, leaky ripe banana peel tissue extract in different solvent: inhibition of human red blood cell (erythrocyte) expressed as percentage value. Ascorbic acid was used as positive control

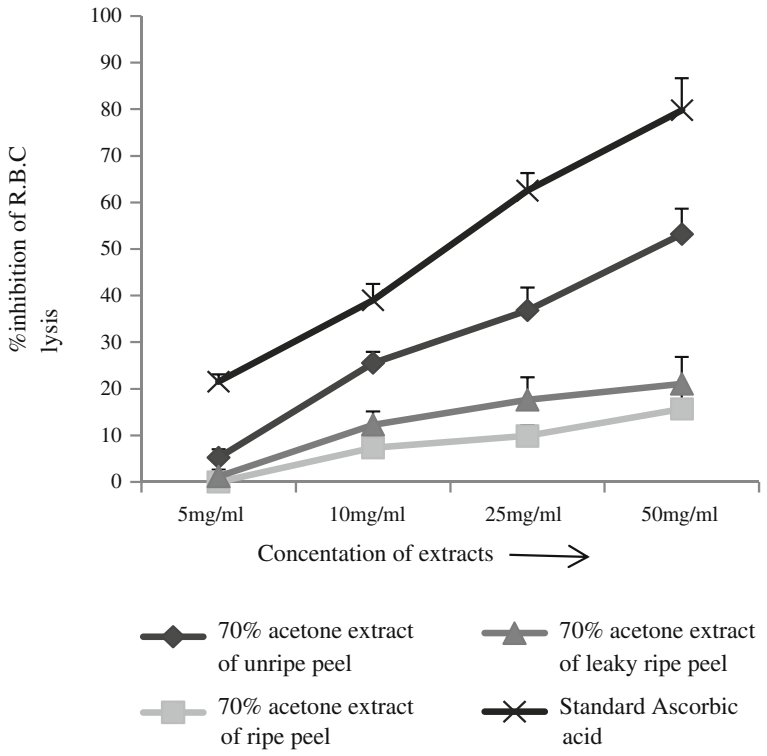

Fig. 9 antioxidant activity of banana peel tissue at different concentration to inhibit RBC lyses in respect of standard ascorbic acid. The data are displayed with mean±standard deviation (*bars*) of three replications

Table 4 Correlation between phenolic content and AOA of banana peel fractionate on dry weight bases

Variation in ripening stage				
Correlation R^2 (%)	Water	Ethyl acetate	Chloroform	Hexane
TPC vs. DPPH	91.0	93.3	74	7.29[a]
TPC vs. SOD	97.0	19.8[a]	88.36	76.5[a]
TPC vs. hemolysis	38.8	65.4	96.8	18.4
DPPH vs. SOD	98.2	3.96[a]	96.4	5.47[a]
DPPH vs. hemolysis	68.7	39.69	57.4	97.0[a]
SOD vs. hemolysis	55.8	78.4[a]	74.9	0.43

Ripening stages in banana peel fractionate in the antioxidant activity, scavenging effects on superoxide anion, DPPH radicals, and H_2O_2-induced hemolysis used for correlation analysis were unripe, ripe, and leaky ripe, respectively

On varying the stage of banana peel, here the correlation in between antioxidant activity on keeping the extraction in solvent constant (on taken a single stage of extraction)

[a] Indicate negative correlation

(dependent factor) in ethyl acetate and water extract (independent factor). Similarly, highest correlation was observed between TPC and SOD in water fractions than that of ethyl acetate.

Conclusion

The present study was conducted with a view to exploit banana peels as a source of valuable components. Antioxidant activities have been detected in peel portion of banana fruit from unripe stages to leaky ripe stages. Unripe banana peel has the highest antioxidant activity and antioxidant enzyme in comparison to the other two stages. Fruit maturation and ripening was accompanied by the decrease in the activities of phenolic compound and antioxidant enzyme. These events may lead to increased oxidative stress and cause many metabolic changes associated with ripening and maturation.

Table 5 Correlation between phenolic content and AOA of banana peel fractionate on dry wt bases

Variation in solvent polarity (extraction condition)			
Correlation R^2 (%)	Unripe	Ripe	Leaky ripe
TPC vs. DPPH	91.39	80.10	31.02
TPC vs. SOD	51.84	92.7	78.85
TPC vs. hemolysis	32.14	96.4	70.22
DPPH vs. SOD	64.80	55.3	9.92
DPPH vs. hemolysis	48.72	92.5	0.102
SOD vs. hemolysis	77.02	80.46	63.2

Solvent to fractionate banana peel extract in the antioxidant activity, scavenging effects on superoxide anion, DPPH radicals, and H_2O_2-induced hemolysis used for correlation analysis were water, acetone, ethyl acetate, chloroform, and hexane respectively

On varying the extraction process in different solvent, here the correlation in between antioxidant activity on taken single stages of banana peel

Higher antioxidant potency of ethyl acetate fraction from 70% acetone extract of banana peel is due to high phenolic content. The chemical activity of polyphenols in terms of their reducing properties as hydrogen or electron donating agents predicts their potential for action as free radical scavengers (antioxidants). Polyphenols possess ideal structural chemistry for free radical scavenging activities and have been shown to be more effective antioxidants in vitro than vitamins E and C on a molar basis. One of the antioxidant compounds in the banana peel was determined to be gallocatechin, which was related to the antioxidant activity of the banana extract show the maximum extraction in non polar solvent. The low recovery of phenolic compounds obtained with water could be caused by the oxidation of phenolic compounds by polyphenol oxidase, whereas in 70% acetone, ethyl acetate, chloroform fraction the enzyme is inactivated. Thus, from the above investigation, it can be concluded that the waste part of banana peel is a potential candidate for nutraceutical preparation for their antioxidant potency. Thus, further work can be carried out to isolate the active moiety responsible for the biological activity, characterize it and commercialize it.

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