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# Banana (*Musa* sp. var. elakki bale) Flower and Pseudostem: Dietary Fiber and Associated Antioxidant Capacity

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**ABSTRACT:** Banana flower (BF) and pseudostem (PS) are byproducts of banana cultivation and are known to have health beneficial effects. The main objective of this study was to evaluate the dietary fiber composition and antioxidant effect of BF and PS. In the present study, BF and PS were found to be rich in dietary fiber (65.6 ± 1.32 and 28.8 ± 0.98%, respectively). Dietary fiber fractions were extracted and characterized in terms of sugar profile, and antioxidant activities were determined. BF and PS fractions were rich in sugars and showed wide diversity with respect to the nature of the sugars. Hemicellulose A fraction of BF showed high amounts of total polyphenols and total antioxidants, which were 121.8 ± 1.9 and 39.03 ± 0.118 μg/mg extract, respectively. HPLC analysis showed the presence of phenolic acids in hemicellulose A and B fractions of BF. These results indicate that BF and PS are rich sources of dietary fiber associated with polyphenols, which could promote health beneficial effects.

**KEYWORDS:** *banana flower, banana pseudostem, dietary fiber, antioxidant activity, polysaccharides*

## INTRODUCTION

Dietary fiber (DF) is one of the most enduring dietary interests of this decade worldwide. Carbohydrates constitute a diverse nutrient category ranging from sugars easily digested by monogastric animals in the small intestine to DF fermented by microbes in the large intestine.[1] DF is defined as complex carbohydrates in foods, which have many effects in the gastrointestinal tract, including altering fluid dynamics, slowing macromolecule digestion, and absorption of nutrients.[2,3] Insoluble DF is made up of cellulose and other nonstarch polysaccharides along with a small amount of cell wall lignin and cutin, whereas pectins, β-glucans, arabinoxylans, galactomannans, and other polysaccharides and saccharides are typical soluble DF constituents.[4] DF components are long polymeric carbohydrate chains containing up to several hundred thousand monomeric units, which differ by the number and type of monomeric units linked together, the order in the chain, the types of linkages between various monomers, the presence of branch points in the backbone, and those having acidic groups, for example, uronic acids.[5]

In recent years, the search for newer sources of DF with beneficial effects has received fresh impetus. This is partly because of the reported beneficial effect exhibited by DF and its associated compounds in various pathological conditions.[6] Furthermore, the nature and components of DF determine its amenability to fermentation by various microflora in the intestine to release short-chain fatty acids to bring about health-beneficial effects.[7] Reports available on the beneficial effects of DF show a link between molecular structure and physiological effects.[8]

The DF content of plants varies not only in accordance with the plant species but also between genotype and cultivar of the same species. Other factors such as agronomic cultivation conditions, environmental conditions prior to harvest, and storage conditions after harvest can influence the DF content in plants.[9] Thus, a systematic study on the nature of fiber components that make up the DF complex will be helpful in predicting its beneficial role, because its consumption is being increasingly advocated in a majority of pathological conditions. Polysaccharides isolated from a variety of sources such as plant cell walls, animals, and fungal cells possess marked immunological properties, including antitumor, antiviral, antioxidant, antimutagenic, and hematopoietic activities.[10]

Bananas are widely cultivated all over the world. Banana flower (BF) and pseudostem (PS), which are byproducts of banana cultivation, are good sources of DF. BF and PS are consumed as vegetables in many countries. In traditional forms of medicine such as Ayurveda, BF and PS are used for the management of various diseases including diabetes.[11] Fruits, leaves, roots, and stalks from banana plant have been used to treat fevers, burns, diarrhea, inflammation, pains, and snakebite in folkloric medicine.[12] Biologically active compounds, such as dopamine, noradrenaline, serotonin, isochronal-4-one derivative, and antihyperglycemic factors, have been identified in different parts of the banana plant.[13−15] In our previous study we showed that BF and PS, when fed at 5% level to diabetic rats, significantly reduce blood glucose levels and ameliorate the diabetic condition.[16] In the present work, we have evaluated the proximate composition and nature of DF components from both BF and PS. Although there are some reports on the nutritional composition of BF and PS, a detailed study on different DF fractions is not reported.[17,18] Furthermore, because polysaccharides are associated with phenolic compounds, which act as a source of antioxidants, an attempt has also been made to evaluate the antioxidant activity of various fractions from both BF and PS.

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## MATERIALS AND METHODS

**Materials.** Banana inflorescence (banana flower, BF) and pseudostems (PS) of *Musa* sp. cv. elakki bale were purchased from a local market and identified by the Department of Horticulture, Government of Karnataka, Mysore, India. Flowers were separated from inflorescence. Both BF and PS were cleaned, cut into small pieces, and dried in an oven at 40 °C. This was powdered and stored at 4 °C until use.

**Extraction of Polysaccharides.** Extraction of polysaccharides was done as described by Swamy et al.[19] Dried flour of BF and PS (100 g) was defatted by extraction with petroleum ether and chloroform (1:1) for 4 h. Free sugars were extracted from the defatted residue with 70% ethyl alcohol. The insoluble residue was subjected to hot water extraction to gelatinize starch, followed by $\alpha$-amylase (EC 3.2.1.1) and glucoamylase (EC 3.2.1.3) digestion. The digestion at 60 °C was continued by adding fresh aliquots of enzyme, if necessary, until negative to the starch $I_2$ test. The digest was centrifuged, and the supernatant was dialyzed overnight with repeated changes of water and lyophilized to obtain water-soluble polysaccharides (WSP). The hot water insoluble residue was then extracted with 0.5% ammonium oxalate solution for 4 h (three times) to obtain pectic polysaccharides (PP). Hemicelluloses (HA and HB) were extracted from the residue by 10% NaOH under nitrogen atmosphere using a three-necked flask. The extract after centrifugation was adjusted to pH 4.5 with 50% acetic acid to get HA, and to the supernatant was added 3 volumes of alcohol to obtain HB. The insoluble residue was desalted by washing with water to get alkali-insoluble residue (AIR).

**Determination of Sugars by Gas−Liquid Chromatography (GLC).** To determine the sugar composition, polysaccharides (10−20 mg) were hydrolyzed with 10% sulfuric acid by placing in a boiling water bath for 6−8 h in air-cooled condenser-fitted tubes. The hydrolysates were neutralized with barium carbonate for 2−3 h, and the sugar composition was determined as alditol acetates[20] by GLC (Shimadzu CR4A chromatograph, Kyoto, Japan) on an OV 225 column (Shimadzu). The temperatures of the column, injector, and detector block were set at 200, 250, and 250 °C, respectively, and the flow rate of nitrogen was maintained at 40 mL/min.

**Antioxidant Assays.** Total phenolic content was estimated by using the Folin−Ciocalteu method with gallic acid as the reference standard.[21] Briefly, Folin−Ciocalteu reagent and 4 mL of sodium bicarbonate solution (100 g/L) were added and mixed. The absorbance was measured at 765 nm using a Shimadzu UV−visible spectrophotometer after incubation for 2 h at room temperature. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram of sample.

Total antioxidants were estimated by FRAP assay.[22] Extract (40 $\mu$L) or standard ascorbic acid was used for the assay. The incubation mixture consisted of 200 $\mu$L of double-distilled water, and 1.8 mL of FRAP reagent (2,4,6-tripyridyl-*s*-triazine (TPTZ)) in 40 mM HCl in 0.2 M acetate buffer containing 20 mM $FeCl_3$. The absorbance was read at 593 nm after 10 min of incubation at 37 °C. Free radical-scavenging activity was estimated according to the previously reported procedure using the DPPH radical.[23] In brief, an aliquot (10−100 $\mu$g) of polysaccharide fraction was mixed with 1 mL of freshly prepared DPPH in methanol (200 mM). The absorbance of the resulting solution was measured spectrophotometrically at 517 nm after 20 min of incubation in the dark. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\%\ \text{inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of test}} \times 100$$

The reducing power of different fractions of BF and PS was determined as detailed by Yen and Chen.[24] Briefly, polysaccharide fractions (10−50 mg/mL) were mixed with an equal volume (2.5 mL) of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide (2.5 mL) and incubated at 50 °C for 20 min. Trichloroacetic acid (10%, 2.5 mL) was added and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with $H_2O$ (2.5 mL) and 0.1% ferric chloride (0.5 mL), and the absorbance was recorded at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ferrous ion chelating ability of the fractions was determined according to the method of Dorman et al.[25] In brief, 1.25−5 mg of polysaccharide fraction was mixed with $FeCl_2$ (2 mM, 0.1 mL) and incubated for 5 min at room temperature followed by the addition of ferrozine (5 mM, 0.2 mL) and further incubated for 10 min. The absorbance was measured at 562 nm against blank.

**Determination of Bound Phenolics by HPLC.** Bound phenolics from HA fractions of banana flower and pseudostem were extracted according to the method of Suresh et al.[23] Sample (1 g) was extracted with 70% ethanol and hexane to remove free phenolics, sugars, and fat. The residue was extracted twice with 1 M NaOH containing sodium borohydride (0.5%) under an atmosphere of nitrogen. The clear supernatants obtained after centrifugation were pooled and acidified with 4 N HCl until the pH reached 1.5. Released phenolic acids in the fractions were identified by HPLC (Shimadzu LC-VP) using a C-18 column and a mobile phase consisting of water/methanol/acetic acid (85:13:2). The flow rate was maintained at 1 mL/min, and the eluates were monitored at 280 nm.

**Other Analyses.** Total sugar was estimated by using the phenol/sulfuric acid method.[19] To the sample solution was added 0.3 mL of 5% distilled phenol followed by 1.8 mL of concentrated sulfuric acid. The absorbance was read at 480 nm in a spectrophotometer after incubation at room temperature for 10 min. Uronic acid was estimated according to the carbazole method.[26] In brief, to the sample solution was added 3 mL of concentrated $H_2SO_4$ by keeping tubes in ice; the mixture was then boiled for 20 min. To this mixture was added 0.1 mL of 0.1% carbazole, and the mixture was kept in the dark for 2 h. Absorbance was read at 530 nm. Estimation of starch was done after hydrolysis with $\alpha$-amylase and glucoamylase, until negative to the starch-$I_2$ test, and released glucose was estimated by using a glucose oxidase/peroxidase (GOD/POD) kit.[27] The glucose value was multiplied by a factor of 0.9 to obtain starch content. The contents of ash, moisture, and total DF were determined by using AOAC[28] methods. Protein content was determined according to the Kjeldahl[29] method. Free sugars in 70% ethanol extract of BF and PS were identified and quantitated by HPLC using a C-18 column.

## RESULTS AND DISCUSSION

The present study deals with the carbohydrate composition of various DF components from BF and PS (*Musa* sp. var. elakki bale) and their antioxidant activities. BF and PS, which are byproducts of banana cultivation, were found to be rich in DF.

**Proximate Composition of BF and PS.** The proximate composition of BF and PS is given in Table 1. The percentages of protein, fat, and DF contents were higher in BF when compared to PS. DF, in particular, was higher in BF by >2-fold compared to PS. Most of the fiber was of insoluble nature in both BF and PS. Starch content, on the other hand, was negligible in BF, whereas PS showed 27% of starch (Table 1). The percentages of free sugars in BF and PS were less, being

**Table 1. Proximate Composition of Banana Flower and Pseudostem[a]**

| analysis | banana flower (%) | banana pseudostem (%) |
|---|---|---|
| protein | 12.5 ± 0.9 | 2.5 ± 0.0 |
| fat | 12.7 ± 1.0 | 1.7 ± 0.0 |
| free sugar | 1.2 ± 0.0 | 3.4 ± 0.1 |
| total dietary fiber | 65.6 ± 1.3 | 28.8 ± 0.9 |
| soluble dietary fiber | 7.3 ± 0.2 | 1.4 ± 0.0 |
| insoluble dietary fiber | 58.3 ± 1.0 | 27.4 ± 0.9 |
| starch | 1.0 ± 0.0 | 27.3 ± 1.1 |
| ash | 0.5 ± 0.0 | 0.3 ± 0.0 |
| moisture | 9.8 ± 1.0 | 15.1 ± 1.9 |

[a]Values are expressed as the mean ± SD ($n$ = 3).


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Table 2. Carbohydrate Composition (Percent) of Banana Flower and Its Isolated Fractions[a]

| fraction | yield | total sugar | uronic acid | Rha | Ara | Xyl | Gal | Glu |
|---|---|---|---|---|---|---|---|---|
| FR | (100) | 71.5 ± 1.5 | 22.7 ± 1.7 | | 22.7 ± 0.1 | 21.4 ± 0.2 | 9.5 ± 0.1 | 46.1 ± 0.5 |
| WSP | 6.5 ± 1.5 | 93.6 ± 1.8 | 20.2 ± 0.6 | | 29.5 ± 0.0 | | 70.3 ± 0.0 | |
| PP | 3.3 ± 0.5 | 58.4 ± 2.0 | 37.8 ± 3.2 | 10.5 ± 0.3 | 37.8 ± 0.1 | 23.4 ± 0.4 | 28.1 ± 0.1 | |
| HA | 3.8 ± 0.2 | 59.8 ± 0.6 | 14.6 ± 0.6 | | 9.3 ± 0.2 | 69.6 ± 0.8 | | 20.9 ± 0.5 |
| HB | 2.5 ± 0.7 | 73.7 ± 1.2 | 31.0 ± 1.2 | | 28.9 ± 0.3 | 24.6 ± 0.8 | 22.6 ± 0.2 | 23.5 ± 0.9 |
| AIR | 22.5 ± 3.1 | 91.5 ± 4.8 | 13.6 ± 0.7 | | 12.5 ± 0.1 | 13.0 ± 0.0 | | 74.1 ± 0.1 |

[a]Values are expressed as the mean ± SD ($n$ = 3). Abbreviations: FR, flour; WSP, water-soluble polysaccharide; PP, pectic polysaccharide; HA, hemicellulose A; HB, hemicellulose B; AIR, alkali-insoluble residue; Rha, rhamnose; Ara, arabinose; Xyl, xylose; Gal, galactose; Glu, glucose.

Table 3. Carbohydrate Composition (Percent) of Banana Pseudostem and Its Isolated Fractions[a]

| fraction | yield | total sugar | uronic acid | Rha | Ara | Xyl | Gal | Glu |
|---|---|---|---|---|---|---|---|---|
| FR | (100) | 87.8 ± 1.9 | 23.3 ± 0.2 | | 4.5 ± 1.9 | 8.3 ± 1.1 | | 87.0 ± 0.8 |
| WSP | 5.1 ± 1.9 | 98.4 ± 4.3 | 36.6 ± 1.0 | | 3.0 ± 0.0 | | | 96.6 ± 0.0 |
| PP | 2.7 ± 0.7 | 59.1 ± 2.1 | 34.0 ± 0.9 | 16.4 ± 0.3 | 32.8 ± 0.1 | 19.1 ± 1.1 | 16.7 ± 0.4 | 14.6 ± 0.8 |
| HA | 1.1 ± 0.0 | 68.7 ± 0.7 | 15.9 ± 0.1 | | 3.9 ± 0.2 | 51.3 ± 0.3 | | 44.5 ± 0.1 |
| HB | 1.5 ± 0.0 | 79.8 ± 1.7 | 35.7 ± 0.1 | | 34.1 ± 0.2 | 55.3 ± 0.5 | | 10.3 ± 0.7 |
| AIR | 9.9 ± 0.3 | 90.1 ± 0.7 | 30.8 ± 0.4 | | 11.2 ± 0.4 | 7.8 ± 0.1 | | 80.7 ± 0.4 |

[a]Values are expressed as the mean ± SD ($n$ = 3). Abbreviations as in Table 2.

1.24 ± 0.09 and 3.46 ± 0.14%, respectively (Table 1). The moisture content was higher in PS (15.1%) in comparison to BF (9.8%). Both BF and PS showed the presence of glucose, fructose, and sucrose as major free sugars. Additionally, maltose was observed in BF. Both BF and PS showed DF, which was rich in different types of complex polysaccharides with a wide diversity in sugar composition.

**Carbohydrate Composition of BF and PS and Their Isolated Fractions.** Various polysaccharide-rich fractions were isolated from BF and PS as detailed under Materials and Methods. The carbohydrate compositions of BF and PS and their isolated fractions are given in Tables 2 and 3, respectively. Both BF and PS yielded higher amounts of cellulosic fraction (AIR) followed by water-soluble polysaccharide (WSP). They also had substantial amounts of PP and HA and HB polysaccharides. All of the isolated polysaccharide fractions were rich in sugars. Acid hydrolysis of polysaccharides followed by gas chromatography showed the presence of arabinose, xylose, galactose, and glucose in BF. One distinguishing feature is that all of the fractions were rich in uronic acid content. WSP from BF had predominantly galactose (70.3%) followed by arabinose (29.5%). PP did not show the presence of glucose, but had rhamnose apart from arabinose, xylose, and galactose. On the other hand, HA had substantial amounts of xylose (69.6%), followed by glucose (20.9%) and arabinose (9.3%). HB had arabinose, xylose, galactose, and glucose in equal measure. AIR had glucose predominantly (74.1%) with significant amounts of arabinose and xylose.

The carbohydrate composition of PS and its fractions revealed that they were rich in sugars, namely, arabinose, xylose, and glucose (Table 3). The fractions also had substantial amounts of uronic acid. In PS, glucose was the predominant sugar followed by xylose and arabinose. WSP had predominately glucose, in the amount of 96.6%, with the rest being arabinose. PP had substantial amounts of rhamnose (16.4%) and galactose (14.6%), apart from arabinose (32.8%), xylose (19.1%), and glucose (14.6%). HA and HB also had arabinose, xylose, and glucose as major sugars with relative differences in their amounts.

Extraction of polysaccharides revealed the presence of arabinogalactan type of polysaccharide in WSP fractions of BF. PP from both BF and PS could be rhamnogalacturonan type with various side chains. Rhamnogalacturonan types of PP have been reported in a wide range of plants.[30] HA and HB could be of arabinoxylan or xyloglucan type of polysaccharides. The AIR showed the presence of glucose, indicating that it is rich in cellulosic polysaccharide. It, however, showed substantial amounts of arabinose and xylose. This could be due to their strong association with cellulosic fibrils through covalent linkages. This kind of strong association has been previously encountered in various cereal brans.[31]

**Polyphenols and Antioxidant Activity.** DF in recent years has been widely recognized as a carrier of antioxidants.[4] A study was therefore undertaken to determine the phenolic content of the polysaccharide fractions and their antioxidant activities. Total polyphenol content was higher in BF fractions in comparison to PS. Among BF fractions, HA showed the highest polyphenol content of 121.8 ± 1.9 $\mu$g/mg extract (Figure 1A) followed by HB. Total antioxidants, as measured by FRAP assay, were higher in BF fractions when compared to PS fractions (Figure 1B). The HA fraction of BF showed high amounts of antioxidants, which measured around 39.03 ± 0.118 $\mu$g/mg extract followed by HB. Various fractions from BF were further evaluated for their DPPH radical-scavenging activity, reducing power, and metal ion chelation. A dose-dependent effect was observed in all fractions of BF, among which HA showed the highest radical-scavenging activity and reducing power compared to other fractions (Figure 2A,B). However, metal-chelating ability was similar in BF flour, HA, and PP fractions and was found to be comparatively less in WSP and HB fractions (Figure 2C). Marked differences were observed with respect to total antioxidants and their antioxidant property in polysaccharide-rich fractions.

To determine the nature of bound phenolic acids, they were isolated from HA and HB fractions of BF and identified by HPLC (Table 4). The HA fraction of BF showed the presence of phenolic acids such as gallic acid, catechol, protocatechuic acid, gentisic acid, vanillic acid, caffeic acid, syringic acid, $p$-coumaric acid, and ferulic acid, whereas HB fraction showed gallic acid, catechol, protocatechuic acid, gentisic acid, vanillic acid, syringic acid, and epicatechin as major phenolic acids.

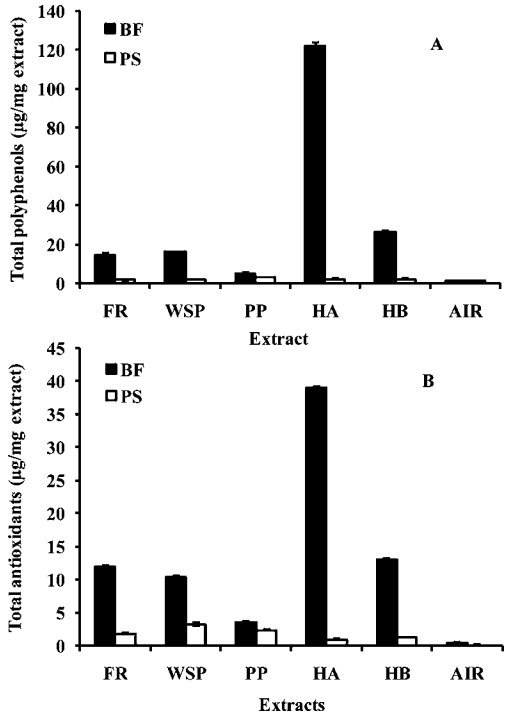


**Figure 1.** Total polyphenols (A) and total antioxidants (B) in different fractions of banana flower (BF) and pseudostem (PS). Values are expressed as the mean ± SD ($n$ = 3). Abbreviations are as in Table 2.

There were quantitative differences in phenolic acids in HA and HB. HA had higher proportions of gallic and ferulic acid, whereas, on the other hand, HB was rich in vanillic and syringic acid and epicatechin. Because the amount of phenolic acid was less in PS fractions, further identification was not done.

Cereal brans have been the main source of DF. With increasing awareness of the beneficial role of DF in relation to its amelioration of pathological conditions, newer sources are being exploited. Previously, our studies have revealed that BF and PS ameliorate the diabetic condition when fed to experimentally induced diabetic rats.[16] It is reported that consumption of resistant starch from banana (*Musa cavendish*), which forms part of the DF complex, lowered body weight and increased insulin sensitivity in obese type 2 diabetics.[32] However, there are very few studies with respect to DF components from BF and PS. In one such study the nutritional composition of BF from two cultivars was studied, which showed that BF was rich in nutritional factors.[17] PS has also been reported to be rich in fiber and polyphenols.[18] These results along with ours show that BF and PS, byproducts of banana cultivation, are nutritionally good.

Apart from the primary function of these polysaccharides as part of the DF complex involved in structural architecture, many other activities are attributed to them. For instance, xyloglucans from the seeds of *Copaifera langsdorffii*, *Hymenaea courbaril*, and *Tamarindus indica* showed enhancement of IL-1$\beta$ and TNF-$\alpha$ production, acting as biological response modifiers.[33] Studies indicate that pectins and xyloglucans have antimutagenic activities against nitroaromatic compounds.[34] Arabinoxylans isolated from wheat bran showed antitumor and immunomodulatory effects in mice.[35] The DF complexes of BF and PS have all of these polysaccharide fractions, which could have potential beneficial effects.

One of the important factors in the pathogenesis of a disease is oxidative stress, which occurs due to the generation of free

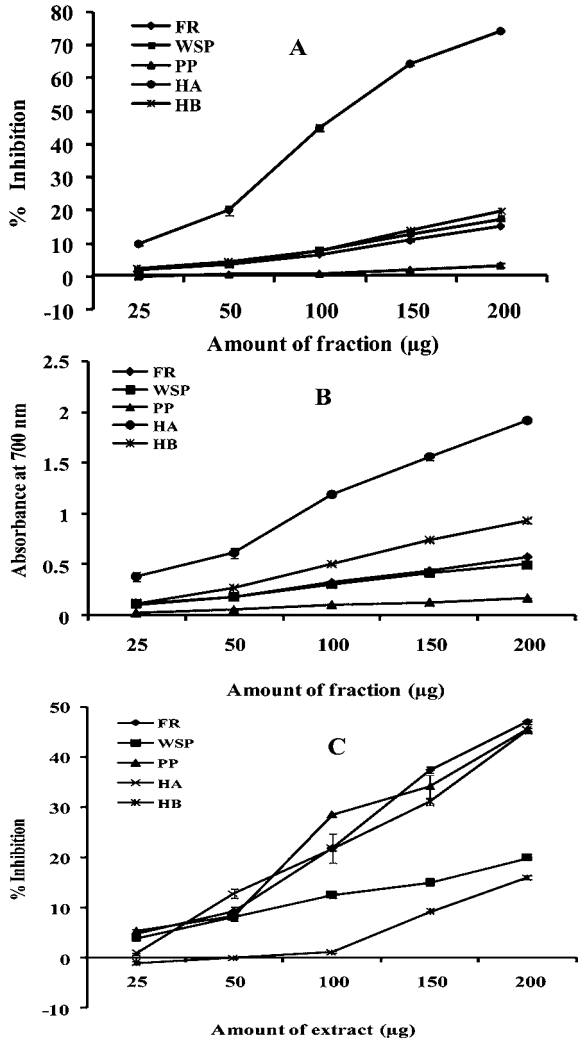


**Figure 2.** Scavenging activity (percent) on DPPH radicals (A), reducing power (B), and metal ion chelation (C) of different fractions of banana flower. Values are expressed as the mean ± SD ($n$ = 3). Abbreviations are as in Table 2.

**Table 4. Bound Phenolic Acids in BF-HA and BF-HB Extracts**[a]

| phenolic acid | BF-HA (μg/mg ext) | BF-HB (μg/mg ext) |
|---|---|---|
| gallic acid | 10.31 ± 0.23 | 0.55 ± 0.01 |
| catechol | 0.86 ± 0.02 | 1.42 ± 0.01 |
| protocatechuic acid | 2.39 ± 0.08 | 0.32 ± 0.03 |
| gentisic acid | 0.47 ± 0.04 | 0.23 ± 0.02 |
| vanillic acid | 1.06 ± 0.04 | 0.84 ± 0.01 |
| caffeic acid | 0.11 ± 0.01 | |
| syringic acid | 5.38 ± 0.02 | 4.88 ± 0.04 |
| epicatechin | 10.02 ± 0.21 | 2.51 ± 0.02 |
| *p*-coumaric acid | 1.44 ± 0.05 | 0.15 ± 0.00 |
| ferulic acid | 5.37 ± 0.04 | 0.36 ± 0.01 |

[a]Values are expressed as the mean ± SD ($n$ = 3). Abbreviations: BF-HA, banana flower hemicellulose A; BF-HB, banana flower hemicellulose B.

radicals.[36] Plants are rich sources of nutraceuticals, which act as potent antioxidants. Some of the fractions of BF, such as HA, are rich in total polyphenols and antioxidants, thereby displaying good antioxidant activity. Polyphenols obtained from


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plants are frequently associated with DF.[37] Additionally, other polysaccharide-rich fractions from BF such as PP along with HA displayed good metal ion (ferrous ion) chelating ability. Chelating agents that form $\sigma$ bonds with metals are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion.[38] PS, on the other hand, displayed comparatively less polyphenol content and thereby displayed less antioxidant activity.

In conclusion, BF and PS are rich sources of DF with associated polyphenols. As banana is a widely cultivated fruit all over the world, its byproducts can be commercially exploited as rich sources of DF. BF and PS can be utilized for formulating functional foods with potential health-beneficial effects.

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