Assessment of Nutritional Quality and Global Antioxidant Response of Banana (Musa sp. CV. Nanjangud Rasa Bale) Pseudostem and Flower

Ramith Ramu, Prithvi S. Shirahatti, K. R. Anilakumar1, Shivasharanappa Nayakavadi2, Farhan Zameer3, B. L. Dhananjaya4, M. N. Nagendra Prasad5

Department of Biotechnology, Sri Dharshathla Manjunatheshwara College of Post Graduate Centre, Dakshina Kannada,1 Food Quality and Assurance Department, Biochemistry and Nutrition Discipline, Defence Food Research Laboratory, Department of Studies in Biotechnology, Microbiology and Biochemistry, Mahajana Life Science Research Centre, Pojja Bhagavat Memorial Mahajana Post Graduate Centre, Department of Biotechnology, Sri Jayachamarajendra College of Engineering, Mysore,2 Toxinnology/Toxicology and Drug Discovery Unit, Centre For Emerging Technologies, Jain University, Bengaluru,3 Department of Veterinary Pathology, Animal Science Section, ICAR-Central Coastal Agricultural Research Institute, Ela, Goa, India

ABSTRACT

Background: The assessment of the nutritional composition and phytochemical screening of banana pseudostem (PB) and flower (FB) advocate this nonconventional food source for routine consumption, considering its various health benefits. Objectives: The aim is to assess the proximate nutrient composition, fatty acids, minerals, amino acid profile, and global antioxidant response (GAR) of PB and FB. Methods: Standard analytical procedures were used to determine the nutritional quality and GAR of PB and FB. Results: The chemical analysis illustrated that functional profile (water holding capacity, oil holding capacity, swelling power, and solubility), and proximate (ash, moisture, protein, fat, dietary fiber, and carbohydrate) contents were substantially high in FB than PB. With a well-proportionate amino acid profile, PB (0.56) and FB (0.54) comprised of a high ratio of essential to nonessential amino acids than those of FAO/WHO requirement (0.38). The mineral analysis revealed that PB and FB were rich in macro and micro minerals in the order K > Ca > Mg > P > Na and K > Mg > Na > Ca > P, respectively. Linoileic acid was found to be the major component in PB and FB. Besides, total antioxidant activity conducted for PB and FB by GAR method, measuring both bio-accessible and insoluble fractions, revealed that the soluble fraction fared better than the chemical extracts. Conclusion: The results revealed high nutritional qualities of the byproducts of banana and the low cost of its production promotes their use as a prospective nonconventional food resource with high nutraceutical value.

Key words: Amino acid, fatty acid, global antioxidant response, mineral element, proximate composition

SUMMARY

• AOAC: Association of Analytical Communities
• FAO/WHO: Food and Agriculture Organization of the United Nations/World Health Organization

Abbreviations Used: Banana flower was more potent than banana pseudostem in terms of its nutritional quality and total antioxidant capacity affirming their usefullness (of both the secondary products) in the pharmaceutical sector as a nutritional supplement due to the health-related properties of dietary fibre and associated bioactive compounds.

INTRODUCTION

The population explosion has exemplified a substantial rise in the demand for food resources which has shifted the attention of the global food market to nonconventional food resources.[1] Several by-products of cultivation, once discarded as wastes, are studied for their nutritive values to advocate their use as routine food sources meeting the expanding demands of the industry. Such by-products have proved to be economical and hence are well-accepted in the global market considering the present scenario.[2] India contributes a major portion of the total banana production in the world, whereas it is a conventional form of food and commercial food as well. Banana cultivation comprises of the secondary products, banana pseudostem (PB), and banana flower (FB) which have been discarded as wastes, fed to cattle or used for composting.

More recently, some studies have reported its use in the production of alcohol, methane, food for livestock, or adsorbents for water purification. A massive quantity (about 40%) of the total fresh weight of banana plant...
comprises of PB and FB, and hence, it can be useful as an alternative food resource.\[^{[3]}\] However, to accomplish the potential health benefits of these, a detailed study of its nutritional value needs to be carried out with special emphasis on the influence of a particular variety on its nutritional composition. In vitro and in vivo studies using the extracts have proven PB and FB as antihyperglycemic,\[^{[14]}\] antimicrobial, hypolipidemic, and anti-hypertensive agents thus upholding their health beneficial properties.\[^{[6]}\] The dietary fiber content, antioxidant compounds and several other macro- and micro-nutrients are responsible for these health benefits, and thus, the present study was designed to assess the composition of PB and FB in terms of its nutritional value.

Further, it is well-known that diseases either acute or chronic induce the generation of reactive oxygen species which are also the main factors responsible for tissue damage and aging.\[^{[20]}\] To ameliorate these damages, a mode of treatment which also has antioxidant properties has improved the condition and hence, this study also aims to evaluate the antioxidant potential of PB and FB. Several procedures are available to assess the antioxidant properties of food, and its potency depends upon either the method of assessment or the method of extraction of samples. In this regard, the total antioxidant capacity (TAC) can be determined after analyzing the mode of action likely to be either radical scavenging or metal ion chelating activity.\[^{[6]}\] In addition, the extraction procedure plays a significant role for its TAC since irrespective of the extraction procedure; some fraction of the extract always remains insoluble and not involved in the activity. More recently, a method developed by Vural et al.\[^{[9]}\] known as Quencher is being widely accepted for TAC evaluation since the method is carried out without extraction and thus the entire sample is tested in its solid state. The acceptance of this method is also affirmed because it resembles the condition similar to the physiological conditions where antioxidants are not extracted and administered directly, but instead, it needs to be released from the food source during digestion. Similar to the physiological conditions, after the enzymatic digestion, the extractable antioxidants play their role, and the nonextracted materials enter the intestine where they are acted on by the intestinal microflora and continue the digestion process. The extracted antioxidants are estimated by conventional methods, and the undigested material is evaluated for its antioxidant capacity using the Quencher method thus attaining a the global antioxidant response (GAR). This method provides a summation of the complete antioxidant potential of the food source the same way as it exists in vivo and hence, the method is widely accepted.\[^{[20]}\]

With this background, the objectives of this study are an evaluation of the nutritional composition and TAC of PB and FB to advocate this nonconventional food source for routine consumption, considering its various health benefits.

**MATERIALS AND METHODS**

**Samples**

Flawless inflorescences and pseudostems of *Musa* sp. cv. Nanjangud rasa bale were harvested from the banana cultivating farms of Nanjangud, Karnataka, India. The specimen was identified by the Department of Horticulture, Government of Karnataka, Mysores, India. Peeling the thick outer leaf-sheath of the tender pseudostems, the inner pith region was collected and flowers were separated from the inflorescences by discarding the spathe. For isolation, pseudostems (PB) and FBs were gutted, chopped and allowed to dry in an oven (40°C). This was pulverized, using a homogenizer and further stored at 4°C until use.

**Proximate analysis**

Moisture (method 44-15A), ash (method 08–01), crude fiber (method 32–10), fat (method 30–25), and carbohydrate content of PB and FB were determined according to the AACC method.\[^{[21]}\] Total carbohydrates were expressed as residual percent weight by the formula: [100-(moisture + ash + fat + fibre + protein)]. Crude protein (method 46–13) was estimated by the procedure described by Kjeldahl.\[^{[22]}\] The total dietary fiber content (method 991.43) in PB and FB was estimated by food-enzymatic-gravimetric method.\[^{[23]}\] The procedure described by Thomas et al.\[^{[24]}\] was used to determine neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, hemicellulose, and cellulose content in PB and FB. Hemicellulose and cellulose were estimated according to the formula: [Hemicellulose = NDF– ADF]; and [Cellulose = ADF– lignin], respectively. Water holding capacity (WHC), oil holding capacity (OHC), swelling power (g of swollen granules/g of dry weight of sample), and solubility (%) of PB and FB were performed as per the method described by Noor et al.\[^{[25]}\] WHC and OHC were expressed as grams of water or oil/grams of dry weight of samples, respectively. Starch and uronic acid content were determined by the method described by Jamuna et al.\[^{[15]}\] The phytochemical analysis and the vitamin content of PB and FB were determined.\[^{[17,19]}\] The sugar composition was performed according to AACC\[^{[26]}\] for PB and FB using high-performance liquid chromatography (HPLC) with differential refractive index detector (RID-10A, Shimadzu, Japan).

**Fatty acid profile by gas chromatography-mass spectrometry**

Before gas chromatography-mass spectrometry (GC-MS) analysis, derivatization was performed for PB and FB samples using BF, methanol as derivatizing reagent.\[^{[26]}\] Once the conversion of non-volatile fatty acids into volatile fatty acid methyl esters (FAMES) through methylation\[^{[28]}\] was achieved, the samples were subjected to GC (Clarus 500, Perkin Elmer, AOC-201 autosampler; Perkin Elmer, California, USA) interfaced with a mass spectrometer equipped with an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused to a capillary column (30 μm × 0.25 mm ID × 0.25 μm film thickness, DF). To achieve a good resolution of FAMES, chromatographic parameters were optimized as per Ramith et al.\[^{[3]}\] with slight modifications in the oven temperature. It was programmed at the rate of 10°C/min (no hold) up to 200°C, later at the rate of 5°C/min up to 280°C for a 9 min hold. In comparison to the acquired mass spectra of PB and FB with the standard mass spectra of NIST Library (NIST 05), the phytoconstituents present were recognized.

**Mineral analysis**

PB and FB were assessed for comprehensive mineral analysis (Li, B, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Cu, Ni, Cs, Zn, and Pb) using inductively coupled plasma atomic emission spectrometry (ICP-AES, Varian Vista MPX, USA) as per the official method 985.01.\[^{[29]}\] Ahead of subjecting to ICP-AES, the dry samples were ashed in a muffle furnace at 400°C–500°C and acid digested.\[^{[22,24]}\] On the other hand, the concentrations of Mo, Se, P, As, Cd, and Sb were determined using flame atomic absorption spectroscopy (AAS, Varian 240, USA) according to the method described by Vikas et al.\[^{[30]}\] Phosphoric acid and boric acid were measured according to the method described by Pearson\[^{[31]}\] and method 970.33.\[^{[32]}\] The elemental analysis of PB and FB was performed on a Perkin Elmer 2400 elemental analyzer.

**Amino acid composition**

Amino acid composition of PB and FB was analyzed according to the standard AOAC procedure (method 994.12).\[^{[28]}\] For hydrolysis, methods of Wong and Peter\[^{[33]}\] were employed. Before the analysis of the samples through automated amino acid analyzer (L 8900, Hitachi, Japan), filtration was performed using a 0.45 mm nylon membrane filter. Subsequently, following the prehydrolysis oxidation with performic
by the filtering of individual extracts from solvents. The three filtrates were then stored at −20°C until used for the analysis of total phenolic content and antioxidant activity. All the samples were analyzed in triplicates. The phenolic component separation of PB and FB extracts was performed on a reverse phase C18 (250 mm × 4.6 mm, Supelco) and the compounds were monitored by PDA (photodiode array) detector HPLC system (Agilent Technologies Inc., USA). Column temperature was maintained at 37°C and flow rate was set to 0.8 ml/min. The solvent system used was 0.1% formic acid (solvent A) and methanol (solvent B). The solvent gradient elution program was: 0–55 min 85% of A and 15% of B; 55–57 min 20% of A and 80% of B; 57–60 min 85% of A and 15% of B. A volume of 20 μl of the sample was injected (auto-injection) into the column and the phenolic acids were detected at 280 nm. The sample was quantified by comparing the retention time/peak areas with those of standards, namely, gallic acid, p-hydroxybenzoic acid, chlorogenic acid, sinapic acid, caffeic acid, vanillin, p-coumaric acid, quercetin, catechin, and epicatechin. The Quencher procedure described by Vural et al. was employed to determine antioxidant activity of the solid sample. In addition, enzymatic dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), and catalase (CAT) activities were determined by following the method of Moaed et al. and Manoj et al.

**Statistical analysis**

All data were expressed as mean ± standard deviation (n = 3). Results were determined using one-way analysis of variance (ANOVA), followed by Duncan’s multiple range test using SPSS Software (version 21.0, Chicago, USA). The results were considered as statistically significant if the P < 0.05.
Table 1: Nutritional composition of banana pseudostem and banana flower

|                      | PB                  | FB                   |
|----------------------|---------------------|----------------------|
| Proximate analysis   |                     |                      |
| Moisture (%)         | 12.30±0.87          | 8.33±0.79            |
| Ash (%)              | 4.93±1.42           | 6.51±1.05            |
| Fat (%)              | 0.98±3.27           | 5.79±1.78            |
| Total carbohydrates (%) | 46.58±2.33    | 53.78±6.58           |
| Starch (%)           | 21.06±0.87          | 6.01±0.79            |
| Energy               | 64.40±1.25          | 63.20±0.86           |
| Uronic acid (%)      | 31.87±3.55          | 27.72±4.10           |
| Protein (%)          | 7.34±3.60           | 19.60±5.08           |
| Total dietary fiber (%) | 61.14±0.34     | 70.07±2.05           |
| Insoluble dietary fiber (%) | 59.10±0.99   | 62.93±1.01           |
| Soluble dietary fiber (%) | 02.04±0.28     | 07.14±0.56           |
| Neutral detergent fiber (%) | 66.25±0.67    | 75.61±1.43           |
| Acid detergent fiber (%) | 51.88±2.35    | 58.78±3.03           |
| Cellulose (%)        | 44.02±0.91          | 47.30±0.87           |
| Hemicellulose (%)    | 24.37±1.57          | 16.83±1.13           |
| Lignin (%)           | 07.86±0.56          | 11.48±0.37           |
| Water holding capacity | 15.40±3.90    | 23.95±2.72           |
| Oil holding capacity  | 04.75±4.33          | 08.00±2.50           |
| Solubility           | 12.52±2.07          | 13.08±1.98           |
| Swelling power       | 12.58±1.67          | 16.02±1.24           |

Phytochemical constituents (mg/100 g)

|                      | PB                  | FB                   |
|----------------------|---------------------|----------------------|
| Phenols              | 188.64±0.88         | 201.12±1.05          |
| Flavonoids           | 78.60±1.18          | 83.49±0.61           |
| Alkaloids            | 62.32±0.39          | 71.09±0.48           |
| Tannins              | 07.86±0.21          | 86.87±2.43           |
| Saponins             | 305.45±0.60         | 387.51±1.79          |
| Oxalates             | 25.56±0.51          | 20.54±2.08           |
| Phytates             | 34.56±3.85          | 28.78±2.72           |

Vitamin (mg/100 g)

|                      | PB                  | FB                   |
|----------------------|---------------------|----------------------|
| Ascorbic acid        | 8.81±0.20           | 9.50±0.05            |
| Riboflavin           | 0.08±0.18           | 0.13±0.07            |
| Niacin               | 0.73±0.19           | 0.90±0.27            |
| Thiamine             | 0.15±0.06           | 0.18±0.04            |
| β-Carotene           | 0.08±0.24           | 0.12±0.16            |
| Vitamin E            | 0.12±0.04           | 0.17±0.12            |
| Pyridoxine           | 0.33±0.16           | 0.28±0.04            |
| Pantothenic acid     | 0.34±0.08           | 0.26±0.02            |

Sugar composition (mg/kg)

|                      | PB                  | FB                   |
|----------------------|---------------------|----------------------|
| Fructose             | 0.01±0.44           | 4.43±0.08            |
| Glucose              | 0.01±0.43           | 5.33±1.14            |
| Sucrose              | 10.27±0.02          | 7.74±0.06            |
| Malto               | 0.02±0.08           | 9.38±0.30            |
| Xylose               | 0.04±0.12           | 0.02±0.18            |
| Arabinose            | 10.60±0.20          | 1.53±0.10            |
| Rhamnose             | 0.62±0.25           | 0.05±0.08            |

Values are expressed as mean±SD (n=3). Means in the same row with distinct superscripts are significantly different (P<0.05) as separated by Duncan multiple range test. PB: Banana pseudostem; FB: Banana flower; SD: Standard deviation

RESULTS AND DISCUSSION

Dietary fiber composition

The proximate composition of PB and FB are defined in Table 1 which suggests a high total dietary fiber content. A diet comprising of high dietary fiber is efficient in generating early satiety signal by increasing the food retention time in the stomach and also reduces risk towards the development of gastric ulcers. Of the total dietary fibers, while both were higher than Musa sapientum and Musa acuminate x balbisiana Colla cv. Awak pseudostem tender core flour (ADF: 32.02; NDF: 43.89) reported by Thomas et al.[16] The present study shows that cellulose was the most abundant component, followed by hemicellulose and then lignin in both PB and FB. These components are considered insoluble and thus are not digested. Overall, the relative amounts of hemicellulose and cellulose in the study were higher than those published by Samrat et al.[15] (14.98% hemicellulose and 31.27% cellulose) and Thomas et al.[16] (18% hemicellulose and 42% cellulose) PF fibers of Musa sapientum and Musa acuminate x balbisiana Colla cv. Awak, respectively. Cellulose constitutes the major component or primary and secondary cell walls, thus explaining their presence at high levels. However, the cellulose content of PB and FB reported in this study is less than the cellulose content in the outer bark material of pseudostems of M. acuminate Colla (40.2%) and pseudostem tender core flour as reported by Cordeiro et al.[18] and Thomas et al.[16] respectively. On the contrary, the lignin content of both PB and FB was higher than banana pulp (6.0%), wheat (0.88%), and soy meal (0.58%), as reported by Jrgen[27] and are considerably lower with other wood-based materials such as sawdust (20.33%), Musa sapientum species (15.07%) and plantain (green banana) (14.3%).

Functional properties

The functional properties such as WHC, OHC, solubility, and swelling capacity (SWC) of banana by-products were measured and are presented in Table 1. From the physiological standpoint, the ability of any material to retain water when subjected to an external centrifugal gravity force or compression is its WHC. The study suggests that FB exhibited highest WHC (23.9 g water/g dry weight of samples) compared to PB (15.4 g water/g of dry weight of samples). Meanwhile, PB and FB exhibited greater WHC than those of cereals which showed <5.5 g water/g, such as rice bran (5.21 g water/g) and durum wheat (1.5–2.1 g water/g). This minute disparity may be due to the structural differences in cell wall components between the stem and flower fibers. Subsequently, the SWC of PB and FB were assessed which is directly attributed to the amount of cellulose in the dietary fiber. The extent of water retained in the swollen granules of PB (16.02 g of swollen granules/g of dry weight of sample) was significantly (P<0.05) greater than PB (12.58 g of swollen granules/g of dry weight of sample) with no statistically significant (P>0.05) differences in solubility between them, suggesting them to be more potent than some of the exotic fruits such as pineapple and mango concentrates (7.2, 6.6 and 4.60 ml water/g sample, respectively). Furthermore, the OHC of PB and FB were assessed which is attributed to the chemical and physical
structure of the plant polysaccharides. With an OHC value of 4.75 of oil/g of dry weight in PB and 8.0 g of oil/g of dry weight of sample in FB, they fared better than dietary fibres obtained from commercial preparations (1.29 g of oil/g of dry matter) and other fibrous residues, such as coconut fibre (5.3 g oil/g fibre or banana fibre-rich powder (2.2 g oil/g fibre). With these results, PB and FB can be advocated for use in stabilizing emulsions and as a dietary fiber reservoir.[14,36]

Sugar
Sugar is the main source of bio-available energy, and hence, it is important to assess the sugar content as well as the type of sugars present in the food. The digestible sugar content in PB was 21.57 mg/kg, and FB was 28.48 mg/kg samples [Table 1] and was not as high as banana fruit and other tropical fruits.[18] Further, the sugar profile of PB revealed the presence of sucrose and arabinose, which contributed 47.6% and 49.1% to total sugars, respectively while FB revealed the presence of several types of sugars, namely, maltose, sucrose, glucose, fructose, and arabinose. Despite the quick metabolism of these sugars, their presence at low levels prevents the use of PB and FB as an alternative energy resource. While the present study revealed the absence of galactose and rhamnose, a previous GC-MS study of polysaccharide fractions of Musa sp. cv. elakkil bale suggested their presence[40] which might be due to the difference in the banana cultivars used in the study. In addition, the level of sucrose was higher than glucose in both PB and FB. Other studies with banana peel exhibited high fructose while banana pulp showed the presence of glucose, fructose, and sucrose with lower sucrose levels as compared to other sugars.[39]

Phytochemicals
A class of alkaloids, flavonoids, tannins, saponins and more complex phenolic, phytoesterols, oxalates, and phytates are collectively known as phytochemicals which not only impart color to the fruits and vegetables but also possess several physiological functions, including antioxidant properties.[40] Table 1 elaborates on the phytochemicals and their amount in PB and FB which reveal that the most abundant phytochemical in this study are phenols and saponins. Furthermore, high tannin content (86.9 mg/100 g) in FB as compared to PB was witnessed. All these phytochemicals are proven to possess antimicrobial, antioxidant, and hormone modulatory activities. The study also revealed high amounts of flavonoids, which are well-known for their antioxidant properties. The higher flavonoids and saponins were present in PB and FB than in banana flowers of two cultivars[41] Baxijiao (saponins: 0.11 g/100 g and flavonoids: 5.90 mg/100 g) and Paradisiacal (saponins: 0.12 g/100 g and flavonoids: 5.27 mg/100 g) and considering these benefits, the potential of PB and FB for their health beneficiary properties is upheld.

Vitamins
Vitamins are the micronutrients required in minute amounts to the body, deficiency of which adversely affect the metabolism of the body. In the present study, Vitamin C (ascorbic acid) was present in the highest quantity with a mean content of 9.50 and 8.81 mg/100 g of FB and PB, respectively. While ascorbic acid is among the most important antioxidants involved in the prevention or minimization of the formation of carcinogenic substances from dietary material by preventing the oxidation of nitrate, its deficiency causes impaired functioning of the intracellular substances in the body including collagen, bone matrix, and tooth dentine.[40] In addition to ascorbic acid, riboflavin, niacin, thiamine, β-carotene, vitamin E, pyridoxine, and pantethenic acid were observed in PB and FB in quantities significant to create a nutritional impact by the food source [Table 1]. Vitamin C content in FB and PB was lower than banana fruit (10 mg/100 g) but higher than other tropical fruits, namely, blueberries (6 mg/100 g) pears (3 mg/100 g), and grapes (3 mg/100 g). Furthermore, the vitamin B complex was present in a significant amount which emphasizes these by-products for their potential in the treatment of various diseases including prostate cancer.[40]

Fatty acids
Fatty acid composition as given in Table 2 suggests that linoleic acid and palmitic acid were the major components in both the parts of the banana. FB contained 84.8% linoleic acid of its total fatty acid content, while PB contained 72.8%, which was followed by palmitic acid, which was high in PB (18.9%) compared to FB (14.8%). These results were similar to banana fruit peels of the Musa Genus: French Clair (FC), Grande Naine (GN), Big Ebanga (BE), pelipita (PPT), Yankambi Km5 (YKm5), and CRBP039039 had high proportions of unsaturated fatty acid, especially linoleic acid.[40] Linoleic acid is a precursor fatty acid for cell membrane components as well as other compounds involved in physiological responses and its presence in this study proves beneficial. Further, some of the less common fatty acids in PB were stearic acid and arachidic acid and FB was eicosenoic acid. Further, the polyunsaturated fatty acid levels were greater in this study as against Musa spp. Baxijiao and Paradisiacial flowers.[41] Such variations may be attributed to the stage of ripening at harvest, changes in the climate, soil conditions, and genetic variations between the sources. The online Dr. Duke's phytochemical and ethnobotanical database-assisted in ascertaining the biological activity of the compounds and the same are tabulated in Table 2.

Minerals
Based on the amount required for the human body, minerals are classified as macro- and micro-elements. The minerals present in PB and FB are given in Table 3 which suggests the presence of Na, K, Ca, Mg, and P with K being the major mineral in both PB and FB. However, minerals in FB were about 2–5-fold higher than the levels in PB. The levels of minerals in FB were in the order K > Mg > Na > Ca > P while that of PB was in the order K > Ca > Mg > P > Na. While Na and K are involved in the ion pumps in several metabolic pathways, Mg regulates over 300 metabolic reactions by acting as cofactors to several enzymes, P is involved in almost every chemical reaction taking place in the body in the form of ATP and Ca along with P forms Ca₃(PO₄)₂ and are essential for bone and teeth formation.[41] Overall, the levels of these minerals were in agreement with that of Musa spp. Baxijiao and Paradisiacial flower variety[41] but slightly lower than the limiting contents found in banana peels and pulps determined by Shaida et al.[43] In summary, the peel had a higher content of minerals than the pulp and the potassium content was lower to banana fruits and other tropical fruits such as pears, blueberries, and grapes.[18]

Along with these macro minerals, the micro minerals in PB and FB were also evaluated which showed the presence of Fe, Mn, Zn, Cu, Al, and several others are listed in Table 3. The levels of these elements in FB were found to be higher than PB and overall higher than those of other tropical fruits, including banana when compared with the data given by The Department of Health (2013).[43] Similar to the various macro-elements, microelements also have several vital biological functions. Zn is involved in various reactions of the body to construct and maintain DNA, required for the growth and repair of body tissues and iron along with manganese, copper, and zinc are constituents of various important proteins and enzymes involved in macro-nutrient metabolism and body function.[42] Considering the several vital functions of the macro- and micro-elements, their high contents in FB and PB could contribute to explain their use in folk medicine.

Further, a thorough analysis to evaluate the elements present in the banana byproducts was performed, and the results are tabulated in Table 3. Carbon was present in the highest amounts in FB (47.1%)
Table 2: Fatty acid profile of banana pseudostem and banana flower by gas chromatography-mass spectrometry

| Compounds detected                        | Trivial name of fatty acid | Total percentage composition | Activitya |
|------------------------------------------|---------------------------|------------------------------|-----------|
| Hexadecanoic acid, methyl ester          | Palmitic acid             | 18.93                        | Lubricant, 5 alpha reductase inhibitor, antiandrogenic and antioxidants |
| 9,12-octadecadienoic acid (Z, Z), methyl ester | Linoleic acid             | 72.85                        | Antiarthritic, anti-inflammatory, hepatoprotective, hypocholesterol and 5 alpha reductase inhibitor |
| Octadecanoic acid, methyl ester          | Stearic acid              | 6.80                         | Cosmetics, lubricant, flavor, hypocholesterol and 5 alpha reductase inhibitor |
| 9-octadecanoic acid (Z)-, methyl ester   | Oleic acid                | 0.47                         | Cancer-preventive, flavor, hypocholesterol and anti-inflammatory ** |
| Eicosanoic acid, methyl ester            | Arachidic acid            | 0.94                         | HB        |
| Hexadecanoic acid, methyl ester          | Palmitic acid             | 14.89                        | Lubricant, 5 alpha reductase inhibitor, antiandrogenic and antioxidants |
| 9,12-octadecadienoic acid (Z, Z), methyl ester | Linoleic acid             | 84.84                        | Antiarthritic, anti-inflammatory, hepatoprotective, hypocholesterol and 5 alpha reductase inhibitor |
| 11-eicosenoic acid, methyl ester         | Eicosanoic acid           | 0.27                         | E         |

Compounds were identified by referring to NIST05 library; aActivities were acknowledged by Dr Duke’s phytochemical and ethnobotanical databases; **Activity not reported; aSaturated fatty acid; yPolyunsaturated omega-6 fatty acid; xMonounsaturated omega-9 fatty acid; PB: Banana pseudostem; FB: Banana flower

Table 3: Mineral composition of banana pseudostem and banana flower

|                      | PB            | FB             |
|----------------------|---------------|----------------|
| **Macroelements (mg/g)**                      |               |                |
| Sodium (Na)          | 0.02±0.02a    | 18.34±0.12b    |
| Potassium (K)        | 10.63±0.10b   | 51.29±0.04c    |
| Calcium (Ca)         | 4.01±0.07b    | 10.65±0.05b    |
| Magnesium (Mg)       | 1.55±0.18a    | 23.55±0.21b    |
| Phosphorus (P)       | 2.09±0.04a    | 4.10±0.16b     |
| **Microelements (ppm)**                   |               |                |
| Iron (Fe)            | 30.65±0.16b   | 405.50±0.04b   |
| Lithium (Li)         | 0.01±0.01a    | 0.03±0.01a     |
| Boron (B)            | 39.88±0.04b   | 34.53±0.01b    |
| Aluminium (Al)       | 7.67±0.01a    | 18.43±0.02b    |
| Chromium (Cr)        | 5.04±0.04a    | 9.33±0.02a     |
| Manganese (Mn)       | 2.78±0.09a    | 133.80±0.06b   |
| Copper (Cu)          | 0.02±0.01a    | 0.52±0.03b     |
| Nickel (Ni)          | 0.46±0.04a    | 0.99±0.02b     |
| Cobalt (Co)          | 3.79±0.01a    | 19.44±0.04b    |
| Zinc (Zn)            | 16.60±0.01a   | 207.90±0.10b   |
| Lead (Pb)            | 0.15±0.01a    | 0.42±0.02b     |
| Molybdenum (Mo)      | 0.028±0.01a   | 0.042±0.01b    |
| Antimony (Sb)        | <0.01         | <0.01          |
| Cadmium (Cd)         | 1.06±0.01a    | 1.50±0.01b     |
| Arsenic (As)         | 0.015±0.01a   | 0.016±0.01a    |
| Selenium (Se)        | 0.010±0.01a   | 0.010±0.01a    |
| Phosphoric acid (mg/g) | 6.72±0.07b   | 13.12±0.03b    |
| Boric acid           | 66.66±0.02a   | 150.0±0.01b    |
| **Elemental analysis (%)** |             |                |
| C                    | 35.53±0.07a   | 47.19±0.02b    |
| H                    | 6.01±0.02c    | 3.21±0.02a     |
| N                    | 1.35±0.01a    | 1.96±0.03b     |
| S                    | 0.07±0.01a    | 0.17±0.01a     |
| O                    | 52.78±0.06b   | 43.08±0.04a    |

Values are expressed as mean±SD (n=3). Means in the same row with distinct superscripts are significantly different (P<0.05) as separated by Duncan multiple range test. SD: Standard deviation; PB: Banana pseudo stem; FB: Banana flower

compared to PB (35.5%) which was contrary to the hydrogen content which was higher in PB (6.01 ± 0.02) as against FB (3.21 ± 0.02). The present findings were on par with the composition of principal elements of banana (Musa acuminate) pseudostem by Ketty et al.,[44] i. e., (carbon: 36.83%, hydrogen: 5.19%, and nitrogen: 0.93%). The composition of hydrogen was higher in PB than FB and this is due to the high moisture composition of that compared to the FB [Table 1]. The moisture content of the present study was higher in case of PB (13.3%) over FB (8.33%) suggesting the difference in the hydrogen content of both the byproducts. Overall, the moisture content of both PB and FB were lower than commercial wheat flour, which had a value of 12.36%[44] and PB and FB of elakki bale cultivar as reported by Jamuna et al.[44]

In support to the above findings, the ash content that is directly proportional to the mineral content was also estimated which suggested the presence of it at high levels. It is clear from our studies that the highest levels of ash content were recorded for Musa sp. cv. Nanjangud rasa bale (PB and FB of 4.9 and 6.5%, respectively), and was comparatively higher than those Musa sp. cv. elakki bale (0.3 and 0.5%, respectively),[16] banana fruit of 1.1%.[18] Whereas it was comparable with banana (Musa acuminate x balbisiana Colla cv. Awak) pseudostem flour (3.03%)[15] and lower than banana peel and pulps (6.4%–12.8%).[19]

Amino acids

The quality of EAAs suggests the nutritional value of dietary proteins, and hence the amino acid content in PB and FB were tested. An overall picture of the amino acid content present in PB and FB is given in Table 4 suggest that all the EAAs according to the FAO classification[21] are present in them with FB having a major amino acid content compared to PB. A high glutamic acid content (63.8 and 152.9 mg/g of protein) followed by aspartic acid, leucine, alanine, proline, arginine, cysteine, serine, and lysine was witnessed in both PB and FB, respectively. The importance of glutamine is learnt during critical illness where it acts as a prime carrier of ammonia to the splanchic area and the immune system. In addition while the sulfur-containing amino acids were above the FAO/WHO,[41] requirement (score ranged from 99 to 240), the other EAAs met FAO/WHO,[41] requirement pattern. Further, the concentration of the amino acids that are lower than the FAO standard protein value is considered as limiting concentration, and in this context, in the present study, lysine was at the limiting concentration and the same has been reported by Thomas et al.[39]

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in fruit peels of the *Musa* Genus: FC, GN, BE, PPT, YKm5, and 039, obtained at three different stages of ripeness, namely, stage 1 (Green), stage 5 (More yellow than green), stage 7 (yellow/a few brown spots). The ratio of essential to non-EAs for PB and FB were 0.56 and 0.54, respectively, which was substantially higher than their requirement in adults (0.38) as recommended by the WHO. In addition, the protein values of PB (7.3%) and FB (19.3%) in the present study were marginally higher than the values reported for *Musa* spp. with the application of the QUENCHER procedure, an *in vitro* gastrointestinal digestion, and the combination of the latter with the application of the QUENCHER procedure to the insoluble fraction [termed henceforth the GAR method] are the methods of extraction employed in the present study. The results provide promising evidence for the need for employing such methods of antioxidant estimation since the chemical extraction method (solvents and aqueous) gave lower results, ranging from 2 to 2.5 times lower in comparison with the Quencher and GAR methods. They are in agreement with the previous reports for 27 fresh and cooked foods, estimated by Pastoriza et al. On the other hand, with regard to the chemical extraction method, both PB and FB extracted with the solvent ethanol fared better than methanol and aqueous counterparts. They were also higher than the Quencher and GAR methods. They are in agreement with the previous reports for 27 fresh and cooked foods, estimated by Pastoriza et al.

### Antioxidants

Antioxidant adjuncts have proven beneficiary in many diseases where they play a protective role in the prevention of ROS mediated damage to the cells and tissues. Hence, in the present study, we have evaluated the antioxidant potential of PB and FB and the results thus obtained are tabulated in Table 5a. Studies have suggested both PB and FB of banana as potent antioxidants extractable with aqueous and organic solvents. Most antioxidant studies involve its evaluation using the reductive antioxidant power of the antioxidant compounds were carried out to assess *in vitro* antioxidant activity of PB and FB. As mentioned previously, along with the method of evaluation, another factor contributing to the antioxidant potential of the samples is the method of extraction and hence, a conventional solvent extraction (with different solvents), a direct measure using the QUENCHER procedure, gave lower results, ranging from 2 to 2.5 times lower in comparison with the Quencher and GAR methods. They are in agreement with the previous reports for 27 fresh and cooked foods, estimated by Pastoriza et al.

Further, to acquire a detailed phenolic composition of the extracts, HPLC analysis was performed and the results are detailed in Table 5c suggesting the presence of diverse phenolic acids, namely, gallic acid, p-hydroxybenzoic acid, chlorogenic acid, sinapic acid, caffeic acid, and green banana Cavendish (AAA) flour (4.1%). Proteins being the source for the supplementation of amino acids, it can thus be suggested that PB and FB are potential sources of EAAs.

#### Table 4: Amino acid profile of banana pseudostem and banana flower

| Amino acids          | Content (mg/g protein) | Reference (mg/g protein) | Score (%) |
|----------------------|------------------------|--------------------------|-----------|
|                      | PB                     | FB                       |           |
| Leucine              | 27.4\(^{a}\)           | 63.2\(^{b}\)             | 66\(^{c}\) | 41 | 96 |
| Phenylalanine + tyrosine | 20.2\(^{a}\)       | 55.6\(^{b}\)             | 63\(^{c}\) | 32 | 88 |
| Lysine               | 12.5\(^{a}\)           | 40.8\(^{b}\)             | 58\(^{c}\) | 22 | 69 |
| Valine               | 10.4\(^{a}\)           | 36.6\(^{b}\)             | 35\(^{b}\) | 30 | 105 |
| Threonine            | 9.4\(^{a}\)            | 33.0\(^{b}\)             | 34\(^{b}\) | 28 | 97 |
| Isoleucine           | 11.1\(^{a}\)           | 28.0\(^{b}\)             | 28\(^{b}\) | 40 | 100 |
| Methionine + cysteine | 24.8\(^{a}\)          | 60.0\(^{b}\)             | 25\(^{b}\) 99 | 240 |
| Tryptophan           | 3.7\(^{a}\)            | 12.5\(^{b}\)             | 11\(^{b}\) | 34 | 114 |
| Valine               | 10.37±0.06\(^{a}\)     | 36.64±0.02\(^{b}\)       |           |   |
| Lysine               | 12.46±0.05\(^{a}\)     | 39.95±0.07\(^{b}\)       |           |   |
| Leucine              | 27.19±0.02\(^{a}\)     | 63.19±0.08\(^{b}\)       |           |   |
| Isoleucine           | 11.05±0.01\(^{a}\)     | 28.02±0.02\(^{b}\)       |           |   |
| Phenylalanine        | 13.52±0.02\(^{a}\)     | 31.03±0.01\(^{b}\)       |           |   |
| Threonine            | 9.43±0.01\(^{a}\)      | 33.01±0.03\(^{b}\)       |           |   |
| Histidine            | 7.51±0.02\(^{a}\)      | 16.60±0.02\(^{b}\)       |           |   |
| Methionine           | 8.14±0.04\(^{a}\)      | 18.11±0.02\(^{b}\)       |           |   |
| Tryptophan           | 3.70±0.03\(^{a}\)      | 12.48±0.05\(^{b}\)       |           |   |
| Arginine             | 14.25±0.04\(^{a}\)     | 41.98±0.02\(^{b}\)       |           |   |
| Proline              | 19.82±0.02\(^{a}\)     | 50.28±0.01\(^{b}\)       |           |   |
| Aspartic acid        | 24.19±0.03\(^{a}\)     | 78.87±0.03\(^{b}\)       |           |   |
| Glutamic acid        | 63.75±0.05\(^{a}\)     | 152.92±0.08\(^{b}\)      |           |   |
| Serine               | 10.50±0.03\(^{a}\)     | 40.65±0.02\(^{b}\)       |           |   |
| Glycine              | 14.64±0.06\(^{a}\)     | 30.58±0.04\(^{b}\)       |           |   |
| Alanine              | 14.05±0.02\(^{a}\)     | 51.25±0.07\(^{b}\)       |           |   |
| Cysteine             | 16.63±0.04\(^{a}\)     | 41.89±0.04\(^{b}\)       |           |   |
| Tyrosine             | 6.65±0.02\(^{a}\)      | 24.56±0.02\(^{b}\)       |           |   |
| Total essential amino acids | 103.37±0.01\(^{a}\) 279.03±0.01\(^{b}\) |           |   |
| Total nonessential amino acids | 184.28±0.02\(^{a}\) 512.98±0.03\(^{b}\) |           |   |
| Ratio (essential/nonessential) | 0.56±0.01\(^{a}\) 0.54±0.02\(^{b}\) | 0.38\(^{b}\) |   |

Values are expressed as mean±SD (n=3). Means in the same row with distinct superscripts are significantly different (P<0.05) as separated by Duncan multiple range test. *Amino acid pattern of preschool children (2–5 years) (FAO/WHO/UNU, 1985). SD: Standard deviation; PB: Banana pseudostem; FB: Banana flower.
vanillin, p-coumaric acid, quercetin, catechin, and epicatechin at different concentrations. Ethanol extracts in both PB and FB were found to contain high concentrations of phenolic acids in comparison to methanol and aqueous extract. p-hydroxybenzoic acid was the most predominant phenolic acid recorded in PB and FB (62.7 µg/mg and 95 µg/mg), followed by gallic acid (31.1 and 61.3 µg/mg, respectively) with varying concentrations. Caffeic acid was predominant in the methanol extract of PB (23.3 µg/mg), whereas gallic acid was predominant in that of FB (73.4 µg/mg). Although methanol and aqueous extract had phenolic acids, the concentration was lesser than the ethanol extract [Table 5c]. However, under physiological conditions, these results cannot be reproduced by administering the extracted antioxidants directly. Irrespective of the extraction method, some amount of the sample always remains insoluble in one or the other solvent and hence Arda et al.[46] developed a direct procedure (QUENCHER) to evaluate the TAC of foods without an extraction step. Since, this method cannot differentiate between the physiologically active fraction and the insoluble one, a combination of enzymatic digestion step for the soluble fraction and the Quencher method for the insoluble fraction thus furnish an optimal antioxidant potential of the given sample.

The results of the antioxidant activity using Quencher method [Table 5a] for PB and FB (ABTS: 17.8 and 24; DPPH: 0.33 and 0.42; FRAP: 1.59 and 2.78 mmol equivalents of the standard Trolox per kg of sample, respectively) were in accordance with the results obtained by Arda et al.[46] for different cereal products. The order of magnitude was same as the GAR method for PB and FB samples despite a 2–3 times reduction in most parts of the results. Such a reduction could be attributed to the absence of the enzymatic digestion step which could otherwise result in different compounds obtained after the enzymatic reactions. Overall, the best results were obtained by the GAR method which exhibited highest antioxidant activity with FB faring better than PB. In particular, the insoluble fraction exhibited about 40%–50% of the total antioxidant potential.

Table 5: Enzymatic antioxidant potential and global antioxidant response of banana pseudostem and banana flower using different methods and distribution of antioxidant activity in soluble and insoluble fractions after in vitro digestion (a); yield, total phenolic content and antioxidant activity of banana pseudostem and banana flower sequential solvent extracts (b) and phenolic acids identification (c)

| (a) Methods | PB | FB |
|-------------|----|----|
| Enzymatic antioxidants* | | |
| Superoxide dismutase | 14.5±0.70* | 19.08±1.60* |
| Catalase | 3.68±0.54* | 7.86±1.01* |
| Ascorbate peroxidase | 0.32±0.44* | 0.49±1.89* |
| Glutathione reductase | 0.76±2.61* | 1.53±0.47* |
| Total | 54.07±0.25* | 70.15±0.55* |
| Soluble | 37.58±1.88* | 45.62±1.38* |
| Insoluble | 15.41±3.33* | 22.99±2.96* |
| Quencherx | 17.86±0.69* | 24.05±1.98* |
| GAR-ABTSx | | |
| Total | 0.78±0.60* | 1.37±2.09* |
| Soluble | 0.49±1.60* | 0.99±1.23* |
| Insoluble | 0.21±0.75* | 0.27±3.08* |
| Quencherx | 0.33±3.38* | 0.42±1.08* |
| GAR-DPPHx | | |
| Total | 3.47±1.65* | 6.53±1.34* |
| Soluble | 2.02±1.03* | 4.99±1.77* |
| Insoluble | 1.26±0.50* | 1.44±1.04* |
| Quencherx | 1.59±0.54* | 2.78±8.20* |
| GAR-FRAPx | | |
| Total | 101.84±0.54* | 4.29 |
| Soluble | 1.95 | 0.32 |
| Insoluble | 0.91 | 0.44 |

| (b) Extracts | Yield (g/kg) | TPC* | ABTS* | DPPH* | FRAP* |
|---------------|--------------|------|-------|-------|-------|
| PB Methanol | 25.4±0.46* | 98.98±0.58* | 11.98±1.87* | 0.22±0.17* | 1.23±1.01* |
| Ethanol | 91.20±0.48* | 211.43±1.98* | 21.87±0.40* | 0.43±0.34* | 3.33±0.33* |
| Water | 89.09±0.58* | 122.34±4.1* | 10.64±1.50* | 0.20±0.67* | 1.06±0.46* |
| FB Methanol | 61.37±0.55* | 121.59±0.58* | 18.80±1.31* | 0.25±1.24* | 1.58±1.79* |
| Ethanol | 126.87±1.74* | 228.87±2.05* | 24.03±1.00* | 0.73±0.27* | 4.83±0.51* |
| Water | 101.84±0.54* | 105.78±0.48* | 18.08±0.94* | 0.24±2.50* | 1.44±2.00* |

| (c) Phenolic acid | Methanol | Ethanol | Water | Methanol | Ethanol | Water |
|-------------------|----------|---------|------|----------|---------|------|
| Gallic acid | 5.82 | 31.13 | 15.88 | 73.44 | 61.20 | 73.76 |
| p-hydroxybenzoic acid | 11.48 | 62.68 | 32.78 | 61.65 | 94.97 | 19.43 |
| Chlorogenic acid | 6.09 | 11.87 | 8.61 | 14.08 | 13.76 | 14.42 |
| Sinapic acid | 14.91 | 37.06 | 3.19 | 2.02 | 3.22 | 9.81 |
| Caffeic acid | 25.33 | 19.11 | 4.06 | 1.59 | 1.07 | - |
| Vanillin | 14.80 | 7.17 | 2.69 | 1.95 | 7.62 | 3.38 |
| p-coumaric acid | 7.58 | 2.09 | 4.29 | 0.25 | 1.45 | - |
| Epicatechin | 3.07 | 0.72 | 5.37 | 0.52 | 0.75 | - |
| Catechin | 9.34 | 4.12 | 1.76 | 0.91 | 2.63 | - |
| Quercetin | 4.39 | 6.06 | 1.19 | 0.32 | 1.67 | - |

*Units/min/mg of protein; †Mmol equivalents of trolox/kg sample; ‡Direct procedure without extraction of PB and FB and expressed as in GAR (mmol equivalents of trolox/kg sample); §Mg equivalents of gallic acid/g. Values are expressed as mean±SD (n=3). Means in the same row with distinct superscripts are significantly different (P<0.05) as separated by Duncan multiple range test. SD: Standard deviation; PB: Banana pseudostem; FB: Banana flower; GAR: Global antioxidant response; TPC: Total phenolic content; ABTS: 2,2’-azino-bis, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric reducing antioxidant power.
activity and since this fraction is excluded during the extraction process, this is the most recommended method for the measurement of TAC. Although the antioxidant role of the insoluble fraction is questioned since they are not extractable, they are expected to exert their effect by the surface reaction phenomenon. Furthermore, some part of the insoluble fraction may undergo digestion by the intestinal microflora thus releasing some substances which can also exert antioxidant properties and considering these; it would be essential to measure the antioxidant capacity of even the insoluble fraction of the digested food.[40]

Further, with respect to the antioxidant assays, the different affinities of the radicals to scavenge various antioxidant groups present in different samples suggest the need to use more than a single assay to determine the antioxidant potential of a particular sample. In this regard, in the present study, the TAC as measured with two radical scavenging assays (ABTS and DPPH) fared differently for both the byproducts. In support of these results, Roger et al.[40] demonstrated that the macromolecules are seldom attacked by the hydrophobic radicals, which could be the reason for the lower activity in DPPH as compared to the ABTS assay wherein DPPH is a hydrophobic radical while ABTS is more of a hydrophilic probe. Furthermore, DPPH being more selective in the reaction with H-donors, it could also be the reason for its lower TAC values in this assay. Further, the FRAP activity which is based on the reduction of the Fe	extsuperscript{3+}-TPTZ complex in the ferrous form at low pH, exhibited 6.5 mmol Trolox Eq./Kg for FB and for PB with a statistically significant difference in the values (P > 0.05). The results, however, in comparison with ABTS were lower, but better than the DPPH assay.[40]

In addition, enzymatic (SOD, CAT, APX, and GR) antioxidant potential has been evaluated for the FB and PB. As evident from Table 5a, FB showed maximum activity of SOD (19.1 U/min/mg protein) followed by catalase (7.9 U/min/mg protein), GR (1.5 U/min/mg protein) and APX (0.49 U/min/mg protein). On the other hand, PB also exhibited enzymatic activities for SOD (14.6 U/min/mg protein) followed by catalase (3.7 U/min/mg protein), GR (0.76 U/min/mg protein), APX (0.32 U/min/mg protein) and found was to be lower in comparison to FB. Higher SOD, APX, and GR enzymatic antioxidant activities in FB and PB clearly indicates their greater ability to detoxify ROS such as superoxide, hydroxyl, and peroxide radicals formed in human cell by endogenous and exogenous factors which in turn could lead to geriatric degenerative conditions, cancer and a wide range of other human diseases.

**CONCLUSION**

In summary, the present study manifests that both PB and FB possess rich nutraceutical properties because of the presence of various bioactive ingredients with numerous benefits. It provides evidence that the two banana byproducts are rich in proximate nutrient composition, minerals, fatty acids, and antioxidants (both enzymatic and nonenzymatic) and hence could be used in the human diet. The beneficiary properties are mainly derived from their minerals, carbohydrates, dietary fibers and proteins together with the low content of fat and calories. Furthermore, as a rich source of phytochemicals, minerals and vitamins reside in PB and FB they can be further evaluated for use as a key ingredient for valuable drugs. To add to these, the high total dietary fiber content and a balanced ratio between insoluble dietary fiber and soluble dietary fiber in both PB and FB are attractive targets for the food industry. These could be used in the development of a nutritional supplement because of their health-related properties of dietary fiber and associated bioactive compounds.

In addition to the strong basis provided by the nutritional aspects of PB and FB, their potential as antioxidants are also confirmed by a series of studies which included different methods of extraction as well as different assays to determine their antioxidant potential. It is demonstrated that the GAR method exhibited antioxidant activity higher than that reported with traditional procedures, which asserts the role of both insoluble as well as soluble fractions of the digested food to possess antioxidant properties. To summarize on the whole, this paper reinforces the concept that PB and FB are potent sources of several biologically active ingredients and also possess rich antioxidant property.

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**Conflicts of interest**

There are no conflicts of interest.

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