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Nutrition and Physiology

Nutritional composition and bioactivity studies on edible soft stem of banana (*Musa spp.*)

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Abstract. *Banana tree (Musa spp.) has a false stem called a pseudostem which, is an edible soft-stem. A study was executed to compare the nutritional composition, antioxidative and anti-hyperglycemic properties of soft-stem of four local varieties namely, Alu-Kesel (AL), Ambul-Kesel (AM), Seeni-Kesel (SE), and Suwandel-Kesel (SU). Banana soft-stem of individual varieties were sequentially extracted with dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) as solvents. The extracts were evaluated for total phenolic content (TPC), total flavonoid content (TFC), anti-oxidative capacity, and anti-hyperglycemic effect using relevant assays. The main constituents of the banana soft-stem, regardless of variety, were carbohydrates (~66-77%), followed by ash content (~9-18%), protein content (~3-11%), and fat content (~1-2%). Palmitic acid (~22-29%), linoleic acid (~21-45%), and arachidic acid (~8-16%) were the dominant fatty acids. K (~27-55 g/kg) was the most abundant mineral, followed by Mg (~1.6-2 g/kg) and Ca (~1.4-2 g/kg). Among the solvent extracts, the EtOAc extract of SE had the highest TPC (20.06±1.97 GAE/g of crude extract) and the highest FRAP value (1.12±0.01 mM FeSO4/g of crude extract). EtOAc extract of AM had the highest TFC (17.54±2.25 CE/g of crude extract) and the highest DPPH (0.18±0.02 mM trolox/g of crude extract) and the highest ABTS (0.29±0.00 mM trolox/g of crude extract) radical scavenging activities. Among all extracts, MeOH of SU exhibited the strongest α-amylase inhibitory potential. Based on the findings, banana soft-stems of the local varieties could be utilized as a potential source for development of nutritionally rich and bioactive products.*

Keywords: Antioxidant activity, Banana soft-stem, Fatty acid profile, Mineral composition, Proximate analysis

Introduction

Sri Lanka is a tropical country with rich fertility and conducive climate to cultivate a wide array of crops. Among them, the banana can be identified as the most extensively grown fruit in Sri Lanka, ranking as the world's fourth most cultivated fruit (Arvanitoyannis and Mavromatis, 2009). This versatile crop can be cultivated throughout Sri Lanka including wet, intermediate, and dry zones. There are about 29 varieties of banana (*Musa spp.*) grown in Sri Lanka, including two wild types. Each of the individual varieties differ from one another in their appearance, morphology and agronomical

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traits. The peel color of various banana varieties displays a range of colors from reddish-purple and yellow to green. Additionally, these bananas show a diverse range of sizes, spanning from 3 to 40 cm. The taxonomy of the *Musa spp.* associated with two terms including banana and plantain. While the sweeter varieties known as bananas are consumed as a dessert, starchier varieties are usually called plantains which are consumed after cooking. According to a previous report by Ediriweera (2008), the common varieties of banana are Seeni, Ambul, Suwandel, Kolikuttu, Nethra Palam, Ambun, Bim Kesel, Nadi, Pulathisi, Puvalu, Anavalu, Kandula, Prasad, Sudu Kochchi, Rata Horadavalu, Wal Suwandel while the different varieties of plantains are Alukesel and Kithala.

The Banana crop is considered as a perennial herb with 4 to 5 years of life cycle. The central part of the tree is the rhizome, which is used to contain several side buds known as meristems. Based on previous scientific investigations carried out across the globe, each component part of the banana tree delivers various health benefits. As a matter of fact, the secondary metabolism of bananas tends to generate various bioactive compounds such as phenols, phytosterols, carotenoids, biogenic amines which are known for their diverse pharmacological effects (Afzal et al., 2022).

In the local scenario, utilization of banana crop is limited mere to consumption of the fruit and using its leaves as a wrap or leafy plate. After the harvest of banana bunch, farmers face a significant challenge in managing the waste or remnants of the tree. The cultivation of this crop has led to generating large volume of wastes including peduncle, pseudostems, leaves, banana peel, corm, etc. As the stem and other parts of the tree become susceptible to contamination by organisms like banana weevil, damages on subsequent banana cultivation is severe (Mohamed, 2017). Banana pseudostem is utilized in a limited scope for human consumption due to lack of knowledge on its nutritional and other biochemical properties. Banana trunks being a pseudostem often go to waste causing greenhouse gas emissions and air pollution when discarded or dumped in yards in various locations. Over the years, the pseudostem is utilized for applications that include extracting fiber, using it as a medium for fertilizing, creating handicrafts, etc. The soft-stem within the pseudostem is also one of the edible parts of the banana crop, containing a high amount of carbohydrates and dietary fiber (Bhatnagar et al., 2015; Subagyo and Chafidz, 2018). According to past investigations, it is low in calories and can be incorporated into various foods like flour, pastries, candies, fruit juice etc. (Palde et al., 2022). Utilization of the pseudostem to recover the soft-stem would be a productive way to either eliminate or control the spread of undesirable organisms. In this study, the aim is to conduct a comprehensive analysis of nutrition and bioactivities of four local varieties of edible banana soft stems, namely Ambul kesel, Alu kesel, Seeni kesel and Suwandel.

Material and methods

Materials

Plant materials

Edible banana soft-stems of four different varieties (AM:Ambul-kesel; SE:Seeni-kesel; SU:Suwandel-kesel; AL; Alu-kesel) were collected from the Bokkawala region of the Kandy district, Sri Lanka from 5th December 2023 to 18th December 2023. The plants were cross-checked by a certified botanist and confirmed these as pure varieties.

Chemicals and reagents

Dichloromethane (99.5%, AR grade), ethyl acetate (99.5%, AR grade), methanol (99.5%, AR grade), hexane (99.9%, AR grade), sulphuric acid (95-97%) were purchased from Sigma-Aldrich, sodium hydroxide (98-100%) was purchased from Research-Lab Fine Chem. Kjeldhal catalyst (Cu, 6.25% in $CuSO_4.5H_2O$) was purchased from AppliChem. The rest of the chemicals were either analytical or HPLC grade unless otherwise indicated.

Sample preparation

Pseudostem layers of the freshly cut banana trees of the above varieties were peeled off to uncover the central white-colored soft-stem. The isolated soft-stem was pulverized into small

pieces and subjected to blanching in a water bath maintained at 85°C. The stem pieces were subsequently dried in an oven (BOV-V230F, China) maintained at 55°C for 16 hours. The dried stem pieces were then transformed into powder form by using an electric grinder (Model MG 2053, India). Powdered soft stems were finally kept under refrigerated conditions for further analysis.

Proximate analysis

Proximate compositional analysis of dry powdered samples of cultivars was performed to determine the moisture, crude fat, total ash, and protein contents using the relevant methods described in AOAC (2019) manual. Moisture content was determined using the oven (BOV-V230F, China) method by drying at 105°C for 3 hours until constant weight was reached (AOAC Official Method 934.06); oil content was determined by soxhlet extraction using hexane (40-60°C) as solvent (AOAC Official Method 948.22); ash content determination was done by dry ashing method (AOAC Official Method 942.05); crude protein content determination was done by micro Kjeldahl method (AOAC Official Method 970.02). The total carbohydrate content was estimated according to the following equation: Total Carbohydrate content (%) = 100 – % (Moisture + ash + protein + fat).

Mineral analysis

Samples of soft-stem powder were digested using a microwave digester (CEM MARS 6, USA), where 0.25 g of flour was treated with 3 mL of 65% nitric acid. In the second step, the digested samples were filtered through whatman No 1 filter paper into 25 mL volumetric flasks and volume was adjusted to the mark using ultra-pure water. The samples were then analyzed for minerals using an Inductively Coupled Plasma spectrophotometer (Thermo Scientific, iCAP 7000 series, USA)

Fatty acid analysis

The constituent fatty acids present in the lipids of banana soft-stem powder were determined by adopting the procedure described by Marasinghe et al. (2019). In brief, fatty acid methyl esters (FAME) were prepared by dissolving 50 mg portion of the oil in 0.8 mL of hexane and adding 0.2 mL portion of 1M solution of sodium methoxide (AOAC, 2019). The mixture was centrifuged to separate out the top layer to analyze on a gas chromatograph (Agilent Technologies, Singapore) connected to a FID detector. The column type and instrumental conditions adopted for the analysis were as described before by Marasinghe et al. (2019). The identification of the peaks of the samples was done with reference to a standard chromatographic profile containing thirty-seven FAME standards (Supelco, Bellefinte, PA). The percentage of the individual fatty acid was calculated as the ratio of the partial area to the total peak area in the chromatogram.

Plant extract preparation

A 100 g portion of powdered soft stems of individual banana variety were sequentially extracted with DCM, EtOAc, and MeOH using an ultrasonicator bath (Rocker ultrasonic cleaner, model-Soner 206H) for 30 min. The extraction was repeated thrice for each of the solvent types. Each extract type was concentrated using a rotary evaporator at 40°C (Heidolph, Laborota 4000) under reduced pressure followed by vacuum drying at ambient temperature (vacuum oven, Heraeus instrument, Germany) for 3-4 hours. The crude extracts were stored at -18°C for further analysis.

Determination of TPC of samples

The determination of TPC was conducted in accordance with the method detailed out by Adekola et al. (2017) with slight modifications. Solutions of crude extracts were prepared by dissolving a known amount of sample in distilled water. A 50 µL portion of the sample solution was mixed with 15 µL of distilled water and 105 µL of 10% Folin-Ciocalteu reagent in a 96-well micro-plate. The mixture was allowed to incubate at room temperature for 3 min. A portion of 80 µL of 7.5% Na_2CO_3 was added to this, and the mixture was allowed to incubate in the dark for 30 min at room temperature. The absorbance value of the solution was recorded at 765 nm against distilled water using the micro-plate reader. The TPC of the individual sample was

read from the calibration curve of gallic acid.

Determination of TFC of samples

The determination of TFC was performed as described previously by Adekola et al. (2017) with minor modifications. Solution of crude extracts were prepared by dissolving a known amount of sample in distilled water. The final concentration of the solution was adjusted to around 1 mg/mL from all 3 extracts. The analysis was carried out by mixing the solutions as described previously using 50 µL of sample solution instead of catechin and the absorbance reading was recorded at 510 nm. The TFC of the individual sample was read from the calibration curve of catechin.

Determination of DPPH radical scavenging activity

Antioxidant activities of crude extracts of DCM, EtOAc, and MeOH from individual soft stems were measured against stable DPPH radical following the method described by Adekola et al. (2017). For this, a solution series ranging from 62.5–2000 µg/ mL was prepared in methanol for DCM, EtOAc, and MeOH extracts by dilution method. The assay was conducted in a 96 micro-well plate by maintaining the final volume at 210 µL. Accurately 150 µL aliquots of each extraction solution were mixed with 60 µL of 0.3 mM DPPH in methanol and incubated for 30 min in the dark. The absorbance was recorded against the control at 517 nm using a microplate reader. The absorbance of the control (0.3 mM, 60 µL), control blank (210 µL) sample blank (150 µL of sample, 60 µL of methanol) were measured. The positive control of this experiment was ascorbic acid. The DPPH radical scavenging activity corresponding to each sample was calculated from the calibration curve of trolox. The values were expressed as mM of trolox per g of crude extract.

Determination of FRAP value

The FRAP values of the crude extracts were analyzed in accordance with the method previously described by Tanko et al. (2017). A 50 µL of aliquot sample solution (reconstituted crude extract with distilled water) was added to 96-well microplate and mixed with 150 µL of FRAP solution and allowed to incubate for 4 min at room temperature. The absorbance values were recorded at 593 nm and the values were expressed as µmol $FeSO_4$ per g of crude extract. In this experiment, ascorbic acid was used as positive controls. The FRAP value corresponding to each sample was calculated from the calibration curve of $FeSO_4$.

$ABTS^+$ radical scavenging assay

The $ABTS^+$ radical scavenging activity of the crude extracts was assessed by using a method described by Adekola et al. (2017). A 50 µL portion of each concentration was mixed with 150 µL of $ABTS^+$ radical solution in a 96-well microplate. After that, the absorbance was measured at 734 nm by the microplate reader against distilled water as the blank. Determination of $ABTS^+$ Radical Scavenging Activity of 12 samples of banana soft-stem crude extracts were prepared by dissolving 4 mg of sample in 2 mL of 3% DMSO. A 50 µL portion of the sample solution was mixed with 150 µL of $ABTS^+$ working solution in a 96-well microplate. The absorbance was measured at 734 nm against distilled water using the microplate reader. Ascorbic acid was used as the positive control. The ABTS radical scavenging activity was calculated from the calibration curve of trolox. The values were expressed as mM of trolox per g of crude extract.

Statistical analysis

All data were taken in triplicates in this study (n=3) and the results were presented as mean ± standard deviation (SD). The experimental results of the study were analyzed using one-way ANOVA with Minitab Software Version 20 package. When the F values were significant ($p<0.05$), mean differences were compared using Tukey's test at a 5% significance level.

Results and discussion

Proximate parameters

The varietal differences in the proximate

parameters of the soft stems are presented in Table 1. Except for moisture content, all other parameters exhibited remarkable ($p<0.05$) differences. Carbohydrate is the most abundant nutritional component in the soft-stem powder ranging from 65.96±0.02% to 77.06±0.21%. The carbohydrate content percentages of the AM, SE, SU, and AL cultivars were 66.87±0.28%, 72.53±0.36%, 65.96±0.02%, and 77.06±0.21%, respectively. The value recorded for carbohydrate content of AL was markedly ($p<0.05$) higher than that of any other cultivar. The carbohydrate content percentage of the SE cultivar was also distinctly ($p<0.05$) lower than that of AL cultivar, but significantly ($p<0.05$) higher than those of AM and SU. However, AM and SU cultivars did not show any significant ($p>0.05$) difference between them. Information from the literature is scanty to compare the inter-varietal differences in the carbohydrate contents of these four cultivars. These values were roughly similar to those found in some literature related to other banana cultivar types. For instance, the carbohydrate content of the soft-stem of *Musa accuminata Cavendish* from Spain was 76.09±5.58% (Ramírez-Bolaños et al., 2021). According to a study by Aziah et al. (2011), the carbohydrate content of native banana soft-stem flour of *M. acuminate* × *balbisiana Colla* cv. Awak of Malaysia was 57.58%.

Ash was the second largest nutrient component of the soft stems of four cultivars of *Musa sp* (Table 1). The ash contents of the AM, SE, SU, and AL were 17.89±0.21%, 9.41±0.05%, 14.29±0.05%, and 10.61±0.14%, respectively. They were found to exhibit remarkable ($p<0.05$) differences. The highest ash content was noticed for the AM cultivars while the lowest for SE. These values were roughly similar to those found in the literature for other banana cultivars. For instance, soft stems of *Musa acuminate Cavendish* from Spain was found to have the ash content of 15.97±2.67% (Ramírez-Bolaños et al., 2021). According to another study by Abdullah et al. (2014) conducted in Malaysia, the ash content of the banana soft stem was 11%. The protein contents of the soft stems among the varieties ranged from 2.62±0.08% to 10.5±0.3% (Table 1). The amounts of protein present in AM, SE, SU, and AL were 4.81±0.15%, 7.87±0.3%, 10.5±0.3%, and 2.62±0.08%, respectively. In fact, there were remarkable ($p<0.05$) differences in these values. SU had the highest protein content among the varieties, followed by SE. AL showed the lowest protein content among these four cultivars. The values found in this study were more or less similar to those reported in the literature for other banana cultivars. For instance, the protein content of the banana pseudostem of *Musa acuminate Cavendish* of Spain was 7.25±2.48% (Ramírez-Bolaños et al., 2021).

Fat was the nutrient lowest in abundance in the soft stems of all four varieties of this study. According to Table 1, the fat content ranged from 1.26±0.05% to 1.87±0.06%. The amounts present in AM, SE, SU, and AL were 1.69±0.26%, 1.87±0.06%, 1.26±0.05%, and 1.38±0.08%, respectively. Based on the statistical analysis, the values were significantly ($p<0.05$) different and the amount of fat in SE was remarkably higher than that of SU. Among all four varieties, SU showed the lowest fat content. Also, no marked ($p>0.05$) difference was noticed between the fat content of AM and AL. The values of fat content found in this study were roughly similar to those reported in literature for some other banana cultivars. For instance, the fat content of the pseudostem of *Musa accuminata cavendish* from Spain was 1.01±0.31% (Ramírez-Bolaños et al., 2021). According to another study by Aziah et al. (2011) in Malaysia, the fat content of the *M. acuminate* × *balbisiana Colla* cv. Awak was 0.24±0.08%.

Table 1. Inter varietal differences in proximate composition of Musa spp. pseudostem of four different varieties (dry matter basis)

Variety	Moisture content (%)	Ash content (%)	Fat content (%)	Protein content (%)	Crude fiber (%)	Other carbohydrate content (%) (by difference)
Ambul Kesel	8.73^{a}±0.34	17.89^{d}±0.21	$1.69^{b,a}$±0.26	4.81^{b}±0.15	13.32^{c}±0.03	53.56^{a}±0.03
Seeni Kesel	8.31^{a}±0.24	9.41^{a}±0.05	1.87^{b}±0.06	7.87^{c}±0.3	9.90^{b}±0.02	62.64^{c}±0.05
Suwandel	7.99^{a}±0.02	14.29^{c}±0.05	1.26^{a}±0.05	10.5^{d}±0.3	8.23^{a}±0.02	57.73^{b}±0.05
Alu Kesel	8.33^{a}±0.01	10.61^{b}±0.14	$1.38^{b,a}$±0.08	2.62^{a}±0.08	13.31^{c}±0.01	63.75^{d}±0.04

Each value in the table represents the mean of three replicates with the standard deviation. Means within each row bearing different superscripts are significantly ($p < 0.05$) different at 95% confident.

Mineral distribution

The data presented in Table 2 compares the distribution of macro and micro minerals in the banana soft stem of four varieties. Minerals are essential nutrients required for the bodily functions and hence knowing the mineral content of various food would help to ensure that individuals meet their daily nutritional requirements (Ramírez-Bolaños et al., 2021; Farag et al., 2023). As shown in Table 2, macro minerals present were K, Ca, Mg, and Na, while micro minerals present were Zn, Mn, Ba, Sr, Fe, and Al. K was the most abundant mineral (26937-55445 mg/kg), followed by Mg (1600-2389 mg/kg) and Ca (1442-2385 mg/kg). The order of abundance in the mineral distribution is in accordance with a previous study by Nadeeshani et al. (2021) who reported the mineral distribution of the fruits of Seeni, Ambul, Kolikuttu, Rathambala, and Puwalu banana types. In the present study, the mineral distribution displayed a remarkable ($p<0.05$) difference regarding the mean values of K content of AL, AM, SE, and SU. The K content obtained for AM was distinctly ($p<0.05$) higher than those of all other varieties. Given its essential role in regulating intracellular fluid volume and transmembrane electrochemical gradients and its close relationship with sodium, it is important to ensure an adequate intake of potassium through diet for overall health and well-being (Lanham-New et al., 2012).

Mg was the next most abundant mineral detected in banana soft stems of the local varieties. The Mg contents of the samples ranged from 1600 to 2389 mg/kg; a remarkable ($p<0.05$) difference was seen among all four varieties. The highest Mg content was detected in AM while the lowest one was detected in SU. According to reports, Mg is a macro mineral which contributes to more than 300 biochemical reactions in the human body. It also aids the nerve and muscle function, boosts immune system, maintains a steady heartbeat, promotes the bone health, and regulates the blood glucose levels (Al Alawi et al., 2018).

Calcium, being a mineral typically linked with strong bones and teeth, plays key functions in blood clotting, muscle contraction, and normal maintenance of heart rhythms (Pravina et al., 2013). As shown in Table 2, a remarkable ($p<0.05$) difference was noticed in the Ca content of all four varieties. Similar to K and Mg contents, Ca was found to occur as the highest in AM. When compared to other macro minerals, sodium occurs in lower quantities (ranging from 63 - 175 mg/kg). The amount of Na detected in AM was extremely higher than those of all other varieties. Regarding Na content, AL did not show a distinct ($p>0.05$) difference when compared to SE and SU, but exhibited a remarkable ($p<0.05$) difference with AM. Sodium plays a crucial role in maintaining cellular balance and regulating fluid, electrolyte levels, and blood pressure. Excessive sodium intake is also bad since it is linked to hypertension, cardiovascular disease, and various other health conditions (Strazzullo and Leclercq, 2014). Fortunately, the Na contents of the soft stems of bananas in this study were moderate.

Micro minerals also play some important roles in human physiology; for instance, Zn as integral part of the immune function and wound healing, Mn supporting bone health, and Fe being essential part of the oxygen transport. Among the micro-minerals, Fe was the highest found among the varieties (79.7 - 180.4 mg/kg) followed by Zn (56.5 - 122.4 mg/kg), Al (22.1 - 94.2 mg/kg), Ba (29.5 - 57.6 mg/kg), Mn (24.9 - 44.0 mg/kg, and Sr (12.7 - 25.9 mg/kg) (Table 2). Regarding Fe content, no distinct ($p>0.05$) difference was noticed between AL and SE. However, remarkable ($p<0.05$) differences were detected between these varieties and AM. Among the varieties, the lowest micro-mineral contents were detected in SU with the exception of Zn. Concerning Mn content, no significant ($p>0.05$) difference was come across among AL, AM and SU except SE ($p<0.05$). With regard to other micro minerals that include Ba, Sr, and Al, significant ($p<0.05$) differences were noticeable among all varieties.

Table 2. Inter-varietal differences in mineral composition of banana soft stems of four different varieties

Minerals	Varieties (mg/kg)			
	Alu Kesel	Ambul Kesel	Seeni Kesel	Suwandel Kesel
Potassium (K)	$26936.5^{a} \pm 12.80$	$55444.6^{d} \pm 4.40$	$30617.8^{b} \pm 7.80$	$36360.1^{c} \pm 9.30$
Calcium (Ca)	$1564.9^{c} \pm 0.84$	$2385.3^{d} \pm 2.71$	$1469.4^{b} \pm 6.85$	$1442.7^{a} \pm 5.34$
Magnesium (Mg)	$2041.2^{c} \pm 6.67$	$2388.9^{d} \pm 2.26$	$1865.6^{b} \pm 5.05$	$1600.4^{a} \pm 1.75$
Sodium (Na)	$66.3^{ab} \pm 0.60$	$175.5^{c} \pm 1.04$	$70.2^{b} \pm 0.91$	$2.5^{a} \pm 1.58$
Zinc (Zn)	$56.5^{a} \pm 0.91$	$122.4^{d} \pm 0.47$	$67.1^{b} \pm 2.01$	$72.8^{c} \pm 0.26$
Manganese (Mn)	$31.8^{a} \pm 0.75$	$29.8^{a} \pm 2.27$	$44.0^{b} \pm 2.39$	$24.9^{a} \pm 0.89$
Barium (Ba)	$57.6^{d} \pm 1.63$	$46.0^{c} \pm 1.19$	$37.4^{b} \pm 0.69$	$29.5^{a} \pm 0.01$
Strontium (Sr)	$17.1^{c} \pm 0.21$	$25.9^{d} \pm 0.22$	$16.2^{b} \pm 0.19$	$12.7^{a} \pm 0.09$
Aluminum (Al)	$33.5^{b} \pm 0.63$	$94.2^{d} \pm 0.47$	$48.9^{c} \pm 1.26$	$22.1^{a} \pm 0.66$
Iron (Fe)	$98.5^{b} \pm 0.32$	$180.4^{c} \pm 1.65$	$106.3^{b} \pm 3.76$	$79.7^{a} \pm 1.07$

Each value in the table represents the mean of three replicates. The means in each row bearing different superscripts are significantly ($p<0.05$) different.

Fatty acid composition

The data given in Table 3 show the distribution of constituent fatty acids present in oils extracted from the soft stems of the four varieties. The total amounts of saturated fatty acid in AL, AM, SE, and SU were 60.32%, 50.72%, 49.64%, and 50.80%, respectively. Likewise, the total unsaturated fatty acid content in AL, AM, SE, and SU were 39.32%, 49.29%, 50.45%, and 49.46%, respectively. As a noteworthy feature, saturated to unsaturated fatty acid ratios of all varieties were more or less similar, with the exception of AL where the saturated fatty acid content was notably ($p<0.05$) higher than the rest of the other three varieties. Among the four varieties, palmitic (21.96 – 28.97%) was the major saturated fatty acid followed by arachidic acid (8.16 – 16.25%) (Table 3) while linoleic (21.23 – 45.02%) was the major unsaturated fatty acid followed by oleic acid (2.74 – 15.84%). Based on the previous study by Ramu et al. (2017), palmitic acid was the most abundant saturated fatty acid in banana pseudostem (*Musa sp.* cv. Nanjangud rasa bale) while linoleic acid was the most abundant unsaturated fatty acid. Additionally, the study highlighted the presence of stearic and arachidic acids in the banana pseudostem. As per the results of the fatty acid composition in our study, these tally with the data reported by Ramu et al. (2017). Occurrence of high amount of linoleic acid in banana soft stem is an adorable feature as it is a polyunsaturated omega-6 fatty acid. Because the human body cannot synthesize it, this

fatty acid is required to be taken through external dietary sources. It also serves as a precursor for formulating cell membrane components and other essential components required for the physiological responses (Jandacek, 2017).

As seen from Table 3, SE contained the highest palmitic and linoleic acids while AL contained the highest arachidic acid ($p<0.05$). Lignoceric acid content of SE was significantly ($p<0.05$) lower than those of the other three varieties. AL was found to have the lowest content of palmitic and stearic acids. The butyric acid content of AL (16.97%) was unusually ($p<0.05$) higher than those of the other three banana varieties. There was no notable ($p>0.05$) difference regarding the stearic acid content between SE and AL or SE and AM, but a significant ($p<0.05$) difference was detected against the value of SU. Oleic acid content of SU was higher when compared to those of the other three varieties, but the arachidic acid content of SU was the lowest. As a special feature, the proportions of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of SU were remarkably ($p<0.05$) higher than those of the other three varieties. As an anomalous feature, docosahexaenoic acid (DHA) was not detected at all in the case of AM and SE.

Table 3. Inter-varietal differences in fatty acid (FA) compositions of soft stem of four banana varieties

Component FA	Alu Kesel	Ambul Kesel	Seeni Kesel	Suwandel Kesel
Butyric acid (C4:0)	$16.97^{c} \pm 0.17$	$1.12^{a} \pm 0.08$	$0.62^{a} \pm 0.23$	$4.52^{b} \pm 0.50$
Caprylic acid (C8:0)	$0.11^{a} \pm 0.02$	$0.18^{a} \pm 0.05$	$0.12^{a} \pm 0.01$	ND
Capric acid (C10:0)	$0.08^{a} \pm 0.01$	$0.25^{a} \pm 0.11$	$0.16^{a} \pm 0.08$	$0.23^{a} \pm 0.13$
Lauric acid C12:0)	$0.69^{a} \pm 0.21$	$2.02^{a} \pm 0.12$	$1.03^{a} \pm 0.09$	$2.11^{a} \pm 0.74$
Myristic acid (C14:0)	$0.56^{b} \pm 0.16$	$1.15^{c} \pm 0.00$	$0.77^{b} \pm 0.08$	$0.12^{a} \pm 0.04$
Pentadecanoic acid (C15:0)	$0.22^{a} \pm 0.03$	$1.13^{b} \pm 0.42$	$0.45^{a} \pm 0.39$	$1.14^{b} \pm 0.13$
Palmitic acid (C16:0)	$21.96^{a} \pm 0.52$	$24.38^{b} \pm 0.50$	$28.97^{c} \pm 0.38$	$28.00^{c} \pm 0.19$
Palmitoleic acid (C16:1)	$0.07^{a} \pm 0.01$	$0.55^{a} \pm 0.46$	$0.21^{a} \pm 0.11$	$0.20^{a} \pm 0.09$
Heptadecenoic acid (C17:0)	$0.26^{a} \pm 0.03$	$0.71^{a} \pm 0.31$	$0.46^{a} \pm 0.06$	$0.69^{a} \pm 0.16$
Stearic acid (C18:0)	$2.20^{a} \pm 0.10$	$3.16^{b} \pm 0.08$	$2.71^{a,b} \pm 0.11$	$4.50^{c} \pm 0.20$
Oleic acid (C18:1)	$6.84^{b} \pm 0.61$	$5.38^{b} \pm 0.37$	$2.74^{a} \pm 0.29$	$15.84^{c} \pm 0.10$
Linoleic acid (C18:2 *cis*)	$27.93^{b} \pm 0.15$	$39.54^{c} \pm 0.58$	$45.02^{d} \pm 0.02$	$21.23^{a} \pm 0.51$
Linolelaidic acid (C18:2 *trans*)	$0.37^{a} \pm 0.32$	$0.39^{a} \pm 0.20$	$0.47^{a} \pm 0.21$	$0.73^{a} \pm 0.29$
Arachidic acid (C20:0)	$16.25^{c} \pm 0.24$	$15.51^{c} \pm 0.51$	$13.96^{b} \pm 0.17$	$8.16^{a} \pm 0.12$
Eicosapentaenoic acid (C20:5)	$2.87^{b} \pm 0.20$	$2.30^{b} \pm 0.22$	$1.26^{a} \pm 0.14$	$7.50^{c} \pm 0.14$
Docosadienoic acid (C22:2)	$0.41^{a} \pm 0.13$	$0.48^{a} \pm 0.44$	$0.58^{a} \pm 0.09$	$1.32^{b} \pm 0.04$
Decosahexaenoic acid (C22:6)	$0.72^{a} \pm 0.05$	ND	ND	$2.03^{a} \pm 0.44$
Lignoceric acid (C24:0)	$1.03^{b} \pm 0.28$	$1.11^{b} \pm 0.18$	$0.40^{a} \pm 0.22$	$1.33^{b} \pm 0.3$
Nervonic acid (C24:1)	$0.12^{a} \pm 0.04$	$0.67^{a} \pm 0.31$	$0.17^{a} \pm 0.15$	$0.61^{a} \pm 0.02$
Total Saturated ($\sum$SFA)	$60.32^{b} \pm 1.75$	$50.72^{a} \pm 2.36$	$49.64^{a} \pm 1.83$	$50.80^{a} \pm 2.48$
Total Unsaturated ($\sum$USFA)	$39.32^{a} \pm 1.52$	$49.29^{b} \pm 2.58$	$50.45^{b} \pm 1.01$	$49.46^{b} \pm 1.62$

Except this study, there is hardly any previous report on the fatty acid composition of soft stem of Sri Lankan banana varieties. Nadeeshani et al. (2021) previously described the fatty acid composition of the fruits of five different Sri Lankan banana types namely Seeni, Ambul, Kolikittu, Rathambala, and

Puhuwalu on a fresh weight basis. By overall, the fatty acid profiles of the banana fruits distinctly differed from those of the soft-stem powder. According to Nadeeshani et al. (2021), Seeni was found to contain the highest level of palmitic, stearic, and oleic acids while Ambul had the lowest level of palmitic, oleic, linoleic and α-linolenic acids. Aside this, lauric, arachidic, and lignoceric acids were not detected in the banana fruits of the above-mentioned varieties.

TPC and TFC

The TPC of banana soft-stem extracts are shown in Table 4. TPC of the banana soft-stem extracts were calculated using the developed calibration curve ($Y = 9.4362 X + 0.1172$; $R^2 = 0.9736$) for Gallic acid, where, X is the concentration of gallic acid while Y is the respective absorbance. Among all extracts, the highest TPC was observed in EtOAc extracts, while comparatively lower TPC was noted in DCM and MeOH extracts. An intervarietal difference in TPC content was in fact noticed in each extract. When considering the DCM extracts, the TPC followed the descending order of SE > SU > AL. A marked ($p<0.05$) difference was noticeable among the extracts except for those of AL and SU. Nevertheless, no TPC was detected in DCM extract of AM. Among the EtOAc extracts, the TPC can be aligned in the descending order of SE>SU>AL>AM; the values were remarkably ($p<0.05$) different from each other. The highest and lowest TPC were observed for SE (20.06 mg GAE/g of crude extract) and AM (1.03 mg GAE/g of crude extract), respectively. In the case of MeOH extracts, TPC was noticed only for SU with a value of 1.21 mg GAE/g of crude extract.

The data presented in Table 4 also showed the TFC of banana soft-stem extracts. TFC of the banana soft-stem extracts were calculated using the developed calibration curve ($Y = 10.38 X - 0.019$, $R^2 = 0.9922$) for catechin, where X is the concentration of catechin and Y is the respective absorbance. EtOAc extracts had the highest TFC content when compared to those of other extracts, except for SU. MeOH extracts were found to contain extremely low TFC, except the case of AM. Among the DCM extracts, SU (12.72 ± 1.95 mg CE/g of crude extract) had the highest TFC followed by SE and AM. In this, the lowest TFC content was displayed by AL (1.51 ± 0.93 mg CE/g of crude extract). Nevertheless, the differences between AM and AL were not remarkable ($p>0.05$). Among the EtOAc extracts, the TFC ranged from 3.51 to 17.54 mg CE/g of crude extract, with the highest and the lowest TFC values recorded for AM and AL. The TFC of EtOAc extracts followed the order of AM>SE>SU>AL and the values were markedly ($p<0.05$) different, except the difference between AM and SE. When we consider the MeOH extracts, TFC ranged from 0.84 to 14.29 mg CE/g of crude extract with the highest and the lowest values noted for AM and SU, respectively. Although AL and SU did not show distinct ($p>0.05$) differences, other varieties showed a clear distinct ($p<0.05$) difference among them.

Many previous studies have demonstrated the presence of phytonutrients that include phenolics and flavonoids in bananas. According to Singh et al. (2016), the phenolics concentration in the banana pulp varied from 11.8 to 90.4 mg of GAE/100 g/ fresh weight. Saravanan and Aradhya (2011) conducted a study to determine the TPC, TFC, and antioxidant activity of different solvent extracts of pseudostem obtained from some Indian banana varieties. Their findings showed that the TPC and TFC content varied among different cultivars, ranging from 7.58 to 291 mg GAE and from 4 to 80 mg CE per gram of crude extract, respectively. Nevertheless, the availability of data on TPC and TFC of the soft-stem of local banana varieties is scanty. Based on the results of the present study, EtOAc extracts of the soft stem had higher TPC as well as higher TFC when compared to those of the extracts obtained with DCM and MeOH. This clearly demonstrated that the TPC and TFC compositions varied according to the medium of extraction.

Table 4. TPC and TFC of dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) extracts obtained from soft stems of four banana varieties

Assay	*Musa spp.*	Type of Extract		
		DCM	EtOAc	MeOH
TPC mg gallic acid equivalent (GAE)/g of crude extract	AL	$0.50^{a} \pm 0.19$	$13.09^{b} \pm 1.88$	ND
	AM	ND	$1.03^{a} \pm 1.31$	ND
	SE	$4.79^{b} \pm 0.90$	$20.06^{c} \pm 1.97$	ND
	SU	$1.50^{a} \pm 0.84$	$14.06^{b} \pm 1.81$	1.21 ± 1.25
TFC mg catechin equivalent (CE)/g of crude extract	AL	$1.51^{a} \pm 0.93$	$3.51^{a} \pm 0.87$	$1.04^{a} \pm 0.57$
	AM	$4.47^{a} \pm 0.81$	$17.54^{c} \pm 2.25$	$14.29^{c} \pm 2.41$
	SE	$9.44^{b} \pm 0.67$	$15.10^{c} \pm 1.44$	$7.28^{b} \pm 1.12$
	SU	$12.72^{c} \pm 1.95$	$10.69^{b} \pm 0.92$	$0.84^{a} \pm 0.80$

Each value in the table represents the mean of three replicates. The means within each column sharing different superscripts are significantly ($p < 0.05$) different at 95% confident. Abbreviations: ND, not detected.

Antioxidant capacity

The data presented in Table 5 show the results obtained from the FRAP assay. The FRAP value of the banana soft-stem extracts were calculated using the developed calibration curve (Y = 0.4634 X - 0.0986, $R^2 = 0.996$) for $FeSO_4$, where X is the concentration of $FeSO_4$ and Y is the respective absorbance. When compared to the extracts obtained with DCM and MeOH, markedly ($p<0.05$) higher FRAP values were recorded for EtOAc extracts. In the case of DCM extracts, the highest and the lowest FRAP values were observed for SE (0.48 ± 0.05 mM $FeSO_4$/g of crude extract) and AL (0.16 ± 0.02 mM $FeSO_4$/g of crude extract), respectively. The FRAP values of DCM extracts followed the descending order of AL>AM>SU>SE and displayed no significant ($p<0.05$) difference between AL and AM. Among EtOAc extracts, the FRAP values tended to follow the order of AL<AM<SU<SE and displayed remarkable ($p<0.05$) differences from each other. The highest FRAP value was observed for SE (1.12 mM $FeSO_4$/g of crude extract) while the lowest value was observed for AL (0.41 mM $FeSO_4$/g of crude extract). When we consider the MeOH extracts, only SU and SE had displayed antioxidant activity in terms of FRAP and the values were remarkably ($p< 0.05$) different between these two. When compared to ascorbic acid (10.12 ± 0.02 mM of $FeSO_4$ /g), the reducing power of the individual plant extract of this study was significantly ($p<0.05$) lower.

The data presented in Table 5 show the results obtained from the ABTS assay. ABTS radical scavenging activity of banana soft-stem extracts were calculated using the developed calibration curve (Y = 352.7 X – 2.1631, $R^2 = 0.9948$) for trolox, where X is the concentration of trolox and Y is the respective percentage radical scavenging activity. When compared to the extracts obtained using DCM and MeOH, the highest ABTS radical scavenging activities were shown by EtOAc extracts of all banana varieties. Among the three EtOAc extracts, the strongest ABTS radical scavenging activity was exerted by the EtOAc extract of AM (0.288 ± 0.002 mM trolox/g of crude extract). Among the EtOAc extracts, the strongest ABTS activity was observed for both AM and SU. The ABTS values of different varieties tended to follow the order of AL≈SE<SU≈AM, but no remarkable ($p>0.05$) difference was observed between AL and SE. Among the DCM extracts, the strongest ABTS activity was exhibited by SE (0.24 ± 0.01 mM trolox/g of crude extract). The ABTS values of different varieties tended to follow the order of AM≈AL<SU<SE, but their values were distinctly ($p<0.05$) different, except those between AL and AM. ABTS values of MeOH extracts ranged between 0.098 to 0.184 mM trolox/g of crude extract and were relatively lower when compared to those of the extracts obtained with DCM and EtOAc. The values tended to follow the order of AL<AM<SE<SU and the differences were significant ($p<0.05$) among the varieties. The results obtained from DPPH radical scavenging activity assay are presented in Table 5. DPPH radical scavenging activity of banana soft-stem extracts were calculated using the developed calibration curve (Y = 279.48 X – 10.836, R^2 =

0.9348) for trolox, where X is the concentration of trolox while Y is the respective percentage radical scavenging activity. Significantly ($p<0.05$) higher DPPH radical scavenging values were recorded for EtOAc extracts while significantly lower values were observed for MeOH extracts. Among EtOAc extracts, AM exhibited the highest DPPH value (0.18 ± 0.02 mM trolox/g of crude extract) while SU displayed the lowest DPPH value (0.14 ± 0.00 mM trolox/g of crude extract). DPPH values of these extracts tended to follow the order of SU<SE<AL<AM. No significant ($p>0.05$) difference was observed between SU and SE, SE and AL as well as between AL and AM. However, a distinct ($p<0.05$) difference was detected between SU and AM as well as between AM and SE.

Among DCM extracts, SE exhibited the highest DPPH value (0.143 ± 0.013 mM trolox/g of crude extract) while AM displayed the lowest DPPH value (0.121 ± 0.012 mM trolox/g of crude extract). No significant ($p>0.05$) difference in values was observed among AM, AL, and SU as well as among SE, AL, and SU. Nevertheless, a clear ($p<0.05$) difference was observed between AM and SE. Among MeOH extracts, SE exhibited the highest DPPH value (0.15 ± 0.00 mM trolox/g of crude extract) while AM displayed the lowest DPPH value (0.07 ± 0.01 mM trolox/g of crude extract). Based on Table 5, DPPH values of MeOH extract can be aligned in the order of AM<AL<SU<SE. Although no significant ($p>0.05$) difference was observed between AM and AL, significant ($p<0.05$) differences were observed between AL, SE, and SU as well as between AM, SE and SU.

For comparison purpose, the availability of data on the antioxidant capacity of banana soft stem of local varieties is scanty. Some previous studies conducted elsewhere have demonstrated the presence of bioactive compounds contributing to antioxidant capacity of banana fruit pulp For instance, Alothman et al. (2009) assessed the antioxidant potential of the banana fruit pulp extracts using FRAP and DPPH assays. Based on their study, DPPH inhibition of 36.8% and 68.0% were recorded for aqueous and acetone extracts, respectively, while recorded FRAP values of 0.59 and 5.26 µmol Fe (II)/g fresh fruit weight. Separately, Bhaskar et al. (2011) observed that the flower and pseudostem of banana had DPPH radical scavenging activity with the values of 9.35 ± 0.54 and 37.2±0.83 µg, respectively. In another study, Ranjan Kumar et al. (2014) found that banana pseudostem extracted in 90% ethanolic system had the higher antioxidant capacity with a value of 34.82 µg/mL.

Table 5. Antioxidant activities of dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) extracts obtained from soft stems of four banana varieties

Assay	*Musa spp.*	Type of Extract		
		DCM	EtOAc	MeOH
FRAP values (mM FeSO4/g of crude extract)	AL	$0.16^{a} \pm 0.02$	$0.41^{a} \pm 0.01$	ND
	AM	$0.21^{a} \pm 0.02$	$0.77^{b} \pm 0.02$	ND
	SE	$0.48^{c} \pm 0.05$	$1.12^{d} \pm 0.01$	$0.07^{a} \pm 0.01$
	SU	$0.29^{b} \pm 0.01$	$1.03^{c} \pm 0.03$	$0.10^{b} \pm 0.01$
ABTS+ radical scavenging activity (mM trolox/g of crude extract)	AL	$0.12^{a} \pm 0.00$	$0.27^{a} \pm 0.01$	$0.10^{a} \pm 0.00$
	AM	$0.12^{a} \pm 0.01$	$0.29^{b} \pm 0.00$	$0.13^{b} \pm 0.00$
	SE	$0.24^{c} \pm 0.01$	$0.27^{a} \pm 0.01$	$0.15^{c} \pm 0.01$
	SU	$0.14^{b} \pm 0.00$	$0.29^{b} \pm 0.00$	$0.18^{d} \pm 0.01$
DPPH radical scavenging activity (mM trolox/g of crude extract)	AL	$0.14^{ab} \pm 0.01$	$0.17^{bc} \pm 0.01$	$0.08^{a} \pm 0.01$
	AM	$0.12^{a} \pm 0.01$	$0.18^{c} \pm 0.02$	$0.07^{a} \pm 0.01$
	SE	$0.17^{b} \pm 0.02$	$0.15^{ab} \pm 0.00$	$0.15^{c} \pm 0.00$
	SU	$0.16^{ab} \pm 0.02$	$0.14^{a} \pm 0.00$	$0.11^{b} \pm 0.01$

Each value in the table represents the mean of three replicates. The means within each column sharing different superscripts are significantly ($p< 0.05$) different at 95% confident. Abbreviations: ND, not detected.

Pearson's correlation analysis

Pearson's correlation coefficients (r) obtained for TPC and TFC with the antioxidant assays for DCM, EtOAc and MeOH extracts of banana soft stems are presented in Table 6. When considering the r values obtained for DCM extracts, TPC showed significantly ($p<0.05$) strong positive correlations with the ABTS radical scavenging activity (r = +0.963) and the FRAP value (r = +0.899), indicating that the phenolic compounds present in banana soft stem extracts have strong influence on the ABTS radical scavenging activity and the ferric reducing antioxidant power. Similarly, TFC also showed significantly ($p<0.05$) positive correlations with the FRAP value (r=+0.742), ABTS radical scavenging activity (r=+0.682) and the DPPH radical scavenging activity (r=+0.656), denoting the influence of TFC on the antioxidant activity of banana soft stem extracts. Referring to the r values obtained for EtOAc extracts, TPC showed a significant ($p<0.05$) negative correlation only towards the DPPH radical scavenging activity (r=-0.602). Whereas, TFC showed a significant ($p<0.05$) positive correlation only with the ferric reducing antioxidant power (r=+0.666). Unlike the DCM and EtOAc extracts, no significant ($p>0.05$) correlations were observed for TPC and TFC with any of these antioxidant assays. These findings suggest that the non-polar and mid-polar phenolic compounds have stronger contribution towards antioxidant activity of banana soft stem extracts than the polar phenolic compounds.

Table 6. Pearson's linear correlation coefficients (r) of TPC and TFC with DPPH, ABTS, FRAP values of DCM, EtOAc and MeOH extracts of banana soft stems

Extract		DPPH	ABTS	FRAP
DCM	TPC	+0.696	+0.963	+0.899
	TFC	+0.656	+0.682	+0.742
EtOAc	TPC	-0.602	-0.563*	+0.368*
	TFC	-0.455*	+0.206*	+0.666
MeOH	TPC	+0.062*	+0.533*	+0.507*
	TFC	-0.342*	-0.152*	-0.451*

* No significant correlation ($p>0.05$). Abbreviations: TPC, total phenolic content; TFC, total flavonoid content; DPPH, DPPH radical scavenging activity; ABTS, ABTS+ radical scavenging activity FRAP, ferric reducing antioxidant power; DCM, dichloromethane; EtOAc, ethyl acetate; MeOH, methanol.

Conclusion

In this study, varietal differences on nutritional composition and bioactivities of the banana soft stem from four local varieties were compared. As a common feature, all four varieties possessed high contents of carbohydrate and ash, but low proportions of fat and protein. Except for moisture, remarkable differences were noticed in the contents of fat (1.26 – 1.69%), ash (9.41 – 17.89%), carbohydrate (53.56 – 63.75%), and protein (2.62 – 10.50%). Regardless of the varieties, K, Ca, Mg and Na were the macro minerals present while Zn, Mn, Ba, Sr, Fe, and Al were the micro minerals. The content of saturated fatty acids was a little higher than the proportion of the unsaturated fatty acids in all varieties except SE. Linoleic (21.23 – 45.02%), palmitic (21.96 – 28.97%), and arachidic (8.16 – 16.25%) were the major fatty acids detected irrespective of the variety. Out of three solvents employed, the highest contents of TPC and TFC were recovered from EtOAc extracts of all four varieties. With regard to antioxidant capacity, remarkably high FRAP values were recorded for EtOAc extracts while significantly lower values were obtained for both DCM and MeOH extracts. The highest antioxidant capacity in terms of DPPH and $ABTS^{+}$ radical scavenging activity was displayed by EtOAc of all four varieties. Since all four varieties could be rich sources of polyphenols and had the ability to act as potent antioxidants, further studies can be carried out on developing food products using banana soft stem flour. In this connection, it is worthwhile to undertake some future studies to see the impact of changing agro climatic conditions on the nutritional qualities and functional attributes of the soft stem flour.

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