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Proximate composition and antioxidant activity of the saba (*Musa acuminata x balbisiana Colla*) banana blossom family Musaceae

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Abstract

This study evaluated the proximate composition, and content of phenolic and flavonoid compounds as well as the potential antioxidant property of saba (*Musa acuminata x balbisiana Colla*) banana blossoms. The banana blossom was dried at 60 °C for 12 hours. The sample was then extracted using 80% ethanol. The ethanolic extract of the sample was subjected to antioxidant and various characterization, including the following: i) nutritional values of the banana blossom powder, which was analyzed according to the Association of Official Analytical Chemists (AOAC), ii) total phenol and total flavonoid content and iii) antioxidant test using, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity.

The results revealed that the sample contains a high amount of ash, fiber, protein, and carbohydrate and a low amount of fat. The Total Phenolic Content and Flavonoid Concentration of extract revealed an abundant reservoir of compounds, the concentration 500 mcg/mL, exhibited the highest radical scavenging activity of banana blossom ethanolic extract, suggesting a dose-dependent activity. Further investigation using other *in vitro* and *in vivo* antioxidant assay is recommended to confirm the potential use of saba (*Musa acuminata x balbisiana Colla*) banana blossom as an antioxidant.

Keywords: Banana blossom; Proximate composition; Phenolic; Flavonoid; Antioxidant property

1. Introduction

Banana is the second largest fruit crop produced and widely consumed all over the world particularly in the Philippines making it one of the leading fruit crops cultivated in the region. In fact, the Philippines is one of the top producers of banana and plantain, together with India, China, Uganda, Ecuador, and Nigeria [1]. The fruit part of banana is widely used and regularly consumed due to its nutritional value and health benefits, while banana blossoms are usually unfamiliar and commonly underutilized in most parts of the world [2]. Banana blossoms, together with banana pseudo stems, are regarded as wastes [3]. Studies show that banana blossoms have great potential for their high nutritional value and medicinal benefits [4]. They are rich sources of dietary fiber that significantly reduce the prevalence of various heart diseases [5]. Furthermore, they possess a high level of antioxidant property that is significant in inhibiting oxidative damage that may lead to cardiovascular diseases. The flowers can also be utilized for bronchitis, ulcers, and dysentery [6].

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Many studies have supported the use of banana by-products like leaves and peels, but not much has been reported on banana blossoms. There is a rise in the need to determine the proximate composition of the banana blossoms to identify their potential as a possible source of nutrients [7].

These days, there is a dramatic increase in the search for antioxidants from local and natural sources which are abundant in the Philippines, in order to reduce potential risk and toxicity to consumers which uses synthetic antioxidants like butyl hydroxyanisole (BHA) and butylhydroxytoluene (BHT) [8].

Plants rich in phenolic compounds like flavonoids are of particular importance because of their ability to scavenge radicals and inhibit peroxidation [9].

With all these studies about the different medicinal uses of saba (*Musa acuminata x balbisiana* Colla) banana blossoms, its antioxidant property is still unexplored. Several researchers have also presented data showing the banana blossoms as a potential source of dietary fibers and proteins in addition to its antioxidant components [10]. Hence, determining the amount of the saba (*Musa acuminata x balbisiana* Colla) banana blossom extract needed to exert the antioxidant property is of great importance. To further strengthen the claim about its antioxidant property, characterizing the extract through total phenol content and flavonoid concentration is highly recommended.

2. Material and methods

Organic solvents used in the study are of reagent grade and were obtained from Centro Escolar University. All drugs and reagents used are of analytical grade, unless specified.

The researchers used an experimental method since they employed several analyses of a sample to determine its composition and the actual content. The descriptive method was also utilized since there are no standard criteria for the proximate composition of saba (*Musa acuminata x balbisiana* Colla) banana blossom samples. The quantitative method was observed as the researchers elicited numerical data from the samples tested.

2.1. Plant Collection and Authentication

Banana blossoms were collected from Alfonso Lista, Ifugao, and subsequently submitted to the University of the Philippines, Diliman, for authentication.

2.2. Preparation of Banana Blossom Powder

Banana blossoms were washed thoroughly with tap water to remove adhering dirt and other extraneous articles. The banana blossoms were then cut into small pieces manually. It was further dried at 60 °C for 12 hours and was grounded using a mixer grinder. It was packed in a sterilized container and then stored at 5 °C before further analysis.

2.3. Proximate Analysis of Banana Blossom Powder

Following the methods of the Association of Official Analytical Chemists (AOAC), banana blossom powder was analyzed for proximate composition namely, moisture content, crude protein, crude fat, total ash, and crude fiber with modification [11].

2.3.1. Moisture Content

Ten (10.0) grams of the sample were weighed accurately. The samples were dried using an oven at 135 ± 2 °C for 2 hours \pm 5 minutes in order to determine the moisture content, followed by cooling at desiccator and individual weighing of the dried samples [12]. The moisture content of the samples was calculated based on loss in weight on drying as an estimate of H₂O by using the formula:

$$\% \text{ Moisture content} = \frac{\text{weight before drying} - \text{weight after drying}}{\text{weight before drying}} \times 100$$

2.3.2. Crude Protein

Protein was determined by Kjeldahl method based on AOAC [11] using copper sulfate and sodium sulfate as catalysts. Two (2.0) grams of the sample were weighed and placed on an N-free filter paper and was transferred to a 250 mL digestion tube. Seven (7.0) grams Na₂SO₄ and 0.8 g CuSO₄ · 5H₂O were added. The tube was placed in a fume hood, and 15 mL H₂SO₄ was added. After the reaction subsided, the tube was placed in a block digester preheated to 420 °C. It was

digested until the mixture was clear. The tube was removed and cooled for 10 minutes. Fifty (50.0) to 75 mL of water was carefully added, and a digestion tube was attached to the distillation unit. The cooled digest was diluted with 15 mL H₂O. The receiver flask was added with 50 mL of 0.1 N H₂SO₄. Fifty (50.0) mL 50% NaOH was added to the tube. The reaction was allowed to settle. It was distilled for 5 minutes and titrated with standardized 0.2N NaOH using methyl red as an indicator. A reagent blank was performed before each batch of samples.

2.3.3. Crude Fat

Crude fat was determined by the Soxhlet extraction method with modification [11,13]. The Soxhlet apparatus was fitted up with a reflux condenser allowing water flow to start. Five (5.0) grams of the sample were weighed. It was placed on a folded Whatman No.1 filter paper into a thimble free from fat with porosity permitting rapid passage of ether. The thimble was placed into the main extraction chamber where 200 mL of petroleum ether was added until it siphoned over the flask. The sample was heated for 4 to 5 hours. At the end of the extraction process, the thimble was removed and further dried. The flask with the fat was dried at 100 °C in the oven for 30 minutes and then cooled. The flask plus fat will be weighed and calculated as follows:

$$\% \text{ crude fat} = \frac{\text{weight of flask and extracted fat} - \text{the weight of empty flask}}{\text{weight of sample}} \times 100$$

2.3.4. Total Ash

Five (5.0) grams of the banana blossom powder were weighed and placed in an empty porcelain crucible. The container was placed in a muffle furnace heated at 600 °C. The process was held for two hours, afterwards the crucible was directly cooled in a desiccator and weighed to compute for the total ash.

2.3.5. Crude Fiber

The determination of the crude fiber content was analyzed using a modified method previously published by AOAC [11]. Five (5.0) grams of the banana blossom powder were weighed individually and placed in a reflux beaker. It was then added with bumping granules, followed by 200 mL 1.25% H₂SO₄ solution in a small stream directly on sample. The beakers were placed on the digestion apparatus and were boiled for exactly 30 minutes. At the end of the refluxing process, the sample was washed using water through a funnel to warm. The sample was filtered to dryness and the residue was again washed with water, filtered after each process. The residue collected was with a 1.25% NaOH for another 30 minutes. At the end of refluxing, the sample was washed in several portions and was allowed to drain, subsequently heated in the oven at 150 °C ± 2 °C for 2 hours, afterwards it was taken to a muffle furnace where it was burn at 550 °C ± 10 °C for 2 hours and was cooled in a desiccator.

2.3.6. Carbohydrates

The percentage of carbohydrates in the sample will be calculated as follows, according to the procedure of Krishnan and Sinija [10].

$$\% \text{ Carbohydrates} = 100 - \text{sum of other proximate components}$$

Where in;

$$\text{Sum of other proximate components} = \% \text{ Protein} + \% \text{ Ash} + \% \text{ Fat}$$

2.4. Preparation of the Banana Blossom Extract

The sample extract equivalent to 100 grams was placed in a 1L Erlenmeyer flask with 80% ethyl alcohol as the solvent, the Erlenmeyer flask was subsequently placed in a mechanical shaker to improve the extract efficiency for 10 hours. The extract was then filtered and concentrated under pressure using a rotary evaporator kept at 50 °C to evaporate the solvent.

2.5. Characterization & Evaluation of the Antioxidant Activity of Banana Blossom Extract

2.5.1. Estimation of the Total Phenol Content

The total phenolic content of the banana blossom extract was determined by a modified Folin - Ciocalteu colorimetric method. The stock aqueous solution of gallic acid was diluted to obtain 10 to 100 µg/mL standard solutions. Exactly 75 µL of distilled water, 25 µL of the extract, and 25 µL of Folin-Ciocalteu reagent were then added to each well and allowed

to stand for minutes. After standing, 100 uL of 20% Na₂CO₃ was added to each well. The plate was shaken for 30 seconds, covered and allowed for incubation in dark place for 90 minutes. The plate was placed on mechanical shaker for 120 seconds prior to absorbance reading using 760 nm wavelength. Gallic acid was used as the standard, and the results were expressed as mg of gallic acid equivalents/g of extract [10].

2.5.2. Estimation of the Flavonoid Concentration

The total flavonoid content of the banana blossom extract was determined using a modified aluminum chloride colorimetric method using a 96-well plate. Exactly 100 uL of distilled water, 25 uL of the extract, and 10 uL of 5% NaNO₂ were transferred to each well and allowed to stand for 5 minutes. After 10 minutes, 20 uL of AlCl₃ TS were added together by 40 uL of NaOH and 40 uL of distilled water. The plate was placed in a mechanical shaker for 60 seconds prior to absorbance measurement at 510 nm. A calibration curve was prepared with catechin and the results of the test was expressed as mg catechin equivalents (CEQ)/g sample [10].

2.5.3. Measurement of DPPH Free Radical Scavenging Activity

DPPH radical was evaluated using modified method published by Yen and Chen [14]. A 1.5 mL sample extract was taken at five concentrations (0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL & 0.5 mg/mL) and 2.0 mL of 0.2 Mm DPPH in methanol (80%) was added to each test tube with different concentrations. The test tubes were subjected in incubation for 1 hour in a dark & controlled room temperature. The absorbance was read against a blank at 517 nm.

$$\% \text{ scavenging activity} = \frac{A_{\text{control}} - AS_{\text{control}}}{A_{\text{control}}} \times 100$$

Where;

A_{control} = Absorbance of the control (solution in which no antioxidant was added)

AS_{control} = Absorbance of the extract solution

3. Results and discussion

The proximate composition analysis of banana blossom powder is presented in Table 1.

3.1. Nutritional Analysis of Saba (*Musa acuminata x balbisiana* Colla) Banana Blossom

Table 1 Proximate composition (g/100g) of Saba (*Musa acuminata x balbisiana* Colla) Banana Blossom Powder

Component	Trial 1	Trial 2	Trial 3	Mean	Std. Deviation
Moisture	15.20	15.19	15.30	15.23	0.06083
Protein	15.28	15.31	15.28	15.29	0.01732
Fat	7.53	7.68	7.62	7.61	0.07550
Ash	14.61	14.81	14.76	14.73	0.10408
Crude Fiber	21.42	20.64	20.98	21.01	0.39107
Carbohydrate	62.58	62.2	62.34	62.37	0.19218

In the proximate composition analysis, the moisture content of the banana blossom powder was considerably low, this in effect will result in the reduction of the perishability of food crops and extension of the shelf life. Other substances including protein and fat were also low in quantity in dried banana blossom powder, this is possible because of the heat treatment as a result of drying process [10]. On the other hand, low lipid content could be due to the occurrence of lipid oxidation in the process. Interestingly, the analysis showed significantly high ash and fiber contents which may be attributed to the removal of moisture, increasing the concentration of nutrients.

3.2. Percentage Yield of Saba (*Musa acuminata x balbisiana* Colla) Banana Blossom Extract

Banana blossom extract obtained from saba (*Musa acuminata x balbisiana* Colla) was computed and resulted in a 17.64 % yield, which is a significant amount in terms of quantity that can be for further analyzed in the research.

3.3. Determination of the Total Phenol & Total Flavonoids from Banana Blossom Extract

The antioxidant activity of saba (*Musa acuminata x balbisiana* Colla) banana blossom extract correlates with the phenolic and flavonoid compounds present in the sample. Phenolic compounds are secondary metabolites and considered to be a common water-soluble antioxidant compounds found in plants, and together with flavonoids these compounds are known to exhibit various mechanisms of antioxidant activity

Table 2 summarizes the major antioxidant components present in Banana Blossom Extract.

Table 2 Antioxidant Components (mg/g) Present in Banana Blossom Extract

Component	Mean Result Banana Blossom Ethanolic Extract
Total phenols	837.60
Total Flavonoids	83.33

Total phenols were expressed as gallic acid equivalents. Total flavonoid concentration was expressed as catechin equivalent. The assay confirmed that saba (*Musa acuminata x balbisiana* Colla) banana blossoms can be a potential rich source of phenolic compounds and flavonoids which are known to be capable of exhibiting antioxidant property as seen in several fruits and vegetables proven with such claims.

3.4. DPPH antioxidant assay of Banana Blossom Extract

Antioxidant activity was measured by DPPH free radical scavenging activity. DPPH is a stable organic nitrogen radical and free radical compound with a purple color which changes into a stable yellow compound when reacting with an antioxidant. In brief, the reduction capacity of DPPH was determined by the decrease on its absorbance at 517 nm, which is reduced by the antioxidant.

The antioxidant activity of the ethanolic extract is shown in Table 3.

Table 3 DPPH Free Radical Scavenging Activity of Banana Blossom and Reference Standard under Different Concentrations

Concentration	% Radical Scavenging Activity			Mean	Std. Deviation
	Trial 1	Trial 2	Trial 3		
Banana Blossom Extract					
100 mcg/mL	54.06 %	53.87 %	53.82 %	53.92 %	0.0012662
200 mcg/mL	54.66 %	54.61 %	54.71 %	54.66 %	0.0005000
300 mcg/mL	56.11 %	55.11 %	54.96 %	55.39 %	0.0062517
400 mcg/mL	54.36 %	56.36 %	56.36 %	55.69 %	0.0115470
500 mcg/mL	56.71 %	56.76 %	56.66 %	56.71 %	0.0005000
Ascorbic acid					
100 mcg/mL	96.36 %	96.41 %	96.26 %	96.34 %	0.0007638
200 mcg/mL	97.36 %	97.36 %	97.31 %	97.34 %	0.0002887
300 mcg/mL	97.41 %	97.46 %	97.36 %	97.41 %	0.0005000
400 mcg/mL	97.56 %	97.61 %	97.56 %	97.58 %	0.0002887
500 mcg/mL	98.75 %	98.80 %	98.70 %	98.75 %	0.0005000

The presence of banana blossom extract into the standard DPPH free radical solution, caused a moderate decrease in the absorbance of the solution, indicating a potential scavenging capacity of the extract, however, not comparable to the standard – ascorbic acid. Five hundred micrograms per milliliter (500.0 mcg/mL) of extract exhibited the highest activity (56.71 %), suggesting a dose-dependent activity, with a significant difference compared to the standard (98.75 %). This result shows that saba (*Musa acuminata x balbisiana* Colla) banana blossom extract in its crude form is inferior to ascorbic acid, however, it still has the potential to be a natural source of antioxidants.

4. Conclusion

The total phenolic and total flavonoid assay showed the presence and abundance of the compounds on the sample, which suggest that these compounds are responsible for the radical scavenging activity of the Saba (*Musa acuminata x balbisiana* Colla) banana blossom and, therefore, could be considered as a potential source of dietary supplement. Further investigation using different *in vitro* and *in vivo* antioxidant assays are recommended to confirm the potential use of Saba (*Musa acuminata x balbisiana* Colla) banana blossom as an antioxidant.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no conflicts of interest to declare.

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