Proximate Composition and Antioxidant Activity of Banana Blossom of Two Cultivars in India

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Abstract

The objectives of the work taken up were proximate composition analysis and extraction and estimation of antioxidant components from banana blossom of two cultivars (Poovan & Monthan) in India. Flower samples were collected and extracted by using ethanol and water according to the method of Association of Official Analytical Chemist (AOAC). Banana blossom powder was prepared by drying banana blossom slices at 60\degree C for 12 hrs in a tray drier. Before drying banana blossom slices were dipped in 0.2\% citric acid solution for 30 min in order to reduce browning. Nutritional composition of the banana blossom samples was studied. Results showed that banana blossom samples of Poovan and Monthan variety contained high amount of fiber (12.42 to 12.82 in fresh samples and 15.32 to 15.48 in banana blossom powder) and ash (2.42 to 3.21 in fresh samples 3.08 to 4.19 in banana blossom powder). Antioxidant activity was found out using DPPH free radical scavenging assay. Ethanol extract of Poovan variety revealed better antioxidant properties than Monthan variety.

Keywords: Banana blossom, Nutritional composition, Antioxidant activity

INTRODUCTION

Novel drug entities continue to be developed through research into their constituents. One such plant family of medicinal importance is Musaceae. Musaceae family has 2 genera and 42 different species and within 42 species, 32 species belongs to musa species [Nuengchhammong \textit{et al.}, 2004] – one of the largest known herbaceous flowering plant in the world. It includes banana and plantains [Evans, 2002]. Banana, an antique fruit crop known as ‘Apple of the Paradises’ has played interesting and important roles in the history of human civilizations.
Banana blossom is usually considered as a byproduct of banana cultivation. India is the world’s largest producer of banana with 14.20 million tons and it is grown almost in every state. Among the states Tamilnadu ranks first in banana cultivation with a production area of 118.04 hectares in the crop year 2013-2014. The by-products of banana cultivation are estimated at about 220 tones of plant mass per hectare. Banana blossom is having tremendous nutritional value and health effects. It is consumed as a vegetable either raw or cooked by some ethnics in the Asian region. Instead of being treated as an agricultural waste banana blossom can be utilized as an ingredient in food formulations.

Although India is the leading country in banana cultivation the nutritional properties and health benefits of banana blossom are less focused by researchers. Banana blossom is considered as an indigenous food and its consumption is limited due to the tedious preparation procedure. Considering all these factors the present study was done to analyze the nutritional composition and antioxidant properties of two cultivars (Poovan and Monthan) in India.

MATERIALS AND METHODS

Banana blossom sample:
Musa spp. “Poovan” and “Monthan” the two most popular and accessible banana flowers were selected for this study. The two varieties were obtained from the local market of Thanjavur, Tamilnadu. Two to three layers of outermost bract were removed and the samples were washed thoroughly in running water. Compared to Monthan variety Poovan variety was small in size and was dark purple in colour. Poovan variety was having an initial weight of 0.463 g approximately and that of Monthan variety was 0.653g. For most of the analysis dried samples were used.

Proximate Analysis of Banana Blossom:
Samples of banana flower were analyzed for proximate composition (moisture, protein, fat, ash and total dietary fiber) following the standard methods published by Association of Official Analytical Chemists (AOAC, 1995). Moisture content was estimated by gravimetric measurement of weight loss after drying the sample in an oven at 105°C until constant weight was obtained. Protein was determined by Kjeldahl method (Kjeldahl, 1883), and thereafter a conversion factor of 6.25 was used to calculate the total nitrogen to crude protein. Crude fat was analyzed by the Soxhlet extraction method. The content of ash was measured by gravimetric measurement of the sample in the furnace at 550°C until the constant weight was achieved. Crude fiber was determined according to the AOAC enzymatic gravimetric method (1995).

Preparation of Banana Blossom Powder (BBP):
The blossoms were cut into a thickness of (≈) 5mm, directly into 0.5% citric acid solution in order to reduce enzymatic browning. The slices were immersed in the citric acid solution for 30 min. After that the water was drained and the banana blossom slices were spread over the trays. Banana blossom slices were dried at 600°C.
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for 12 hrs, ground in a mixer grinder into a particle size of 40 mesh, packed in polyethylene bags and then stored at 50°C prior to further analysis.

**Proximate Analysis of Banana Blossom Powder:**
Banana blossom powder was analyzed for proximate composition (moisture, protein, fat, ash and crude fiber) following the standard methods published by Association of Official Analytical Chemists (AOAC, 1995).

**Preparation of Banana Blossom Extract (BBE):**
Banana blossom extracts were prepared by using two different solvents-Water and Ethanol. Water and ethanol extracts of both the varieties were prepared by the following method. Fifty gram of BBP (both Poovan and Monthan variety) was shaken in the solvent medium (water or ethanol) for 6 h in a shaker. After shaking water and ethanol extracts were filtered by using a watman filter paper. The residues obtained after filtration were dried overnight and were extracted twice with the solvent medium (Water or ethanol) by shaking for 1 h. The obtained extracts were again filtered by using watman filter paper. The combined extracts were concentrated by evaporating the solvent in a rotary evaporator. The obtained extracts were stored in amber colored air-tight containers at -4°C, until further use.

**Antioxidant Activity of BBE:**
DPPH radical scavenging was monitored according to the method of Yen and Chen (1995) with minor modification. The prepared ethanolic and water extracts were used for free radical scavenging activity test. Extracts were taken at concentrations of 0.1, 0.2, and 0.3 ml and 6 ml of 0.004 % of DPPH in 80% methanol was added to all test tubes. The test tubes were incubated for 30 min at room temperature in dark. The absorbance was read against a blank at 517 nm.

\[
\% \text{ Radical scavenging activity (RSA)} = \frac{A \text{ control} - AS \text{ A control}}{} \times 100
\]

Where, A control is the absorbance of the control (solution to which no antioxidant was added) and AS is the absorbance of the extract solution.

**Estimation of Total Phenol content:**
The total phenol content of extracts was determined by the Folin-Ciocalteau colorimetric method (Singleton et al., 1999). One ml of the extract solution was mixed with the Folin–Ciocalteau reagent (1 ml) and 7.5% Na2CO3 (3 ml). After 1 h of incubation at room temperature, the absorbance was measured against water at 760 nm (UV-Spectrophotometer). Gallic acid was used for establishing the standard curve and the results were expressed as mg of gallic acid equivalents/g of extract.

**Estimation of Flavonoid concentration:**
The determination of flavonoid was performed according to the colorimetric assay of Kim et al., (2003). Distilled water (4 ml) was added to 1 ml of disopropyl fluorophosphates extract. Then, 5% Sodium nitrite solution (0.3 ml) was added
followed by 10% Aluminum chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1 M Sodium hydroxide were added to the mixture. Immediately after, the volume of reaction mixture was filled upto 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink colour developed was determined at 510 nm. A calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents (CEQ)/100 g sample.

**Estimation of Vitamin E:**
Estimation of vitamin E was carried out according to the method described by Rosenberg (1992). Each banana blossom extract (1.5 ml), standard (10 mg/litre of α – tocopherol dissolved in ethanol) and water was pipetted out in three centrifuge tubes (test, standard and blank). To the test and blank 1.5ml of ethanol was added and to the standard 1.5 ml of water was added. The mixture was centrifuged and 1.5 ml of xylene was added to each tube. One ml of xylene layer was transferred into another stopper tube without including any protein or ethanol and 1 ml of 2,2-dipyridyl reagent was added. The mixture (1.5 ml) was pipetted out into cuvette and the extinction of test and standard was read at 460 nm against blank. After that, beginning with the blank, 0.33 ml of ferric chloride solution was added and the test as well as standard was read at 520 nm exactly after 15 min against blank.

**Statistical Analysis:**
Triplicate analyses were conducted for each sample. The experimental data were expressed as mean ± standard deviations of three separate determinations. One-way analysis of variance (ANOVA) was carried out on the experimental results using flowers species as an independent variable. The significance of differences between means was compared by Tukey's multiple tests at p < 0.05. All calculations were performed using an ANOVA package, IBM SPSS version 21.0 from statistical analysis systems.

**RESULTS AND DISCUSSION**
**Proximate Composition Analysis of Banana Blossom Samples:**
The nutritional composition analyses of banana blossom as well as banana blossom powder are presented in Table 1.
Table 1: Proximate composition (g/100 g) of banana flowers of two cultivars (Poovan and Monthan)

<table>
<thead>
<tr>
<th>Component</th>
<th>Poovan</th>
<th>Monthan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>90.1±1.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.23±0.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein</td>
<td>1.99±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.43±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>0.43±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>3.21±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.42±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>12.82±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.42±0.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>95.23±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.61±0.98&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±S.D, Means in rows with different letters (a-b) are significantly different (p<0.05), based on ANOVA.

The banana flowers of two cultivars were having the similar moisture content (above 90%) implying a very short shelf life of banana blossom. The content of protein varied from 1.43 – 1.99 g/100g and the higher protein content was found in Poovan variety. Fat concentration was generally low in both the samples (0.43 to 0.54 g/100g). The total ash content of both the samples was found to be significantly different (P<0.05) and it varies from 2.42 – 3.21 g/100g. The fiber content of both the flower samples was found to be higher and no significant difference (P>0.05) was found between them. A higher content of fibers in banana flowers indicates that the flowers can be consumed as dietary fiber supplements. Total carbohydrate was calculated by %carbohydrates = 100 - (%protein + %fat + %ash) for purposes of comparison (Sheng et al., 2010)

Table 2: Proximate composition of banana blossom powder of two cultivars (Poovan and Monthan)

<table>
<thead>
<tr>
<th>Component</th>
<th>Poovan</th>
<th>Monthan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>1.76±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.89±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein</td>
<td>1.98±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.29±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>0.41±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>4.19±0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.08±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>15.48±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.32±1.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>93.42±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.17±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±S.D, Means in rows with different letters (a-b) are significantly different (p<0.05), based on ANOVA.

Banana blossom powder was prepared according to the method described in the section 2.3. Proximate analysis of banana blossom powder was also determined (Table 2). The banana blossom samples were dried at 60°C in a tray drier for 12 hrs. In the nutritional analysis, the moisture content of the powdered samples was 1.76 – 1.89 g/100g. Reduction in the moisture content decreases the perishability of food
crops, adds value, and also extends the shelf life (Demirel and Turhan, 2003 and Emperatriz et al., 2008). The levels of protein and fat are found to be low in dried banana blossom powder (1.29 – 1.98 g/100g and 0.41 – 0.46 g /100g respectively) than the fresh sample. Decrease of these macronutrients due to drying may be due to the effect of heat treatment. Losses of these macronutrients by the application of heat have also been reported by Hassan et al., (2007), Akpan and Umoh (2004), and Morris et al., (2004). Carbohydrate content is also lowered in dried samples (93.42 – 95.17 mg/100g). Decrease in protein and carbohydrate contents probably occurred as a result of Maillard reaction; which results in complex changes in food due to the reaction between carbohydrate and protein (Boumendjel and Boutebba (2003), Wiriya et al., (2009)). The decrease in lipid content of the dried samples could be as the result of lipid oxidation. Nutrients have been reported to be lost as a result of chemical changes such as oxidation. Lipid oxidation is known to be increased by many factors such as heat, light and radiation (Savage et al, 2002). The ash and fiber content of the dried banana blossom powder was increased (4.19 – 3.08 mg/100g and 15.48 – 15.32 mg/100g respectively) due to drying, than the fresh sample. The increase in the ash and fiber contents could be as the result of the removal of moisture which tends to increase the concentration of nutrients (Morris et al, 2004).

**Antioxidant Activity (AOA) of Banana Blossom:**

Antioxidant activity was measured by DPPH free radical scavenging activity as explained in section 2.6. The assay of the scavenging of DPPH radical is widely used to evaluate the antioxidant capacity of extracts from different plant materials (Amarowicz et al., 2004). DPPH is a stable organic nitrogen radical and free radical compound with a purple colour which change into a stable yellow compound on reacting with an antioxidant. In brief, the reduction capacity of DPPH was determined by the decrease in its absorbance at 517 nm, which is reduced by the antioxidant (Duh, 1998).

Water and ethanol were used for the extraction of antioxidant components since solvents with higher polarity are much favorable towards extracting the antioxidants from banana inflorescence. Similar results were also obtained by Padam et al (2012). Water, alcohols (methanol, ethanol and isopropanol), acetone and ethyl acetate are proved to be better extracting solvent compared to chloroform, hexane and petroleum ether in which the extracts show almost no antioxidant activity.

The antioxidant activity of ethanolic extract of both Poovan and Monthan variety is shown in Fig 1 and 2. The addition of the flower extract into the DPPH solution caused a rapid decrease in absorbance at 517 nm indicating the excellent scavenging capacity of the flower extracts. High antioxidant activity was shown by ethanolic extracts of both Poovan and Monthan variety than the water extracts and among all the ethanolic extract of Poovan variety was having highest antioxidant activity (82%).
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**Figure 1:** DPPH radical scavenging activity under different concentrations in two flower extracts using water (PW-Water extract of Poovan variety, MW-Water extract of Monthan variety)

**Figure 2:** DPPH radical scavenging activity under different concentrations in two flower extracts using ethanol (PE-Ethanol extract of Poovan variety, ME-Ethanol extract of Monthan variety)

**Determination of Antioxidant Components from Banana Flower Extracts:**

The antioxidant activity of plant materials strongly correlates with their content of the phenolic and flavonoid compounds (Velioglu et al., 1998). Phenolics are plant secondary metabolites which are very important in chelating redox-active metal ions, inactivating lipid free radical chains, and preventing hydro peroxide conversions into reactive oxyradicals as they have been generally recognized. The most common water-soluble antioxidant compounds in plants and foods are the phenolic compounds (Macheix and Fleuriet, 1990). Flavonoids are classified as one of the important group of antioxidant component which is commonly found in fruits and vegetables. Vitamin E is a major lipid-soluble antioxidant in the cell antioxidant defense system and is exclusively obtained from the diet. Table 4.4 summarizes the major antioxidant components present in BBE.
Table 3: Antioxidant components present in banana flower extracts of two cultivars (Poovan and Monthan)

<table>
<thead>
<tr>
<th>Component (mg/g)</th>
<th>Poovan (Ethanol extract)</th>
<th>Poovan (Water extract)</th>
<th>Monthan (Ethanol extract)</th>
<th>Monthan (Water extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenols</td>
<td>13.45±0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.42±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.3±0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.24±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>6.4±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.53±0.42&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.2±0.5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin E (mg/Kg)</td>
<td>1.42±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.04±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.42±0.41&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±S.D. Means in rows with different letters (a-d) are significantly different (p<0.05), based on ANOVA.

Total phenols are expressed as gallic acid equivalents which varied between 9.3±0.36 mg/g and 13.45±0.35 mg/g. Regarding the antioxidant components present in ethanol extracts of banana blossom, the flower sample of Poovan showed a higher phenolic content (13.45 ± 0.35 mg/g) than samples extracted from the Monthan variety. Total flavonoid content is expressed as catechin equivalents, and it varied from 3.2±0.5 mg/100g to 6.4±0.2 mg/100g. The results confirmed that banana flowers are good sources of phenols and flavanoids. Phenolic acids and flavonoids have been reported to be the main phytochemicals responsible for the antioxidant capacity of fruits and vegetables (Bahramikia et al., 2009). The concentration of vitamin E in Monthan variety was found to be significantly lower than Poovan variety (p < 0.05) (Table 4.4). The value found in banana flowers in this study was higher than those reported in other tropical plants (Ching and Mohamed, 2001). The results showed that total phenol content is higher than flavonoid content in the ethanol extract of banana flower samples (Table 3). Therefore, the higher free-radical-scavenging activities of the ethanol extract of banana flower samples may be due to the higher amounts of phenolic compounds in those samples.

Water extracts of both Poovan and Monthan variety were showing a low yield of antioxidant components. There is a significant difference in antioxidant components between the two extracts of banana blossom. However the water extracts of Poovan variety contains a significantly higher amount of antioxidant components than the Monthan variety. This indicates that ethanol is the best solvent for extracting antioxidant component from banana blossom than water extract.

CONCLUSION
This research work has comprehensively investigated the proximate composition and antioxidant properties of banana blossom, which is considered as a by-product of banana cultivation. *Musa* spp. ‘Poovan’ and ‘Monthan’ the most popular and accessible varieties in Tamilnadu were chosen for this study. The analysis of banana blossom revealed their considerable antioxidant properties and nutritional value. Also,
banana blossom powder was found to contain a significant nutritive complement based on their high fiber content. In view of their high nutritional and antioxidant properties, banana blossom can be used in diets in the form of dehydrated flour, and can be easily incorporated into food formulations. The results obtained signify the potential of banana blossom as a source of natural antioxidants including phenols and flavonoids, and among the two varieties, ethanol extract of Poovan variety was having highest antioxidant properties.

REFERENCES


