CHAPTER II

Analysis of bananaplant (*Musa balbisiana*) pseudo-stem juice

II.1 Introduction

Banana plant juice is a transparent fluid which runs out from the pseudo-stem when cut and it represents 95 percent of the pseudo-stem weight. The juice becomes pink when exposed to air and after some time changes to light brown. The pseudo-stem juice is not only important for its medicinal value but also for its other applications in different fields.

Traditional medicine is still practiced and used by about 75-80% of the world population, mainly in the developing countries. India, having a very old and rich tradition of folk medicine for centuries, has provided very simple and effective remedies to various ailments using plants and plant derived compounds.\(^1\) Further herbal products are often perceived as safe and have fewer side effects than synthetic compounds because they are "natural" and their effectiveness can be improved by modern pharmacological methods. In India, research on traditional ayurvedic herbal drugs is being increasingly emphasized since recent past and efforts are being made to explore their known effectiveness in the treatment of ailments for which they have been traditionally applied.\(^2,3\)

Banana pseudo-stem juice is beneficial to health, according to Ayurveda. To make the juice, chopped banana stem and water are ground until it becomes homogeneous. Banana stem juice combined with buttermilk and taken on an empty stomach helps in weight reduction. The juice also heals ulcers, reduces burning sensation and acidity. Its astringent quality helps in blood coagulation. Banana stem is believed to have a cooling effect on the body and hence, is recommended in tropical climates. Other traditional uses of banana trunk juice are

- In China, juice of the trunk applied to scalp to increase hair growth and prevent hair fall.
- In West Africa, used for diarrhea.
- In Cambodia, Java and Malaya, juice from trunk used for dysentery and diarrhea.
The whole plant, roots, stem and flowers of *Musa Sapientum* (MS) are extensively used in traditional system of medicine for various ailments including convulsions. The plant is known to possess diuretic, anti-oxidant, anti-ulcer, hypocholesterolaemic, antioxidant and hypoglycemic. The juice recovered from the banana plant has nutraceutical properties and has a potential use in pharmaceutical industry. It is also taken to cure hemorrhages, cholera, epilepsy and hysteria. The aqueous and ethanol extract of stem of *Musa sapientum* Linn possess potential analgesic activity. An extract obtained from juice expressed from the stem of the plantain banana tree (*Musa sapientum* L., var. *paradisiaca*) induces twitch augmentation in skeletal muscles. The mechanism of this action was investigated in the mouse hemi-diaphragm preparation. Directly evoked twitches and potassium induced (K⁺) contractures were both increased by the extract. Twitch augmentation was partly dependent on extracellular Ca²⁺. The results are consistent with an action of banana pseudo-stem juice on the molecule responsible for excitation-contraction coupling in skeletal muscle resulting malabilization of intracellular Ca²⁺. The juice recovered from the banana plant is taken to cure hemorrhages, cholera, epilepsy and hysteria. The aqueous and ethanol extract of stem of *Musa sapientum* Linn possess potential analgesic activity.

Fresh pseudo-stem juice of *Musa sapientum* (AMS) has anticonvulsant activity which might be due to antioxidant potential of phyto-constituents present in it. Pseudo-stem juice has been shown to contain peroxidase activity of the order of 0.1 enzyme unit/ml. At low pH *Musa paradisiaca* stem juice exhibits lignin peroxidase type activity. Herbal formulated drug named as MTEC consists of aqueous methanol extract of *Musa paradisiaca, Tamarindus indica, Eugenia jambolana and Coccinia indica* treatment of Streptozocin-induced diabetic rat at the ratio of 2:2:1:1 at the dose of 60mg/day for two times a day for 14 days resulted in a significant protection in fasting blood glucose and serum insulin levels along with the testicular function towards the control levels (p<0.05).

Banana pseudo-stem (BPS) was found to be a potential source of polyphenols or natural antioxidants, which can be used as natural antioxidants in the food, nutraceutical, and pharmaceutical industries. The multiple antioxidant property may be
an impetus to increase the consumption of BPS either in fresh or in processed form. The total phenolics (TP) and total flavonoids (TF) in various solvent extracts of pseudo-stem (PS) of different banana cultivars varied from 7.58 to 291 mg gallic acid equivalent (GAE/g of extract) and from 4 to 80 mg catechin equivalent (CE/g of extract), respectively. Acetone extract showed high antioxidant activity (AOA) in all of the *in vitro* models tested, whereas methanol extract exhibited high metal chelating activity. Antioxidants, present in food or in the body at low concentrations, are substances that markedly retard or prevent the oxidation of that substrate. Uses of the synthetic antioxidants are restricted due to their carcinogenicity. Hence, interest has increased in finding naturally occurring antioxidants to replace synthetic antioxidants. Natural antioxidants have the capacity to improve food quality and can also act as nutraceuticals to terminate free radical chain reactions in biological systems. These benefits have been attributed to some of the phytochemical constituents and, in particular, to polyphenols. Preliminary phytochemical analysis reveals the presence of vitamin B, oxalic acid, vitamin C, tannin, glycosides, phenolic compounds, gum etc. in fresh stem juice of *Musa sapientum* (AMS). It is reported that AMS prevents the convulsions possibly through prevention of inhibition of vitamin B₆ metabolism. The anti-convulsion effect of fresh steam juice of *Musa sapientum* might be due to antioxidant potential of phyto-constituents present in AMS.

The juice of the banana pseudo-stem produces a non-depolarizing neuromuscular block. Oxygenation of the extract enhances its potency. Reversals with anti-cholinesterases are transient. Partial reversals in isolated preparations indicate these could be both specific and non-specific binding which could account for blockade after washing. It could be specifically bound to Ach (acetylcholine) receptors in an irreversible way since its action appears similar to that of alpha-BuTX (alpha.-bungarotoxin).

Steel reinforcement could give effectively the deleterious effects of aggressive ions in the environment surrounding the concrete when the latter has been mixed with banana plant juice as anti-corrosive materials. Generally, the percentage of chemical oxygen demand (COD), suspended solids (SS), and turbidity removal by using banana stem juice showed tremendous potential as a plant-based natural coagulant in the
treatment of spent coolant wastewater. High COD, SS, and turbidity removal percentages by the banana stem juice were observed for effluent at pH 7 where percentages were 80.1, 88.6, and 98.5%, respectively.

II.2 Materials and Methods

II.2.1 Materials

Amongst the different species of banana plants available in Assam, the trunk of *Musa balbisiana* (*Bhimkol* or *Athiakol* in Assamese), the seedy variety of banana plant has been selected for this experiment because the plant of this species grows easily in Assam, has resistance to diseases and flood, nutritious fruit and a stout trunk. The post-harvest banana plant (*Musa balbisiana*) was randomly selected and collected from a banana farm at Dhakuakhana, district Lakhimpur, Assam. The plant specimen was authenticated by the Department of Botany, Dhakuakhana College.

II.2.2 Methodology

II.2.2.1 Separation of fresh pseudo-stem juice of banana plant

A fresh pseudo-stem of post-harvest banana plant was taken and the leaf sheaths and tender core (floral stalk) were manually separated from the pseudo-stem. The separated leaf sheaths and tender core were washed well in running water. Juices were extracted from the leaf sheath and tender core separately with the help of a sugar-cane juicer machine by squeezing again and again to extract the juice as much as possible. The volume of the juices were measured after filtration and kept it in an air tight container for chemical and spectroscopic investigation. The juices were preserved in a refrigerator at 7 °C to ensure its freshness. The fibres were dried in the sun light and weighed.

II.2.2.2 Measurement of pH of juice samples

The pH of the juice samples were recorded with the help of a digital pH meter (Eutech Instrument, pH 510, pH/mV/°C meter). Procedure given in the manual was followed. The meter was calibrated using buffer capsules of pH 4, pH 7 and pH 10. The cell of the meter was directly immersed in to the experimental solution and pH was recorded at ambient temperature (24 °C).
II.2.2.3 Methods of inorganic analysis

II.2.2.3.1 Procedures involve in chemical and spectroscopic investigation on juice

Qualitative analysis of the juice samples were done by standard procedure of chemical tests and the presence of the following acid and basic radicals as the major components was confirmed.

Acid radicals: Oxalate (C$_2$O$_4^{2-}$)

Chloride (Cl$^-$)

Nitrate (NO$_3^-$) and

Phosphate (PO$_4^{3-}$)

Basic radicals: Sodium (Na$^+$) and

Potassium (K$^+$)

The concentration of potassium and sodium was determined by flame photometric method, chloride and oxalate by gravimetric quantitative analysis, and nitrate and phosphate by UV-visible spectrophotometric method. The concentration of trace metals was determined by atomic absorption spectroscopy.

II.2.2.3.2 Estimation of oxalate ion (C$_2$O$_4^{2-}$)

The oxalate ion (C$_2$O$_4^{2-}$) in banana pseudo-stem juice was estimated quantitatively by gravimetric method. The pseudo-stem juice was filtered through a Whatman-40 filter paper using vacuum pump. 50 ml of such filtrate was taken in a 200 ml clean beaker and acidified with acetic acid. The acidic solution was heated to boil and precipitated with boiling calcium chloride solution assisted by little ammonium chloride solution for effective precipitation. The solution was allowed to cool, treated with one third of its volume of 90% ethanol, and allowed to stand for 30 minutes. The precipitate was filtered through a pre weighed sintered glass crucible and washed with warm water (50-60°C) for several times until the chloride tested negative in the filtrate. The calcium oxalate was then washed with cold water, five times with ethanol, and several times with small volume of anhydrous diethyl ether. The precipitate was sucked dry at the pump for 10 minutes and outside of the crucible was wiped dry with a clean cloth and kept in a vacuum desiccator for 10 minutes. The weight of the precipitate was regarded as CaC$_2$O$_4$·H$_2$O. Since this rapid method yields result of moderate accuracy, so the crucible with CaC$_2$O$_4$·H$_2$O was heated at 475-525 °C in an electric muffle.
furnace for one hour. Then the crucible was allowed to cool in a desiccator and the precipitate was weighed as CaCO$_3$. This method is most satisfactory, since CaCO$_3$ is non-hygroscopic.

\[
\text{CaC}_2\text{O}_4 \rightarrow \text{CaCO}_3 + \text{CO}_2
\]

The quantity of oxalate ion (C$_2$O$_4^{2-}$) in 50 ml of banana pseudo-stem juice was calculated as

\[
\text{Total weight of C}_2\text{O}_4^{2-} \text{in 50 ml juice} = \frac{\text{Weight of CaCO}_3 \times 88}{100.08} \text{ g}
\]

\[
\text{Concentration of C}_2\text{O}_4^{2-} \text{in the juice} = \frac{\text{Weight of CaCO}_3 \times 88 \times 20 \times 1000}{100.08} \text{ ppm}
\]

II.2.3.3 Quantitative estimation of chloride ion (Cl$^-$)

The quantitative estimation was carried out by gravimetric method. 50 ml of juice was taken in a 250 ml beaker and acidified (tested with litmus) with 0.1 M nitric acid. A silver nitrate solution of 0.1 M was added drop wise with constant stirring to the acidified sample solution until the precipitation completed and then a few drops in excess. Completion of precipitation was tested by allowing the precipitate to settle down and added a few drops of AgNO$_3$ solution carefully to the supernatant solution. The mixture was heated at near boiling point with constant stirring until the whole precipitate coagulated and the supernatant liquid was clear. The mixture was then allowed to cool down to room temperature, and filtered through pre weight gooch crucible. The precipitate was dried in a hot air oven for one hour at temperature maintained in between 130°C to 160°C. The precipitate along with the crucible was cooled to room temperature in a desiccator and weighed. From the difference of this weight and the weight of empty crucible, the weight of AgCl was known from which weight of Cl$^-$ was calculated. The whole process of chloride estimation was carried out in subdued light as AgCl is sensitive to light.

Hence the weight of chloride (Cl$^-$) in 50 ml juice = \[
\frac{\text{Weight of ppt} \times 35.5}{143.5} \text{ g}
\]

Concentration of Chloride ion in the juice = \[
\frac{\text{Weight of ppt} \times 0.24739 \times 20 \times 1000}{\text{ppt}} \text{ ppm}
\]
II.2.3.4 Estimation of nitrate ion by spectroscopic method

The concentration of nitrate ion (NO$_3^-$) in juice sample was estimated using UV spectrometer (U-3210 spectrometer, Hitachi). The nitrate ion absorbs ultraviolet radiation at 220 nm but not at 275 nm. Organic matter, if present, absorbs at both 220 nm and 275 nm. To distinguish between the absorbance of nitrate and the other organic matter, the sample’s absorbance at 275 nm is also measured and an empirical correction factor is applied to the 220 nm measurements to distinguish the nitrate from the organic matter.

**Procedure:**

The juice samples were diluted to ten times of its original volume and acidified using 1 N HCl to prevent interferences due to the absorption of either OH$^-$ or CO$_3^{2-}$ both of which can absorb at 220 nm. Hydrochloric acid is used because Cl$^-$ does not absorb light in 250–290 nm region of the spectrum. The absorbance reading was taken at 220nm and 275 nm on a spectrometer using a distilled water blank with the same amount of HCl.

The value for the experimental absorbance due to NO$_3^-$

\[ \text{Absorbance at 220 nm} - 2 \times \text{absorbance at 275 nm} \]

The standard curve was prepared in the range of 0.0 to 50 mg/L of NO$_3^-$ at the interval of 10 following the same method described for sample. The concentration of nitrate ion NO$_3^-$ was calculated from the following relation obtained from calibration curve.

![Figure II.9: Calibration curve ([NO$_3^-$] against absorbance)](image-url)

\[ y = 0.1021x + 0.9295 \]
\[
x = \frac{y - 0.9295}{0.102} \text{ ppm}
\]

Hence, the concentration of nitrate ion (NO\textsubscript{3}\textsuperscript{-}) in the original juice sample in terms of ppm was calculated as:

\[
\text{Nitrate ion (NO}_3\text{) in juice} = \frac{y - 0.9295 \times 10}{0.102} \text{ ppm}
\]

II.2.2.3.5 Estimation of phosphate by spectroscopic method

The phosphate content in juice samples were estimated by spectroscopic method using UV-visible spectrometer\textsuperscript{31} as per following procedure.\textsuperscript{32} For this experiment, the required reagents were prepared as follows.

a. Ammonium molybdate solution.
   i) Dissolved 5.0 g of ammonium molybdate in 35 ml of distilled water.
   ii) Added 56 ml of concentrated H\textsubscript{2}SO\textsubscript{4} to 80 ml distilled water and cooled.
       The above two solutions were mixed and the volume was made up to 200 ml with distilled water and cooled.

b. Stannous chloride solution
   0.5 g of stannous chloride was dissolved in 20 ml glycerol by heating on a water bath.

c. Standard phosphate solution
   2.194 g of pre dried anhydrous potassium hydrogen phosphate, K\textsubscript{2}HPO\textsubscript{4} was dissolved in distilled water and the volume was made up to 1 lit. The solution was then diluted 100 times (10 ml to 1000 ml). This was standard phosphate solution containing 10 mg P/L (1ml = 0.01 mg P).

Procedure:

First of all, 50 ml of the juice was digested in concentrated HNO\textsubscript{3} and evaporated to dryness. Then 40 ml distilled water was added and filtered. The volume of the filtrate was made up to again 50 ml by adding distilled water. This solution was taken for the experiment. The sample solution was diluted to 5 times of its original volume by adding 40 ml distilled water in 10 ml juice solution. 50 ml of such juice
sample was taken and added 2 ml of ammonium molybdate solution and 5 drops of stannous chloride solution to it. A blue coloured solution appeared. It confirms the presence of phosphate. The optical density reading was taken at 690 nm on a spectrometer using a distilled water blank with the same amount of the chemicals. The reading on the spectrometer was taken after 5 minutes of the addition of the last reagent. The concentration of phosphate was found out with the help of standard curve. The standard curve was prepared in the range of 0.0 to 5.0 mg/L of PO$_4^{3-}$ at the interval of 0.5 following the same method described for the sample.

The concentration of phosphate (PO$_4^{3-}$) in the juice sample was calculated using following relation obtained from calibration curve.

$$x = \frac{y - 0.0777}{0.0331} \text{ ppm}$$

![Calibration curve](image)

**Figure II.9: Calibration curve ([PO$_4^{3-}$] against optical density)**

Hence, the concentration of phosphate ion (PO$_4^{3-}$) in the original juice solution in terms of ppm was calculated as:

Concentration of phosphate ion (PO$_4^{3-}$) in juice = \( \frac{y - 0.0777 \times 5}{0.0331} \) ppm

**II.2.3.6 Estimation of sodium (Na$^+$) and potassium (K$^+$) by Flame Photometry**

Before going to estimate Na$^+$ and K$^+$ ions by Flame Photometry, the juice was subjected to digestion with concentrated nitric acid as in the case of phosphate ion estimation (II.3.3.5). Estimation was carried out using Systronics, Flame Photometer.
128. The sample for Flame Photometry was prepared by diluting 100 times of its original volume by taking 5 ml of sample solution in a 500 ml volumetric flask and volume was made up to the mark with redistilled water. The standard solutions were prepared as follows:

100 ppm solution of Na\(^+\) was prepared by dissolving 0.0254 g of NaCl (GR, 99.5%) in 100 ml of distilled water in a volumetric flask. Similarly 100 ml of 100 ppm K\(^+\) solution was prepared by dissolving 0.0191 g of KCl (GR 99.5%) in 100 ml distilled water. Four other standard solution viz. 40 ppm, 20 ppm, 10 ppm and 5 ppm were prepared from 100 ppm standard solution by dilution technique.

The concentration of Na\(^+\) and K\(^+\) in the original juice in ppm is

\[ \text{Na}^+ \text{ and K}^+ \text{ in the original juice} = \text{Flame Photometer reading} \times 100 \text{ppm} \]

II.2.2.3.7 Estimation of trace metals in juice by Atomic Absorption Spectroscopy

The presence of a few metals in juice was estimated by Atomic Absorption Spectroscopy (Varian Spectra AA-220). The samples for Atomic Absorption Spectroscopy were prepared as follows:

50 ml of juice was made strongly acidic with 6 ml concentrated HNO\(_3\) and evaporated to near dryness. Then, it was cooled to room temperature and dissolved in distilled water and filtered. The filtrate was transferred to 50 ml volumetric flask and the volume was made up to the mark with distilled water. The solution so prepared was taken for the estimation of trace metals by Atomic Absorption Spectroscopy.

II.2.2.4 Methods of organic analysis

II.2.2.4.1 Preparation of ethyl acetate extract and isolation of crude extract

200 ml banana leaf sheath juice was treated with 100 ml ethyl acetate and shaken well and the organic layer separated. This process was repeated twice with 50 ml ethyl acetate each. Combined organic phase was dried over anhydride sodium sulphate for two hours. After filtration, the solvent was removed under vacuum. Traces of solvents were further removed under high vacuum and the residue weighed.
II.2.2.4.2 Isolation of organic components from the crude extract

In the crude extract there were at least five components present (TLC in iodine chamber) out of which two were major. These two components were isolated by column chromatography over silica gel (60-120 mesh) using 15% ethyl acetate in petroleum ether.

II.2.2.4.3 General experimental procedures for the identification of the isolated components

The separated compounds were characterized by melting point, and spectroscopic investigation. Melting points were recorded in a melting point apparatus (Scientific Device, India, type MP-D in open capillary) and were uncorrected. IR spectra were recorded with a FT-IR (Model Perkin Elmer spectrum RX I FT-IR system) as a KBr pallet. All $^1$H NMR spectra were recorded on Bruker-300 MHz spectrometer in CDCl$_3$ as the solvent using TMS as the internal reference. All GC-MS spectra were recorded on Perkin-Elmer-Clarus 600 spectrometer with Elite 5 MS column with dimension 30 m × 250 µm. The injection temperature was fixed at 280 °C. The oven temperature was initially held at 250 °C for 2 minutes, increased to 270 °C at 2 °C/ min and held at 270 °C for 2 min. Helium was used as the carrier gas.

II.3 Results and discussion

II.3.1 Results of inorganic analysis

Banana pseudo-stem is highly rich in water which accounts for about 95% of mass. A sample of raw pseudo-stem weighing 25.00 kg yielded 17.26 litres of juice containing some phyto-chemicals. The analysis has been done for the juices of both the leaf sheath and the tender core separately, and the results are shown in Table II.1

<table>
<thead>
<tr>
<th>Entry</th>
<th>Material</th>
<th>Raw weight (kg)</th>
<th>Volume of juice (L)</th>
<th>Weight of dry fibre (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaf sheath</td>
<td>25.000</td>
<td>17.26</td>
<td>0.831</td>
</tr>
<tr>
<td>2</td>
<td>Tender core</td>
<td>9.900</td>
<td>5.30</td>
<td>0.340</td>
</tr>
</tbody>
</table>
Table II.2: Results of chemical and spectroscopic investigation of leaf sheath juice

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ion</th>
<th>ppm</th>
<th>Methods of determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Na⁺</td>
<td>619.50</td>
<td>Flame photometry</td>
</tr>
<tr>
<td>2</td>
<td>K⁺</td>
<td>2250.00</td>
<td>Flame photometry</td>
</tr>
<tr>
<td>3</td>
<td>Cl⁻</td>
<td>549.60</td>
<td>Gravimetric analysis by silver nitrate</td>
</tr>
<tr>
<td>4</td>
<td>NO₃⁻</td>
<td>136.00</td>
<td>UV spectrometry</td>
</tr>
<tr>
<td>5</td>
<td>C₂O₄²⁻</td>
<td>1820.30</td>
<td>Gravimetric analysis by calcium chloride</td>
</tr>
<tr>
<td>6</td>
<td>PO₄³⁻</td>
<td>25.00</td>
<td>UV spectrometry</td>
</tr>
</tbody>
</table>

Table II.3: Results of estimation of trace metals in the juice of leaf sheath by Atomic Absorption Spectroscopy

<table>
<thead>
<tr>
<th>Entry</th>
<th>Metal</th>
<th>ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Al</td>
<td>1.599</td>
</tr>
<tr>
<td>2</td>
<td>Ca</td>
<td>4.883</td>
</tr>
<tr>
<td>3</td>
<td>Cd</td>
<td>0.016</td>
</tr>
<tr>
<td>4</td>
<td>Co</td>
<td>0.011</td>
</tr>
<tr>
<td>5</td>
<td>Cr</td>
<td>0.009</td>
</tr>
<tr>
<td>6</td>
<td>Cu</td>
<td>0.079</td>
</tr>
<tr>
<td>7</td>
<td>Fe</td>
<td>5.536</td>
</tr>
<tr>
<td>8</td>
<td>Mg</td>
<td>1.450</td>
</tr>
<tr>
<td>9</td>
<td>Mn</td>
<td>0.205</td>
</tr>
<tr>
<td>10</td>
<td>Ni</td>
<td>0.010</td>
</tr>
<tr>
<td>11</td>
<td>Pb</td>
<td>0.032</td>
</tr>
<tr>
<td>12</td>
<td>V</td>
<td>3.349</td>
</tr>
<tr>
<td>13</td>
<td>Zn</td>
<td>1.995</td>
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Table II.4: Results of chemical and spectroscopic investigation of tender core juice

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ion</th>
<th>ppm</th>
<th>Methods of determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Na⁺</td>
<td>642.00</td>
<td>Flame photometry</td>
</tr>
<tr>
<td>2</td>
<td>K⁺</td>
<td>2350.00</td>
<td>Flame photometry</td>
</tr>
<tr>
<td>3</td>
<td>Cl⁻</td>
<td>578.89</td>
<td>Gravimetric analysis by silver nitrate</td>
</tr>
<tr>
<td>4</td>
<td>NO₃⁻</td>
<td>11.80</td>
<td>UV spectrometry</td>
</tr>
<tr>
<td>5</td>
<td>C₂O₄²⁻</td>
<td>1539.80</td>
<td>Gravimetric analysis by calcium chloride</td>
</tr>
<tr>
<td>6</td>
<td>PO₄³⁻</td>
<td>22.00</td>
<td>UV spectrometry</td>
</tr>
</tbody>
</table>

Table II.5: Results of estimation of trace metals in the juice of tender core by Atomic Absorption Spectroscopy

<table>
<thead>
<tr>
<th>Entry</th>
<th>Metal</th>
<th>ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Al</td>
<td>0.714</td>
</tr>
<tr>
<td>2</td>
<td>Ca</td>
<td>1.994</td>
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<tr>
<td>3</td>
<td>Cd</td>
<td>0.002</td>
</tr>
<tr>
<td>4</td>
<td>Co</td>
<td>0.012</td>
</tr>
<tr>
<td>5</td>
<td>Cr</td>
<td>0.015</td>
</tr>
<tr>
<td>6</td>
<td>Cu</td>
<td>0.078</td>
</tr>
<tr>
<td>7</td>
<td>Fe</td>
<td>4.536</td>
</tr>
<tr>
<td>8</td>
<td>Mg</td>
<td>0.011</td>
</tr>
<tr>
<td>9</td>
<td>Mn</td>
<td>0.079</td>
</tr>
<tr>
<td>10</td>
<td>Ni</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>Pb</td>
<td>0.042</td>
</tr>
<tr>
<td>12</td>
<td>V</td>
<td>0.983</td>
</tr>
<tr>
<td>13</td>
<td>Zn</td>
<td>2.391</td>
</tr>
</tbody>
</table>
II.3.2 Results of organic analysis

A brown coloured crude was isolated after ethyl acetate extraction of 200 ml juice from leaf sheath. Further experimental results are shown below.

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of crude extract</td>
<td>2.0018 g</td>
</tr>
<tr>
<td>Weight of crude extract used for column separation</td>
<td>0.5394 g</td>
</tr>
<tr>
<td>Weight of component, code SRN-2</td>
<td>0.239 g</td>
</tr>
<tr>
<td>Weight of component, code SRN-3</td>
<td>0.146 g</td>
</tr>
</tbody>
</table>

II.3.2.1 Spectral data

i) Compound code : SRN-2

State : Yellow solid
Melting point : 72 ºC

IR, $\nu$ cm$^{-1}$ : 521, 760, 814, 981, 1007, 1119, 1169, 250, 1315, 1350, 1431, 1504, 1593, 1674, 2353, 2847, 2932, 3044

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ : 2.36 (s, 3H, Me), 3.84 (s, 3H, ArOMe), 6.60 (d, $J$ = 16.2 Hz, 1H, olefinic), 6.92 (d, $J$ =8.7 Hz, 2H, aromatic), 7.45-7.50 (m, 2H, aromatic, 1H, olefinic).

$^{13}$C NMR (75 MHz, CDCl$_3$)$\delta$ : 27.39, 55.39, 114.43, 125.00, 127.03, 129.97, 143.27, 161.60, 198.43.

MS, m/z : 176 (M$^+$ ~ 50%), 161 (100%), 145 (15%), 133 (45%) 118 (20%), 89 (20%), 77 (15%), 63 (10%), 43 (10%)

ii) Compound code : SRN-3

State : Light yellow crystalline solid
Melting point : 128 ºC
IR, $\nu$ cm$^{-1}$ : 517, 826, 980, 1026, 1173, 1250, 1508, 1597, 1678, 2361.
\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) : 3.86 (s, 6H, ArOMe), 6.94 – 6.99, (m, 6H, 2H olefinic and 4H Aromatic), 7.58 (d, \(J = 7.8\) Hz, 4H, Aromatic), 7.71 (d, \(J = 16.8\), 2H, olefinic)

\(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) : 55.40, 114.07, 123.98, 127.61, 130.09, 142.69, 161.54, 188.87

MS, m/z : 294.1(M\(^+\)~ 100%), 185.9 (50%), 160.9 (40%), 133 (50%), 88.9 (30%), 76.9 (36%)

II.3.2.2: FT-IR, \(^{13}\)CNMR, \(^1\)HNMR and GC-MS spectra of the isolated compounds

Figure II.1: \(^1\)H NMR spectrum of the compound I (Code No. SRN-2)
Figure II.2: $^{13}$C NMR spectrum of the compound I (Code No. SRN-2)

Figure II.3: IR spectrum of the compound I (Code No. SRN-2)
Figure II.4(a)

Figure II.4(b)

Figure II.4(a) and Figure II.4(b): GC-MS of the compound I (Code No. SRN-2)
Figure II.5: $^1$H NMR spectrum of the compound II (Code No. SRN-3)

Figure II.6: $^{13}$C NMR spectrum of the compound II (Code No. SRN-3)
Figure II.7: IR spectrum of the compound II (Code No. SRN-3)
Figure II.8(a) and Figure II.8(b): GC-MS of the compound II (Code No. SRN-3)
II.3.3 Inorganic analysis - Discussion

Quantities of juice extracted from leaf sheath and tender core of banana pseudo-stem of *Musa balbisiana* are shown in the Table II.1. Chemical investigations on the two samples of juice of leaf sheath and the juice of tender core of banana (*Musa balbisiana*) reveal that oxalate (C$_2$O$_4^{2-}$) and chloride (Cl$^-$) are the two major anionic constituents present (Table II.2 and Table II.3). Juice samples also contain nitrate and phosphate, which are present in lesser amounts with compared to the amounts of chloride and oxalate ions. Both the juice samples are rich in oxalate. Higher quantity of oxalate is found in the juice of leaf sheath (1820.3 ppm) but both samples have almost equal quantities of chloride ion. In case of phosphate, it is almost same in both the solutions but present in least quantity (about 25 ppm) among the anionic radicals. The amount of nitrate ion (NO$_3^-$) is almost twelve times higher in the juice of leaf sheath (136 ppm) than that in the juice of tender core (11.8 ppm). Potassium is the major cationic constituent in both the juice samples but potassium is slightly higher in quantity in juice of tender core (2350 ppm) than in the juice of leaf sheath (2250 ppm). Other major cation sodium is present in both the juices almost in equal quantities. Among the trace metals, Fe, Ca and V are the majors followed by Al, Mg and Zn (Table II.4). Fe is the major among the trace metals present in the juice of tender core followed by Zn, Ca and V. Other trace metals detected in the both the juice samples are Co, Cr, Cu, Mn, Pb and Cd.

II.2.4 Organic analysis - Discussion

Two major organic compounds isolated from the leaf sheath juice of *Musa balbisiana* plant are

Compound I: (E)-4-(4-Methoxyphenyl)but-3-en-2-one or (E)-p-methoxybenzylideneacetone (SRN- 2)

Compound II: (1E,4E)-1,5-bis(4-Methoxyphenyl)penta-1,4-dien-3-one or (1E,4E)-bis(p- methoxybenzylidene)acetone(SRN-3)

Their structure and quantities are given in the Table II.6
Table II.6: Isolated organic compounds from juice with relative amounts

<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>Structure</th>
<th>% in crude extract</th>
<th>% in plant juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>(E)-p-Methoxybenzylideneacetone</td>
<td><img src="image" alt="Structure" /></td>
<td>44.34</td>
<td>0.443</td>
</tr>
<tr>
<td>II</td>
<td>(1E,4E)-Bis(p-methoxybenzylidene)acetone</td>
<td><img src="image" alt="Structure" /></td>
<td>27.1</td>
<td>0.271</td>
</tr>
</tbody>
</table>

The $^1$H NMR spectrum of the compound I is shown in Fig II.1. The singlet at $\delta$ 2.36 ppm represents the methyl proton at $\alpha$ position. The singlet at $\delta$ 3.84 ppm indicates the methoxy proton attached with aromatic ring. The doublet at $\delta$ 6.60 ppm (d, $J$ =16.2 Hz) indicates the olefinic proton as trans-isomeric form. Doublet at $\delta$ 6.92 ppm (d, $J$ =8.7 Hz) represents the aromatic proton. The multiplet signal at $\delta$ 7.45-7.50 ppm is due to aromatic and olefinic proton present in the component.

The $^{13}$C NMR spectrum of the compound I is shown in the Fig II.2. The signal at $\delta$ 27.39 ppm represents the methyl carbon attached to the carbonyl carbon and the signal at $\delta$ 55.39 ppm is due to the methoxy carbon attached with the aromatic ring. The olefinic carbons appear at $\delta$ 125.00, 143.27 and the aromatic carbons appear at $\delta$ 114.43, 127.03, 129.97, 161.60 ppm. The signal at $\delta$ 198.43 ppm is due to carbonyl carbon.

The IR spectrum of the compound I is shown in Fig II.3. The IR spectrum of the compound shows a strong band at 1593 cm$^{-1}$ due C=O stretching vibration of $\alpha$, $\beta$ unsaturated ketone and C−O stretching bands at 1119, 1169 and 1250 cm$^{-1}$. The weak signal at 1504 cm$^{-1}$ may be due to C=C stretching frequency. The signals at 2847 and 2932 cm$^{-1}$ are due to C−H stretching frequency.

In GC-MS(Fig II.4), the molecular ion peak at $m/z$ = 176 is nearly 50% of the base peak intensity. Base peak at $m/z$ =161 indicates CH$_3$COCH=CH(C$_6$H$_4$)O$^+$ as the dominating species among the host of ions.
The $^1$H NMR spectrum of the second compound II is shown in Fig II.5. The singlet at $\delta$ 3.86 ppm indicates the six methoxy protons attached to the aromatic rings. The multiplet at $\delta$ 6.94 – 6.99 ppm indicates the two olefinic protons and four aromatic protons. Doublet at $\delta$ 7.57 ppm ($d, J =7.8$ Hz) represents the four aromatic protons. The doublet at $\delta$ 7.71 ($d, J = 16.8$) ppm is due to two olefinic protons present in the compound as trans-isomeric form.

The $^{13}$C NMR spectrum of the compound II is shown in the Fig II.6. The signal at $\delta$ 55.40 ppm is due to the methoxy carbons attached to the aromatic rings. The olefinic carbons appear at $\delta$ 123.98 and 142.69 ppm and the aromatic carbons appear at $\delta$ 114.07, 127.61, 130.09, 161.54 ppm. The signal at $\delta$ 188.87 ppm is due to carbonyl carbon.

IR spectrum of the compound (SRN 3) is shown in the Fig II.7. The IR spectrum of the compound shows a strong band at 1597 cm$^{-1}$ due C=O stretching vibration of $\alpha,\beta$-unsaturated ketone and C–O stretching bands at 1173 and 1250 cm$^{-1}$. The weak signal at 1508 cm$^{-1}$ may be due to C=C stretching frequency. The signals in between 2800 and 3000 cm$^{-1}$ are due to C–H stretching frequency.

In GC-MS(Fig II.8), the molecular ion peak at m/z = 294.1 (100%) is the dominating species among the host of ions.

The two compounds isolated from banana pseudo-stem juice ($E$)-$p$-methoxybenzylideneacetone and ($1E,4E$)-bis($p$-methoxybenzylidene)acetone are known as synthetic compounds, but no natural sources have been reported yet. Both belong to the class of $\alpha,\beta$-unsaturated acyclic ketones. $\alpha,\beta$-Unsaturated ketones have been found useful in the preparation of a wide variety of nitrogen heterocycles. These compounds have been used as substrates for the preparation of anti-cancer, cell-specific triarylpyridines via immobilized bismuth nitrate catalysed cascade reactions. They are extensively used in the preparation of cardiovascular Hantzsch products, many of which are prescribed drugs. $\alpha,\beta$- Unsaturated ketones are easily elaborated to anti-anxiety diazepines which also regulate our central nervous system. When treated with hydrazine, $\alpha,\beta$-unsaturated compounds yield substituted pyrazoles which have a wide spectrum of bioactivity.$^{34}$
\(\alpha,\beta\)-Unsaturated ketones have been widely applied as useful key reagents in organic synthesis. Their use as substrates for a number of reactions such as Michael addition, hydrogenation, epoxidation, cycloaddition, Morita Baylis-Hillman reaction, etc., has stimulated their synthetic advancements. The most common access to \((E)-\alpha,\beta\)-unsaturated ketones is by the Claisen-Schmidt condensation of aldehydes and ketones under basic conditions.  

4-Methoxybenzylideneacetone possesses active pharmacological properties. It has in vitro anti-tumor promoting activities which were evaluated by determining the inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-\(O\)-tetradecanoylphorbol-13-acetate (TPA) in Raji cells and was found to be about one-half of that of benzalacetone (IC\(_{50}\) is 129). 4-Methoxybenzylideneacetone is an active antioxidant and the activity test as a hydroxyl radical scavenger was conducted in vitro by using Halliwell method.

The compound \((1E,4E)\)-bis(p-methoxybenzylidene)acetone, is an analogue to curcumin. Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is a yellow compound isolated from the rhizome of the herb Curcuma longa L., which possesses multifunctional pharmacological properties including apoptosis in a variety of tumor cells. Although curcumin is remarkably non-toxic and has promising anti-inflammatory and anti-cancer activities, its poor bioavailability and pharmacokinetic profiles have limited its application in anti-cancer therapies. During the last decade, synthetic modifications of curcumin, which were aimed at enhancing its bioactivities, have been intensively studied. One of such modified synthetic compound is \((1E,4E)-1,5\)-bis(4-methoxyphenyl)penta-1,4-dien-3-one and can be synthesized by reacting 4-methoxy benzaldehyde with acetone in presence of solid sodium hydroxide. No natural sources of bis(p-methoxybenzylidene)acetone have been reported yet. Bis(p-methoxybenzylidene)acetone has less anti-tumor activity and chemo-preventive activity (IC\(_{50}\) 9.0) than curcumin (IC\(_{50}\) 6.0) in HCT116 \(\mu\)M. It is a low active antioxidant (IC\(_{50}\) 1812.7) where the activity test was carried on as a hydroxyl radical scavenger.

II.4 Conclusion

Banana plant is a potential source of potassium. The juice obtained from banana pseudo-stem (Musa balbisiana) contains considerable amount of potassium along with
sodium and some trace metals such as Fe, Ca, V, Zn, Al, Mg and Mn. Nitrate (NO$_3^-$) and phosphate (PO$_4^{3-}$), which are needed in fertilizer, are also present. Therefore, banana plant juice can be used as an organic fertilizer in the cultivation of different crops.

Harmful effects of synthetic drugs can be avoided if they are replaced by extracts from medicinal plants. Active constituents are present in different plant parts of banana plants like fruit, flower, bark, root etc. *Musa* species, one of the useful plant species, carries a number of beneficial pharmacological effects and comes with a set of variety. Banana plant (*Musa balbisiana*) is an important natural source of $(E)$-$p$-methoxybenzylideneacetone and $(1E,4E)$-bis($p$-methoxybenzylidene)acetone which are better to known only as synthetic compounds. $(E)$-$p$-Methoxybenzylideneacetone is a useful key reagent in organic synthesis and has been used as a substrate for the preparation of anti-cancer, cell-specific triarylpyridines. $(1E,4E)$-1,5-Bis(4-methoxyphenyl)penta-1,4-dien-3-one exhibits anti-angiogenic properties and is useful in the treatment of cancer.

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