Analysis of Fatty acid and Determination of Total Protein, Alkaloid, Saponin, Flavonoid of Bangladeshi Bombax ceiba Linn Leaves and Seeds

Shahin Aziz*1, Shahal Ahmed2, Sharmin Akter Lisa3, Tanzima Parvin2

1Chemical Research Division, BCSIR, Dhaka, Bangladesh
2Departments of Applied Chemistry and Chemical Technology, Islamic University, Kushtia, Bangladesh
3Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Dhaka, Bangladesh

ABSTRACT
Bombax ceiba Linn belongs to the family of Bombacaceae and is an important medicinal plant. In Bangladesh, Bombax ceiba Linn is locally known as “Shimul tree”. The whole part of the plant used as traditional folk medicine for the treatment of antidysentric, anti dihorreal and antipyretic effects. The present communication attempts to evaluate fatty acid analysis by GC-MS spectrophotometer, total protein content by Kjeldahl method and to quantify some active constituents i.e. alkaloid, saponin and flavonoid. The fatty acid compositions of the petroleum ether extract of leaves and seeds of Bombax ceiba grown in Bangladesh were determined by gas chromatography- mass spectrophotometer. 8 compounds were identified from leaves and 13 compounds were identified from the seeds. For both cases Palmitic acid showed higher value. The findings from present study showed the protein content for seeds have higher value (18.89%) than leaves of Bombax ceiba. The present investigation showed that both leaves and seeds of Bombax ceiba contain phytochemicals such as flavonoids, alkaloids and saponins in appreciable quantities. The flavonoid content of leaves was 5.97% and for the case of seeds (5.72%), the alkaloid content for leaves was (9.73%) and for seeds (31.44), the saponin content for the case of leaves (13.90%) and for the case of seeds was (43.58%).

Introduction
Traditional knowledge of various indigenous communities is based on their necessities, instincts, observations, trial and error and long experiences. In this regard, multi-located and multi-ethnic medicinal use of any plant, is a good criterion which definitely increases the credibility of the plant to posses medicinal potential against particular disease. [1] Herbal remedies are gaining their revival as many sufferers shifting from modern drugs and embracing complementary medicine. World wide most clinical useful prescription drugs are of plant origin.[2] Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grown in different parts of the country [3]. In recent years, there has been a gradual revival of interest in the uses of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs. Thus a search for new drugs with better and cheaper substitutes from plant origin are a nature choice.[4] Bombax ceiba is commonly known as silk cotton tree which belongs to the family of Bombacaceae. It is one of the important medicinal plants in tropical and subtropical region in Asia especially in India, Sri Lanka, Pakistan, Malaysia, Myanmar and in Bangladesh. It has number of traditional and medicinal uses in the traditional system of medicine such as Ayurveda, Siddha and Unani. [5]. The plant is known by different names such as red Cotton tree, Indian kapok tree (English), shalmali (Sanskrit), semal (Hindi), Shimul (Bengali), mullilavu (Malyalam) in different languages[6]. According to Ayurveda, the plant has stimulant, astringent, haemostatic, aphrodisiac,
diuretic, antidiarrhoeal, cardiotonic, emetic, demulcent, antisynergic, alternative and antipyretic properties [7,8]. The different parts of the plant, Bombax ceiba Linn are used for medicinal purposes for thousands of years in India, or subcontinent. A paste of leaves and flowers of this tree is employed as external application for skin trouble.[9] Seed oil is used for the manufacture of soaps and lubrication substances. [10]. Seeds are applied on the skin in small pcox and chicken pox [11]. Leaves are used in laxative, haematinic.[12]. The tree is a strong light-demand and fast growing. It grows best on deep sandy loams or other well-drained soils, particularly in valleys, in regions receiving 50 to 460 cm annual rainfall well distributed throughout the year[13]. This plant has the compound leaves which are palmate, digitate, large, spreading, glabrous which has common petiole and the size of leaf is 15-30 cm long. Five leaflets are common in one leaf but sometimes up to the seven leaflets could be found. Within the capsule the plant has many seeds which are obvoid, smooth, 6-9 mm long in size. The plant has bright red flowers which appear in January to March. Theses seeds are oily and surrounded by a thick mass of long silky hairs or floss, hence easily blown about by wind. Floss isolated from its fruits is an excellent material for making padded surgical dressings, insulating material for refrigerators, sound proof covers and walls and as being vermin proof: it is the most suitable for making cushions, pillows and upholstery.[14] The plant is best matchwood resource and useful for reclamation of wastelands and mine spoils. So it can also be utilized to improve the barren soil and gain the economic benefits simultaneously.[15,16]. Several studies have been carried out on the isolation of pharmacologically active compounds on leaves and seeds of Bombax ceiba L[6] but no systematic work has been reported about fatty acid composition analysis of the leaves and seeds of the plant by GC-MS, protein content of leaves and seeds by Kjeldahl method and quantification of some active constituents i.e. alkaloid, saponin and flavonoid content of leaves and seeds of this medicinal plant was done so far. Keeping in mind the wide application of different plant parts of Bombax ceiba Linn in traditional medicine and ayurvedic preparation, fatty acid analysis, total protein content determination and quantification of some secondary metabolites i.e. alkaloid, saponin and flavonoid content of leaves and seeds was carried out.

Materials and Methods:

Collection of plant material: Fully matured fresh leaves and seeds of Bombax ceiba L were collected from chamta village of Natore district, Bangladesh in the month of June 2015 and identified by the taxonomist of Bangladesh national Herbarium, Dhaka, where a voucher specimen (No. 41877) has been deposited. The leaves and seeds of Bombax ceiba Linn were separately air dried. These dried samples of leaves and seeds were powdered using 20 mesh screen in Willey mill and then used for subsequent analyses.

Reagents and Standards

All reagents used were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Petroleum ether (b.p 40-60°C, Merck, Germany) of AR grade, under normal atmospheric pressure was employed for extraction of plant material. Solvent from extract were recovered under distillation and the dried extracts were preserved in a refrigerator.

Determination of Protein Content (Kjeldahl Method)

A known amount of fat free sample (after hot extraction with 40-60°C petroleum ether) of leaves and seeds of Bombax ceiba L was heated separately with conc. H2SO4 in presence of K2SO4 and CuSO4 (98:2) in a long necked Kjeldahl flask. When nitrogen present in the sample, it was converted into (NH4)2SO4. The ammonium sulphate (NH4)2SO4 thus obtained was boiled with excess of 60% NaOH solution (ca.11-16 ml) to liberate NH3 gas which was passed through in a conical flask containing 10 ml of 2% H3BO3 solution until 20-40 ml of distillate was collected (about 15 minutes). Excess NH3 (in the receiving flask) were titrated against 0.1 N sulphuric acid with mixed indicator methyl red and methylene blue (2:1). The end point was obtained at the reversion to the original greenish blue color. A similar procedure was followed in the blank determination[17].

Calculation of Nitrogen and Protein Content

\[
\text{Nitrogen} \% = \frac{(\text{ml.standard acid} - \text{ml Blank}) \times \text{N of acid} \times 14 \times 100}{\text{Weight of sample taken in grams}}
\]

\[
\text{Protein content} \% = \text{Nitrogen content} \% \times 6.25
\]

Extraction of fatty acids and preparation of methyl ester (FAMEs)

The fresh plant material of leaves and seeds of Bombax ceiba L was collected and washed individually from running tap water to remove soil particles and other dust. Then they were dried at room temperature and powdered by Fritsch mortar grinder, Germany. The natural fatty acids were extracted separately from the powder (50 gm) of both leaves and seeds of the plant with petroleum ether (b.p 400C-600C) in a Soxhlet apparatus for 72h. The extracts were concentrated under reduced pressure in a rotary evaporator. The extracts were filtered using Whatman No.1 filter paper and then vacuum distilled to remove solvent completely. The extracts from the leaves of Bombax ceiba was (8.92 % w/w) and seed
oil of *Bombax ceiba* L was (15.44 % w/w). Petroleum ether extracts for both the leaves and seeds of *Bombax ceiba* L were kept in a nitrogen atmosphere in a refrigerator. The fatty acids present in the extracts were converted to fatty acid methyl esters (FAMEs) first and analyzed according to the method reported by Griffin[18] for GC-MS analysis.

The fatty acid composition was determined by analysis of their methyl esters. The fatty acid methyl esters (FAMEs) were prepared by esterification reaction by using BF3-MeOH complex according to AOAC method [19]. 10 mg of extract of leaves/ seeds were taken in a screw capped glass tube. 1 ml of BF3-MeOH complex were added and then heated at 100°C for 1 hour in a water bath. After that it was cooled at room temperature and 1ml of deionized water & 2 ml of hexane were added. The glass tube was vortexed and centrifuged at low RPM for two minutes. The upper layer was collected by means of syringe and kept in closely tight glass vial in refrigerator. Then the prepared FAMEs were ready to analyze.

**Gas Chromatograph-Mass Spectrum Analysis**

GC-MS analysis of the fatty acids of leaves and seeds of *Bombax ceiba* L from petroleum ether extract were carried out on a Agilent 7890A system equipped with mass Spectrophotometer detector and split less injection system. The GC was fitted with a HP-5MS capillary column (30 m X 0.25mm: film thickness: 0.25µm). The temperature program was as follows: injector temperature 260°C, initial oven temperature at 70°C, then increased at 10°C/min to 150°C for 5 min., then 12°C/min to 200°C for 15 min. and then12°C/min to 220°C for 15 min. Helium was used as the carrier gas at 17.69 psi pressure with flow 0.6 ml/min. Samples were dissolved in methanol and 1µl aliquot was injected automatically. MS was set in scan mode. The ionization was electron ionization. The mass range was set in the range of 50-550 m/z. MS spectra of separated components were identified on NIST libraries for fatty acid compositions.

**Alkaloid Determination [20]**

5 gms of leaves and seeds of *Bombax ceiba* L powder sample were weight separately into a 250ml conical flasks and 200ml of 10% acetic acid in ethanol were added, cover the contains by aluminium foil and allow to stand for 2 days then filter. After filtration, the extract was reduced to 1/4th of its original volume on a water bath. To the reduced volume of the extract concentrate ammonium hydroxide was added in drops until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, dried and weighed.

**Saponin Determination[21]**

5 gms of each plant samples (leaves and seeds) of the plant were taken separately in a 250 ml conical flask, 250 ml of 25% ethanol was added, the suspension was heated with continuous stirring on a water bath at about 60°C for 4 hrs. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol and then filtered. The combined extracts were reduced to 40ml over water bath at 90°C. The concentrate mixture was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered into 250 ml conical flask while the ether layer was discarded. The purification process was repeated thrice; 60 ml of n-butanol was added. The combined n-butanol extracts was washed with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated in percentage.

**Flavonoid Determination[22]**

5 gms of each plant samples (leaves and seeds) of the plant were extracted separately in 250 ml conical flasks with 150 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper . 42 (125mm). The filtrate was then transferred into a crucible and evaporated to dryness over a water bath and weighed.

**Result**

In *Bombax ceiba* L, the value of total protein content for the case of leaves was (12.07%) and for the case of seeds (18.89%). The storage of protein of different parts of the plant provided amino acids that are readily used for germination and swelling growth. GC-MS analysis of fatty acids of leaves and seeds of *Bombax ceiba* L from petroleum ether extract showed the presence of 8 compounds for the case of leaves and 13 compounds in the case of seeds. GC analyzed results which include the active principles with their retention time, molecular formula, molecular weight and composition of the fatty acids of leaves and seeds of *Bombax ceiba* L from petroleum ether extract are presented in table-1 and table-2.

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Retention time(min)</th>
<th>Name of the compound</th>
<th>Molecular weight</th>
<th>Molecular Formula</th>
<th>Conc. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>4.07</td>
<td>Capric Acid</td>
<td>172.26</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2.81</td>
</tr>
<tr>
<td>2.</td>
<td>14.11</td>
<td>Myristic Acid</td>
<td>228.37</td>
<td>C&lt;sub&gt;14&lt;/sub&gt;H&lt;sub&gt;28&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.76</td>
</tr>
<tr>
<td>3.</td>
<td>22.33</td>
<td>Palmitic Acid</td>
<td>256.42</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>35.32</td>
</tr>
<tr>
<td>4.</td>
<td>30.86</td>
<td>Stearic Acid</td>
<td>284.47</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;36&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1.20</td>
</tr>
<tr>
<td>5.</td>
<td>31.61</td>
<td>Oleic Acid</td>
<td>282.46</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;34&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>15.38</td>
</tr>
<tr>
<td>6.</td>
<td>33.62</td>
<td>Linoleic Acid</td>
<td>280.44</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>7.85</td>
</tr>
<tr>
<td>7.</td>
<td>36.11</td>
<td>α-Linoleic Acid</td>
<td>278.43</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;30&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>13.98</td>
</tr>
<tr>
<td>8.</td>
<td>37.85</td>
<td>Arachidic Acid</td>
<td>312.53</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;40&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>21.81</td>
</tr>
</tbody>
</table>

Table 1: GC-MS analysis of fatty acids from petroleum ether extract of leaves of *Bombax ceiba* L.
Total 8 fatty acids were identified in the case of leaves and 13 fatty acids were identified from seed oil of *Bombax ceiba* L. The analysis of fatty acid for both cases revealed the presence of saturated (Capric acid, Myristic acid, Palmitic acid, Stearic acid, Arachidic acid, Caprylic acid) and unsaturated fatty acids (Oleic acid, Linoleic acid, α-Linoleic Acid, γ-Linoleic Acid, Palmitolic acid, Elaidic acid, Vacenic acid). For both the case, the major constituents was Palmitic acid (35.32%) with retention time 22.33 and in the case of seed oil, the major constituents was Palmitic acid (26.15%) with retention time 22.88 were identified respectively.

**Figure 1:** Structure of the saturated fatty acid identified from GC-MS analysis of petroleum ether extract of leaves and seed oil of *Bombax ceiba* L.

**Figure 2:** Structure of the unsaturated fatty acid identified from GC-MS analysis of petroleum ether extract of leaves and seed oil of *Bombax ceiba* L.
The present investigation showed that both leaves and seeds of *Bombax ceiba* contain phytochemicals such as flavanoids, alkaloids and saponins in appreciable quantities. The flavonoid content of leaves is (5.97%) and for the case of seeds (5.72%), the alkaloid content for leaves is (9.73%) and for seeds (31.44%), the saponin content for the case of leaves (13.90%) and for the case of seeds is (43.58%).

**Conclusion**

Protein analysis is of great importance in the nutritive determination of different parts of medicinal plants. In *Bombax ceiba* L, the value of total protein content for the case of leaves was (12.07%) and for the case of seed was (18.89%) which indicates seeds contain nutritive value in appreciable quantity. This is the first report of total protein content for different parts of *Bombax ceiba* L, and it can be seen as a potential source of useful drugs. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent. We therefore, suggest further the isolation, purification, and characterization and of the bioactive compounds of the leaf and seed of *Bombax ceiba* L with a view to obtain useful chemotherapeutic agents.

**Acknowledgement**

We are grateful to IFST, BCSIR for giving us the opportunity to do GC-MS analysis of plant materials. We are also thankful to the Director, BCSIR Laboratories, Dhaka for providing necessary facilities to carry out this research work.

**References**