

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Proximate Compositions and Elemental Analysis of Leaf, Root, Stem, and Fruit of *Brideliastipularies*.

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ABSTRACT

The purpose of this present study was to investigate the proximate composition and elemental analysis of *Brideliastipularies* leaf, root, stem, and fruit. The whole part of the plant is used for the treatment of asthma, sexual disease, fever, bronchitis, anemia, abdominal pain, diabetes, diarrhea, dental carries, and rheumatism. During present study, various parts (leaf, root, stem & fruit) of *Brideliastipularies* used to investigate their proximate and mineral composition. Standard protocol was used for the proximate analysis and the quantitative analysis. Atomic absorption spectrophotometer was used for quantitative analysis of various elements. Total 11 important elements were analyzed from different parts of the plant indicated that the plant are enriched in some micro and macro nutrients like Ca, K, Mg, Zn, Na and Mn which are very important for biological metabolic system as well as human health. Among the elements, Ca, Zn, Mn & Cu concentration was found higher in leaf part than other parts. Root part showed high concentration of Mg, stem part showed higher concentration of Fe whereas fruit part showed higher concentration of K and Na than the rest parts.

Keywords: *Brideliastipularies*, Proximate analysis, Atomic Absorption Spectrophotometer, mineral compositions.

<https://doi.org/10.33887/rjpbcs/2021.12.3.4>

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INTRODUCTION

Human beings have depended on nature for their simple requirements as being the sources for medicines, shelters, food stuffs, fragrances, clothing, flavors, fertilizers and means of transportation throughout the ages. For the large proportions of world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, where herbal medicine has continuous history of long use. The development and recognition of medicinal and financial aids of these plants are on rise in both industrialized and developing nations. The foundations of typical traditional systems of medicine for thousands of years that have been in existence have formed from plants. The plants remain to offer mankind with new medicines. Definition, 'traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines'¹. Some of the beneficial properties ascribed to plants have recognized to be flawed and medicinal plant treatment is based on the experimental findings of hundreds to thousands of years. The earliest reports carved on clay tablets in cuneiform date from about 2600 BC are from Mesopotamia; among the materials that were used were oils². Medicinal plants have become a worldwide topic. Herbal medicine is playing a crucial role in healthcare system. The traditional use of medicine based on medicinal plant kingdom present a strong relationship related to health issue, diet & folk healing practice recognized by different culture³. Now a days, the people of developing countries especially the rural people are suffering for malnutrition. Food contains essential ingredients for sustenance of plants and animals⁴. *Brideliastipularies* (*B. stipularies*) is an evergreen climber tree of the family phyllanthaceae⁵. In Bangladesh *Brideliastipularies* popularly known as "patkholi". The synonymous are *clutiastipularies*, *B. scandens*. It grows in shady, moist forest floors. Generally, found in central & eastern forest areas & also found in India, Myanmar. The Trees may grow at place up to a height from the ground level. This tree commonly⁶ found in the hilly areas of Bangladesh, India, Nepal, Srilanka, Myanmar, Iran, Egypt and Turkey.

Brideliastipularies is a climbing shrub or small tree. Leaves small and it is much reduced in the flowering twigs, elliptic oblong. Flowers small, monoecious, and greenish in numerous axillary clusters. Fruit is drupe, small, reddish and turn in to blue black when ripe, generally with the enlarged calyx. Small but mighty, seeds can supply various life-enhancing nutrients such as protein, iron, fiber, and vitamins that can help the body fight diseases and promote good healthy living. Studies have shown that leaf, root, stem, and fruit contain nutritionally important bio-compounds, are also sources of other phyto-compounds which at certain critical levels have significant anti-nutritional effects⁷.

Various parts of *Brideliastipularies* (*B. stipularies*) is used in Ayurvedic Materia medica for treatment of asthma, sexual disease, fever, bronchitis, anemia, abdominal pain, diabetes, diarrhea, dental carries, and rheumatism⁸.

Considering the traditional and medicinal uses of this important medicinal plant *B. stipularies* creates sufficient interest to carry out research on proximate analysis and mineral compositions of the different parts of the plant, since no work has been reported about proximate analysis and mineral compositions of leaf, root, stem & fruit part of the plant so far as a comparative way. So the purpose of this investigation is therefore to determine the proximate analysis and to estimate the mineral composition on leaf, root, stem & fruit part of the plant, *B. stipularies* to justify its uses in traditional and ayurvedic medicinal system.

MATERIALS AND METHODS

Collection of plant material

Fully matured fresh leaf, root, stem, fruit of *B. stipularies* were collected from Jahangirnagar university, Bangladesh in the month of April 2019 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. = 47695) has been deposited. Different parts of the plant, leaves, roots stems, and fruits were separately air dried. These dried samples of leaves, roots stems, and fruits were powdered using a grinder and then used for subsequent analysis.

Proximate analysis

Standard protocols were used for proximate analysis of various parts of the plant *B.stipularies* (leaf, root, stem and fruit).The proximate analysis including moisture, organic matter, ash, acid insoluble ash, water soluble ash, crude fibre, carbohydrate, food energy were determined by the method as described in “ A manual of Laboratory Techniques.”

Mineral Compositions

Chemicals

Chemical used here for the digestion of plant samples such as Nitric acid (69%, Merck India) and Perchloric acid (70%, Merck India). For standard calibration of respective elements Na, K, Ca, Mg, Cr, Fe, Zn, Al, Ni, Cd, Mn standard solution (100mg/ml) were purchased from Hach (Germany). The respective desired standard from the stock solution were prepared using double distilled water.

Ashing and digestion of plant parts

Plant samples were taken separately in a porcelain crucible and heated to about 650C and cooled and was weighed. In each case 2.0g of sample was used. The crucible was placed in the Bunsen burner (at low flow rate gas) until the smoke ceased. Then it is placed in a muffle furnace at 525C for about 8-10 hours to get carbon free white ash. It was then cooled in desiccators and weighed. This procedure was repeated till the color of the ash was changed to almost white as well as constant weight was obtained. About 1.0g ash sample for leaves, stems, flowers and seeds were taken separately in 50 ml volumetric flask and then 15ml 1M HNO₃ acid was added. Then the flask was placed on magnetic stirrer heater in fume hood for four hours at 250C. When the color of the solution was changed to milky solutions, it was cooled for 10 minutes and then 7.5ml concentrated perchloric acid (HClO₄) was added. Then it was heated until colorless solution was obtained. The sample was filtered through 0.45 micron filter paper. The pH of the sample for all cases was maintained and verified to be less than 2.0 prior to analysis. The standard working solution of interest was prepared to make the standard calibration curve. Absorption for a sample solution used the calibration curve to determine the concentration of element in that sample.

Analytical procedure

Among all elements only Sodium (Na) and Potassium (K) were estimated by using flame photometer (Model AnA-135, OSK, Japan). Most of the elements like Calcium, Magnesium, Chromium (19), Iron (Fe), Zinc (Zn), Aluminum (Al), Copper (20), Nickel (Ni), Lead (Pb), Cadmium (Cd) and Manganese⁹ in leaves and seeds were analyzed by using Atomic Absorption Spectrophotometer (Varian, AA 240FS, Australia) which was equipped with flame and graphite furnace. For the experiment, air acetylene flame mode was used. The condition fixed with acetylene 1.8 l/min and air 15 l/min, argon gas flow for inert atmosphere. The instrumental default temperature parameters were automatically fixed for each element analysis.

Determination of Protein Content (Kjeldahl Method)

Protein content was determined through Micro-Kjeldahl method (Ranganna, 1986) using 2 gm of dried powder sample of leaves, roots stems, and fruits of the plant separately and according to the following equation¹⁰.

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

Protein content (%) = Nitrogen content % × 6.25

RESULTS AND DISCUSSION

Proximate analysis

Proximate composition of leaves, roots, stem and fruits of *B.stipularies* were recorded and the results are presented in Table 1.

Table 1: Proximate composition (%) of *B.stipularies* Leaf, Root, Stem &Fruit.

| Test parameters | Leaf | Root | Stem | Fruit |
|--------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | Percent (%) Composition | Percent (%) Composition | Percent (%) Composition | Percent (%) Composition |
| Moisture | 7.39 ±0.23 | 2.06±0.23 | 13.49±0.22 | 5.00±0.21 |
| Total Ash | 7.53±0.20 | 7.17±0.21 | 3.84±0.21 | 3.93±0.20 |
| Acid Insoluble ash | 5.68±0.02 | 3.21±0.05 | 4.43±0.05 | 3.15±0.05 |
| Water soluble ash | 93.82±0.02 | 93.41±0.02 | 91.86±0.01 | 93.26±0.02 |
| Crude fiber | 9.05±0.50 | 32.27±0.50 | 6.01±0.50 | 4.94±0.01 |
| Nitrogen Content | 1.50±0.01 | 0.82±0.23 | 0.46±0.23 | 0.90±0.23 |
| Protein content | 9.40±0.02 | 5.10±0.02 | 2.90±0.02 | 5.60±0.02 |
| Carbohydrate | 14.38±0.30 | 10.96±0.30 | 15.11±0.05 | 15.42±0.05 |
| Food energy | 203.12±.02cal/g | 163.24±.02cal/g | 189.04±.02cal/g | 156.08±.02cal/g |

Data are expressed as Mean ± SD (n=3)

The present study showed the moisture content for leaf, root, stem, fruit (7.39%, 2.06%, 13.49%, 5.00%) respectively. Moisture content is among the most vital factors considered in food processing, preservation and storage¹¹. The low percentage of moisture obtained indicates that *B.stipularies* leaf, root, stem & fruit have low shelf-life, implying that its long storage could lead to spoilage due to susceptibility to microbial attack¹². Ash content is useful in assessing the quality grading of leaf, root, stem, fruit and also gives an idea of the amount of mineral element present in the plant part¹³. The total ash is particularly important in the evaluation of purity of drug *i.e.* the presence or absence of foreign organic matter such as metallic salts or silica¹⁴. For the case of leaf part the total ash content was found higher (7.53 %) than the rest of the parts of the plant. Crude fiber recorded in the present study (32.27%), indicates the level of non-digestible carbohydrate and lignin in *B.stipularies* root^[14,15]. Fiber is characterized by low or no nutritional value however, its effect on digestive system may help to fight diabetes and lower high blood cholesterol level^[16,17]. Low level crude fiber is considered appropriate¹² because high level can cause intestinal irritation, lower digestibility and decreased nutrient usage¹⁸.

Proteins are major source of energy. It contains essential amino acids responsible for growth and repair of worn-out tissues in humans^[19-22]. From the present study it was found that the leaf part showed higher percentage of nitrogen (1.50%) and protein content (9.40%) than the other part. Proteins should possess the requisite functionality for their successful utilization in various food products. These functional properties are intrinsic physio-chemical characteristics that affect the behavior of properties in food systems during processing, manufacturing, storage and preparation^[23,24].

Acid insoluble ash is an indication of silicate impurity and water soluble ash indicates the content of soluble minerals²⁵. In both cases leaves of the plant showed the higher values than stems, flowers and seeds. Also in the current studies, the amount of acid insoluble ash was found low compare to water soluble ash in all cases.

Carbohydrate gives ready source of energy to the body^[26,27]. The sample (leaf, root, stem & fruit) contains (14.38%, 10.96%, 15.11%, 15.42%) carbohydrate.

Elemental analysis

The result for the mineral composition for different parts of the plants (leaf, root, stem & fruit) are shown in Table 2. The most abundant mineral found in the sample is calcium with the concentration of (3.27,

2.37, 2.20, 2.10) mg/g for leaf, fruit, root & stem, respectively. Calcium is a constituent of bones and helps the body to contract correctly, blood to clot and the nerves to convey messages²⁸.

Table 2: Mineral composition of *B.stipularies* (Leaf, Root, Stem, Fruit).

| Elements | Leaf (dry weight basis, mg/g) | Root (dry weight basis, mg/g) | Stem (dry weight basis, mg/g) | Fruit (dry weight basis, mg/g) |
|----------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|
| K | 2.46±0.05 | 2.78±0.06 | 2.18±0.05 | 2.86±0.06 |
| Ca | 3.27±0.27 | 2.20±0.18 | 2.10±0.17 | 2.37±0.19 |
| Mg | 1.97±0.10 | 2.00±0.10 | 0.5±0.02 | 0.25±0.01 |
| Na | 1.25±0.142 | 2.23±0.254 | 2.00±0.23 | 3.00±0.34 |
| Zn | 1.75±0.20 | 1.01±0.11 | 1.01±0.12 | 0.75±0.09 |
| Fe | 1.86±0.15 | 1.001±0.08 | 2.01±0.16 | 1.75±0.14 |
| Mn | 1.29±0.09 | 0.008±0.05 | 1.15±0.08 | 0.99±0.06 |
| Cu | 1.11±0.08 | 0.75±0.054 | 1.00±0.0721 | 0.70±0.0504 |
| Ni | ND | 0.001±0.002 | 0.005±0.001 | ND |
| Pb | 0.00080±< 0.001 | 0.0008±<0.001 | 0.007±<0.001 | 0.005±<0.001 |
| Cd | 0.0012±<0.001 | 0.002±<0.001 | 0.001±<0.001 | 0.001± <0.001 |

Measured values are mean ± Standard Deviation (24) of three replicate analysis

The 2nd most abundant mineral found in different parts of *B. stipularies* plant is potassium. High concentration of potassium in the body was reported to increase iron utilization²⁹ and beneficial to people taking diuretics to control hypertension and suffer from excessive excretion of potassium through the body fluid³⁰. In the present study potassium concentration was found higher for the case of fruit part (2.86 mg/g) than the rest of the part.

Sodium regulates fluid balance in the body and helps in the, proper functioning of muscles and nerves³¹. In the present study the highest concentration of sodium was found for the case of fruit is (2.37 mg/g). However, there is a need to judiciously consider this samples, especially in sodium and potassium restricted diets. This is important since high dietary sodium is implicated in cardiovascular and renal disorders³².

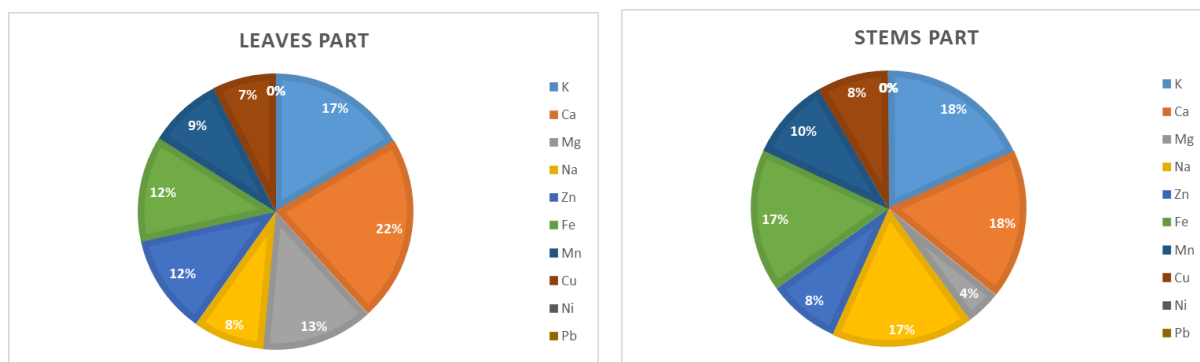


Figure 1: Mineral compositions (mg/g) of Leaves and Stems of *B.stipularies*.

Magnesium plays a key role to improve insulin sensitivity, protects against diabetes and its complications and reduces blood pressure³³. Also Mg involves in many enzymatic reactions of oxidative metabolism of nutrients and cell constituents synthesis, transmission of nerve impulses, body temperature regulation, detoxification, energy production and the formation of health bones and teeth³⁴. The highest average concentration of Mg was found in root part (2.00 mg/g) than the other parts, (1.97 mg/g) for leaves, 0.50 mg/g for stems and for fruit 0.25 mg/g in the present study.

Iron helps in the formation of blood and in the transfer of oxygen and carbon dioxide from one tissue to another³⁵.The highest average concentration of Fe was found in the present study for stem part (2.01 mg/g) than the rest of the part. Zinc boosts the health of our hairs, plays a role in the proper functioning of some sense organs such as ability to taste and smell³⁶, helps in carbohydrate and protein metabolism and also assists in metabolism of vitamin A from its storage site in the livers and facilitates the synthesis of DNA and RNA necessary for cell production³⁶. Leaf part showed the highest average concentration of Zn (1.75 mg/g) than the other parts.

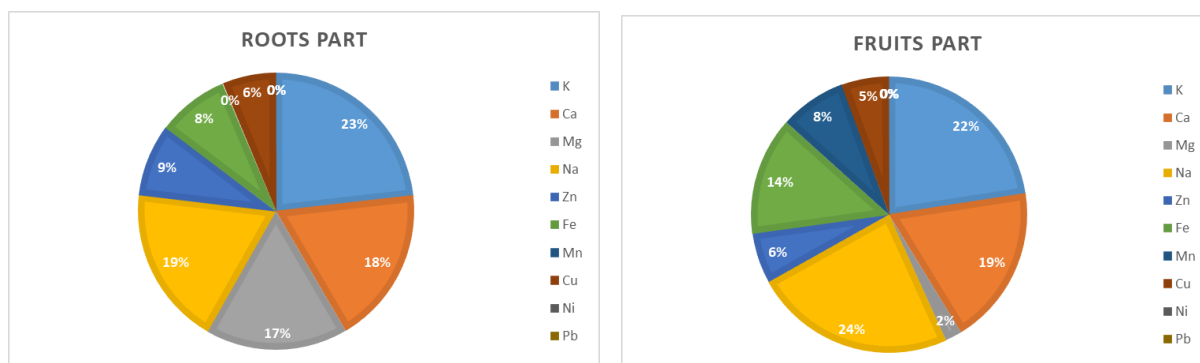


Figure 2: Mineral compositions (mg/g) of Roots and Fruits of *B.stipularies*.

Manganese (Mn) helps the diabetic to metabolize carbohydrates and in treating diabetes³⁷. Also Mn³⁸can help to assist the body in metabolizing protein. The average concentration of Mn was 1.29 mg/g for leaves, 1.15 mg/g for stems, 0.99 mg/g 0.007 mg/g for fruits & 0.008mg/g for root respectively in the present study.

Copper³⁹has a role in energy production, wound healing, taste sensation, skin and hair color⁴⁰. The average concentration of Cu was 1.11 mg/g for leaves, 1.00 mg/g for stems, 0.75 mg/g for roots & 0.70mg/g for fruits in the present study.

The health benefits of nickel (Ni) are optimal growth, healthy skin, bone structure and involved in iron metabolism but it is required in low quantity. Otherwise it may cause toxicity⁴¹. The average concentration of Ni was 0.001 mg/g for roots, 0.005mg/g for steams in the present study.

Lead (Pb) is toxic metal and nonessential element for human body. Pb causes a rise in blood pressure, kidney damage, miscarriage and subtle abortion, brain damage, declined fertility of men through sperm damage, diminished learning abilities of children and disruption of nervous systems^[41-42].The average concentration of Pb was 0.0008 mg/g for leaves& roots, 0.007 mg/g for stems and 0.005 mg/g for fruits in the present study.

The value of toxic element Cd for the case of leaf was 0.0012 mg/g, 0.002mg/g for root and 0.001 mg/g for stem & fruit respectively. The maximum limit for Cd is 0.3 mg/g in herbal medicines and products while the dietary intake limit is 10.3 mg/g which is prescribed by WHO⁴³.

The variation of elemental concentration in different part of the plant due to the environmental factors including atmosphere and pollution, season of collection samples, age of plant and soil condition in which plant grows. These factors may affect the concentration of elements in the plant region to region^[44-45].

CONCLUSION

Plants have contributed immensely to the medical field. It has been the source of most drug sused for combating infections. The proximate compositions of *B.stipularies* suggest that the seeds contain the important constituents needed to combat various kinds of infections in human beings. The high level of minerals elements in various parts of the plant make it useful as human diets or livestock feed and also be as raw materials in pharmaceuticals formulation. Further investigations on the chemical compositions and possible isolation of the active ingredients for specific functions in order to standardize the formulation for efficient medical use would be carried out.

Conflict of interest

The authors declare no conflict of interest with regards to this study.

ACKNOWLEDGEMENT

We are grateful to BCSIR for giving us the opportunity to do mineral compositions analysis by AAS of plant materials. We are also thankful to the Director, BCSIR Laboratories, and Dhaka for providing necessary facilities to carry out this research work.

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