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# Proximate Analysis of Primary Metabolites in Different Parts of *Clitoria ternatea* L. A Comparative Study

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**ABSTRACT**
*Use of Medicinal plants as lifesaving drugs is as old as human civilization. Laboratory evaluations were made to assess the study of primary metabolites of various plant parts in selected plant species Clitoria ternatea L. in the present investigation. Plants of same age group were collected from local areas of Barpeta district of Assam and used for estimation of the primary metabolites (total ash, crude protein, total lipid, crude fiber, soluble carbohydrates, insoluble and soluble minerals). Estimation was done following the AOAC guidelines, 1990. The highest amount of total ash, crude protein, total lipid, crude fiber, soluble carbohydrates, insoluble and soluble minerals was found in leaves, seeds, stem, roots, leaves and stem respectively.*
***Key Words:*** *Medicinal plants, primary metabolites, total ash, crude protein, total lipid, crude fiber, soluble arbohydrates, soluble and insoluble ash.*

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## INTRODUCTION

Evidence of the use of plants for medicinal purposes dates back 60,000 years ago in both Western and Eastern cultures; in both developed and undeveloped countries. Medicinal plants exert their actions on human body by the substances present in various tissues. Many of the plant species have been scientifically evaluated for their possible medicinal applications. India is endowed with a rich wealth of medicinal plants. India recognizes more than 2500 plant species which have medicinal values [1]. A great number of chemicals are biosynthesized by the plants and hence they may be considered the most important source of chemical compounds necessary for the pharmaceutical world. The interest in plants as medicines is now directed towards producing therapeutically semi-purified forms, which are quantifiable, with no serious toxicities and low in cost. This would be of significant value to countries that are poor in financial resources, but are rich in biodiversity.

The North- Eastern Region of India is one of the richest biodiversity hotspots, with more than 300 species of medicinal plants used by different tribal population groups for curing their primary healthcare. Herbal medicine practice is a traditional sign for many of the ethnic communities of this region. *Clitoria ternatea* L. is a medicinal plant used by many of the herbal practitioners to treat many diseases. It is a vigorous, strongly persistent, herbaceous perennial legume. Almost all parts of this plant are reported to have medicinal properties. Flowers of this plant has been using in a number of religious purposes since the ancient times. The plant has been using traditionally to treat infertility, worm infestation, skin disease, tonsillitis, appetizer, digestant, vermicide, cough, asthma etc. Many of the medicinal values are evaluated by many workers such as Anthelmintic [2]; Anti hyperglycemic [3]; Anti-inflammatory [4]; Anti diarrhoeal [5]; Anti-oxidant [6]; hepatoprotective [7]; Immunomodulatory [8]; Anti histamic [9]; cholinergic activity [10] and many more. *C. ternatea* L. is reported to be a good "Medhya" (toning the brain) drug mainly used in the treatment of "Masasika" roga (mental illness), but it is also said to be useful in hectic fever, severe bronchitis, asthma and remedy for snakebite and scorpion sting [11]. A preliminary study using fresh flowers of *C. ternatea* L. showed hypoglycemic and hypolipidemic effects [12].

Primary metabolites are of prime importance and essentially required for metabolic process such as photosynthesis, respiration and nutrient assimilation. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in of pharmaceutical compounds. The present work was designed for proximate analysis of primary metabolites in different parts of the *Clitoria ternatea* L.

## MATERIALS AND METHODS

**Plant Material:**

Whole plant of *C. ternatea* L. was collected from local gardens of Barpeta district, Assam, India in the month of August 2011. For scientific identification of the plant, whole plant of *C.ternatea* was collected, prepared herbarium, authenticated in the department of Botany, Gauhati University, Assam and the voucher no 09193 was collected.

**Estimation of Primary Metabolites:**

Primary metabolites were estimated on dry matter (DM) basis following the AOAC guidelines [16]. Following parameters were studied.

**a. Estimation of total ash:**

A known quantity of sample ranging between 2-5 g was taken in a pre-weighed basin. The content was shared over a heater and then burnt in a muffle furnace for 2 hours at 600±10°c. The desiccated, cooled basin was then weighed with the ash.

$$\% \text{ Total Ash: } \frac{\text{Weight of the ash} \times 100 \times 100}{\text{Weight of the sample} \times \% \text{ DM.}}$$

**b. Estimation of crude protein (CP):**

A known quantity of sample ranging between 2-5 g was transferred to a Kjeidahl flask added 30-35 ml of conc. $H_2SO_4$ and 10-15 g of digestion mixture (anhydrous $Na_2SO_4$ or anhydrous $K_2SO_4$:$CuSO_4$ = 20:1) and kept for overnight avoiding contamination. Next day, the content was heated until a bluish solution was obtained. The Kjeidahl flask was rotated time to time to make proper digestion. The content is then cooled down for solidification to a whitish to off white mass. The mass was then dissolved in distilled water and transferred to a volumetric flask of 250 ml. The volume of aliquot flask is adjusted in the next day with distilled water at 250 ml homogenized and 5 ml aliquot is transferred to micro- Kjeidahl steam distillation assembly. The aliquot was made alkaline with 45% NaOH and the content was subjected for steam distillation. The generated $NH_3OH$ was then collected in a few quantities of 2% Boric acid. Distillation was continued for 10 min. The distillation product was then titrated against N/10 $H_2SO_4$.

$$\%\text{Nitrogen} = \frac{\text{Titer vol. of N/10 } H_2SO_4 \times 0.0014 \times \text{ vol. of aliquot prepared} \times 100 \times 100}{\text{Vol. of aliquot distilled} \times \text{ wt. of sample} \times \% \text{ DM}}$$

**Crude protein % was calculated as follows:**

$$\% \text{ Crude Protein (CP)} = \% \text{ Nitrogen} \times 6.25.$$

**c. Estimation of ether extract (EE) or total lipid:**

A known quantity of sample ranging between 2-5 g was taken in a thimble. The thimble was then transferred to a soxhlet ether extractor and the content was run with Petroleum ether at a temperature between 40°-60°C in winter and 60°-80C in summer. The assembly was then run for at least 90 cycles. The extract was evaporated on moist heat and finally kept in oven for at least 15 min to evaporate the last trace of petroleum ether.

$$\% \text{ Ether Extract} = \frac{\text{Wt. of ether extract} \times 100 \times 100}{\text{Wt. of sample in g} \times \% \text{ DM}}$$

**d. Crude Fiber (CF):**

The fat free sample which was inside the thimble was then transferred to an elongated spouted beaker of 1 lit capacity pre-marked at 200 ml. The sample was then fortified with 25 ml of 10% $H_2SO_4$ and the volume was adjusted at 200 ml with distilled water (1.25% $H_2SO_4$). Stopper the beaker with round bottom flask filled with cold water to act as condenser. Reflux the content for 30 min over a sand bath. The content was washed with water to make acid free. Transferred the content to the beaker and added 25 ml 10% NaOH and adjusted the volume at 200 ml with distilled water. Again reflux the content and made it alkali free. Then the content was dried at 100°C for 8 hours in an oven. Then the content was burnt in a muffle furnace at 600°C±10°C. Desiccate the basin and took the weight.

$$\% \text{ Crude Fiber} = \frac{\text{(Wt. of the dry sample - wt. of the ash)} \times 100 \times 100}{\text{Wt. of the sample taken for ether extract} \times \%\text{DM}}$$

**e. Nitrogen Free Extract or total soluble carbohydrate =**

Nitrogen Free Extract or total carbohydrate was calculated as follows:

100 - (% CP + % EE + %CF + % Total Ash)

**f. Estimation of insoluble ash:**

The total ash possesses the minerals in their oxide forms along with the insoluble ashes. Insoluble ash includes silica, sand etc. It is estimated from the total ash obtained from the burning of the plant material. The total ash obtained in the first experiment was soaked in a few drops of HCl and the content was heated to evaporate the acid. In the next step, few ml of HCl was again added and heated the content till a yellow coloured paste was obtained and allowed to cool. Then a few ml of distilled water was added and heated the content to 60-70$^0$ C. The content was then filtered with distilled water so that the acid is completely removed from the material. The residue was collected and heated at a temperature of 600$^0$C for two hours. The content contained the insoluble ash was weighed after cooling.

**g. Estimation of soluble ash:**

The soluble ash content was estimated as: Total ash – insoluble ash.

## RESULTS AND DISCUSSION

In the present investigation, various plant parts (stem, flower, leaves, seeds and root) of *Clitoria ternatea* L. was used for estimation of proximate constituents viz. total ash, insoluble ash, soluble minerals, crude protein, total lipid, crude fiber and soluble sugar (Table-1). The various plant parts of the plant varied in composition of primary metabolites studied. Maximum content of total ash was found in the leaves and the minimum was recorded in the seed (Fig-1). Insoluble ash content was highest in the leaves whereas there was no insoluble ash recorded in the stem and seed (Fig-2). Soluble mineral content was recorded highest in Stem and lowest in the seeds (Fig-3). Crude protein content was found highest in the seeds and lowest in the roots (Fig-4). The total lipid content was highest in the stem and lowest in roots (Fig-5). Crude fiber content was highest in the roots whereas it was lowest in the leaves (Fig-6). Soluble carbohydrate was observed highest in leaf and lowest in stem (Fig-7).

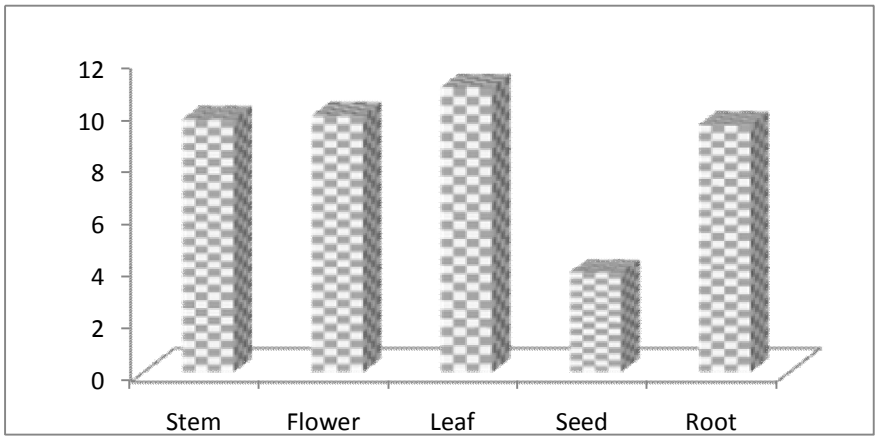


Fig-1: Total Ash concentration in different parts of *C. ternatea* L. (mg/100 g DM)

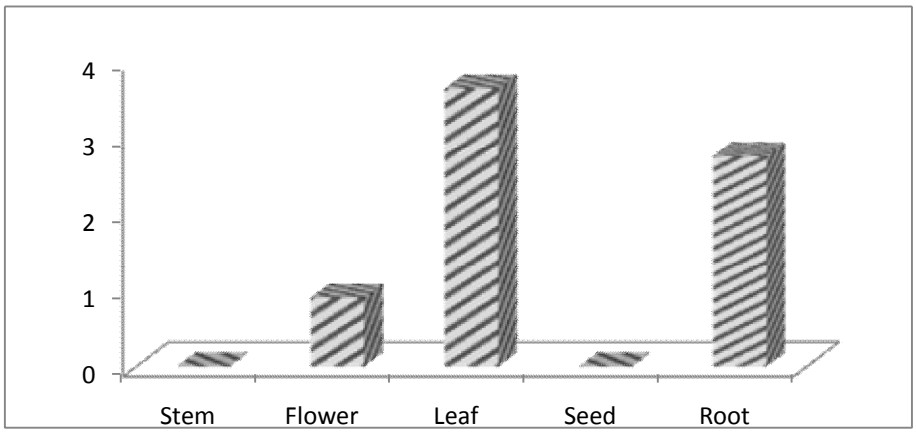


Fig-2: Insoluble Ash concentration in different parts of *C. ternatea* L. (mg/100 g DM)

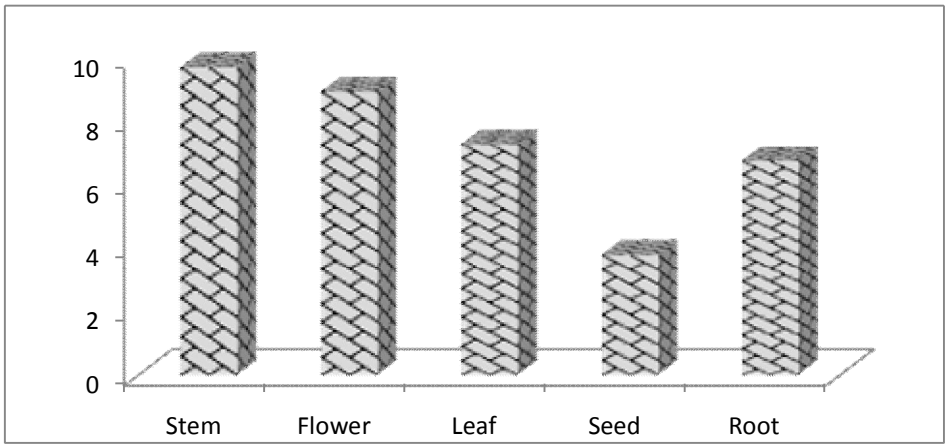


Fig-3: Soluble Mineral concentration in different parts of *C. ternatea* L. (mg/100 g DM)

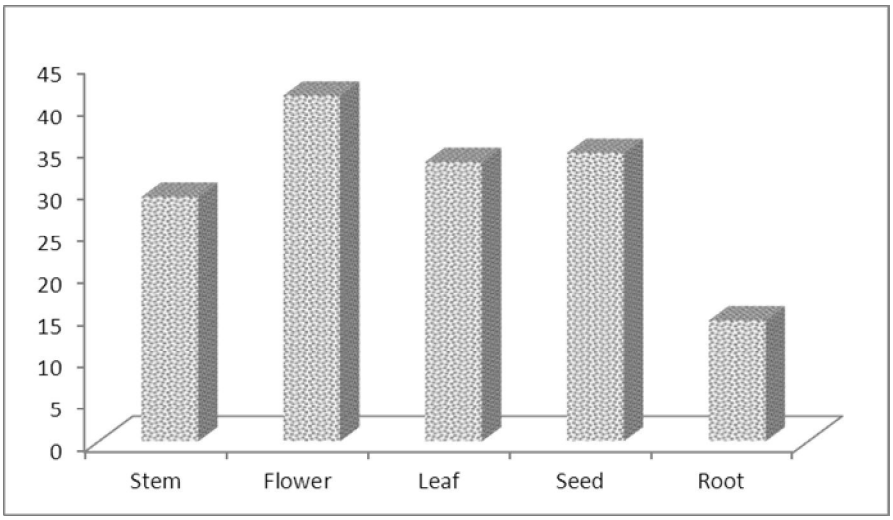


Fig-4: Crude Protein concentration in different parts of *C. ternatea* L. (mg/100 g DM

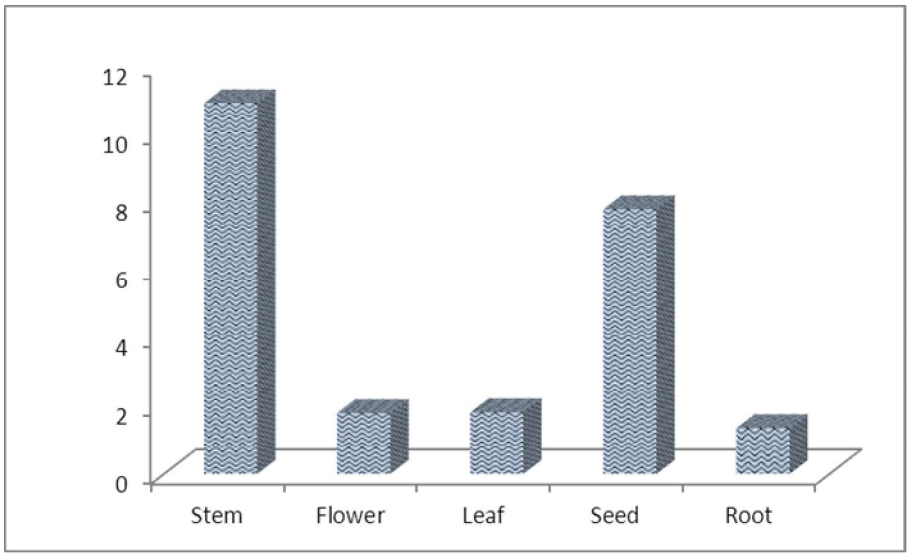


Fig-5: Total Lipid concentration in different parts of *C. ternatea* L. (mg/100 g DM)

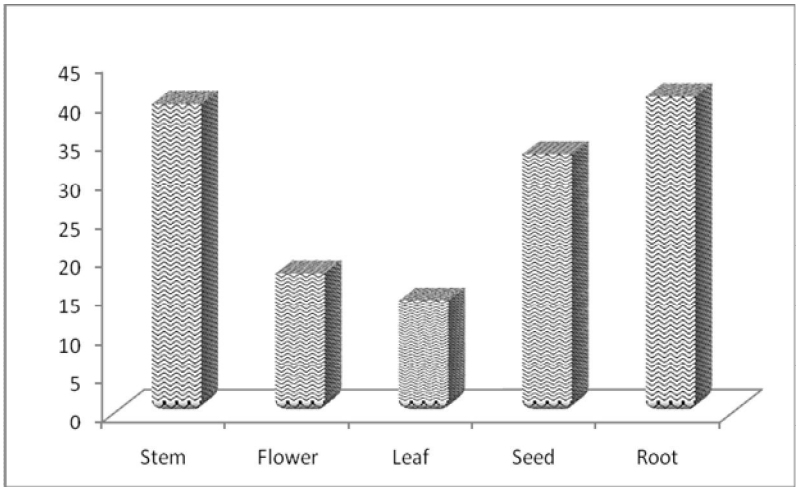


Fig-6: Crude Fiber concentration in different parts of *C. ternatea* L. (mg/100 g DM)

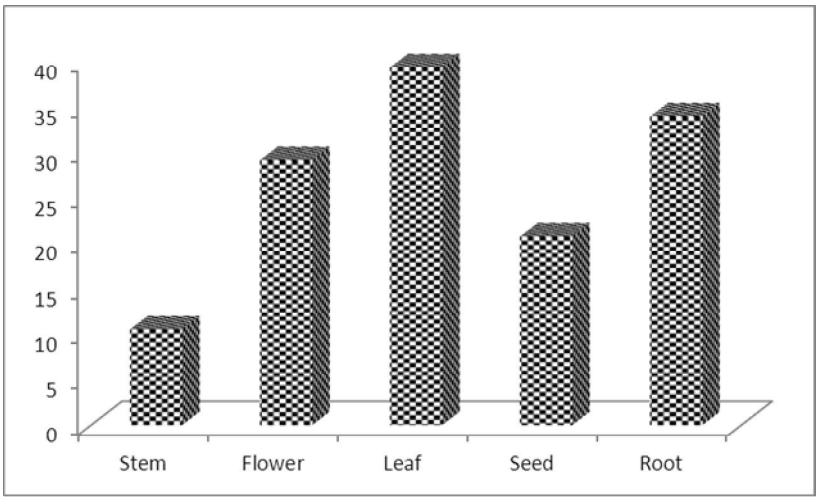


Fig-7: Soluble carbohydrate concentration in different parts of *C. ternatea* L. (mg/100 g DM)

Primary metabolites are of prime importance and essentially required for growth of plants for example; sugars, protein, lipids, starch, Ash. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in of pharmaceutical compounds such as antipsychotic drug .The total ash content of a plant contains both soluble and insoluble minerals. The soluble minerals include all the minerals necessary for the growth and development of the plant and also for the animals. The insoluble ashes are silica, sand etc. in the present investigation it was found that there was no insoluble ash in the stem and seed whereas highest soluble minerals was recorded in the stem. These minerals can be used in the production of some pharmaceutical product. Proteins are the primary components of living things. The presence of higher protein level in the plant indicates the possibility to isolate protein base bioactive compound for the production of protein base drugs besides the food value [13]. Lipid a diverse group of primary metabolites, include reserve plant material such as fats, essential oils, waxes terpnoids and oleoresin**.** Lipids are the supporters and storage molecules of cells. Lipids are being used by industry as highly stable lubricant and as a renewable source of fuel [14]. Plant lipids have developed products that work with diverse requirements, be it culinary, medicinal or cosmetic [15]**.** In the present study total lipid profile showed the highest amount in the stem of the plant indicating the value of the stem. Fibers are the important component for digestion of food materials in animals and in plants it is very important for the strength of the body. Roots of the plants are the main part for which plants are able to withstand with the natural hazards. In the present study maximum amount of fiber has been found in the roots. Almost all organisms use carbohydrates as building blocks of cells and as a matter of fact, exploit their rich supply of potential energy to maintain life. Plant sugars can be used as artificial sweeter and they can even help in diabetes by supporting the body in its rebuilding**.** In the analysis, highest amount of soluble

carbohydrates are found in the leaves of the plant. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. The analysis of different primary metabolites of the plant *Clitoria ternatea* L. showed varied amount in its different parts and thus the plant may be used in the production of pharmaceutical products.

**Table 1:** Concentration of primary metabolites in different parts of *Clitoria ternatea* L**.** (Data expressed in mean **±**SD)

| Plant parts | Name of the metabolites(mg/100g DW) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Total Ash** | **Insoluble Ash** | **Soluble minerals** | **Crude Protein** | **Total Lipid** | **Crude Fiber** | **Soluble Carbohydrates** |
| Stem | 9.71±0.39 | 00.00 | 9.71±0.39 | 29.17±0.66 | 10.91±0.08 | 39.68±0.27 | 10.53±0.04 |
| Flower | 9.84±0.09 | 0.90±0.95 | 8.94±0.52 | 41.27±0.23 | 1.79±0.07 | 17.92±0.06 | 29.18±0.15 |
| Leaf | 10.93±0.29 | 3.64±0.03 | 7.29±0.16 | 33.36±0.23 | 1.81±0.05 | 14.45±0.09 | 39.45±0.25 |
| Seed | 3.80±0.42 | 00.00 | 3.80±0.42 | 43.41±0.14 | 7.78±0.11 | 33.22±0.04 | 20.79±0.08 |
| Root | 9.5±0.50 | 2.75±0.034 | 6.75±0.27 | 14.424±0.45 | 1.351±0.22 | 40.722±0.06 | 34.003±0.74 |

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