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BIOCHEMICAL ESTIMATION OF PRIMARY METABOLITES AND MINERAL COMPOSITION OF *CLITORIA TERNATEA* LINN ROOTS

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ABSTRACT
Use of plant based drugs and chemicals for curing various ailments are as old as human civilization. In recent times, emphasis on plant research has increased and collection as well as evaluation of the traditional use of plants as medicine gets prime importance in the present day scenario. *Clitoria ternatea* Linn has been using since the ancient times for its medicinal values. Almost all the parts of the plant have medicinal property. The root of the plant is reported to have anti -diarrheal, Anti histamic, anti-diabetic, cholinergic activity etc. Traditionally the root has been using for the treatment of many diseases like leucorrhoea, diarrhea, urinary problems, diuretic, impotency, infertility stomach trouble etc. The present work was designed to estimate primary metabolites such as total ash, crude protein, crude fiber, total lipids and carbohydrates along with the mineral composition of the root of the plant *Clitoria ternatea* Linn. Results revealed highest amount of carbohydrate among all the primary metabolites and highest value was recorded for Magnesium among all the minerals under study.

INTRODUCTION

Plants have been an integral part of traditional medicine since the time immemorial. Medicinal plants have their values in the substances present in various plant tissues with specific physiological action in human body. Many of the plant species that provide medicinal herbs have been scientifically evaluated for their possible medicinal applications. *Clitoria ternatea* 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 (Nahar *et al.*, 2010) Anti hyperglycemic (Daisy *et al.*, 2009); Anti-inflammatory (Devi *et al.*, 2003); Anti-diarrhoeal (Upwar *et al.*, 2010); Anti-oxidant (Sarumathy *et al.*, 2011); Hepatoprotective (Solanki *et al.*, 2011); Immunomodulatory (Yogendrasinh *et al.*, 2010); Anti-histamic (Dnyaneshwar *et al.*, 2011); cholinergic activity (Vyawahare *et al.*, 2011) and many more. *C. ternatea* 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 (Chopra *et al.*, 1982). A preliminary study using fresh flowers of *C. ternatea* showed hypoglycemic and hypolipidemic effects (Rajathi and Daisy, 2000). Primary metabolites and minerals directly involved in growth and development of plants. Primary metabolites viz, chlorophyll, amino acid, nucleotides and carbohydrates have a key role in metabolic process such as photosynthesis, respiration and nutrient assimilation. They are used as raw material and food additives. The present study was done to quantify different primary metabolites and minerals in the roots of *Clitoria ternatea* Linn.

MATERIALS AND METHODS

Plant sample collection and identification

Roots of *Clitoria ternatea* were collected from the local gardens of Barpeta district of Assam. The roots were washed properly and dried in shed. For scientific identification of the plant, whole plant was collected, prepared herbarium and submitted in the department of Botany, Gauhati University, Assam. A voucher no.09193 was collected against the submitted herbarium.

Estimation of Primary Metabolites

All the primary metabolites were estimated following AOAC (1990) guidelines.

Estimation of total ash

A known quantity of sample ranging between 2-5g was taken in a Basin. The content was shared over a heater and then burnt in a muffle furnace for 2h at 600 ± 10ºC The desiccated, cooled basin was allowed to cool and the ash content was then weighed. Total ash was calculated as follows:

$$\% \text{ Total Ash} = \frac{\text{Weight of the ash x 100 x 100}}{\text{Weight of the sample x \% DM}}$$

Estimation of crude protein

A known quantity of sample ranging between 2-5g was transferred to a Kjeidahl flask added 30-35mL of conc. H_2SO_4 and 10-15g 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 250mL. The volume of aliquot flask is adjusted in the next day with distilled water at 250mL homogenized and 5mL 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 10min. The distillation product was then titrated against N/10 H_2SO_4.

$$\% \text{ Nitrogen} = \frac{\text{Titer vol. of N/10 } H_2SO_4 \text{x0.0014 x vol. of aliquot prepared x 100 x 100}}{\text{Vol. of aliquot distilled x wt. of sample x \% DM}}$$

The crude protein content was calculated as follows

% Crude Protein (CP) = % Nitrogen × 6.25.

Estimation of total lipid

A known quantity of sample ranging between 2-5g 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 40º-60ºC in winter and 60º-80ºC 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.

$$\% \text{ Total Lipid} = \frac{\text{Wt. of ether extract x 100 x 100}}{\text{Wt. of sample in g x \%DM}}$$

Estimation of crude fiber

The fat free sample which was inside the thimble was then transferred to an elongated spouted beaker of 1L capacity pre-marked at 200mL The sample was then fortified with 25mL of 10% H_2SO_4 and the volume was adjusted at 200mL 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 25mL 10% NaOH and adjusted the volume at 200mL with distilled water. Again reflux the content and made it alkali free. Then the content was dried at 100ºC for 8h in an oven. Then the content was burnt in a muffle furnace at 600ºC±10ºC. Desiccate the basin and took the weight.

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

Estimation of soluble carbohydrates

Soluble Carbohydrate was calculated as follows:

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

Estimation of metals in the plant sample by atomic absorption spectroscopy

The metals Copper (Cu), Magnesium (Mg), Manganese (Mn), Iron (Fe) and Zinc (Zn) were estimated using Flame Atomic Absorption Spectrophotometer (Perkin Elmer, 2380) with air acetylene flame. The aliquot for the estimation were obtained from ash analysis. The standard solutions for calibration and all other required solutions were prepared with distilled water. Pre - reduction was carried out with KI, ascorbic acid and 5 mol/L HCl solutions. Radiation source was electrode less discharge lamp (EDL) and argon gas and 0.5% sodium borohydride ($NaBH_4$) were used for hydride generation.

RESULTS AND DISCUSSION

Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds (Sharma *et al.,* 2012). Plant synthesizes primary metabolites (lipid, protein, starch, sugars, phenol etc). *C.ternatea* roots were evaluated quantitatively for Total ash, Protein, Fat, Fiber and Soluble sugars. Total ash contains both the soluble and insoluble minerals in the sample. It is very important to quantify the amount of mineral in any plant sample. Mineral composition of a plant gives the idea of possibility whether the plant should be used for any medicinal purpose. Plant sample rich in mineral content is a good source of medicine. In the present study total ash content was 9.50%. Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in future (Thomsen *et al.*, 1991). In the present study, protein level was fund to be 14.424% which is a higher level of protein in plants. Lipids are the store of energy which provides mechanical support also. With a strong foundation in research and development, plant lipids have developed products that work with diverse requirements, be it culinary, medicinal or cosmetic (Yadav *et al.*, 2006). In our study, we found 1.351% fat content in the root of the plant under study. Crude fiber is essential for the mechanical strength of plants and it is very essential for the digestion of food materials in the food canal of animals. In the present study, 0.722% fiber was recorded. Almost all organisms use carbohydrates as building blocks of cells and

Table1: Results of the estimation of primary metabolites

S. no	Name of the metabolite	Amount (%)
1.	Total Ash	9.50 ± 0.497
2.	Crude Protein	14.424 ± 0.379
3.	Total Fat	1.351 ± 0.218
4.	Crude Fiber	0.722 ± 0.086
5.	Soluble Carbohydrates	64.003 ± 0.803

Table2: Results of the estimation of plant minerals

S. no	Name of the mineral	Conc. (μg/mL)
1.	Iron	0.251
2.	Magnesium	3.608
3.	Zinc	2.051
4.	Copper	0.230
5.	Manganese	0.453

as a matter of fact, exploit their rich supply of potential energy to maintain life. In the present investigation, 64.003 % soluble carbohydrate was recorded, which is the highest value among all the primary metabolites studied (Table 1), results shown as Mean ± SD). Among the minerals studied, highest value was found for magnesium (Table 2). Iron is a vital mineral for life. It plays an integral role in the transportation of oxygen through the bloodstream. Iron is useful in prevention of anemia and other related diseases (Oluyemi *et al.,* 2006). Iron can also affect brain function as it aids in the synthesis of neurotransmitters. Those with low iron often deal with many symptoms, most commonly fatigue or tiredness, so it is important to keep iron levels up. It is also very essential during pregnancy (http://www.google.co.in. 2012). In the present study, 0.251μg/mL iron was recorded. Magnesium is an essential mineral for staying healthy and is required for more than 300 biochemical reactions in the body. Multiple health benefits of magnesium include transmission of nerve impulses, body temperature regulation, detoxification, energy production and the formation of healthy bones and teeth. Health specialists have always emphasized the importance of including adequate amounts of vitamins and minerals in our daily diet. Zinc, calcium and magnesium are three of the most important minerals essential for good health. Magnesium aids in the absorption of calcium by the body, while zinc actively supports the body's immune system (http://www.google.co.in. 2012). In the present study, highest amount of magnesium among all the minerals was recorded. Zinc is useful for protein synthesis, normal body development and recovery from illness (Muhammad *et al.,* 2011). Zinc is also a powerful antioxidant, helping to prevent cancer, but zinc also is directly involved in proper endocrine function and the maintenance of ideal hormone levels. Zinc deficiency makes both men and women infertile and causes low libido (http://www.google.co.in. 2012).The present study showed 2.051μg/mL zinc in the roots. Copper is an essential trace element that is vital to the health of all living beings. For human beings copper have many uses like working with iron, helping in the creation of red blood cells and in maintaining healthy nerves, blood vessels, bones and immune system. It is essential for the normal growth and development of fetus. In the present investigation, 0.230μg/mL copper was recorded (http://www.google.co.in. 2012). Manganese is a mineral and trace element that plays many essential roles in the body. It aids in the metabolism of food, normal functioning of the nervous system, in the formation of the thyroxine hormone for the thyroid gland, and in the production of sex hormones. Manganese works as an antioxidant to help prevent cancer and heart disease (http://www.google.co.in. 2012). It plays a role in energy production and in supporting the immune system (Muhammad *et al.*, 2011). It also works with vitamin K to support blood clotting and with B complex vitamins to control the effects of stress (Muhammad *et al.,* 2011). In the present study, 0.453μg/mL manganese was recorded. Deficiency of these nutrients and minerals are known to affect the performance and health of poultry (Merck, 2005).

CONCLUSION

The present investigation was designed to investigate the primary metabolites and mineral content of the roots of the plant *Clitoria ternatea* Linn. The study revealed highest amount of soluble carbohydrates and magnesium in the sample. The root has been traditionally used for many diseases like infertility, tonsillitis, cough, worm infections asthma etc. Presence of the mineral content supports the possibility of the plant to be used as a safe medicine. Further investigation is necessary to quantify other plant chemicals or any toxic effects of the plant.

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