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Biochemical study of *Enicostemma Hyssopifolium* (Willd.) Verd

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Abstract
Enicostemma hyssopifolium, a useful medicinal herb was subjected to antimicrobial as well as phytochemical investigations. The petroleum ether, chloroform, n-Butanol, ethanol and aqueous extracts of the whole plant of Enicostemma hyssopifolium were studied for in vitro antimicrobial activity against Human pathogenic microorganisms. The antimicrobial potential of *Enicostemma hyssopifolium* against human pathogenic microorganisms was investigated. Percentage of sodium decreased from roots to seed, although it showed more concentration in leaf than in stem. Phosphorus showed increasing percentage from base towards apex with a slight dip in stem.

Keywords: Biochemical, *Enicostemma hyssopifolium*

1. Introduction
Enicostemma hyssopifolium belongs to family Gentianaceae commonly known as Nava. Its common use in ayurvedic medicine is in antimalarial, abdominal discomfort, colic and for promoting digestion the collection were made from different area of Ajmer district i.e. Rathara, Sagara, Kuthulia and Chorhata for the study of antibacterial and antifungal activity. Medicinal plants have been used for the treatment of various human ailments since long. A revolution came in the medicinal world with the discovery of antibiotics, for treatment of various bacterial infections. However, their indiscriminate use has led to an alarming increase in antibiotic resistance among microorganisms, giving rise to multiresistant strains, which has become a global concern (Shariff, 2001) [5]. Thus, there is a renewed interest in exploring natural resources for such compounds. The need of the hour is to screen a number of new medicinal plants for promising biological activity and there in vitro propagation to conserve the biodiversity (Mathur, *et al.* 2008, Shekhawat, 2001 and Shekhawat, 2009) [3, 6, 7].
The chemical study of plants was made during last 150 years. In several countries wide phytochemical surveys have been carried out for testing the presence of alkaloids, saponin, tanin, carotenoides, terpenes and essential oil etc. in local flora (Fransworth 1966) [2]. About 9000 economic species in world where as India has 5000 species alone, including over 1500 species of medicinal importance. These are used for various kinds of ailments since time immemorial.
The substance which change the character of living protoplasm is called a drug. The science which deals with the study of drugs in called pharmacology and the science which deals with drugs required for prevention and treatment of diseases called pharmacotherapeutics.
India posses the richest source of economic plants in the world. "Ayurveda" has a very authentic treasure of drugs which has undergon chemical and pharmacological trials and many of these tested plants are now used in modern medicine. The plant *Enicostema hyssopifolium* which is called chota kirayata in Hindi is a source of a local Indian myth. It has been a part of Ayurvedic medicine for thousands of the years. From very ancient time it was used cold and flu, Anti-inflammatory, Anti- tumor, Dibetes, HIV, Immune system, Anti-oxidant and liver prodtection, Anti-malerial activity, Respiratory Tract infections and Antimicrobial activity.

2. Material and Methods
Seeds and seedlings were subjected to biochemical analysis. The analysis of protein, soluble nitrogen and amino acids were carefully done in the present investigation.
The estimation of protein and soluble nitrogen was done by the usual (TCA) trichloracetic acid precipitation of proteins and micro-kjeldhal determination (Thimann and Lalorya 1960).
In a small pestal and mortar, the material was kept homogenised in 10 ml of 10% T.C.A. at 4ºC and then the same temperature was maintained for 2 hours to ensure complete precipitation. To get the protein supernatant for the analysis of soluble-N, the precipitate was centrifuged at 400 rpm for 30 minutes. The proteins precipitate was once washed with 5 ml. of 5% T.C.A. and then again centrifuged. The supernatant was added to the Soluble-N fraction which was collected earlier.
In a micro-Kjeldhal flask, soluble-N fraction and protein fraction were digested separately for 8 hours in 10ml. digestion mixture containing 40 gm. of K_2SO_4 10 gm. of $CuSO_4$ and 200 ml. of H_2SO_4 with double distilled water added to make the volume 1000 ml. when water in the flask was evaporated and acid started fuming, a pinch of metal selenium was added as a catalyst. The digestion process was continued for more than 4 hours after the solution had become colourless.
The solution was transferred quantitatively in the distillation apparatus after digestion. 40% Sodium hydroxide was used to liberate ammonia which was trapped in 5 ml. of 2% boric acid containing two drops of indicator. To obtain the indicator, Vol./Vol. 5 parts of 0.2% alcoholic bromocresol green was mixed with one part of 0.2% alcoholic methyl red. Distillation was continued for 5 minutes after the boric acied turned green. The estimation of ammonia trapped in boric acid was done by titration with N/100 H_2SO_4. The nitrogen

content was calculated and expressed as mg protein. The calculation was done by using the following formula.

$$\text{mg N} = \frac{14}{X} \text{ X Volume of } H_2SO_4 \text{ used.}$$

Where N/X = Normality of H_2SO_4, N being replaced by the equivalent weight of nitrogen.

3. Results and Discussion

During the course of present investigation the author has tried to analyse of various parts of *Enicostema hyssopifolium* with the help of authority of Central Drug Research institute Lucknow. The different plant parts, root, stem and leaves were analysed for their nitrogen, sodium, potassium, phosphorus, calcium and magnesium contents. The amino acids separation and identification was also tried during this study.

Table 1: Analysis of moisture and Ash percentage of *Enicostema hyssopifolium* (Willd.) Verd.

Locality	Fresh weight (gm)	Dry weight (gm)	Moisture %	Ash (gm)	Ash %
A	42.373	22.450	47.02	15.03	35.46
B	45.450	24.340	46.44	14.98	32.95
C	43.890	22.850	47.93	15.56	35.45
D	45.750	22.755	46.80	14.55	35.39

Notation: A = Rathara, B = Sagara, C = Kuthulia, D = Chorhata

Analysis of plant material (gm/100 gm) of dry weight.

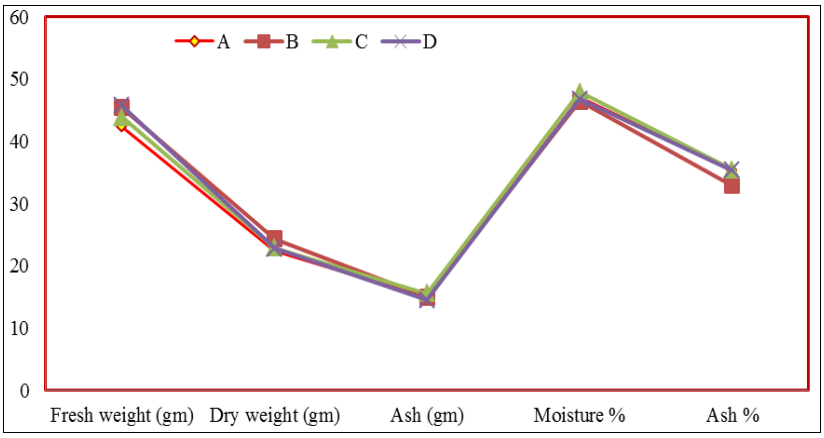

Fig 1: Graphics analysis of moisture and Ash percentageof *Enicostema hyssopifolium* (Willd.) Verd.

Table 2: Chemical analysis of Root, Stem and Leaves of *Enicostema hyssopifolium* (Willd.) Verd.

Plant parts	N	K	Na	P	Ca	Mg
Root	3.302	3.708	0.891	2.348	2.283	2.136
Stem	4.682	8.135	0.345	0.450	3.455	2.253
Leaf	4.631	4.648	0.278	3.378	2.102	1.568

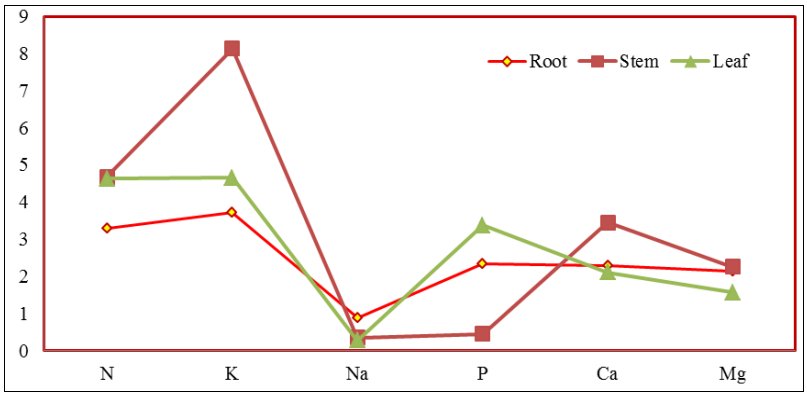

Fig 2: Graphics chemical analysis of Root, Stem and Leaves of *Enicostema hyssopifolium* (Willd.) Verd.

Estimation of Nitrogen

Total nitrogen, protein nitrogen and soluble nitrogen of soaked seeds, seedlings were estimated. Data are given below:

Table 3: Nitrogen contents of *Enicostema hyssopifolium* (Willd.) Verd. per 500 mg of fresh weight.

Seed/ seedling	Fresh weight gms.	Weight			Protein soluble/ Nitrogen ratio
		Total Nitrogen	Protein Nitrogen	Soluble Nitrogen	
Seeds	500	38.852	30.82	8.032	3.83
Seedlings	500	45.703	35.08	10.628	3.30

Values of total nitrogen calculated by adding values of protein and soluble nitrogen.

The results reveal that total nitrogen is maximum in seedlings than seeds. The protein/soluble nitrogen ratio is also high in seedlings than seeds.

During present investigation the author tried to put information in a logical and useful way. The *Enicostema hyssopifolium* is analysed for moisture and ash percentage of differetn localities and also for presence of Nitrogen, Potassium, Sodium, Phosphorus, Calcium and Magnesium in Root, stem and leaves (Tables 2 & 3).

Plant analysis revealed that among major plant nutrients the percentage of potassium showed the highest level in all the parts of the plant. Pallaniappan and Woon (1973) [4] reported similar findings on *Curculigo latifolia*. The nitrogen showed about equal percentage. The maximum level of potassium was found in stem. Percentage of sodium decreased from roots to seed, although it showed more concentration in leaf than in stem. Phosphorus showed increasing percentage from base towards apex with a slight dip in stem. The highest concentration was noted in seed. Calcium is found in increasing order from root to seed. The highest percentage of calcium is found in seed.

Magnesium showed highest level in stem and leaves. The requirement of potassium seems to be the greatest. The nutrients may be arranged in an order of decreasing demand K>N>P>Ca>Mg>Na. This order indicates a preference for potassium by the plant as against an order of N>Ca>K>P, reported for Sehima grassland community (Dakwale, 1975) [1].

4. Acknowledgement

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