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Precursory phytochemical screening and study of physicochemical parameters of Enicostemma axillare (Namme)

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ABSTRACT: It is known that from the last few decades phytochemistry has been the most challenging research topic. Owing to importance of this research field in medicinal chemistry it is necessary to do intensify research in the same.
The 250,000 species of higher plants were the main source of drugs for the world's population. In present world about 119 drugs used in allopathic medicines are still extracted from plants. Phytochemicals are naturally occurring chemicals in plants that provide flavor, color, texture, and smell. They have potential health effects, as they may boost enzyme production or activity, which may, in turn, block carcinogens, suppress malignant cells, or interfere with processes that can cause heart disease and stroke.
This research paper is about togive information of primary phyto-constituents and physicochemical parameters of **Enicostemma axillare (Namme) Family**: Gentianaceae.
KEYWORDS: Phytochemistry, Physicochemical

I. INTRODUCTION

It is belongs to family –Gentianaceae, common name- Indian whitehead, chota chirayata, Namme, Hahli etc. This is a perennial herb found throughout India having Leaves opposite, decussate, sessile, flowers white or bluish, known for its medicinal property from the earlier literature used in folk medicine to treat diabetes mellitus, rheumatisum, abdominal ulcers, hernia, swelling, itching and insect poisoning, anti-inflammatory etc.
The taxonomic position of Enicostemma axillare is as follows
Subdivision**Angiospermae**
Class **Dicotyledonae**
Subclass**Gamopetalae**
Genus **Enicostemma**
Sub Species **Axillare**

Plant Material :-

The whole plants of Enicostemma axillare were collected in fresh condition from Bhilwara (Rajasthan) in drizzling season. Plant indentified by Dr. B.L. Yadav, Rtd. Faculty and Researcher (Taxonomist) (Department of Botany, M.L.V. Govt. College, Bhilwara). The moisture content was removed with the help of soaking paper and then dried under shed to avoid direct loss of phytoconstituent from sunlight. After proper drying whole plant pulverize to a uniform powder using a blender and stored in polythene bags at room temperature. This powder (100gm) ready for further extraction process with the Soxhlet apparatus. The solvent used here was Chloroform and methanol for 15-17 hours and the preliminary study was done (Table C).
Here in this plant the percentage yield of extraction was calculated (Table A)

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Table AShowing percentage yield of Plant Powder of Enicostema axillare by soxhlet extraction method

S. No	Solvent Used	Index of Polarity	Viscosity	Percent yield
1	Methanol	5.5	0.5 (CP)2 ^{0} 0 C	17%
2	Chloroform	4.1	0.3 (CP)2 ^{0} 0 C	4%

Determination of Physical Properties of Extract of Enicostema axillare:-

(1) **Total Ash**: by burning about 3 g accurately weighed, of the finally ground dried plant in a silica dish at a temperature not exceeding 400^{o} C until free from carbon, cool and weigh.

(2) **Determination of Acid Insoluble Ash**: Add 20 ml of dil HCL to the above total ash containing crucible. The matter which doesn't dissolve collect on ash less filter paper and wash with hot water many times for making the filtrate neutral. Transfer this filter paper to crucible and dry on hot plate and ignite to constant weight. Kept the residue in desiccators for one hour and weigh exactly.

(3) **Determination of Water Soluble Ash** : first boil the ash with 25 ml of water and collect insoluble matter on ash less filter paper and burn it for 20 min. now by subtracting the weight of the insoluble matter from the weight of the ash get the weight of water soluble ash. Now one can calculate the percentage of water soluble ash**.**

(4) **Determination of Alcohol Soluble Extractive**: crush 5 g of the air dried drug, roughly powdered, with 100 ml of Ethyle alcohol in closed flask reserved this for 24 hours and filter rapidly. Take 25 ml from the filtrate and dry it in flat bottom dish (at 100^{O} C) and weigh. Now calculate the alcohol soluble extractive with reference to air dried drug

(5) **Determination of Water Soluble Extractive :** crush 5 g of the air dried drug, roughly powdered, with 100 ml of Chloroform-water in closed flask reserved this for 24 hours and filter rapidly. Take 25 ml from the filtrate and dry it in flat bottom dish (at 100^{O} C) and weigh. Now calculate the water soluble extractive with reference to air dried drug

Certain physical parameters given below in Table B

S .NO.	Physical Property measured	Percentage Content
1.	Total Ash	8.50 %
2.	Ethanol Soluble Matter	36%
3.	Acid-Insoluble ash	2.3%
4.	Water Soluble ash	15%
5.	Foreign Organic matter	.25%
6.	Water soluble extractive	35%

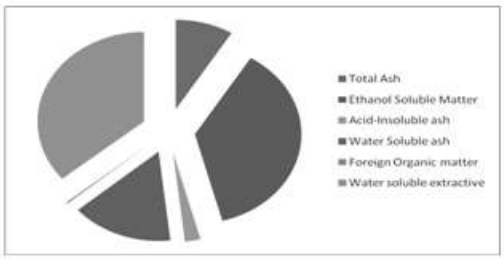

Figure 1 Graphical representation of Physical Properties measured

Preliminary Phytochemical Analysis of Enicostema Axillare:-

1. Steroid:- Steroid in the Extract analyzed by Salkowski Test. First prepared chloroform solution of the extract and shake it with Con. H_2SO_4it gives red colour after standing for few minutes and confirms steroid in the plant.

2. Carbohydrate: - Carbohydrate in the Extract analyzed by Barfoed's Test. Barfoed's reagent (actually a mixture of Acetic Acid and Copper(II) acetate) and extract mixed and boiled. The resultant Cu(II) oxide precipitate is formed which is of red colored confirms the reducing monosaccharide sugars in the plant.

3. Flavonoids: Flavonoids confirmed qualitatively by two methods in Present work

A. H_2SO_4 test: In this I treat the extract fraction with Con. H_2SO_4 the resultant solution shows Orange Colour.

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B. $Pb(C_2H_3O_2)_2$test: In this test, extract treat with lead acetate which gives white precipitate.

Both test was positive and gives confirmation of the Flavonoids in Plant.

4. Proteins:-. Biuret test: To 2 ml of the test solution added 5 drops of 1% copper sulphate solution add to the extract and then mix 5ml of 10% NaOH and mix thoroughly there is Purple colour which confirmed the protein in plant extract.

5. Tannins and Phenolic: - For confirming this Braymer's test applied in which 10 % alcoholic Ferric Chloride solution prepared freshly and treat with plant extract there is observation of sharp blue colour and confirm the preseance of Tannins.

6. Fats and oils: Two experiments were carried out to confirming the presence of fats and oils
A. Stain test: This experiment is little bit trivial, here just press the small quantity of extract between two filter paper and because there was stain on filter paper, it states that there is oil and fat present in plant.
B.Saponification test: Mix few drops of phenolphthalein in small quantity of extracts and then mix few drops of N/2 of Alc. KOH and heat on water bath for 1 hours. There is formation of soap which confirms presence of Oil and Fat.

7. Glycosides :- Two experiments were carried out to confirming
A. W**ith NaOH** : Take a small amount of extract in 2 ml water and add NaOH solution. There is sharp yellow colour appears which indicates presence of Glycosides in the plant.
B. Kellar Killani's test: Mix the extract in water with Glacial acetic acid and $FeCl_3$ and add Con. H_2SO_4 there is brown coloured ring found at the junction which confirms Glycosides in plant.

8. Alkaloids: - approximately 25 gm of extract taken and dissolve in 5 ml of dis. water and finally 2M HCl added to it till acid reacts and filtered. The filtrate was tested for presence of alkaloids.
A. Wagner's test: Add Two drops of Wagner's reagent (Solution of Iodine in KI) to 2ml of the above solution slowly. There is yellow precipitate formed which confirmed alkaloid.
B. Hager's Test: Here also the above filtrate gives yellow precipitate and confirms the presence of alkaloids in plant.

9. Saponins:- there are two test carried out to confirms Saponins

A. Foam Test: Few amount of Extract mix with water and shaken well for 5 minutes. There is formation of stable foam shown which confirms
B. Olive oil test: - There is emultion formation if we add fee drops of Olive oil to extract and shake well. That confirms presence of Saponins in plant.

Qualitative Preliminary Phytochemical Examination of Enicostema Axillare With CH_3OH and $CHCL_3$Chloroform extracts

P=Positive
N=Negative
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S. No.	Pytochemical	Test Used in Present Study	Methanol Ex.	Chloroform Ex.
1.	Steroids	Salkowski	N	P
2.	Carbohydrate	Barfoed's Test	P	P
3.	Flavonoids	H_2SO_4 test Lead Acetate Test	P	N
4.	Proteins	Biuret test	P	P
5.	Tannins & Phenolic	Braymer's test	P	P
6.	Fats and oils	Stain test Saponification test	P	P
7.	Glycosides	By NaOH Kellar Killani's test	P	P
8.	Alkaloids	Wagner's test Hager's test	P	P
9.	Saponins	Foam test Olive oil test	P	N

II. RESULTS & DISCUSSION

The physical properties and preliminary phytochemical test were performed on the extracts of plant of Enicostemma axilare. The plant having number of phytochemicals like Steroids, Flavonoids, Proteins, Tannins, Glycosides, Alkaloids, Saponins and Carbohydrates etc in their extracts. Due to presence of active phytochemicals, Plants can be used medicinally in future.

- In case of Enicostemma axilare the percent yields of methanol and chloroform extracts were found to be 17% and 4% respectively. The physicochemical parameters total ash, ethanol soluble matter, acid-insoluble ash, water soluble ash and foreign organic matter value were found to be 8.50%, 36%, 2.3%, 15%, .25% respectively.
- With the above results we can conclude that total ash value of plant material indicates the amount of minerals and earthy material. The moistures content was found to be very less in both case which ultimately suggest the prevention from bacterial, fungal and yeast growth.
- The present study on physicochemical parameters, preliminary phytochemical analysis, provides important information which may be help in authentication and adulteration for quality control of raw material. The present study adds to the existing knowledge of Enicostema axillare. Although this study is going on in our laboratory to isolate and indentified the pure phytochemicals

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