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Pharmacognostical and Physico-Chemical Evaluation of *Ecbolium viride* (forssk). Alston

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Abstract

Ecbolium viride (Forssk).Alston (Acanthaceae) is a perennial woody under shrub commonly known as Green Shrimp plant. Traditionally different parts of the plant like roots, leaves, stem and whole plant which are used in folklore medicine for several medicinal purposes like cancer, jaundice and rheumatism. The usefulness of this plant is described in many folk books including Ayurveda and is scientifically evidenced and various phytoconstituents were isolated. But no scientific evidences regarding Microscopy, Macroscopy and physic-chemical profile of the plant are available; hence present study is an attempt to investigate the necessary pharmacognostic parameters. The study includes organoleptic characters along with estimation of its physicochemical parameters such as loss on drying, p^H, ash values, extractability in different solvents and Histo-Chemical colour reactions. The generated information of the present study will provide data which is helpful in the correct identification and authentication of this medicinal plant and may help in preventing its adulteration.

Keywords: *Ecbolium viride*, Microscopy, Pharmacognostic evaluation, Physico-chemical Parameters.

INTRODUCTION:

The Indian traditional system of medicine, lays emphasis on promotion of health promotive, disease preventive and rejuvenation approach. In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularize in developing and developed countries owing to its natural origin and lesser side effects. In olden times, patients were treated by preparing drugs according to the requirement. But the scene has been changed now; herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters and etc[1-3]. *Ecbolium viride*(Forssk).Alston (Acanthaceae) is a perennial woody under shrub (Fig.1). It is commonly known as Green Shrimp plant. They can be easily identified by their intense green leaves and greenish blue flowers.The native range of *Ecbolium viride* extends from South and North eastern peninsular part of the country found occasionally in plains and forests of India[4] .Traditionally different parts of the plant like roots, leaves, stem and whole plant which are used in folklore medicine for several medicinal purposes like cancer, jaundice and rheumatism[5-7]. It also possesses pharmacological properties such as anti-microbial, Cytotoxic, Antidiabetic, Analgesic, Anti-trypanosomal, Anti-inflammatory, Anti-Plasmodial, anti-diarrhoeal, hepatoprotective and antioxidant properties. From the above literature, it is clear that no pharmacognostic work is carried out. The present study was therefore undertaken to investigate the pharmacognostical characters, fluorescence analysis and phytochemical analysis of the plant was carried out.

MATERIAL AND METHODS

Plant material:

Fresh aerial parts of plant were collected from the vicinity of Tirumala hills, Chittor district of Andhra Pradesh, India in June 2013 and were authenticated by Dr. M. Venkaiah, Department of Botany. Voucher specimen has been kept in herbarium and preserved for future identification. (EVAU/2013/BGR).

Chemicals and reagents:

All the reagents used were of analytical grade obtained from Sigma Chemical Co. St. Louis, USA and Fine Chemicals Ltd., Mumbai, India.

Microphotography:

The photographs were taken with the help of a Microscope (Nikon Microscope Eclipse 80i).

Macroscopic studies:

The aerial parts of the plant were studied for their macroscopic characters such as size, shape, margin, apex, surface, color, odour, taste, nature and texture.

Microscopic studies:

Free hand transverse sections of leaf and stem were, studied for different microscopic characters and photographs of the sections were taken. All the quantitative parameters were determined following WHO guidelines on quality standards for herbal drugs.

Free Hand Sectioning:

The midrib of the leaf was cut using a sharp razor including a small portion of lamina. The portion of midrib was put between the pith and fine sections were cut with the help of a sharp blade. Fine sections were taken for root and stem also.

Staining:

The cleared sections were transferred to a watch glass containing staining solution (Safranin 1% solution).The sections were allowed to stain for 2-3minutes. The sections

were then transferred to a watch glass containing plain distilled water to wash away excess of stain. The sections were then transferred to a clean micro slide and observed under microscope.

Powder analysis:

The shade dried aerial parts of the plant were powdered and powder was passed through 100 #sieve. A small amount of powder was taken onto a microscopic slide, in 50% v/v glycerol in water. This was then observed under microscope to study the characteristic features.

Physicochemical parameters:

The physicochemical constants such as Organoleptic characters, Fluorescence analysis[8], pH[9], Extractive values[10] , Ash values and Loss on drying were performed according to the officinal methods prescribed in Indian pharmacopeia[11] ,Swelling index, Foaming index, foreign organic matter were performed as per Quality control methods for medicinal plants material by WHO guidelines.

Histochemical color reactions[12]:

The different histo-chemical color reactions were performed on the leaf transverse sections to differentiate the different cell compositions and identification.

Behavior of leaf powder with different chemicals / reagents[13]:

Behavior of leaf powder with different chemical reagents was studied to detect the presence of phytoconstituents with color changes under daylight by reported method.

Preparation of Powder:

The aerial parts of plant were collected, washed in tap water, rinsed with distilled water. Then shade dried in room temperature till there is no loss of weight. The dried material was mechanically powdered, sieved using 80 mesh and stored in air tight container and used for further physicochemical, Phytochemical and fluorescent analysis.

RESULTS AND DISCUSSION:

Macroscopical characteristics:

The morphological studies showed that the plant is a perennial woody under shrub about 1-3 m tall and had a very bitter in taste with characterstic odour. Stems are erect glabrous, with erect branches. Leaves are large (11.5-15cm), elliptic-ovate to obovate, entire or undulate, acute, pubescent, base attenuate tapering to base. Calyx lobes 5, unequal, decurrent, lanceolate, valvate. Flowers are large, sessile, present in opposite pairs, spikes nearly sessile, 5-25 cm long; corolla tube is 3.8 cm long, slightly dilated and laterally compressed at throat. Corolla is tubular, 2-lipped, lobes are 5. Fruits are ovoid and capsule consists of two seeds. They can be easily identified by their intense green leaves and greenish blue flowers shown in **Fig.1**.

Fig.1. A twig of Ecbolium viride (Forssk) Alston.

Microscopical characteristics:

The T.S. of Stem showed a single layered epidermis as outermost covering with numerous non glandular trichomes. The trichomes were unicellular, wavy and few with swollen base were shown in **Fig.2**. Transverse and longitudinal sections of young stem reveals cork cells which are compact ranging from 2-5 layers which are more or less rectangular in shape. Cortex consists of 2-4 layers of chlorenchyma cells followed by parenchyma cells. Groups of lignified pericyclic fibres were scattered in form of ring throughout the cortex. Vascular bundles and pith are large containing compact parenchyma was observed in **Fig.3.**

The T.S. of leaf showed the outermost single layer of rectangular shaped epidermal cells on both the surfaces. These are followed by a single layer of green tubular shaped palisade cells which were absent in the midrib region and on the lower side of lamina. Spongy parenchyma cells are present in the mesophyll. Numerous unicellular and bicellular trichomes are present on both, dorsal and ventral surfaces. Collenchyma cells (2-4 layers) are present in midrib region both below and above upper and lower epidermis, respectively. Stomata and glandular trichomes are present only on lower surface of leaf when observed in surface view were shown in **Fig.4**.

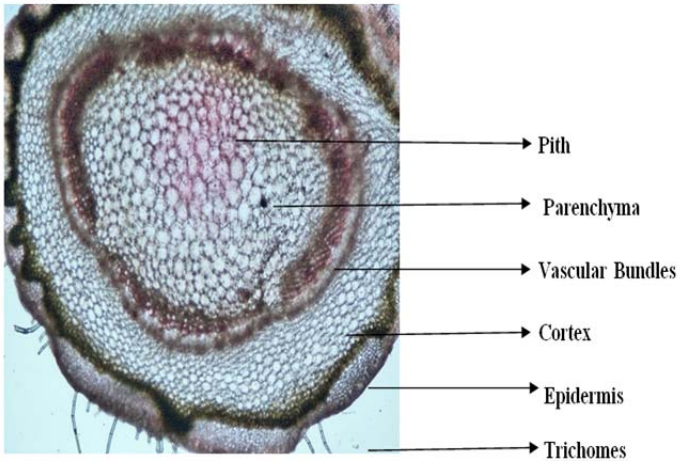

Fig.2. T.S. Of Stem

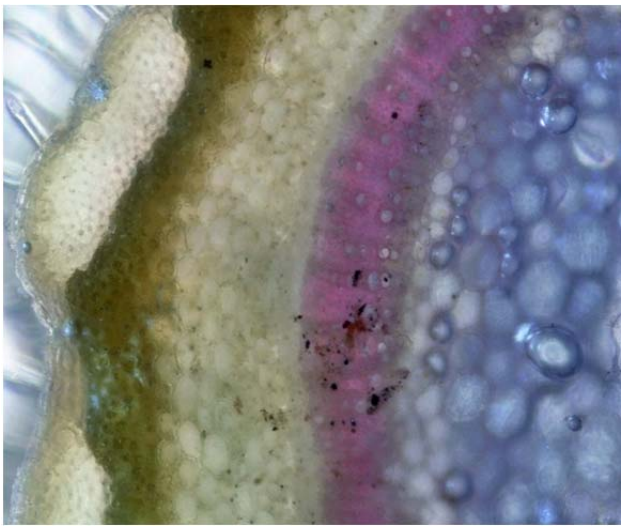

Fig.3. Vascular bundles in Stem

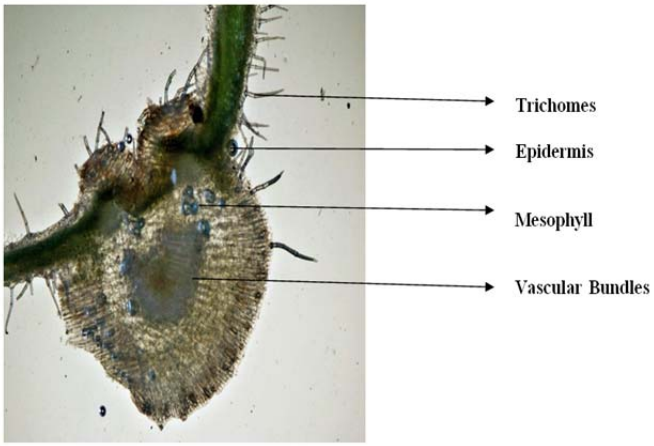

Fig.4. T.S. Of Leaf

Powder microscopy:

The powder of the plant (aerial parts), passed through 100 # sieve, appeared dark green in color. The powder microscopy **(Figure 5)** study showed the presence of stone cells, Calcium oxalate crystals, Sclereids, both anomocytic and anisocyticstomata, covering and multicellular glandular trichomes and non-glandular trichomes, sclerenchymatous layers, fibres and xylem vessels. Vessels were lignified having bordered pits on the surface.

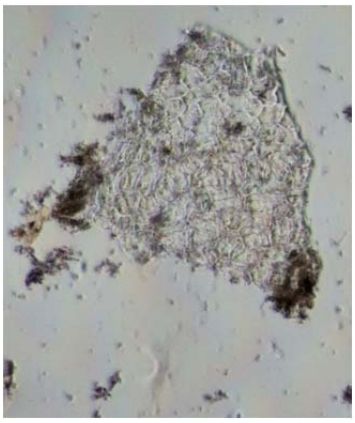

Fig.5. Sclerenchymatous layer

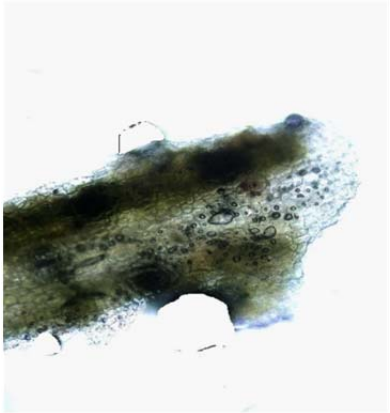

Fig.6. Stem with Idioblast Cells

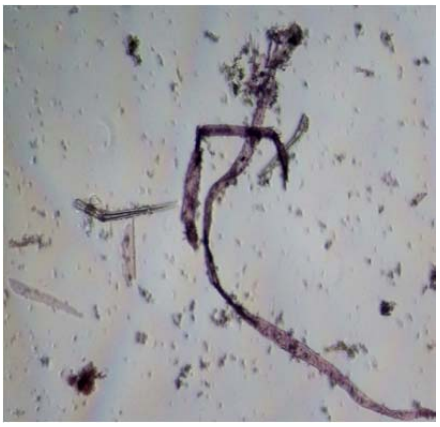

Fig.7. Fibres and Trichomes

Fig.8. Fibre

Physicochemical parameters:

The powder of the plant (aerial parts), was analyzed for various physico-chemical constants and loss on drying (LOD).

Organoleptic characters

The powder was tested to determine organoleptic characters are given in **Table 1.**

Table: 1 Organoleptic Characters

S.NO	Part of the Plant	Color	Taste	Odour
1.	Stem	Green	Acrid	Normal
2.	Leaf	Green	Light bitter	Normal
3.	Root	Brownish yellow	No characteristic taste	No characteristic odour

Fluorescence analysis

The powder was treated with various reagents and the mixture was observed under UV light (254 nm and 365 nm) to see the type of fluorescence and results are given in **Table 2**.

p^H:

1% and 10% aqueous solutions of the powder were prepared to determine the pH range by using standard simple glass electrode p^H meter and results are given in **Table 3.**

Table: 2 Fluorescence Analysis of Powder of Aerial parts

S.NO	Treatment	Day light	Short UV(254nm)	Long UV (365nm)
1.	Dry Powder	Pale green	Green	Green
2.	1N NaOH (Aqueous)	Brownish Green	Green	Brown
3.	1N NaOH (Alcoholic)	Light Green	Green	Brown
4.	5% NaOH	Green	Fluorescent Green	Fluorescent Green
5.	10% NaOH	Green	Dark Green	Brownish Green
6.	Conc. HCl	Brownish Yellow	Brownish Yellow	Black
7.	Conc. H_2SO_4	Slight Brown	Brown	Brown
8.	Conc. HNO_3	Green	Light Green	Black
9.	50% HCl	Green	Pale Green	Brown
10.	50% H_2SO_4	Pale Green	Fluorescent Green	Brown
11.	50% HNO_3	Green	Greenish Black	Black
12.	5% $FeCl_3$	Bluish Green	Black	Black
13.	Picric acid	Green	Fluorescent Green	Fluorescent Green
14.	Acetic acid	Pale Green	Fluorescent Green	Brown
15.	Dil. NH_3	Pale Pink	Violet	Black
16.	Bromine water	Yellowish Brown	Brown	Brown
17.	Iodine solution	Brown	Black	Black

Table: 3 p^H of Powder of Aerial parts

S.NO	Concentration	p^H
1.	1%	10.10
2.	10%	9.60

Table: 4 Extractive values of Powder of Aerial parts

S.NO	Solvent	EV
1.	Hexane	2.569
2.	Dichloromethane	3.449
3.	Chloroform	2.934
4.	Ethyl acetate	2.335
5.	Petroleum ether ($60\text{-}80^0$)	4.27
6.	Methanol	8.125
7.	Water	18.025

Extractive values:

Extracts were prepared with various solvents. Percentages of the extractive values were calculated with reference to air-dried drug and results are given in **Table 4**.

Ash Values, LOD, Swelling index, Foaming index, Foreign organic matter were performed as per the reported methods and the results are summarized in **Table 5**.

Table: 5 Ash values of Powder of Aerial parts

S.NO	Parameter	Values on dry weight basis
1.	Total Ash	23.16% w/w
2.	Acid insoluble Ash	16.97% w/w
3.	LOD	1.66% w/w
4.	Swelling Index	+ 0.73ml
5.	Foaming Index	< 100 (not significant)
6.	Foreign Organic matter	0.05%w/w

Histochemical color reactions:

The different histo-chemical color reactions were performed on the leaf transverse sections to differentiate the different cell compositions and results were given in **Table 6**.

Behavior of leaf powder with different chemicals / reagents:

Behavior of leaf powder with different chemical reagents was studied to detect the presence of phytoconstituents with color changes under daylight and the results were shown in **Table 7**.

Table: 6 Histochemical color reactions of Leaf, Stem and Root Powders of *Ecbolium viride*

Reagents	Leaf		Stem		Root	
	Color	Constituent	Color	Constituent	Color	Constituent
Conc. H_2SO_4	Green	Cellulose +				
Weak I_2 solution	Green	Starch -	Precipitate	Starch -	Pale Brown	Starch -
Millon's Reagent	Pale green	Proteins -	White	Proteins +	Reddish brown	Proteins -
Dragendroffs reagent	Reddish Orange	Alkaloids +	Yellow ppt	Alkaloids -	Brownish Yellow	Alkaloids +
Dilute H_2SO_4	Needles	Calcium Oxalate +			Brownish black	Calcium Oxalate -
5% Aq. KOH	Pale Green	Anthraquinone glycosides -	Whitish brown ppt	Anthraquinone glycosides -	Light green	Anthraquinone glycosides -

'+' indicates Present ; '-' indicate absent

Table: 7 Behavior of Leaf, Stem and Root Powders of *Ecbolium viride* with different Chemical Reagents

Reagents	Leaf		Stem		Root	
	Color/ ppt	Constituent	Color	Constituent	Color	Constituent
Picric acid	Yellow ppt with crystals	Alkaloids (+)	Yellow crystals	Alkaloids +	Yellow ppt with crystals	Alkaloids +
Conc. H_2SO_4	Reddish brown	Steroids/ Triterpenoids (+)	Red-Brown	Steroids/ Triterpenoids (+)	Brown	Steroids/ Triterpenoids (+)
Aq. $FeCl_3$	Black green ppt	Tannins (+)	Light green ppt	Tannins (-)	Black ppt	Tannins (+)
I_2 Solution	Reddidh brown ppt	Starch (+)	Red black ppt	Starch (+)	Brown green	Starch (+)
NH_3	Green ppt	Anthraquinone glycosides (-)	Whitish green	Anthraquinone glycosides (-)	Light green ppt	Anthraquinone glycosides (-)
5% Aq. KOH	Pale green	Anthraquinone glycosides (-)	Pale green	Anthraquinone glycosides (-)	Light brown	Anthraquinone glycosides (-)
Mayers Reagent	Ppt	Alkaloids (+)	Greenish white	Alkaloids (+)	Light Brown-green	Alkaloids (+)
Aq. $AgNO_3$	White ppt	Proteins (+)	White ppt	Proteins (+)	ppt	Proteins (+)
Aq. NaOH	Light yellow-red	Flavonoids (+)	Yellow-green ppt	Flavonoids (+)	Brown-red	Flavonoids (+)
Mg-HCl	Magenta	Flavonoids (+)	Magenta	Flavonoids (+)	Magenta	Flavonoids (+)
Dragendroff reagent	Red-Orange	Alkaloids (+)	Red-Orange	Alkaloids (+)	Red-Orange	Alkaloids (+)
Aq. Lead acetate	White ppt	Tannins (+)	White ppt	Tannins (+)	Greenish White ppt	Tannins (+)

'+' indicates Present ; '-' indicate Absent

CONCLUSION:

Microscopic analysis and qualitative parameters are carried out in order to establish appropriate data that can be used in identifying crude drugs. The physical constants such as Total Ash value (23.16% w/w), Acid insoluble ash (16.97% w/w) are specific identification. The soluble extractive values with solvents such as Hexane, Dichloromethane, Chloroform, Ethyl acetate, Petroleum ether ($60\text{-}80^0$), Methanol and Water were (2.569% w/w, 3.449% w/w, 2.934% w/w, 2.335% w/w, 4.27% w/w, 8.125% w/w and 18.025% w/w) respectively, which indicates the nature of constituents present. The behavior of the plant powder upon treatment with different chemical reagents and histo-chemical color reactions were observed and reported. Fluorescence studies of powder with various reagents were observed under UV light. As there is no pharmacognostical work on record, the present work could be therefore be used as one of the tool for standardization of crude drug to identify and decide the authenticity of drug in herbal industry.

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