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PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL EVALUATION OF ROOT OF *ECBOLIUM VIRIDE* [FORSK.] ALSTON.

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ABSTRACT

In ethnomedicinal practices the traditional healers use the roots of *Ecbolium viride* in the treatment of various ailments. Scientific information on their pharmacognosy is very scant. Scientific parameters are not yet available to identify the true plant material and to ensure its quality. Therefore the present work has been undertaken to establish preliminary phytochemical profile and the necessary pharmacognostic standards for evaluating the plant material. Various parameters like morphology, microscopy, powder analysis, fluorescence characteristics and physico-chemical constants of the roots were studied and the salient diagnostic features are documented. Obvious morphological features and the microscopic characteristics were found in the tissue structures of the roots, many diagnostic elements and preliminary phytochemical profile were found to be useful evidences for further scientific investigations of this medicinal plant.

KEY WORDS: *Ecbolium viride*, Ethnomedicine, Microscopy, Pharmacognostical Parameters, Preliminary Phytochemical.

INTRODUCTION

Ecbolium viride (Forsk) Alston. (Acanthaceae) locally known as "*Nilambari*", is a perenial woody undershrub found occasional in plains of India and also found in Arabia, Srilanka and tropical Africa. In folk medicine, aqueous extracts of dried roots of the plant are used for menorrhagia, rheumatism and jaundice (Datta and Maiti, 1968; Kirtikar and Basu, 1987). The rural people in Tirunelveli district of Tamilnadu are used juice of the root of this plant to the treatment of jaundice by the vaidhiyars. Most of the cases of accidental herbal medicine misuse start with wrong identification of a medicinal plant prescribed. Many of the traditional systems have records where one common vernacular is supplied in place of two or more entirely different species. However, no scientific parameters are available to identify the true plant material and to ensure its quality. For this all reasons we take a plant to bring out an official manner by the through investigation on this plant such as pharmacognostical and phytochemical studies of roots of *Ecbolium viride*, which could serve as a valuable source of information and provide suitable standards for the future identification of this plant.

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MATERIALS AND METHODS

Plant materials

Fresh plant was collected from Wastelands of Kadyanallur, Tirunelveli (District), Tamilnadu, India. The plant specimen was authenticated by Dr.P.Jayaraman, M.Sc., Ph.D, Plant Anatomy Research Centre (PARC), Chennai Tamil Nadu, India (Voucher specimen No. PARC/2008/495). All the reagents used were of analytical grade obtained from Sigma Chemical Co, St. Louis, USA or Fine Chemicals Ltd., Mumbai, India.

Collection of Specimens

The roots of this plant were cut and removed from the plant and fixed in FAA (Formalin 5ml+ Acetic acid 5ml+70%Ethyl alcohol 90 ml) for histological studies; transverse sections (T.S) of the different organs of the plant material. After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary–butyl alcohol (TBA) as per the schedule given by Sass, 1940. Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-68˚C) until TBA solution attained supersaturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were section with the help of rotary microtome. The thicknesses of the sections were 10-12 µm. Dewaxing of the sections was performed by customary procedure (Johansen, 1940). The sections were stained with toludine blue as according to the method prescribed by O'Brien *et al.*, 1964. Wherever necessary, the sections were also stained with saffranin and Fast-green. The microphotographs of the sections were made using Olympus BX 40 microscope attached with Olympus DP12 digital camera.

Physico-chemical constants

Physico-chemical constants such as consistency and organoleptic characters (Pratt and Chase, 1949), fluorescence (Kokashi *et al.*, 1958) and the percentage of total ash, acid-insoluble ash, water-soluble ash and alkalinity of water soluble ash values and loss on drying (LOD) were calculated as per the Indian Pharmacopoeia (Anonymous, 1985) **.**

Method of extraction and Preliminary phytochemical screening

The roots were dried in shade at room temperature and screened for the presence of foreign matter. The roots were ground to a moderately coarse powder in a mechanical grinder. About 200g of the powder was extracted successively with petroleum ether (60 - 80º C), benzene, chloroform and ethanol (95%) using soxhlet apparatus. The extraction with each solvent was carried for 24 h. Finally, the marc left was extracted with water by digesting on a boiling water bath. The extraction was continued till a few drops of the last portion of the extract left no residue on drying. The extracts were taken in a tarred porcelain dishes and evaporated to dryness on a water bath and dried at 105º C to a constant weight. The percentage extractives were calculated with reference to air dried drug. The phytochemical examination of each extract of was performed by the standard methods (Harbone, 2005).

Powder microscopy

The root of the plant *Ecbolium viride* were powdered well and then powder was passed through sieve No: 60 and then proceeded for powder analysis (Wallis, 1985; Trease and Evan 1985).

RESULTS AND DISCUSSION

The External features of the plant; (fig 1)

The plant is a shrub growing up to 2.5m height. The leaves are elliptic-ovate to ovate. The lamina is thin and coriaceous; leaf apex is gradually acute. Spikes terminal and axillary (fig 1.1-3). Bracts and bracteoles leafy. Calyx: 5 sepals imbricate; petals: 5 lobed, bluish green. 2 -lipped, upper lip two lobed, lower lip three lobed and spreading. Stamens: 2, attached at the base of the upper lip; Anthers - two lobed lobes unequal. Ovary: Bicarpellary syncarpous, 2-ovuled; Fruits- 2 seeded Capsules; seeds circular flat. Root: the thin root measures 1.2mm thick, circular with dark fissured surface. The thick root is more than 2mm in diameter. The general structure is similar to that of thin root. It has dark, rough and fissured surface

Microscopical features of the root

Both thin and thick roots were studied.

Thin root; The epidermal and sub epidermal layers are crushed into dark surface layer. The cortex is 100µm wide. It consists of four or five layers of radially oblong elliptical, loosely arranged parenchyma cells with small air-chambers. Some of the cortical cells are dilated and posses cylindrical cystoliths (fig.2). The cystolith containing cells are idioplasts and are 150 -200µm in size. The cystoliths are 50 -150µm in size.

Phloem occurs in narrow, continuous zone around the xylem cylinder (fig.2). The phloem elements are narrow, angular, thin walled and are arranged in this radial files. The phloem zone is 30µm wide.

Secondary xylem is in the form of a circle with even line. It is 650µm in diameter. It consists of vessels, fibres and narrow straight rays (fig 2, 2.1and 2.2). The vessels occur in uni seriate radial lines which are widely separated from each other. They are circular, solitary, thick walled and are 15-20µm in diameter. Xylem fibres are thick walled and lignified with wide lumen. They occur in regular radial lines. Xylem rays are narrows and less prominent.

Thick root: The thick root is more than 2mm in diameter. The general structure is similar to that of thin root. It has dark, rough and fissured surface followed by a thin layer of periderm are wide aerenchymatous cortex. The cortical zone is 350µm wide and comprises of tangentially stretched cylindrical cells and the cells have undergone radial divisious, wide, irregular air-chambers are seen in the cortex (fig.3, 3.1). Some of the cortical cells are dilated into cystolith bearing idioplasts.

Secondary phloem consists of narrow continuous cylinder of radial files of small phloem elements (fig.3.2). The sieve elements are rectangular with lateral companion cells. Secondary xylem is a dense, solid, smooth circle. It exhibits less prominent growth-ring which is demarcated by narrow thick walled fibres (fig.3). The vessels are diffuse in distribution. They are solitary and are in radial chains. The vessels are circular and thick walled, measuring 20µm in diameter. Xylem fibres thick walled and lignified. The lumen of the fibres is wide and angular. The fibres are in radial rows. Xylem rays are thin and straight.

Powder microscopy (fig.4)

Root Powder includes fibres, vessel elements and xylem parenchyma. The fibres are bibriform type; they are needle like with tapering ends. Some of the fibres have wide lumen and others have narrow lumen (fig. 4a and 4b). The wide fibres (fig. 4a) are 20µmwide and up to 400 long. The narrow fibres [shown by asterisk in fig.1.1 are 10µm thick and 500µm long. The lateral pits are not evident (fig. 4b).

Xylem parenchyma [Ray parenchyma] cells are common in the powder. The parenchyma cells narrow and oblong; they are scale-like in out line with blunt or conical, semicircular ends (fig. 4c). These cells have thin walls and dense cell inclusions. The cells are 40µm wide and 240µm long.

Vessel elements (fig. 4d and 4e). The vessels elements are narrow and cylindrical. They have long or short tails. When the vessel element has long tails, it is gradually tapering into the tail. The lateral walls have dense, circular pits. The perforation plate is simple, circular and slightly oblique. The vessel elements range in length from 150-320µm. The long and narrow vessel elements with long tails resemble very much to the wide fibre, excepting that the vessel elements have dense elliptical or circular bordered pits and perforations at the end walls.

Physico-chemical constants of root powder

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

Organoleptic characters

The root powder was tested with various solvents and chemicals to determine consistency and organoleptic characters are given in Table 1.

Ash values

Total ash, water-soluble ash, alkalinity of water soluble ash and acid-insoluble ash values of the root powder was done and the results are tabulated in Table 2.

Fluorescence analysis of root *powder*

The powder of root is examined in daylight, short (at 254nm) and long UV (at 365nm) to detect the fluorescent compounds and the observations are given in Table 3.

Preliminary phytochemical screening

The results of phytochemical examination of each extract are given in Table 4.

Table 1: Organoleptic characters of *Ecbolium viride* (Forsk) Alston. root powder

1.Colour :	Pale brownish yellow
2. Appearance:	Coarse powder
3. Odour:	No characteristic odour
4.Taste:	No characteristic taste

Table 2: Ash values of *Ecbolium viride* (Forsk) Alston. root powder

1. Total ash value:	20.08 **%**
2. Water-soluble ash value:	12.36 **%**
3. Alkalinity of water soluble ash value:	1.89 ml
4. Acid-insoluble ash value:	0.451 **%**

Table 3: Fluorescence characteristics of *Ecbolium viride* (Forsk) Alston. root powder

Treatment	Day light	UV light	
		254nm	365nm
Powder	Pale green	Pale green	Black
Powder + 1N NaOH (aqueous)	Pale-brown	Pale green	Black
Powder + 1N NaOH (alcoholic)	Pale-brown	Pale-brown	Black
Powder + 1N Hydrochloric acid	Pale-brown	Pale-brown	Black
Powder + 50% Sulphuric acid	Brown	Yellow	Black
Powder + 50% Nitric acid	Brown	Pale green	Yellow
Powder + Picric acid	Pale green	Green	Black
Powder + Acetic acid	Brown	Pale green	White
Powder + Ferric chloride	Brilliant green	Greenish yellow	Black
Powder +Con. Nitric acid	Yellowish green	Green	Black
Powder + Nitric acid + Ammonia	Greenish brown	Greenish yellow	Black

Table 4: Phytochemical profiles of extracts of root of *Ecbolium viride* (Forsk) Alston.

Solvent extracts	Chemical constituents							
	Alkaloids	Sterols	Protein & Amino acids	Carbohydrate	Glycosides	Tannins	Saponins	Flavonoids
Petroleum ether (60-80°C)	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-	-	-
Ethyl alcohol	+	-	-	+	-	+	-	-
Water	+	-	-	+	+	+	+	-

(+)= Present; (-)= Absent

Figure 1: A twig of *Ecbolium viride* (Forsk) Alston. *viride* (Forsk) Alston.

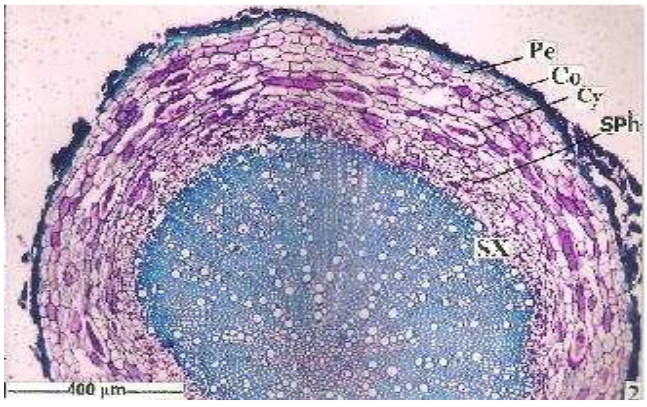


Fig .2: T.S of thin-root (Half section enlarged): Co, Cortex; **Cy,** Cystolith; **Pe,** Periderm; **SPh,** Secondary phloem; **SX,** Secondary xylem.

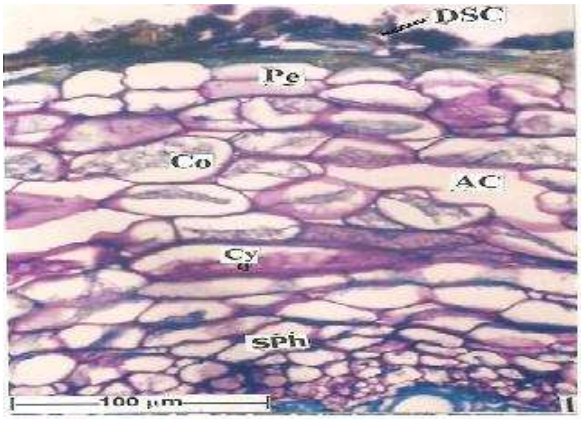


Fig 2.1: T.S of thin-root showing Periderm: Co, Cortex; **Cy,** Cystolith; **Pe,** Periderm; **SPh,** Secondary phloem; **AC,** Air-chambers

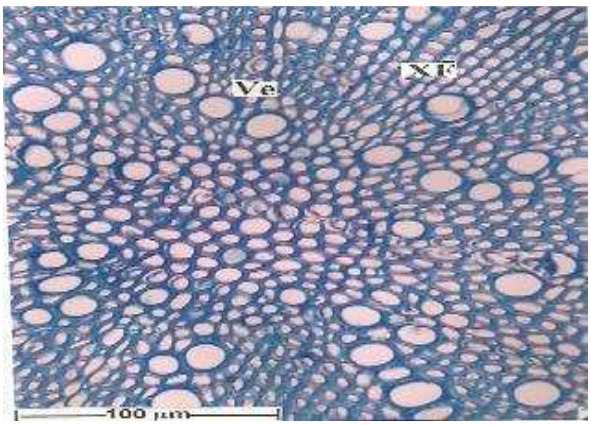


Fig 2.2: T.S of thin-root showing Secondary xylem: XF, Xylem fiber; **Ve,** Vessel.

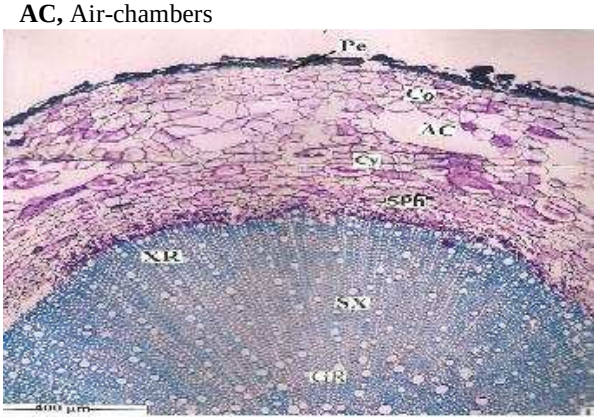


Fig .3: T.S of thick-root (Half section enlarged): Co, Cortex; **Cy,** Cystolith; **Pe,** Periderm; **AC,** Air-chambers; **SPh,** Secondary phloem; **SX,** Secondary xylem; **XR,** Xylem rays; **GR,** Growth-ring boundary.

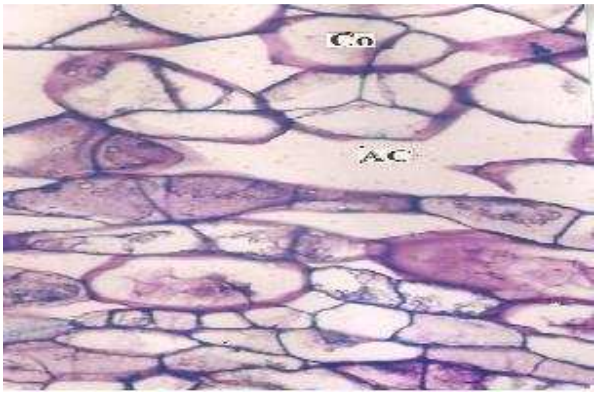


Fig 3.1: T.S of thick-root showing Aerenchymatous cortex: Co, Cortex; **AC,** Air-chambers.

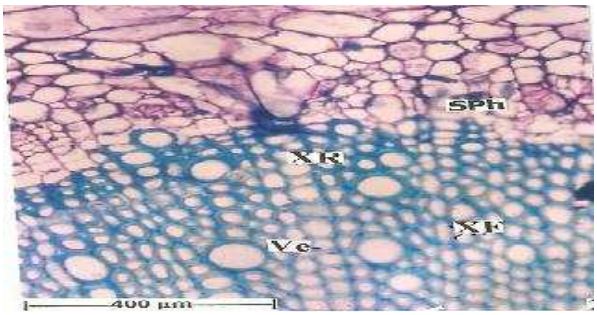
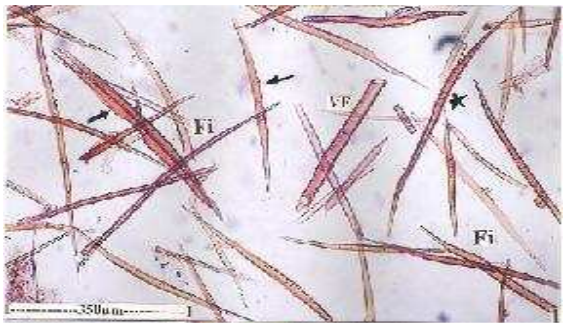
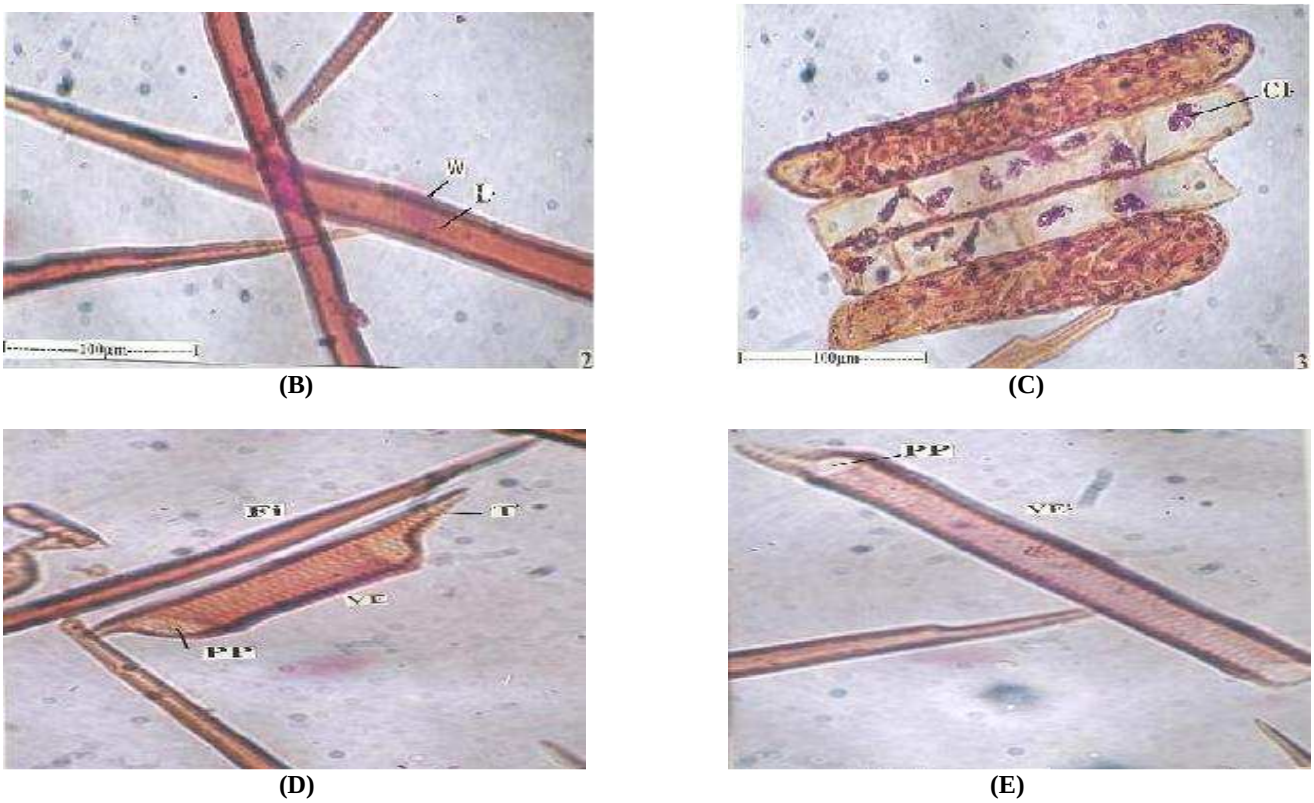


Fig 3.2: T.S of thick-root showing Secondary phloem and Secondary xylem:
SPh, Secondary phloem; **XF,** Xylem fibre; **XR,** Xylem rays; **Ve,** Vessel



(A)



(B) **(C)**

(D) **(E)**

Fig .4: Diagnostic features for the powder microscopy of the root of\ *Ecbolium viride*: Vessel elements and fibres (fig.4a); Fibres enlarged (fig.4b); Parenchyma cells with cell inclusions (fig.4c); Tailed vessel elements with fibres (fig. 4d and 4e).**CI,** Cell inclusions; **Fi,** Fibre; **L,** Lumen; **Ve,** Vessel element; **W,** Wall; **PP,** Perforation plate; **T,** Tail.

CONCLUSION

In ethnomedicinal practices the traditional healers use *Ecbolium viride* in treatment of various ailments, menorrhagia, rheumatism and jaundice.

As per WHO norms, botanical standards are to be proposed as a protocol for the diagnosis of the herbal drug. Macroscopic as well as microscopical studies of any phytodrug are indispensable tool for identification of medicinal herbs to establish its botanical quality control before going to other studies. The above mentioned parameters are helpful for the future identification and authentification of the plant in the herbal industry and in factories. The physico-chemical standards, such as ash values and fluorescence analysis, will be useful to identify the authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing root of this plant in future. The powder constants can be included as microscopical standards in Indian herbal pharmacopoeia. Phytochemical study is also useful to isolate the pharmacologically active principles present in the drug.

In conclusion, the present study on pharmacognostical characters and phytochemical profiles of root of *Ecbolium viride* (Forsk) Alston. will be providing useful information for the future identification of this plant.

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