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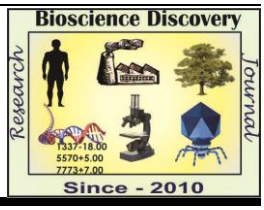

Morpho-anatomical studies of *Euphorbia heterophylla* Linn. leaves

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Abstract

Euphorbia heterophylla Linn. Plant belongs to family Euphorbiaceae grows in tropical and subtropical regions of India, used in traditional and folklore medicine. The usefulness of this plant is scientifically evidenced, and different biologically active phytoconstituents were isolated from plant. But no reports are available on morphoanatomy, and phytochemical studies, hence present attempt was undertaken to investigate the microscopic and preliminary phytochemical studies. The study reveals the midrib is biconvex and lamina is dorsiventral, shows presence of nonglandular trichome, anomocytic stomata, prismatic calcium oxalate crystals. It shows presence of steroids, triterpenoids, cumarines, and flavonoids.

INTRODUCTION

Euphorbia heterophylla Linn. (EH) is a plant belongs to family Euphorbiaceae. It is a hardy, ruderal species, growing between 30 and 70 cm in height, nears 4-5 lobed leaves and stem with milky exudation. The fruits are small, segmented capsules. It is distributed worldwide and has been used as a folk medicine. *Euphorbia heterophylla* leaf is used in traditional medical practices as laxative, antigonorrheal, migraine and wart cures. The plant lattices have been used as fish poison, insecticide and ordeal poisons (Rodriguez *et al*., 1976; Falodun *et al*., 2003). Its leaves are commonly used for antioxidant, an anti-inflammatory and a laxative agent (Falodun &Agbakwuru, 2004; Falodun *et al*., 2006). In some parts of Kogi State, Nigeria , the leaves are used as anticonvulsant and cough remedy. The leaves of *E. heterophylla* have been reported to contain quercetin (Falodun *et al*., 2006). Diterpenoids have been reported in the root of *E. heterophylla*. The skin irritant, tumor promoting anti-tumor/anti-cancer and recently anti-HIV activities of Euphorbia species have also been reported in *E. heterophylla* leaf (Williams *et al*, 1995). The leaves extract also shown antibacterial, antioxidant, nutritive anti-diabetic potential (Falodun *et al.*, 2003; Francis *et al.*, 2016; Annapurna & Hatware, 2014). Ethanol and aqueous extract *E. heterophylla* leaf has shown cytotoxic potential and wound healing ability in experimental animals (James & Friday, 2010). The recent study concluded that the aqueous extract of *E. heterophylla* leaf can be used as anticoagulant for long storage of whole human blood (Ughachukwu *et al.*, 2013)

However, available literature revealed that no pharmacognostic study has been carried out on the leaves; hence the present investigation was undertaken. The objective of the present study is to evaluate various pharmacognostic standards like macroscopy and microscopy of leaves; ash values, extractive values, microscopical characteristics of powdered fruit and preliminary phytochemical analysis of *Euphorbia heterophylla* Linn leaves.

MATERIALS AND METHODS

Fresh leaves of *E. heterophylla* were collected from Shirpur region in rainy season and Identified and confirmed from head of the department, Department of Pharmacognosy R C Patel Institute of Pharmaceutical education and Research, Shirpur, Dhule, the voucher specimens were preserved in the institute herbarium library.

Macroscopy

The leaves part was separated from other parts, washed, cleaned and dried for further use. The following macroscopic characters of the fresh leaves were noted: color, odor, taste, size and shape, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture (Wallis, 1985).

Fig: 1. *Euphorbia heterophylla.*

Microscopy

The free hand thin transverse sections of the fresh leaves through the lamina and the midrib were treated with different staining agent and observed for the general and specific microscopic characteristic. The small quantity of the powdered leaves was cleared, mounted and observed for diagnostic powder characteristics

Physicochemical investigations

The dried leaf powder material was used for the determination of ash values, extractive values, and preliminary phytochemical investigation. The chemomicroscopic examination and behaviour of powder with chemical reagents were also studied (Kalaskar, 2010).

RESULTS AND DISCUSSION

Morphology

The leaves at the upper end of the stalk, close to the cyathium, have a striking, scarlet red coloration. Leaves are mainly 2-4 lobed and 4–7 cm long by 1.5–3 cm wide. Leaves are ovate in shape with obtuse apex and cuneate base. The leaf margin was found to be undulate, the leaves has prominent petiole and stipule. The stalk exudes a toxic milky white latex. Regarding the leaf venation, the multicoasted divergent reticulate type observed in *Euphorbia heterophylla.* All these characteristics were compared with standard references available and the correct species (*Euphorbia heterophylla* Linn.) were confirmed.

Microscopy

The Transverse section of *Euphorbia heterophylla* leaf can be best discussed in two parts (fig 5(i &ii))

1. Lamina region

Midrib region

Upper epidermis **-** Single layered rectangular cells, non-gladular trichomes and stomata are present.

Mesophyll **-** Dosriventral, single layered palisade, 3-4 layered compact and redially elongated cells spongy paranchymatous layer shows 4-6 layered, loosely arranged with intercellular spaces, presence of calcium oxalate prisms.

Lower epidermis -Resembles to upper epidermis but number of trichomes and stomatal pores are more

Covering trichomes **-** multicellular uniserrate covering trichomes are present. Anomocytic stomata are presents.

Collenchyma - 7-9 layers of compactly arranged present below the upper epidermis while 5-7 layers are present above the lower epidermis.

Vascular bundle **-** These are arc shaped collateral, in which xylem are lignified and phloem are non-lignified.

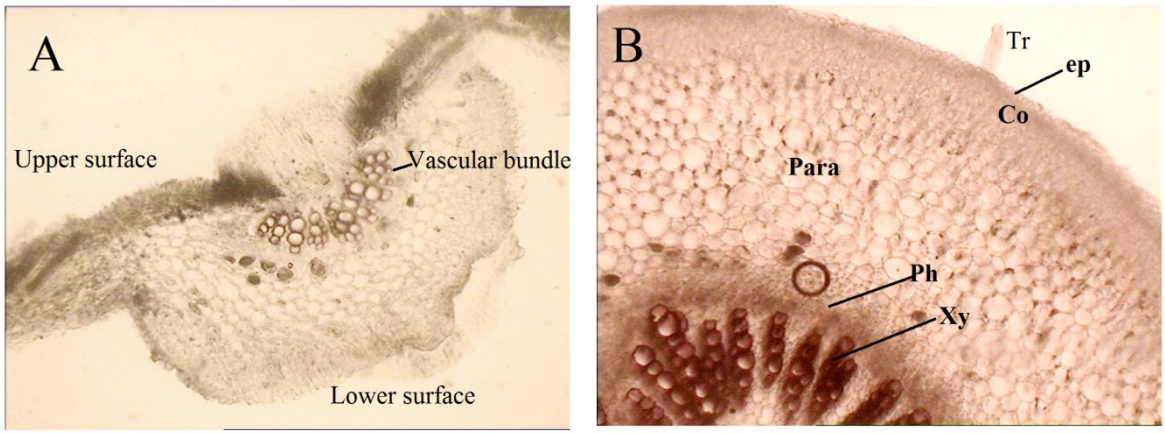

Fig 2. Midrib structure of *Euphorbia heterophylla* (A) Transection of the midrib, showing collateral vascular bundles arranged as closed arc; (B) Trichomes in the midrib; Co: collenchyma; ep: epidermis; Para: ground parenchyma; ph: phloem; Tr: trichome, ph: phloem ; xy: xylem.

Lamina:

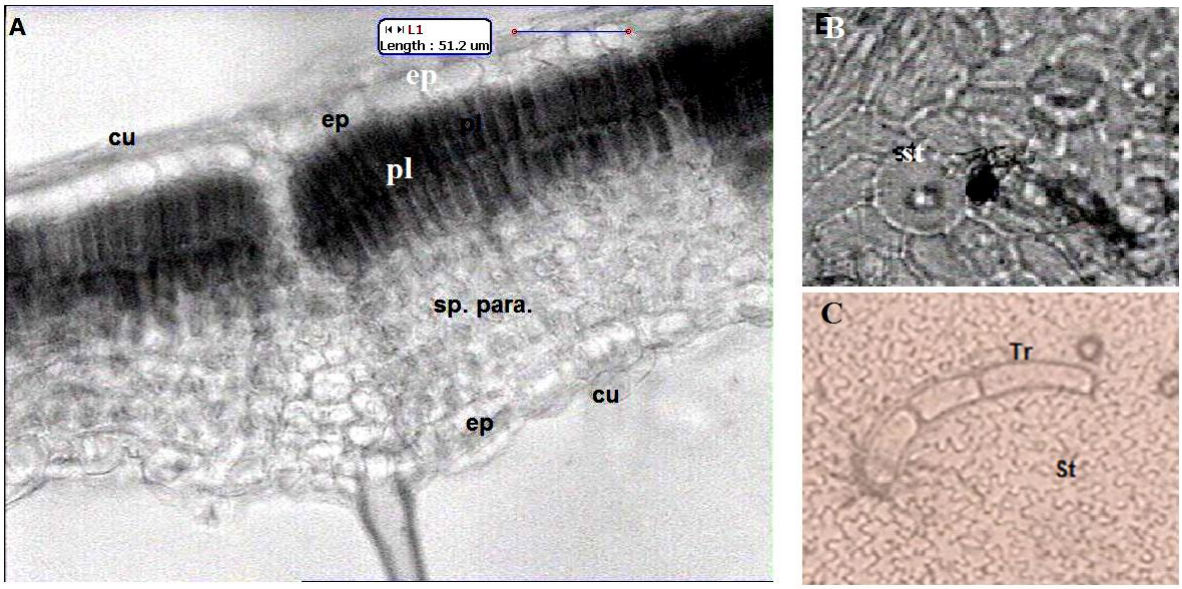

Figure 3. A: Transverse section of lamina region showing dorsiventral leaf; cu: cuticle, ep- epidermis, pl: palisade cell, Sp para: spongy parenchyma B and C: surface preparation showing the presence of multicellular covering trichome (Tr) and anomocytic stomata (St)

Midrib:

The multicoasted divergent reticulate leaf venation besides being involved with the transport of substances, the venation system is also related to the leaf mechanical stabilization, owing to the presence of the lignified xylem and multilayered collenchyma. From Woodward's point of view (Woodward, 1998), although the presence of stomata increases photosynthetic potential, protects xylem from cavitation, favouring water flow and promotes heat dissipation by water loss, herbaceous species maintain low stomatal densities and hydraulic conductances, maximizing the control on loss of water to a dry atmosphere. This control is optimized by a well-developed cuticle, a barrier which contributes to the maintenance of plant water status (Riederer & Schreiber).

Concerning the calcium oxalate crystals, they are by far the most prevalent and widely distributed mineral deposits in higher plants and typically develop within intravacuolar membrane chambers of specialized cells in any organ or tissue (Webb, 1999).

They are formed from environmentally derived calcium and from biologically synthesized oxalate (Nakata, 2003). The functions assigned to calcium oxalate crystals are varied, since they can be related to ionic balance and osmoregulation, storaging form for either calcium or oxalate, mechanical support and protection against foraging animals.

Trichomes are epidermal outgrowths of considerable value for taxonomic purposes. environmental conditions influence more the length, size and density than the types of trichomes. These outgrowths play a role in plant defense especially with regard to phytophagous insects, avoiding insect feeding and oviposition responses, and thenutrition of larvae (Metcalfe & Chalk, 1988). They may be involved in the regulation of temperature and water repellency as well (Neinhuis & Barthlott, 1997).

Powder drug analysis

a) Unicellular, uniserrate covering trichomes are abundant, pointed toward the apex and broader at base, thin measure 240 - 415 microns in length. (fig 3, i)

b) Fragments of paranchyamatous tissue containing spiral vascular strands measures 25 – 48 micron in diameter. (fig 3, ii)

c) Numerous anomocytic stomata meaning thereby that the cells surrounding the stomatal pores are irregularly arranged. (fig 3, iii)

d) The prismatic calcium oxalate of 7 – 10 microns in diameter are less abundant and observed as free or in fragments of parenchymatous cells. (fig 3, iv)

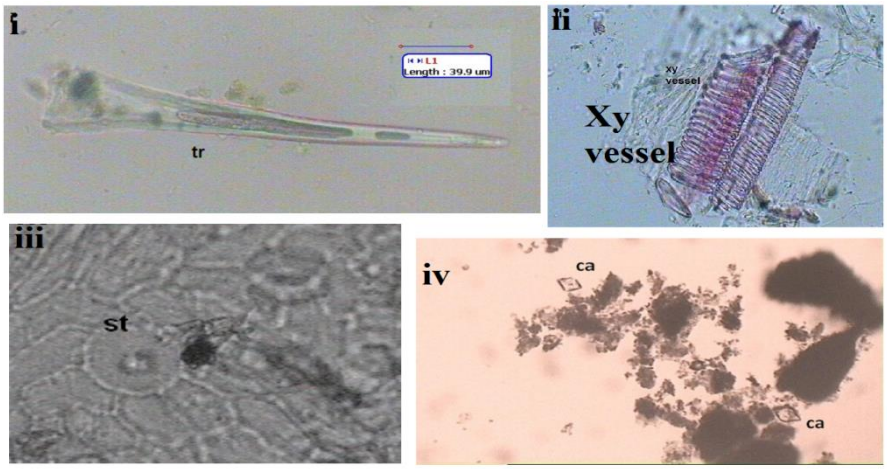

Figure 3. Powder analysis (i) unicellular uniserratenon-glandualr trichomes (ii) spiral lignified xylem vessel (iii) anomocytic stomata (iv) prismatic calcium oxalate crystals

The different histo-chemical color reactions were performed on the leaf transverse sections to differentiate the different cell compositions and identification, results were tabulated in Table 1

The histochemical color reaction showed the deposition of different chemical at different anatomical parts of leaves.

Physicochemical analysis

The fluorescence analysis and behaviors of powder with different chemical reagents were studied [Tables 2 and 3]. The physicochemical standards are important to check the quality, purity, and adulteration of given crude drug. The foreign matter, loss on drying (LOD), ash, and extractive values were determined and summarized in Table 3.

The fluorescence analysis being specific chemical reaction of chemical present in the plant part and different chemicals and combination of chemical. Certain biological active constituents gives the fluorescence, is its marked characteristic. The distinct color appearance in day light and uv light can be used for preliminary identification of specific plant. The ash values of a drug give an idea of the earthy matter or inorganic composition and other impurities present along with the drug. Extractive values are useful for determination of exhausted or adulterated drug. Thus ash, extractive values, fluorescence analysis will be helpful in the identification and authentication of plant material (Kalaskar & Surana, 2012).

Table 1 Histochemical color reactions of *Euphorbia heterophylla* leaf powder.

Reagents	Constituent	Color	Histological zone	Degree of intensity
Aniline So_4 + H_2SO_4	Lignin	Yellow	Xylem,	++
Phloroglucinol + HCl	Lignin	Pink	Xylem, Sclerenchyma	+++
Conc. H_2SO_4	Cellulose	Green	Mesocarp	+
Weak Iodine solution	Starch	--	--	--
Millons reagent	Proteins	white	Spongy paranchyma	+
Dragendorffs reagent	Alkaloids	---	--	--
H_2So_4	Ca. Oxalate	Needles	Mesophyll, and midrib paranchyma	+
$SbCl_3$	Steroids/Triterpenoids	Reddish pink	Mesocarp	+++
5% Aq. KOH	Anthraquinone glycosides	--	--	--

+++ High, ++ Moderate, + Slight, - Negative.

Table 2 fluorescence analysis of *Euphorbia heterophylla* leaf

Color reaction	Day light	Uv light 365nm
Powder + NaOH	Green color	Dark greenish fluorescence
Powder + Methanol + nitrocellulose	Yellowish green	Yellowish green fluorescence
Powder + nitrocellulose	Purple green	Strong yellow green fluorescence
Powder + NaOH in water	Green	Faint yellowish green fluorescence
Powder + nitrocellulose +HCl	Grayish green	Faint green fluorescence
Powder + Hcl	Yellowish green	Dark grey with faint yellow fluorescence
Powder + H_2SO_4	Blackish	Black
Powder + HNO_3	Brownish black	Black
Powder	Green	Greenish yellow florescence

Table 3 Behavior of *Euphorbia heterophylla* leaf powder with different chemical reagents

Regents	Color/ppt	Constituents
Picric acid	No precipitations	Alkaloids absent
Conc. H_2SO_4	Reddish brown	Steroids/triterpenoids present
Aq. $Fecl_3$	No change	Tannins absent
Iodine solution	No change	Starch absent
Ammonia present	No change	Antroquinone glycosides absent

Table 4. Physicochemical analysis of *E. heterophylla* leaves.

Types of ash value/ extractive values	% w/w
Ash value	
Total ash	11.50
Acid insoluble ash	1.50
Water soluble ash	2.50
Sulphated ash	12.50
Extractive values	
Pet-ether	12.0
Ethyl acetate	2.4
Ethanol	28.0
Water	20.0

The preliminary phytochemical analysis of bark extracts showed the medicinally potential constituents include carbohydrates, amino acid, coumarins, tannins, flavonoids, glycoside, steroids, and triterpenoids which were in aggrement with previous study (James & Friday, 2010; Falodun *et al*., 2006)..

The present study provides in-depth microscopical features; physicochemical characteristics and preliminary phytochemical study provide pharmacopoeia standards for easy identification of the *E. hetrophylla* and hence differentiating it from closely related species.

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