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Pharmocognostical and phytochemical evaluation of leaf and stem of *Euphorbia hirta*

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Abstract
Objective: To study detailed pharmacognostic profile and preliminary phytochemical investigation and TLC profiles to evaluate the characters of leaves and stem of *Euphorbia hirta* (Euphorbiaceae) which is often used traditionally in Ayurveda for female disorders, respiratory ailments (cough, coryza, bronchitis, and asthma), worm infestations in children, dysentery, jaundice, pimples, gonorrhoea, digestive problems, and tumours.
Methods: Leaf and stem samples of *E. hirta* were studied by Macroscopical, Microscopical, Physicochemical, Phytochemical analysis of powder of the plant and other methods for standardization recommended by WHO.
Results: Macroscopically, the leaves are simple, opposite, faintly toothed, Elliptic, oblong, oblong-lanceolate, pinnate, bluntly pointed apex, partly fused base, dark green above pale beneath, Mild Characteristic odour, bitter and peppery taste. A small, erect or ascending annual herb reaching up to 50 cm with hairy stems. Microscopically, the leaf showed the presence of Ground tissue, Kranz tissue, Collateral vascular bundles, Hypo stomatic lamina, Anomocytic stomata, Lipriform type of xylem fibres, pith, are the diagnostic features noted from anatomical study. The salient features of stem were small epidermal cells with papillate outer tangential walls, the outer ground tissue to homogenous, parenchymatous and the cells are circular and compact. The central ground tissue is the pith. Vascular tissues comprises short radial rows of narrow xylem elements and thick walled lignified fibers. Phloem occurs both on the outer portion of the xylem cylinder and on its inner portion. Powder microscopy of leaf revealed the presence of parenchyma cells, xylem fibres and epidermis with anomocytic stomata. The investigations also included leaf surface data; quantitative leaf microscopy. Physiochemical parameters such as loss on drying, extractive values and ash values were also determined. Preliminary phytochemical screening showed the presence of steroids, carbohydrates, flavanoids, proteins, alkaloids and saponin.
Conclusions: The results of the study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigations and applications.

Keywords: *Euphorbia hirta*, Ayurveda, microscopy, macroscopy, stomata phytochemical evaluation

Introduction
Medicinal plants are playing very active role in traditional medicines for the treatment of various ailments [1]. However a key obstacle, which has hindered the promotion in use of alternative medicines in the developed countries, is no evidence of documentation and absence of stringent quality control measures. There is a need for the record of all the research work carried out on traditional medicines in the form of documentation. With this drawback, it becomes extremely important to make surety about the standardization of the plant and parts of plant to be used as a medicine. For the process of standardization, we can use different techniques and methodology to achieve our goal in the stepwise manner likes pharmacognostic and phytochemical studies. These steps and processes are helpful in identification and standardization of the plant material. Correct characterization and quality assurance of starting material is an essential step to ensure reproducible quality of herbal medicine which will help us to justify its safety and efficacy [2-5]. The World Health Organization (WHO) estimates that more than 80% of the populations in developing countries rely on traditional medicine for their primary health care [6]. The value of ethnomedicine and traditional pharmacology is these days achieving great appreciation in modern medicine, as the search for new potential medicinal plants is frequently based on an ethnomedicinal basis (Muthu *et al*., 2006; Parveen *et al*., 2007; Upadhyay *et al*., 2010). Ethnobotanical studies of different areas of Rajasthan state has been carried out by many workers in this field (Singh and Pandey, 1998; Mishra and Kumar, 2000, 2001; Katewa *et al*., 2004; Parveen *et al*., 2007; Upadhyay *et al*., 2010.In India and other parts of the world, several ethno botanical surveys were performed and result have shown that

Euphorbia hirta Linn., is a ruderal plant, known and widely used in the treatment of various ailments such as ear aches, boils and promotes wood healing AKE-ASSI (2011). Also this species is known to have analgesic, antipyretic, expectorant, anti-syphilis and antiviral (AKEASSI, 2011). This species has been the subject of many scientific studies highlighting various activities. The majority of these works concern antimicrobial, anti malarial, antioxidants, anti-diabetic, anti-hypertensive, anthelmintics, anti-allergic, anti-tumour, anti-anxiety, sedative and immunomodulatory, anti-inflammatory, antitetanus, galactogenic), diuretic, cytotoxic, cardiovascular and vasodepressor activities (LANHERS *et al*., 2005).. This important medicinal plant is also used in India, Philippines and around the world to treat various contagious and noncontagious diseases. LANHERS *et al*. Recently, modern pharmacological investigations showed that *E. hirta* and its active components possessed wide pharmacological actions, such as anti-inflammatory, antifungal, antibacterial, anti diarrheal, sedative, anxiolytic, analgesic, antipyretic, antioxidant, antiasthmatic, antitumor, antimalarial, larvicidal, diuretic, and increases electrolytes, among others (Lanhers *et al*, 1990; Lanhers *et al*, 1991; Johnson *et al*, 1999. *E. hirta* is an Annual plant growing to 0.3m by 0.25m. The plant prefers light (sandy) and medium (loamy) soils and requires well drained soil. According to survey, different parts of *E. hirta* are used for curing various ailments. The aerial parts of the plant are harvested when in flower during the summer and dried for later use. The stem is used as a treatment for asthma, bronchitis and various other lung complaints. The whole plant is decocted and used in the treatment of athlete's foot, dysentery, enteritis, and skin conditions (Upadhyay *et al*, 2010). It has been used in the treatment of syphilis. The sap is applied to warts in order to destroy them, and treatment needs to be repeated 2 - 3 times a day over a period of several weeks to be fully effective. Literature survey did not provide sufficient information about pharmacognostical studies of leaf and stem of this plant. The current work aims to contribute in solving the problems of controversial drugs prevalent in Ayurveda besides helping in laying down pharmacopoeial standards. Therefore, keeping above view in mind various Macroscopic, Histological and physiochemical and quantitative microscopical studies and Preliminary phytochemical investigation and TLC studies on leaves and stems of *E. hirta* were carried out in present study.

Materials and Methods

Collection and Authentication:

Euphorbia hirta leaf and stem was collected, from Palakkad, Kerala, India and authenticated by taxonomist and the plant authenticated specimen is deposited in the Department of Pharmacognosy Sanjo college of pharmaceutical studies Palakkad. Authentication specimen number is SCPS/P.COG/004/2017 the fresh leaves and stemswere kept for shade drying. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Pharmacognostic Standardization

Organoleptic characters such as shape, size, colour, odour, taste of stem were determined. Microscopic studies were carried out by preparing thin hand section of leaf and stem with Chloral hydrate solution, stained with Phloroglucinol-hydrochloric acid (1:1) and mounted in glycerine [7]. Histochemical studies and powder microscopy were carried out to know about the inclusions and detailed anatomical characters of the material [8].

Physico-chemical Evaluations

The parameters were done to evaluate the proceedings of total ash; water soluble ash; acid insoluble ash and sulphated ash were calculated as per Indian Pharmacopoeia [9]. Extracts of the powdered stem was prepared with different solvents for the study of extractive value. Fluorescence analysis was also carried out for the powder and for extract as per standard procedure [10].

Preliminary Phytochemical Screening

The methanolic, Ethanolic, petroleum ether, Benzene, chloroform and aqueous extract of *E. hirta* Linn. was subjected to tests for the presence or absence of the major class of compounds by standard methods [11].

Extraction of Plant material

For preliminary phytochemical analysis, extract was prepared by weighing 1kg of the dried powdered aerial parts were subjected to hot successive continuous extraction with different solvents as per the polarity, petroleum ether, benzene, chloroform, methanol, ethanol and finally with aqueous. The extracts were filtered in each step using Whatman filters paper. The filtrate was concentrated using a rotary evaporator at low temperature (40-45^{o}C) and pressure. The presence or absence of the primary and secondary phytoconstituents was detected by usual prescribed methods [12].

TLC profile

Powdered *E. hirta* Linn., 10 g was extracted by refluxing with aqueous and methanol (50 ml × 3) sequentially for a period of 30 minutes each and the combined extract of each were filtered and concentrated to 10 ml. Apply 10 ml each extract as bands at a height of 10 mm from the base of a 5 × 10 cm coated silica gel plate 60 F254 using CAMAG Automatic sampler (ATS4) and developed up to 80 mm from the base of the plate in an Automatic Developing Chamber (CAMAGADC2) using the mobile phase toluene: ethyl acetate: acetic acid (3:7:1) v/v for aqueous, Toluene: Ethyl acetate: Formic acid (3:1:1) v/v for methanol extract. Dry the plate in air and profile pictures were taken after derivatization with anisaldehyde sulphuric acid reagent (ANS)

Fluorescence analysis

Powder drug was treated with different reagent and was observed for Fluorescence under UV light.

Powdered drug reaction with different reagent

Powder drug was treated with different reagent and was observed from naked eye.

Results

Macroscopical characters

It is slender stemmed annual hairy plant with many branches. Spreading up to 40 cm in height reddish or purplish in colour. Leaves are simple, opposite,elliptic,oblong,oblong-lanceolate in shape, Faintly toothed margin,, pinnate venation, bluntly pointed apex, partly fused base,,0.5 to 2.5 cm in length and 0.8-1,5 cm width colour dark green in above pale in beneath, mild characteristic odour, bitter and peppery taste, Stems are small, erect or ascending annual herb reaching up to 50 cm with hairy. Flowers are small, numerous and crowded

together in dense about 1cm in diameter. The fruits are yellow, three celled, hairy,, keeled capsules,1-2mm in diameter containing three brown, four sided, angular wrinkled seeds. Seeds are about 0.7 to 0.8 mm diameter.

Fig 1: Macroscopy of *E. hirta*

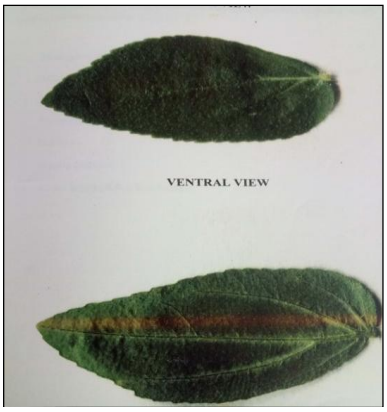


Fig 2: Macroscopy of the Leaf

Histological characters
The detail and systemic Pharmacognostical evaluation would give valuable information for the future studies.

Leaf
The leaf is dorsi ventral with prominent adaxial midrib and uniformly thin lamina (fig 3) Mid rib has wide shallow concavity on the adaxial side and semicircular thick adaxial part. It is 300 µm thick and 390 µm wide. The epidermal layer consists of fairly thick cylindrical, thin walled cells which are 10 µm thick. The cuticle is not prominent. The ground tissue is homogenous and parenchymatous.The cells are circular to angular, thin walled and compact. Some of the cells have mucilage content The vascular strand is single and is flat on the adaxial side, while it is conical on the adaxial side. The strand includes several thin radial rows of xylem elements and an adaxial band of phloem elements. Along adaxial part of the xylem strand occurs on one of the dilated cells with dense chloroplasts. These cells are called 'kranz-tissue' (fig 4) Lateral vein (fig 5) is similar to the midrib. It is planoconvex with flat adaxial side and semicircular adaxial side. It has prominent epidermal layer of spindle shaped cells and parenchymatous ground tissues. There is a prominent collateral vascular bundle with adaxial arc of bundle sheath.

Kranz tissue
The smaller vein lets are circular with wide, dilated bundle sheath cells enclosing small vascular strand (fig 6). The leaf margin is slightly bend down (fig 6).The marginal portion is 120µm thick. It has thicker epidermal layer and small circular vascular strand with bundle sheath cells. The lamina has thick spindle shaped adaxial epidermal cells measuring 10µm thick. The adaxial epidermis is thin and the cells are narrowly cylindrical. The mesophyll tissue includes adaxial layer of short, cylindrical, loosely arranged palisade cells and adaxial network of spongy parenchyma cells (fig 7)

Epidermal cells and stomatal type
The epidermal cells are thin walled, the anticlinal walls are highly wavy with deep folds so that the cells appear amoeboid in outline (fig 8).Stomata occur only on the adaxial side of lamina (hypo stomatic). They are anomocytic type and have no subsidiary cells. The guard cells are small and elliptical in shape. They have wide stomatal pore (fig 9).

Venation pattern
As seen in surface view, the venation is densely reticulate. The major and minor veins are equally thick. They are wavy. The vein islets are wide, rectangular or squarish in outline. The vein boundaries are thick and distinct. The vein terminations are either simple (unbranched) or branched twice or more. Repeatedly terminations are dendroid (tree like) in outline. The veins have central rows of xylem and phloem elements and single layer of kranz-cells forming thick sheath all around the veins (fig 10)

Petiole
The petiole is semi circular and Plano convex in sectional view (fig 11), It is 950µm wide and 750 µm thick. The petiole has a thin, less prominent epidermal layer of small cells. The ground tissue is homogenous and parenchymatous. The cells are thin walled, circular and compact. There is a shallow arc of four vascular bundles with narrow gaps in between. The bundles have short, thin rows of xylem elements and narrow bands of phloem situated on the lower side. (Fig 12)

The young stem (Fig 13, 14)
It is circular and 1.7mm thick. It consists of small epidermal cells with papillate outer tangenttial walls. The outer ground tissue to homogenous, parenchymatous and the cells are circular and compact. The outer ground tissue is 180 micro meter wide. The central ground tissue is the pith; it includes slightly large, circular thin walled parenchyma cells. The vascular tissues occur in wide closed cylinder belonging the central ground tissue. It comprises short radial rows of narrow xylem elements and thick walled lignified fibers. Phloem occurs both on the outer portion of the xylem cylinder and on its inner portion. The inner phloem is in small isolated masses.

Old stem (Fig 15)
It is nearly 3mm thick. The epidermis broken due to formation of thin periderm which is 3 or 4 layered with narrow tabular cells. There is a narrow cortex; the cortical cells are tangentially elongated compact and narrow. Secondary phloem is wide continuous cylinder of sieve elements and parenchyma cells. Secondary xylem is thick and dense. It consists of circular, thick walled, diffusely distributed solitary vessels and radial files of xylem fibres which posses thick lignified walls. Mucilage containing cells are common in the cortex, phloem and pith. The mucilage cells are not different in shape and size from the neighboring cells.

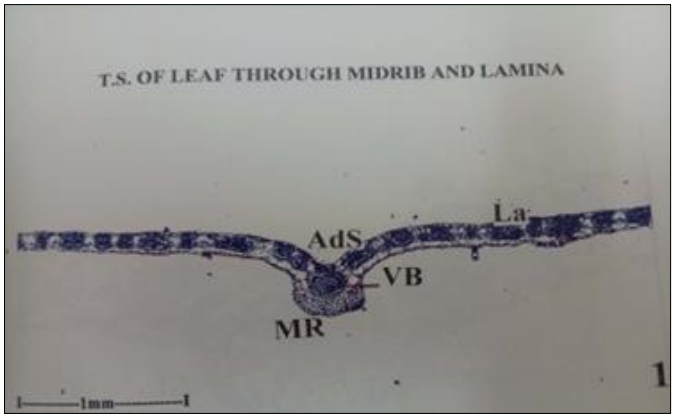


Fig 3: T.S of leaf through midrib and lamina

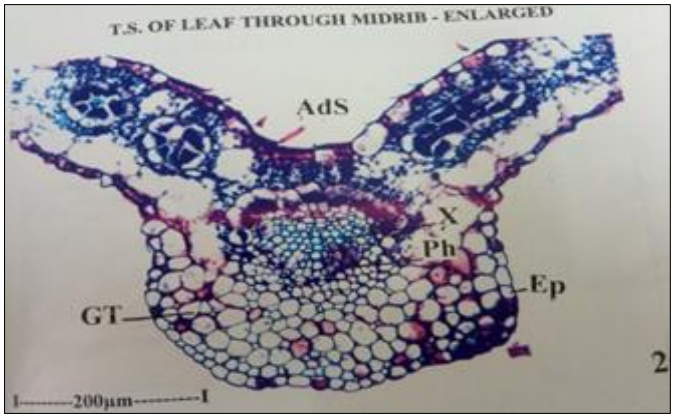


Fig 4: T.S of leaf through mid rib enlarged

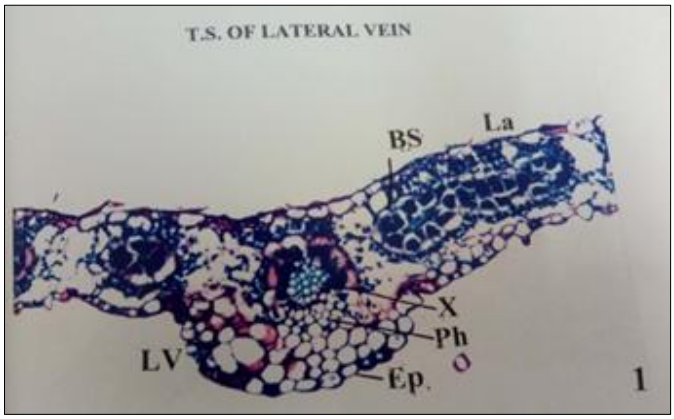


Fig 5: T. S of lateral vein

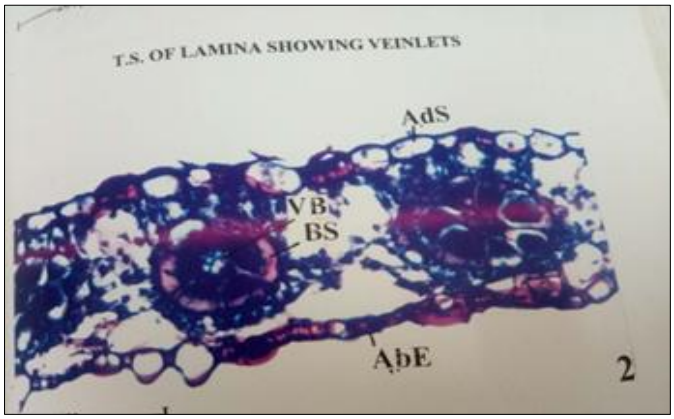


Fig 6: T.S of lamina showing vein lets

Fig 3, 4, 5, 6: Ads-Adaxial side, M.R-Mid Rib, V.B-Vascular bundles, La-Lamina, GT-Ground tissue, Ph-Phloem, X-Xylem, Ep-Epidermis, B.S-Bundle sheath, AbE-Abaxial epidermis.

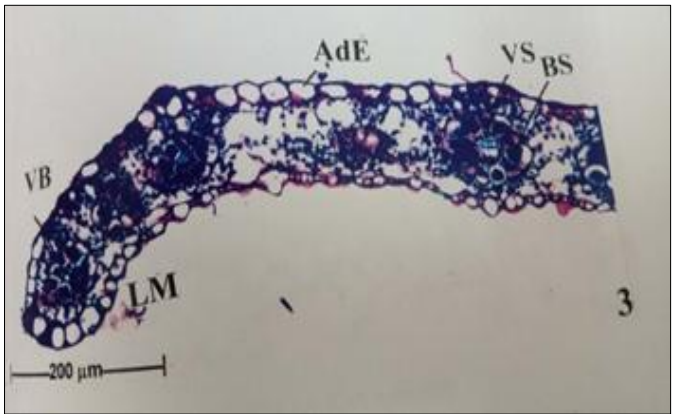


Fig 7: T.S of lamina showing vein lets enlarged

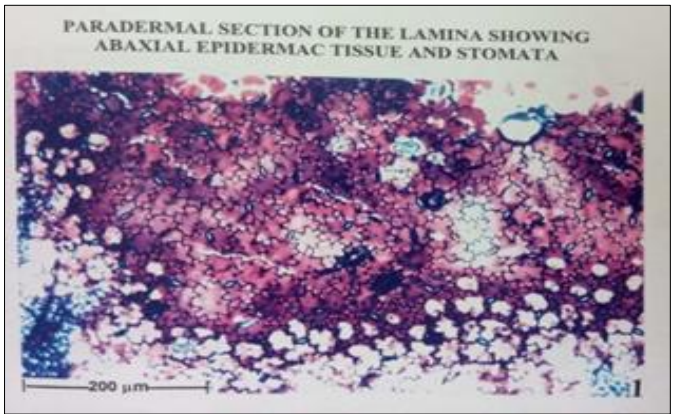


Fig 8: Epidermis and stomata

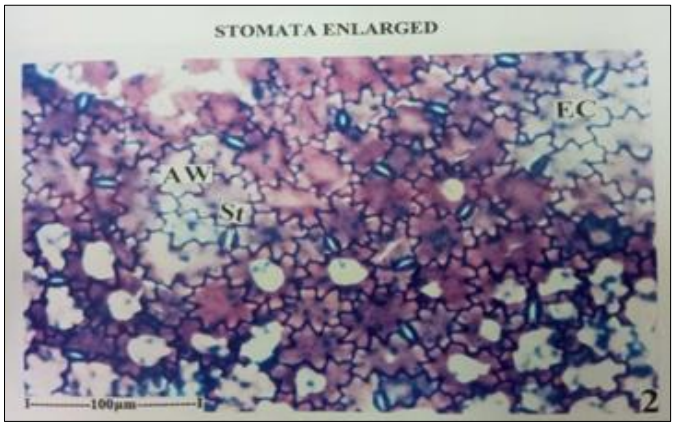


Fig 9: Stomata enlarged

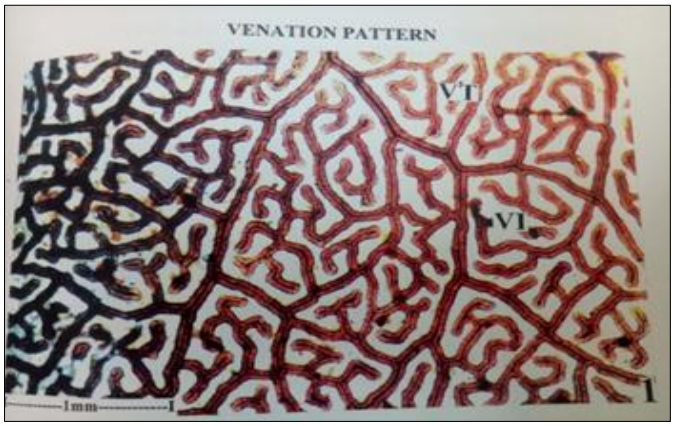
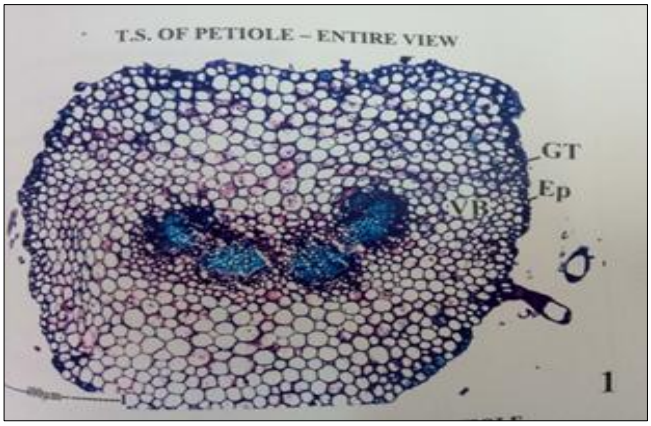


Fig 10: Venation pattern



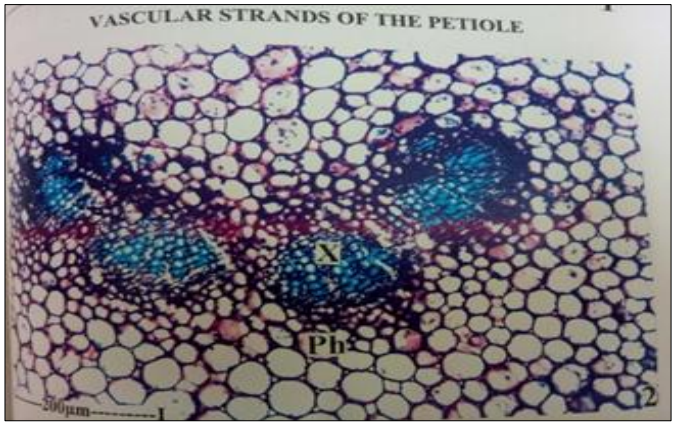
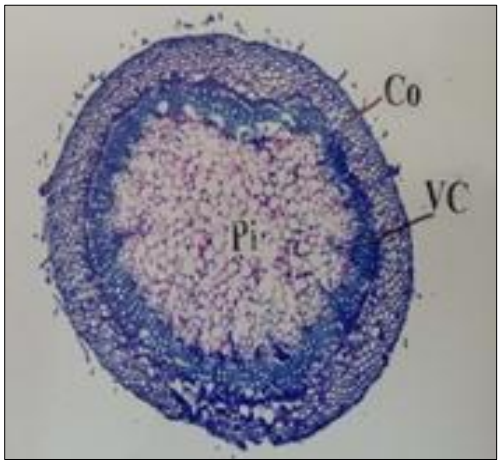


Fig 11: TS of Petiole **Fig 12:** Vascular strands of petiole



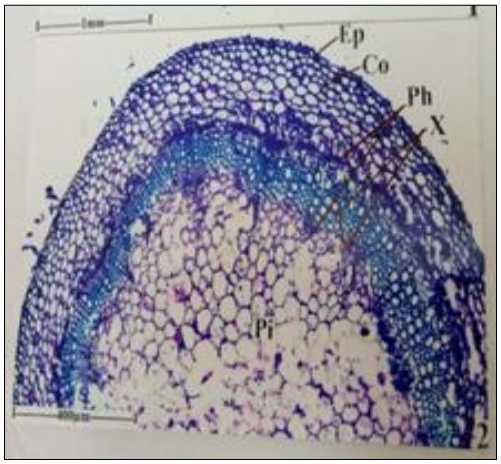


Fig 13: T. S of young stem **Fig 14:** T.S of young stem enlarged

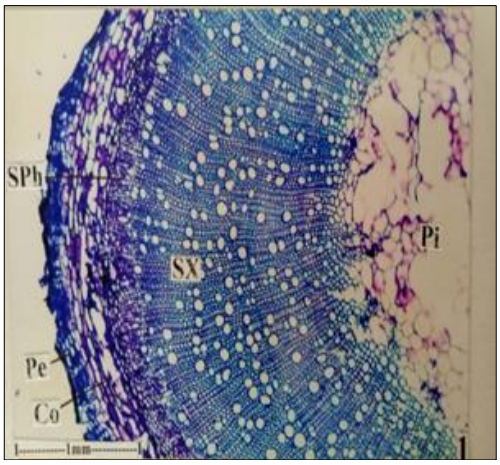


Fig 15: T.S of old stem en

Fig 7=15: EC-Epidermal cell, St-stomata VT-Vein termination, VI-Vein islets, Pi-Pith, Co-Collenchyma, SPH-Secondary phloem, SX-Secondary xylem.

Powder microscopy

Powder characteristics revealed the presence of starch granules, sclariform vessels, covering trichomes, lignified fibres, pericyclic fibres, epidermal cells with trichomes and kranz tissue.

Quantitative microscopy

The quantitative microscopy such as vein- islet number, vein-terminal number, stomatal number and stomatal index were determined and the results were tabulated. (Table 1)

Table 1: Quantitative Evaluation of the Crude Drug of Leaf of *Euphorbia hirta*

Standardization parameters	
Vein islet no	8/sqmm
Vein termination no	6/sqmm
Stomatal number (upper)	16.66
(Lower)	28.66
Stomatal index (upper)	6.391
(Lower)	8.835

Physiochemical parameters

The powdered drug was evaluated for its physico-chemical parameters like total ash values, acid insoluble ash, water soluble ash and loss on drying, and the results were tabulated (Table 2). The ash contents showed the amount of inorganic matter present in the sample and the acid insoluble ash almost within 2.2 % which expresses low siliceous matter present in the sample.

Table 2: Physico Chemical Evaluation of *E. hirta*

Standardization parameters	% W/W
Total Ash	7.8±0.03
Acid Insoluble Ash	2.20±0.02
Water Soluble Ash	4.0±0.023
Loss on Drying	8.5

Extractive values

The extracts were prepared according to the polarity and they were concentrated and their values were calculated with reference to air dried drug and the results were tabulated (Table 3).

Table 3: Extractive Values of Aerial Parts of Extracts of *Euphorbia hirta* with Different Solvents

S. No.	Extracts	Extractability (%)
1.	Petroleum Ether Extract	3.2
2.	Benzene Extract	2.8
3.	Chloroform Extract	2.45
4.	Methanol Extract	12.2
5.	Ethanol Extract	11.2
6.	Aqueous Extract	16.6

Preliminary phytochemical analysis

The powdered drug and various extracts such as petroleum ether extract, benzene extract, chloroform extract, methanolic extract, ethanol extract and aqueous extract were subjected to preliminary phytochemical screening of their presence or absence of the constituents and the results were tabulated (Table 4).

Table 4. Preliminary Phytochemical Tests for Drug Powder and Various Extracts of *Euphorbia hirta*

Test	Drug powder	Petroleum ether	Benzene	Chloroform	Methanol	Ethanol	Aqueous
Sterols	+	+	+	+	+	+	+
Terpenoids	-	-	-	-	+	+	+
Carbohytrates	-	-	-	-	-	-	-
Flavanoids	+			+	+	+	+
Proteins	-	-	-	-	-	-	-
Alkaloids	+	-	-	+	+	+	+
Glycosides	-	-	-	-	+	+	+
Saponins	-	-	-	-	+	+	+
Tannins	+	-	-	-	+	+	+
Mucliage	-	-	-	-	-	-	-
Resins	-	-	-	-	+	+	-
Oil/Fats	-	-	-	-	-	-	-

Fluorescence analysis

The powdered drug and they were treated with solvents and the colour changes were observed under Ultra Violet light in different wave length and the results were tabulated (Table 5)

Table 5: Fluorescence Analysis of *E. hirta*

Solvents used	Day light	U.V light 254nm 366nm	
Powder as such	Dark green	Slight green	Blackish green
1 N HCl	Greenish brown	Brownish green	Dark green
50% HCl	Light green	Green colour	Dark green
50%HNO_3	Green	Brownish green	Light green
50%H_2SO_4	Slightly green	Brownish green	Light green
1 N NaOH	Light yellow	Green	Moderate green
Alcoholic NaOH	Light green	Green	Dark green
Methanol	Light green	Dark green	Dark green
Benzene	Slightly yellow	Slightly buff	Green
FeCl3	Brownish yellow	White	Green
1% KOH	Brownish black	Light buff	Dark green
Lead acetate	White	Florescent white	White
Distilled water	Clear	Green	Green

TLC profile: Well resolved TLC profiles were recorded:

For the future reference and identity of the plant material. Aqueous extract TLC profile showed four spots under UV 254 nm, after derivatization in visible light). Methanolic extract the profile showed three spots under UV 254 nm, All the above parameters, which are being reported for the first time in this plant, are significant towards establishing the pharmacognostic standards for future identification and authentication of genuine plant material. The result obtained from TLC are depicted in (Table 6)

Table 6: TLC analysis of Aqueous and methanolic extract of *E. hirta L.*

S. no	Extract	Solvent system	No of spots	Rf values
1	Aqueous extract	Toluene:Ethyl acetae:Acetic acid (3:7:1)	4	0.52 0.73 0.84 0.86
2	Methanolic extract	Toluene:Ethyl acetate:Formic acid (3:1:1)	3	0.28 0.45 0.74

Powdered drug reaction with different reagent:

The powdered drug treated with different reagents and the observe the colour change**.** The result are tabulated in (Table-7)

Table 7: Powdered drug reaction with different reagent

Treatment	Observation
Conc. HCl	Dark green
Conc. HNO_3	Light brown with whitish foam
Conc. H_2SO_4	Greenish yellow
Glacial acetic acid	Light yellow
Iodine solution	Dark brown
NaOH in methanol	Light green

Discussion

Our study has focused on examining Pharmacognostic and Preliminary phytochemical study of *Euphorbia hirta*. Leaves and stem. Normalization of the macroscopic and microscopic characteristics of the *Euphorbia hirta*. Drug remains essential in other to identify and avoid falsification. Thus comparing the cross section of the leaf and stem anatomy showed structural similarities. Both sections have a spinal cord parenchyma, a phloem, xylem and collenchyma. It is observed in a thin sheet cuticle on the upper epidermis and the lower epidermis. Also palisade tissue above the spongy parenchyma. In the stem, secreting pockets are visible on the surface of the medullary parenchyma, as well as supporting cells sclerenchyma primary tissue (primary phloem). The distinct cortical parenchyma can be seen towards the periphery of the cut. Organoleptic characteristics are important in drugs because they play a role in the detection of adulterated or substituted drugs [13].Thus leaves green in colour, emit a very fragrant and aromatic mintyodor when bruised. The powdery appearance of the crushed leaves, with a coarse texture. The micrograph performed on the powder has highlighted a number of characteristic elements namely: the epidermal cells, the anomocytic type of stomata, the spiral beams, the cystoliths, the trichomes, spiral wooden beam, oily cells, are diagnostic substances for drugs of plant origin. These diagnostic elements are consistent with botanical standards and WHO guidelines [14-15].The study of physicochemical parameters such as moisture content and ash values are useful as it determines the physiological and nonphysiological state of ash, this will help to determine the possibility of microbial growth and lastly contaminant or impurities. The moisture content of the drug studied had a rate of 8.5 ± 0,1, which is below 10%. This result comply with the standards established by the International Pharmacopoeia, because this water content rate, prevent oxidation reactions, fermentation and give less chance to microbial growth and contamination in drugs [16]. Therefore, for proper conservation of drugs made from the leaves of *Euphorbia hirta*, it would be desirable to use those whose water content is less than or equal to 10%.The determination of total ash gave us a rate of 7, 48 ± 0,03. This value indicates the level of minerals in drugs. Insoluble ash in hydrochloric acid gave a rate of 2.2 ± 0,02. Indeed, the ash insoluble in hydrochloric acid tells us about the contamination of the drug by siliceous elements [17]. This result is in agreement with Srikanth *et al*. [18] who found rate of 0.97% and 0.5% respectively. The maximum extractive value was found in distilled water (16.6%) followed by methanol (12.2%) Ethanol (11.2%), Petroleum ether (3.2%), Benzene (2.8%) Chloroform (2.45%). All the extracts of the drug was subjected to different tests for detecting the presence of various phytoconstituents present in the drug, which revealed the presence of alkaloids, flavanoids, saponins, terpenoids and tannins. Preliminary phytochemical analysis indicated a high percentage of quercetine and flavonoids and this may be one of the reasons behind the anti diabatic and hepatoprotective activity of this plant. TLC profile of Aqueous extract showed four spots under UV 254 nm, The Rf values of these spots are 0.52, 0.73,0.84 and 0.86 after derivatization in visible light). Methanolic extract the profile showed three spots under UV 254 nm and the Rf values of the spots are 0.28,0.45 and 0.74. These parameters, which are being reported for the first time in this plant, are significant towards establishing the pharmacognostic standards for future identification and authentication of genuine plant material. Though *Euphorbia hirta* is a weed, it is a highly reputed drug used in Ayurveda. Barring the anatomical details and preliminary phytochemical screening, rest of the pharmacognostical parameters, gives us the clue that it can be cashed economically as well to improve the standard of health in the developing countries.

Conclusion

WHO has emphasized the need to ensure quality control of the raw materials used for Ayurvedic medicines by using modern techniques and by applying suitable parameters and standards. In the present study various standardization parameters such as macroscopy, microscopy (histochemical and powder), physicochemical standards, preliminary phytochemical investigation and TLC profiles in aqueous and methanol extracts were studied, which are being
reported for the first time in this plant and could be helpful in authentication and preparation of a
suitable monograph for the proper identification of *Euphorbia hirta* for the future.

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