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Comparative Pharmacognostic and Phytochemical Standardization of *Euphorbia hirta* L. and *Euphorbia thymifolia* L.

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ABSTRACT

The genus *Euphorbia* is unique, which covers highly reputed medicinal plants useful in various treatment of diseases like asthma, bronchitis, hay fever, cancer and inflammation. The present study includes comparative pharmacognostic and phytochemical standardization of aerial parts of two *Euphorbia* species i.e. *E. hirta* L. and *E. thymifolia* L. The comparisons of both species were carried out by various standardization parameters such as macroscopy, microscopy, powder study, leaf constant, physicochemical analysis, mineral content, qualitative and quantitative phytochemical screening and TLC study. The results of the present study showed various distinguished characters in two species which could serve as an indicator for authentification and identification of *E. hirta* and *E. thymifolia* based on their standardization parameters.

Keywords: *Euphorbia hirta, Euphorbia thymifolia,* pharmacognostic, phytochemical and standardization.

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INTRODUCTION

The largest genus of family Euphorbiaceae is *Euphorbia* with about 1600 species characterized by the presence of white milky latex which is more or less toxic. The genus has diverse chemical entities with a lot of structural diversities. The *E. hirta* L. (Euphorbiaceae) commonly known as *dudheli* in vernacular language (Gujarati). It is widely found in most parts of India and other tropical countries, especially on roadsides and on wasteland.[1] It is erect, slender plant or sometimes found diffused through the soil and white milky juice is exhausted upon cutting of stem.[2] The *E. hirta* L. (Figure. 1a) is often used traditionally for female disorders, respiratory ailments (cough, coryza, bronchitis, and asthma), worm infestations in children, dysentery, jaundice, pimples, gonorrhea, digestive problems, and tumors.[3] The stem sap is used in the treatment of eyelid stye and a leaf poultice is used on swelling and boils.[4] Root decoction is mainly beneficial for nursing mothers deficient in milk and useful for snake bites.[5]

Fig. 1a. *Euphorbia hirta* L Fig. 1b. *Euphorbia thymifolia* L

Figure 1: Morphology of plants

The literature survey reported that *E. hirta* contains alkanes, triterpenes, phytosterols, tannins, polyphenols, and flavonoids.[6] The *E. thymifolia* L. (Euphorbiaceae) usually called as *nani dudheli* in vernacular language (Gujarati). The *E. thymifolia* L. (Figure. 1b) is a prostrate, annual herb; stems many, spreading on the ground. Leaves are opposite, obliquely oblong, small, unequal sided at base, entire or crenulated, glabrous and green or reddish above, glaucous and pubescent.[2] The *E. thymifolia* is traditionally used as a blood purifier, sedative, haemostatic, anthelminthic and also used as an antiviral in bronchial asthma and paronychia.[7] The reported pharmacological indications

of *E. thymifolia* are antispasmodic, bronchodilator, antiasthmatic, diuretic, anthelmintic, hepatoprotective, antioxidant, antiarthritic and galactagogue.[8] The *E. thymifolia* contains hydrolysable tannins, flavonoids, triterpenoids and essential oils.[9-11]

According to Gupta et al.[12] *E. thymifolia* is substituents and adulterant of *E. hirta.* Because *E. hirta* bears same regional name *Dudheli*, while *E. thymifolia* called *nani dudheli.* Sereena and Shahida[13] reported very little work on comparative anatomical and histochemical studies of *E. hirta* and *E. thymifolia* stem. In account of this, the aim of the present study was to perform comparative pharmacognostic characters, physicochemical analysis and phytochemical evaluation including thin layer chromatography (TLC) finger printing profile of aerial part of *E. hirta* and *E. thymifolia*.

MATERIALS AND METHOD

Collection and authentication of plant materials

Fresh plants of *E. hirta* and *E. thymifolia* were collected from Amargadh village, Rajkot, Gujarat, India. The plants were identified and authenticated by CSIR-NISCAIR, New Delhi, India. Voucher specimens (DP/SVU/PHCOG/Herb/02 & DP/SVU/PHCOG/Herb/03) of same have been deposited in Sumandeep Vidyapeeth, Vadodara for future references.

Macroscopic analysis

Details macroscopic and taxonomic features of the leaves viz. size, shape, colour, surface, venation, apex, margin, base, lamina and texture were studied according to the reported procedures.[14,15]

Microscopic analysis

The microscopic studies were done for both species by taking free hand sections and stained with phloroglucinol and concentrated HCl.[16] The stained sections were mounted with glycerin and observed under microscope (Olympus BX10, Tokyo, Japan) at 10X and 45X.

Powder studies

Aerial part of plants were dried in the shade and finely powdered. Powders were passed through a sieve #180 and #125. The sample was treated with phlorogucinol: conc. HCl and mounted with glycerin for micrsocsopic examination.[17]

Leaf constants

Fresh mature leaf was washed and small fragment was taken from the middle region of the lamina to study the venation pattern. Washed leaf fragments were first boiled in 90% alcohol for about 3-5 min to remove chlorophyll, and then washed 2-3 times with water, then boiled again with 10% KOH solution for 2-3 min and washed 4-5 times with water. The epidermal layer was peeled off

using the help of pointed forceps and washed with water. The margins of the cover slip were sealed with DPX (Hi-Media laboratories Pvt. Ltd, Bombay), and the slides were observed under the microscope. Stomatal index, vein termination number and vein islet number were calculated.[17]

Physicochemical parameters

Physicochemical parameters such as total ash, acid insoluble ash, water soluble ash and extractive values were performed to determine quality and purity of the plant material.[18]

Preliminary screening of secondary metabolites

The shade dried plant material was powdered using mixer grinder, and extracted with petroleum ether, benzene, ethyl acetate, methanol and distilled water by using soxhlet apparatus for 8 h. The extracts obtained were evaporated till dryness and weighed to calculate % yields. The extracts were used for preliminary phytochemical analysis for the presence of different phytochemicals like steroids, alkaloids, glycosides, flavonoids and tannins etc. by using standard procedure.[19]

Heavy metal and mineral content

The heavy metals (Pb, Cd, Ni and Zn) in plants were estimated by wet digestion methods.[20] A 1.0 g plant material was first digested with 5 mL of conc HNO_3, followed by application of 15 mL of the tri acid mixture (HNO_3, $HClO_4$, and H_2SO_4, 10:4:1, v/v), heated at 200°C and concentrated to 1 mL. The aforementioned solution was filtered and diluted to 100 mL. This solution was used for the estimation of minerals. Macro minerals viz. Na, K, Ca and Li were estimated by Flame photometer (AIMIL, New Delhi, India) while microelements viz. Fe, Cu, Mn, Zn, and Co were estimated by Atomic absorption spectrophotometer (Perkin-Elmer 200, USA).

TLC study

2 g of each powdered material was extracted with water and methanol separately on water bath for 25 min (3 times). The extracts were collected and filtered. The filtrates were dried at 40°C under reduced pressure in a rotary evaporator and stored in desiccator. Known quantities of the extracts were dissolved separately in methanol and water for TLC. Gallic acid (1 mg/mL) was used as reference standard. Samples were spotted on TLC plates (10 × 10 cm) pre-coated with silica gel 60 F_{254} (Cat. No. 1.05554.0007, E. Merck, Darmstadt, Germany). Plates were developed in a glass twin-trough chamber (20 × 10 × 4 cm) with mobile phase (Toluene: Ethyl acetate: Methanol: Formic acid, 3:3:0.2:0.8, v/v) at 25 ± 2°C and 40% relative humidity. The post chromatographic derivatization was done using Folin-Ciocalteu reagent (FCR) and the R_f value was calculated at 254 nm and 366 nm.[21]

RESULTS AND DISCUSSION

The macroscopic character was useful in quick identification of plant material and also serves as an important standardization parameter. The comparative macroscopical characters of leaf and stems are described in Table 1.

Table 1: Comparative macroscopic characters of *Euphorbia* species

E. hirta	***E. thymifolia***
• Annual herb with 15-70 cm height • Leaves are opposite, leaf blades are oblong-lanceolate, elliptic, serrulate or dantate margin. The apex is almost acute. Midvein is often purple in colour. • Stem is branched, prostrate to ascending with branches reddish or purplish in color, with abundant latex and is covered with short hairs. • Inflorescences called cynathium, several cyathia densely clustered into cyme. • Flowers are pedunculate • Fruits are three lobed covered with short hairs • Capsules minute, hairy	• Annual prostate herb with 3-10 cm length • Leaves are opposite, elliptic, oblong or ovate. The apex is obtuse or rounded. The leaf blades are oval-oblong or obliquely oblong with dentate margin. Petiole is small, thin and often pink in color. • Stem is slender, cylindrical and spreading on the ground. Stem branches radiating, pubescent and pink in colour. • Involucres axillary, solitary or in axil. Cyathia in auxillary cluster • Flowers are campanulate • Fruits are ovoid globose, acutely 3-lobed and short hairy. • Capsule minute, hairy

The microscopic study serves as essential parameters for the identification of plants and provides useful information in monograph preparation. The transverse section (TS) of *E. hirta* and *E. thymifolia* leaf shows trichome, stomata, and arrangement of vascular bundle while stem shows trichomes, lignified xylem vessels elements, endodermis and pericyclic fibres as distinguished characters described in Table 2 and shown in Figure 2.

Table 2: Comparative microscopic characters of *Euphorbia* species

Part	*E. hirta*	*E. thymifolia*
Leaf	• The TS section from the midrib of *E. hirta* leaf possesses thick cutinized upper as well as lower epidermis layer. 2-3 layers • Sclerenchyma were present over lower epidermis. • The Vascular bundle contains xylem and phloem. The arrangements of xylem are found to be endarch. • Vascular bundle covered with pericyclic fibres. • Latex ducts are found in mesophyll region. • Multicellular covering trichomes are abundantly present mainly on the lower epidermis. • Surface preparation bears anisocytic stomata.	• TS of *E. thymifolia* shows a layer of the palisade. The upper epidermis of lamina shows anticlinal wall. • The meristele consists of the collateral vascular bundle. • Trichomes were absent on *E. thymifolia* leaf surface. • Laticeferous ducts are also found in mesophyll region. The surface view of *E. thymifolia* leaf showed the presence of anomocytic type of stomata.
Stem	• The TS of the stem of *E. hirta* shows cork, cortex, stele region and central pith. • Cork bears 2-3 layers of parenchymatous cells covered with a thick cuticle. • Cortex region is wide which contains calcium oxalate crystals and starch grains. • Latex ducts are mainly present in cortex region. Endodermis is not clearly visible. • Non-lignified pericyclic fibers are mainly found around the stele region. • Meristele region of stem contains phloem and xylem vessels and tracheid vessels. • Xylem elements composed of radially running vessel of various sizes, associated with trachieds, parenchyma and fibres. • Medullary rays are uni seriate, lignified. • The lignification of xylems is spiral in shape. Pith region of the stem is highly spongy and parenchymatous.	• The TS of stem of *E. thymifolia* cutinized epidermal cells are elongated, compactly arranged, bearing comparatively more number of trichomes which are unicellular. • Cork; 4-6 layered cortex distinct endodermis and lignified pericyclic fibers. Radially arranged xylem vessel associated with fibres and tracheids; medullary rays are uni-biseriate, lignified. • Oleoresin containing cells are present. • Phloems are narrow, contains latex duct followed by cambium and ring of xylem consisting of radially arranged vessels. • Numerous primary xylem groups are seen towards the pith region. • Pith is wide and parenchymatous and starch grains are present in the pith region.

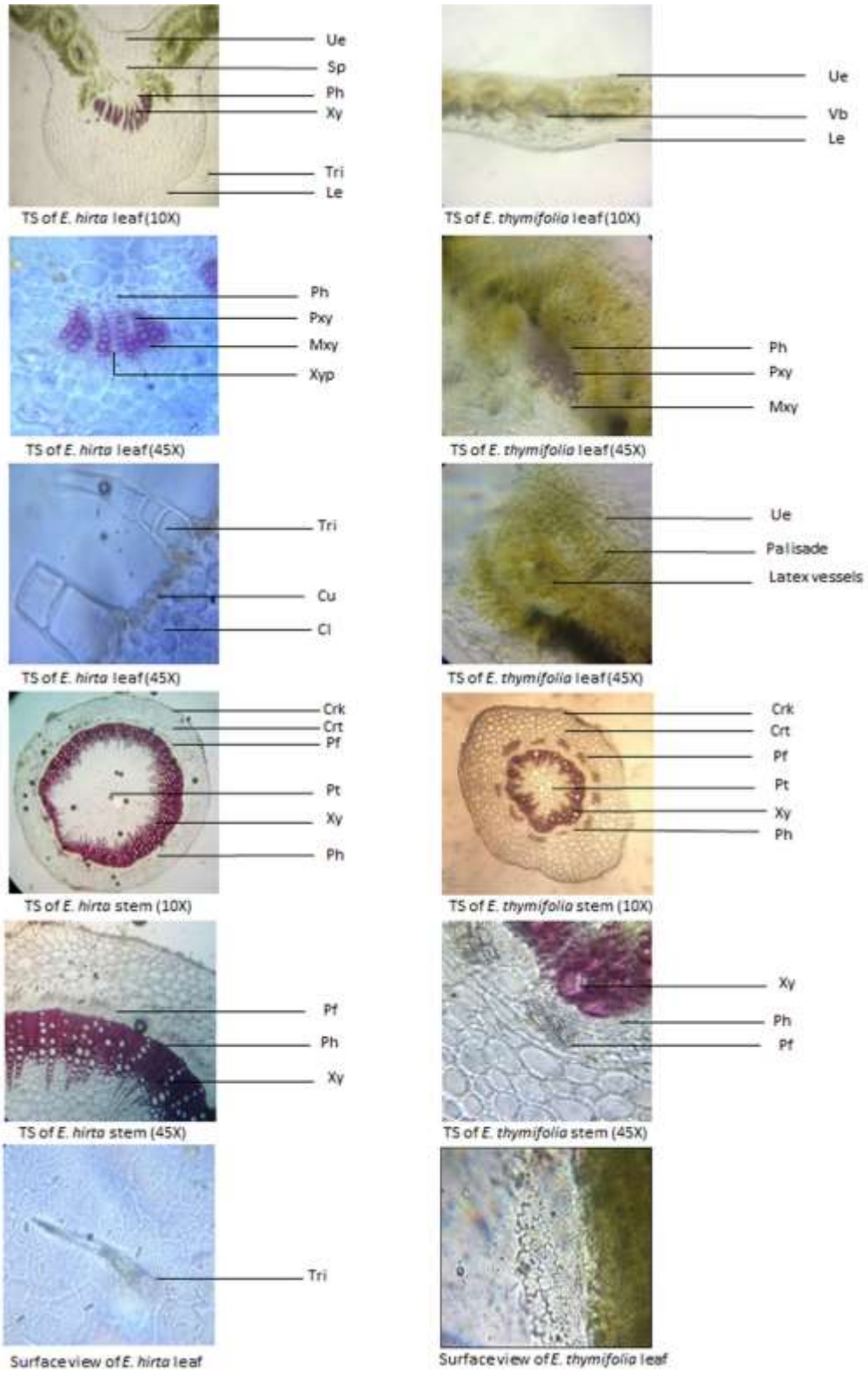

Figure 2: Comparative microscopic descriptors of *Euphorbia* species.

Abbreviation: **Leaf**-Le-Lower epidermis, Ue-Upper epidermis, Cu-Cuticle, Sp- Spongy parenchymatous, Tri-trichome, Mxy- Metaxylem, Pxy- Protoxylem, and Xyp- Xylem parenchyma. **Stem** - Ct-Cortex, Cu- Cuticle, Pt-Pith, Xy-Xylem, Ph-Phloem, and Pf- Pericyclic fibres.

Powder study of aerial part of *E. hirta* and *E. thymifolia* illustrated in Figure 3A-3O. The *E. hirta* shows presence of numerous multicellular covering trichomes having 5-6 cells with bulbous base. It also shows the presence of lignified xylem vessel element, pericyclic fibres, and tracheid's vessels with spiral lignification. However, *E. thymifolia* contain fewer unicellular trichomes, xylem vessel element with pitted lignification, lignified pericyclic fibres and starch grains.

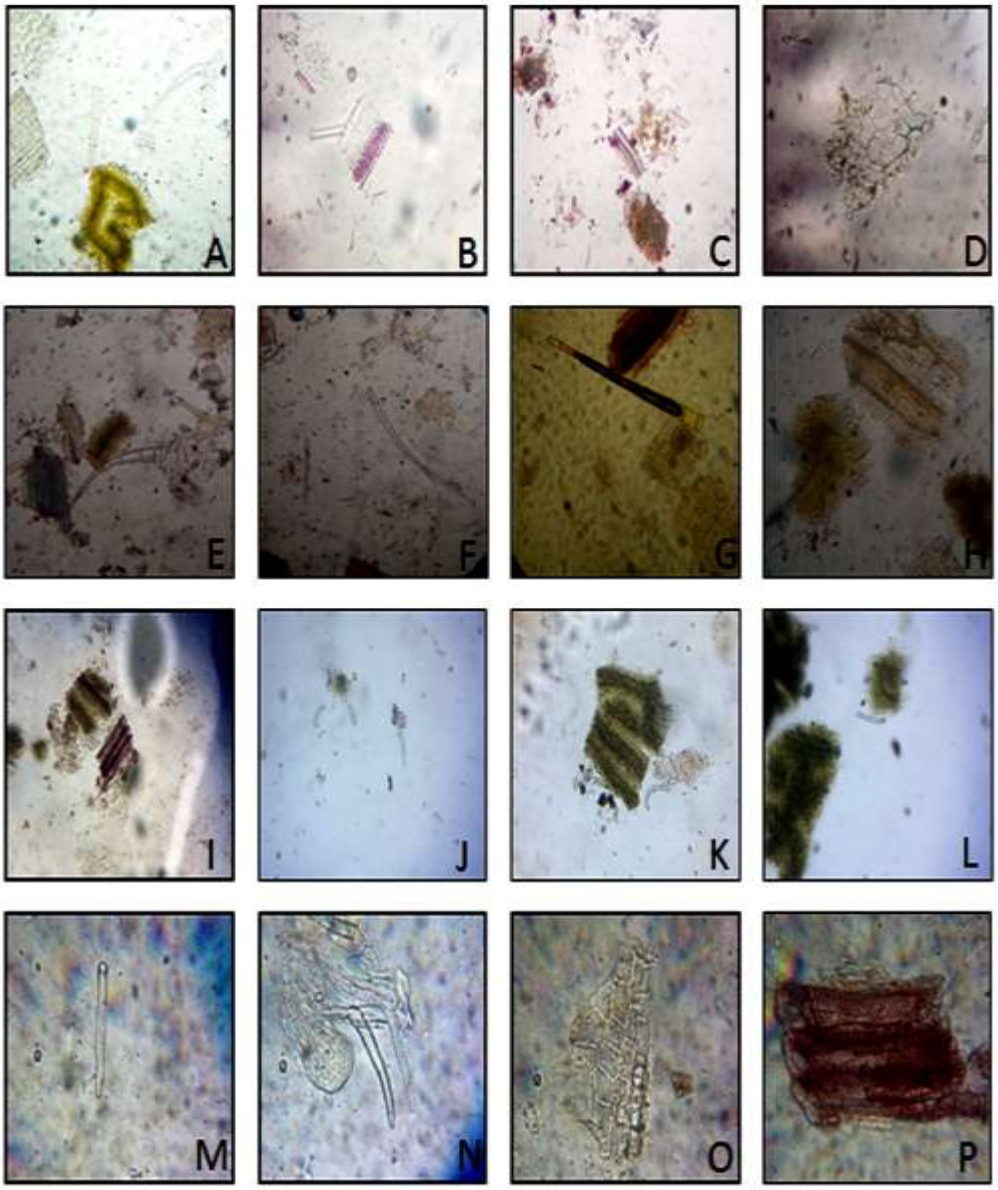

Figure 3: Comparative powder study of *Euphorbia* species.

E. hirta: (A) Multicellular covering trichome, (B) Lamina, (C) Spiral shape xylem vessels, (D) Anisocytic stomata, (E-G) Covering trichome in varying numbers of cells, and (H) Fragment of epidermal cells.

E. thymifolia: (I) Pitted xylem vessels, (J) Unicellular trichome of stem, (K) Fragment of lamina, (L-N) Unicellular trichome of stem and (O) Anomocytic stomata and (P) Pitted xylem vessels (45X)

Leaf constants, one of the parameters of quantitative microscopic studies, were determined to establish the identity of authentic plant material. Stomatal number, vein islet and vein termination numbers for *E. hirta* and *E. thymifolia* showed in Table 3a-c. The surface of *E. hirta* leaf showed two, three, four and five cells covering trichomes with 87.5 to 500 µm length while *E. thymifolia* devoid of trichomes. There are 3-7 numbers of trichomes distributed per square mm area of lamina portion of *E. hirta* leaf.

Table 3: Pharmacognostic characters of leaf surface in *Euphorbia* species

Comparative microscopic characters of *Euphorbia* species

Determination	***Euphorbia hirta***		***Euphorbia thymifolia***	
	Range	**Mean[a] ± SD**	**Range**	**Mean[a] ± SD**
Upper epidermal stomatal index	13-15	14 ± 1.6	12.7-19.7	16.2 ± 2.5
Lower epidermal stomatal index	15-17	16 ± 2.3	18.6-27.3	22.95 ± 4.6
Vein islet number	5-7	6 ± 1.2	3-6	4.5 ± 1.3
Vein termination number	21-23	22 ± 3.4	6-14	10 ± 4.1

[a]n=3, SD= Standarad deviation

Distribution of covering trichome with varying number of cells

Number of cells	***E. hirta***	***E. thymifolia***
One cell	Nil	
Two cells	04	
Three cells	15	Absent
Four cells	16	
Five cells	12	

Relative frequency of trichome of different length

E. hirta		***E. thymifolia***	
Range(µm)	No. of trichome	Range(µm)	No. of trichome
87.5	1	Nil	
229.3	24		
500	22		

Phytochemical screening is an important parameter used to identify different classes of the active constituents present in the plants.[22] The results of phytochemical screening of *E. hirta* and *E. thymifolia* showed the presence of tannins, saponin glycosides, phenols, coumarins, triterpenoids and flavonoids in different extracts (Table 4). In addition, anthraquinone glycosides were found in both plants but *E. hirta* showed modified Bontrager's test positive while *E. thymifolia* showed Bontrager's test positive. Moreover, *E. thymifolia* shows higher amount of tannins as compared to *E. hirta.* Thus, preliminary phytochemical screening and quantitative estimation supporting the reason for their wide range of biological activities of both species.

Table 4: Preliminary phytochemical screening of *Euphorbia* species

Extract	Chemical constituents	*E. hirta*	*E. thymifolia*
Petroleum ether	Triterpenoids	++	++
	Resins	+	+
Benzene	Steroids	++	++
	Triterpenoids	++	++
Ethyl acetate	Alkaloids	-	-
Alcohol	Alkaloids	-	-
	Anthraquinone glycoside	+	+
	Coumarins	+	+
	Saponins	+	+
	Flavonoids	+	+
	Tannins	++	++
Water	Alkaloids	-	-
	Flavonoids	+	+
	Coumarins	+	+
	Anthraquinone glycoside	+	+
	Tannins	++	++
	Reducing sugars	+	+

Physicochemical evaluation is useful to ascertain the quality of medicinal plants. The extractive values are useful for the determination of exhausted or adulterated drugs. The water soluble extractive values of *E. thymifolia* and *E. hirta* was found to be 24.43 ± 0.58 % w/w and 23.86 ± 0.35 % w/w respectively. Alcohol soluble extractive values of both plants were found to be 15.41 ± 0.52 and 12.75 ± 0.67 % w/w, respectively. The results indicated that drug contains higher amount of polar components viz. tannins, flavonoids, glycosides, and carbohydrates which was also confirmed by the phytochemical analysis. Ash values provide an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Total ash content of *E. hirta* and *E. thymifolia* was 9.5 ± 1.00 % w/w and 8.64 ± 0.97 % w/w, respectively. Water soluble ash of *E. hirta* and *E. thymifolia* is 1.27 ± 0.28 % w/w and 3.83 ± 0.38 (% w/w) respectively, while acid insoluble ash of *E. hirta* and *E. thymifolia* is found to be 1.18 ± 0.32 and 1.41 ± 0.52 (% w/w) respectively. The result showed insignificant difference in ash value for both species.

The results of heavy metals and mineral contents were found under limits[18] are shown in Table 5. Both the medicinal plants are good sources of iron, magnesium and potassium. The minerals present in *E. thymifolia* and *E. hirta* can plays an important role in human nutrients.

Table 5: Heavy metals and mineral content of *Euphorbia* species

Metal	PPM	
	***E. hirta*([a]Mean ± SD)**	***E. thymifolia*([a]Mean ± SD)**
Heavy metals		
Pb	1.44 ± 0.01	2.22 ± 0.01
Cd	0.11 ± 0.01	0.55 ± 0.01
Ni	1.15 ± 0.004	0.76 ± 0.01
Cr	0.77 ± 0.01	0.66 ± 0.01
Mineral content		
Mn	188.95 ± 0.089	92.79 ± 0.101
Cu	30.49 ± 0.015	34.16 ± 0.291
Zn	151.78 ± 0.193	64.16 ± 0.478
Fe	2225.19 ± 1.038	1211.13 ± 0.808
Mg	5487.11 ± 23.57	4976.88 ± 2.449
K	33001.15 ± 1.009	47865.66 ± 3.055
Ca	21001 ± 0.550	2424.85 ± 0.251
Na	558.55 ± 0.485	754.62 ± 0.545

[a]n=3, SD= Standarad deviation

The fingerprinting analysis is nowadays getting momentum for the quality control of multi-component herbal medicines and has been widely accepted as a useful tool to determine authenticity and reliability of chemical constituents of herbal drug and formulations.[23] TLC study of methanolic extracts of *E. hirta* and *E. thymifolia* gave 6 and 3 bands respectively. The band with R_f value of 0.51 and 0.52 were observed in both *E. hirta* and *E. thymifolia* respectively, corresponds with standard gallic acid R_f at 0.51 when observed in 254 nm, 366 nm and derivatized with FCR (Figure 4). Hence, on the basis of these cumulative characters these two species can easily be differentiated or identified.

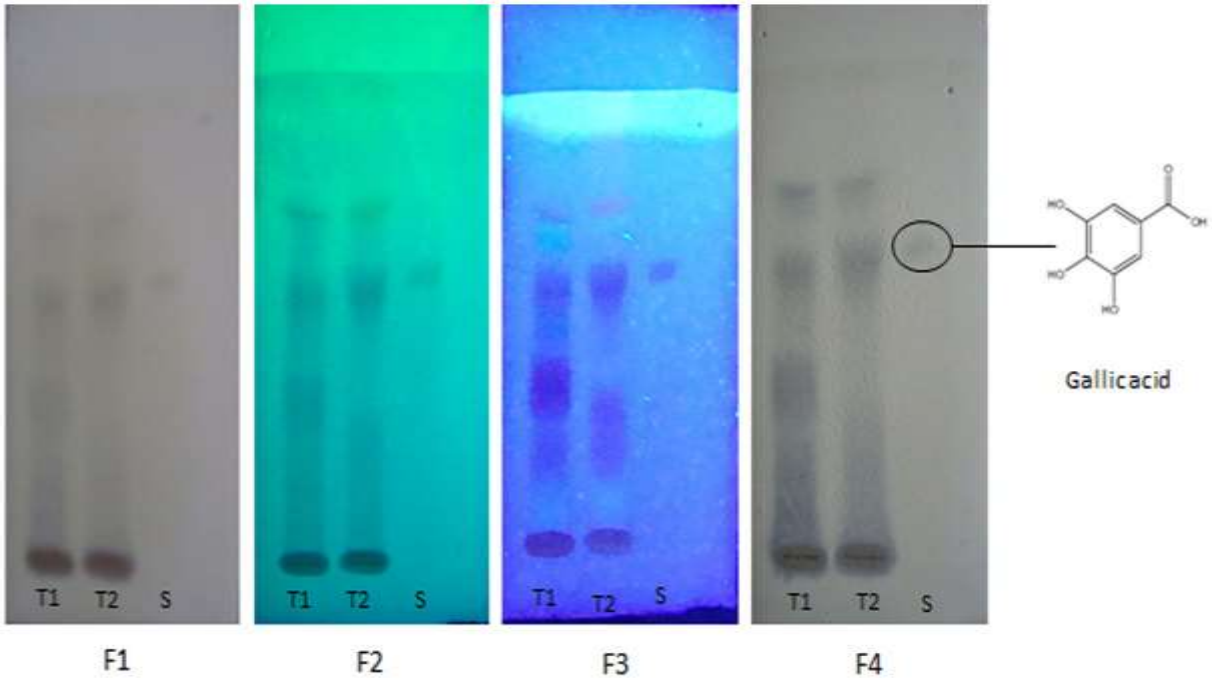

Figure 4: Comparative TLC profiles of *Euphorbia* species along with gallic acid

T1 - Methanol extract of *E. thymifolia*, T2 - Methanol extract of *E. hirta*, S - Standard Gallic acid, F1. Day light, F2. UV 254 nm, F3. UV 366 nm and F4. Folin–Ciocalteu reagent (FCR)

CONCLUSION

The current study assists in identification and authentification of two species of *Euphorbia i.e. E. hirta* and *E. thymifolia* based on diagnostic macroscopic and microscopic charcaters, powder characters, leaf constant, physicochemical parameters, mineral content, and phytochemical screening including TLC study. The result of present study could be useful in selecting authentic plant material and may also be serve as reference in preparation of monograph.

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