

## Pharmacognostical & Physicochemical Studies of *Euphorbia Tithymaloides* (L.) Poit

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**Abstract:** *Euphorbia tithymaloides* (L.) Poit (Euphorbiaceae) leaves are extensively used in traditional medicine to cure asthma, mouth ulcers, persistent coughing, venereal troubles & ringworms. Besides this, it is also known to possess antiprotozoal, mitogenic, anti-inflammatory, anti-plasmodial, anti-mycobacterial, anthelmintic and antimicrobial activities. The present study is an attempt to provide detailed information on pharmacognostical & physicochemical features of *E. tithymaloides*. Fresh plant was selected for macroscopical and microscopical studies while air-dried powdered sample of whole plant was used for physicochemical evaluation and powder microscopy. The macroscopical study of the plant showed that leaves are dark-green, alternate, ovate in shape having entire margin, acute apex and attenuate base, root is externally smooth, light brown tap root with few branches & numerous small lateral roots and has got a thin bark featured with few cracks & fissures while stem is very long, non-woody, greenish & cylindrical in shape which has anisotomous branching. Similarly, the microscopy of leaves revealed that it consists of single layered epidermis with cuticle and trichomes, 2-3 layered collenchyma near the upper epidermis followed by 6-8 layered spongy parenchyma and a centrally placed C-shaped bicollateral vascular bundles. Ash values, extractive values, loss on drying, foaming index like physicochemical parameters were also found out. The results of the study can be used as markers in the identification and standardization of this plant and also towards monograph development on the plant.

**Keywords:** *Euphorbia tithymaloides*, Microscopy, Macroscopy, Pharmacognostical study, Physicochemical study

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### I. Introduction

*Euphorbia tithymaloides* (L.) Poit (Family: Euphorbiaceae), commonly known as devil's backbone, is widely distributed throughout tropical and subtropical North America and Central America and some areas of South Asia (India: Assam, Bihar, Gujarat, Madhya Pradesh, Maharashtra, Odisha, UP).<sup>[1]</sup> The soil must be sandy & nutrient-rich, particularly in boron, copper, iron, manganese, molybdenum and zinc for its better growth. This plant, which is often known by its old scientific name *Pedilanthus tithymaloides*, is relatively intolerant of high soil salinity levels.<sup>[2][3]</sup> As it has got a very impressive variegated foliage, it is mostly used as an ornamental plant. The plant is 0.4 to 3m tall & 40-6cm wide whose fleshy tubular, zigzag stems produce thick, dark-green, ovate leaves and peculiar beak shaped flowers. Leaves are sessile, glabrous and acuminate in shape having length of about 1.4-3 inches while flowers whose color vary from bright white to pink are arranged in dichotomous congested cyme fashion. Fruits are sulcate, deeply 3-lobed having sub globose grey-brown seeds.<sup>[4]</sup> Although flowering in flushes year-round in warm tropical regions, it blooms most heavily in summer. When flowering or chilly winter temperatures occur, the leaves may blush pink. An extended drought or winter cold spell may cause the leaves to completely drop off.<sup>[5][6]</sup>

It is widely used in traditional medicine to treat asthma, persistent coughing and mouth ulcers. The plant is also reported to possess various antioxidant principles like kaempferol 3-O-b-D-glucopyranoside-6'-(3-hydroxy-3-methylglutarate), quercitrin, isoquercitrin, scopoletin and other phytochemicals viz. steroids, tannins, triterpenes, coumarins and saponins which have been shown to possess anti-diabetic, anti-viral, hemostatic, anti-microbial, anti-helminthic and mitogenic activity.<sup>[7]</sup> Its sap has been traditionally used to treat callouses, ear ache, insect stings, ringworm, skin cancer, toothache, umbilical hernias, and warts, though none has been scientifically proven as effective.<sup>[8]</sup>

### II. Materials and Methods

**Chemicals and Instruments:** Microscope, Camera Lucida, stage and eye piece micrometer, black chart paper, blade, watch glass, slide, pipette, HCl, Phloroglucinol, Chloral hydrate, Ethanol, Chloroform & Sulfuric acid

**Plant Collection:** The fresh plant *E. tithymaloides* for quantitative microscopy and physicochemical studies was procured from New Bridge Chouraha, Prayagraj with the help of local tribe and field botanist. The healthy leaves of the plant were first collected and stored properly. They were then washed with water and dried in sunlight for one hour and thereafter dried in shade. The dried leaves along with the dried plant were powdered with the help of grinder and were passed through the sieve no 60. The pharmacognostical evaluation and physicochemical studies of the fine powder and that of healthy leaves were then performed.

**Macroscopical Studies:** The morphological characters like condition, type, size, shape, apex, margin, venation, base, petiole, surface, phyllotaxy, color, odor, taste of leaves, length, branching, color, shape, internodal length of stem and type, shape, rootlets, odor, taste, presence or absence of bark of root of *E. tithymaloides* were studied.

**Microscopical Study:** The fresh leaf collected from the plant was washed with clean water. A small part of leaf from the midrib was then cut off with the help of blade. Then, a slice of a potato was cut into two parts not fully (last end was united). The leaf section was placed in between the slices which was held with thumb and index finger (Middle finger was kept down the potato slice). Then, the blade was driven parallel to the surface of the index finger. The cut off sections were put in a watch glass containing mixture of phloroglucinol & HCl (1:1) & then the better one was chosen for microscopical study. For the study of morphology of stomata, the surface of the leaf was prepared.<sup>[9]</sup>

**Quantitative Microscopy:**

**Stomatal Number:** It is the average no of stomata per square mm of the epidermis of the leaf. First of all, a fresh leaf collected from the plant was washed with clean water. The epidermis was then separated from the leaf by breaking the leaf into pieces and placed carefully on a slide with the help of a brush along with 1-2 drops of chloral hydrate. A square was then drawn on a drawing sheet. With the help of camera lucida, the stomata & epidermal cells were traced in the square. The epidermal cells and the stomata outside the square were traced to completion on two adjacent sides of the square, for counting purpose. Then the no of complete epidermal cells and the stomata within the square and the cells that are more than half on two adjacent sides are counted and stomatal no is calculated.<sup>[9]</sup>

**Stomatal Index:** It is calculated by using this formula:

$$I = \frac{S}{E + S} \times 100$$

I= Stomatal Index, S=No of stomata per unit area, E= No of epidermal cells in the same area.

**Powder Microscopy:** The healthy plants were first collected and then stored properly. It was then dried under sunlight for 4 hours and then at a shade for one whole day. The dried plant was then dried in a grinder and passed through sieve no 60. The fine powder was then stained with phloroglucinol + HCl (1:1) and observed under microscope .

**Physicochemical Studies:** The physicochemical parameters such as Total ash, Water Soluble Ash, Acid insoluble Ash, Sulphated Ash, Solvent extractive value, Loss on drying, Foaming Index.<sup>[9][10][11]</sup>

**Total Ash:** 2g of air-dried powdered drug was accurately weighed and put into the tared silica crucible. The crucible was supported on a pipe-clay triangle placed on a ring of retort stand. Then, it was heated with a burner, using a flame about 2cm high and supporting the crucible about 7 cm above the flame heat till vapors almost ceased to be evolved; then it was lowered and heated more strongly until all carbon was burnt off. It was then cooled in a desiccator. The ash thus produced was weighed and the percentage of total ash with reference to the air-dried sample of the crude drug was calculated.

**Water Soluble Ash:** The ash obtained while calculating total ash value was washed using 25 ml of distilled water into 100ml beaker. It was boiled for 5 minutes and then filtered through an 'ashless' filter paper. The residue thus obtained was washed twice with hot water. The crucible was ignited, cooled and weighed. The filter paper containing the residue was put into the crucible which was then heated gently until vapors ceased to be evolved and then more strongly until all carbons has been removed. After the ignition, it was cooled in a desiccator. The residue was weighed and water-soluble ash was calculated by subtracting the weight of residue from weight of total ash. The % of water-soluble ash was then calculated with reference to air-dried powdered drug.

**Acid Insoluble Ash:** First of all, the total ash was washed using 25ml of 2N HCl into 100ml beaker. It was then boiled for 5 minutes and then filtered through an 'ashless' filter paper. The residue thus obtained was washed twice with hot water. The crucible was ignited, cooled and weighed. The filter paper containing the residue was put into the crucible which was then heated gently until vapors ceased to be evolved and then more strongly until all carbons have been removed. After the ignition, it was cooled in a desiccator. The residue was weighed and acid- insoluble ash was calculated by subtracting the weight of residue from weight of total ash. The % of acid insoluble ash was then calculated with reference to air-dried powdered drug.

**Solvent Extractive Values:** 5gm of drug was macerated with 100ml of different solvents (90% alcohol-alcohol soluble extractive, 90% chloroform water- water soluble extractive) in a dry 250 ml conical flask for 24hrs. It was shaken frequently during first 6 hrs. and allowed standing for 18hrs. Thereafter it was filtered. 25ml out of filtrate was evaporated to dryness on a water-bath. The drying was then completed in an oven at

100°C. The weight was then measured and the percentage w/w of extractive was subsequently calculated with reference to the air-dried drug.

**Loss on drying:** Firstly 3.0 gm of powdered drug was weighed accurately and put in a tarred porcelain dish which was earlier dried at 105°C using hot air ovens at constant weight. Using the difference in weight, the percentage loss of drying with reference to the air-dried substance was calculated.

**Foaming Index:** About 1 gm of coarsely powdered drug was weighed accurately and transferred to 500ml conical flask containing 100ml of water maintained at moderate boiling at 80-90°C for about 30 min. After 30 min of boiling, it was cooled, filtered into a volumetric flask and sufficient water through the filter to make the volume up to 100ml (V<sub>1</sub>). Clean 10 stopper test tubes were then taken and marked as 1, 2, ..., 10. The successive portions of 1ml, 2ml, ..., 10ml drug was taken in separate tubes and then remaining volume was adjusted with the liquid up to 10ml in each. After closing the tubes with stoppers, they were shaken for 15 seconds and allowed to stand for 15 min. Then the height was measured. If the height of the foam in each tube is less than 1cm, the foaming index is less than 100 (not significant). While, if the foam is more than 1cm height after the dilution of plant material in the sixth tube, then corresponding number of the test tube was the index sought. If the height of the foam in every tube is more than 1cm, the foaming index is more than 1000. In this case, 10ml of the first decoction of the plant material needs to be measured and transferred to a 100ml volumetric flask (V<sub>2</sub>) and volume is to be maintained up to 100ml and follow the same procedure. Foaming Index was calculated by using this formula:

Foaming Index = 1000/a in case of V<sub>1</sub>;

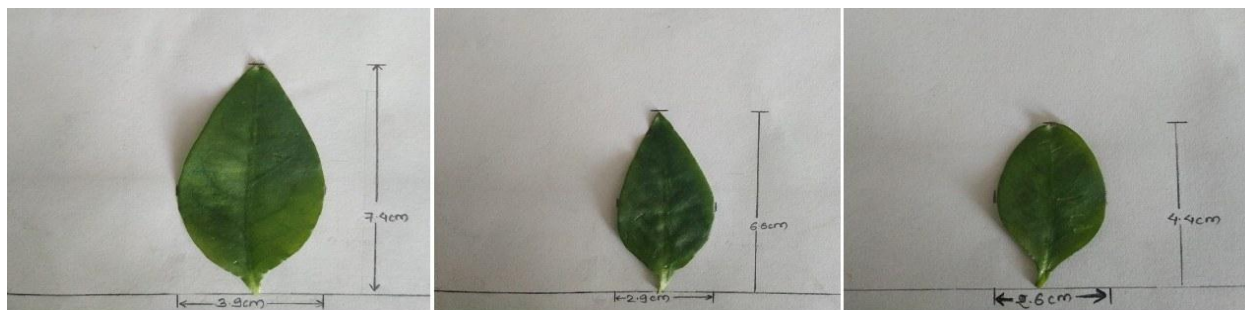
Foaming Index = 1000 \* 10/a in case of V<sub>2</sub>

Where, a = volume (ml) of decoction used for preparing the dilution in the tube where exactly 1cm or more foam was observed.

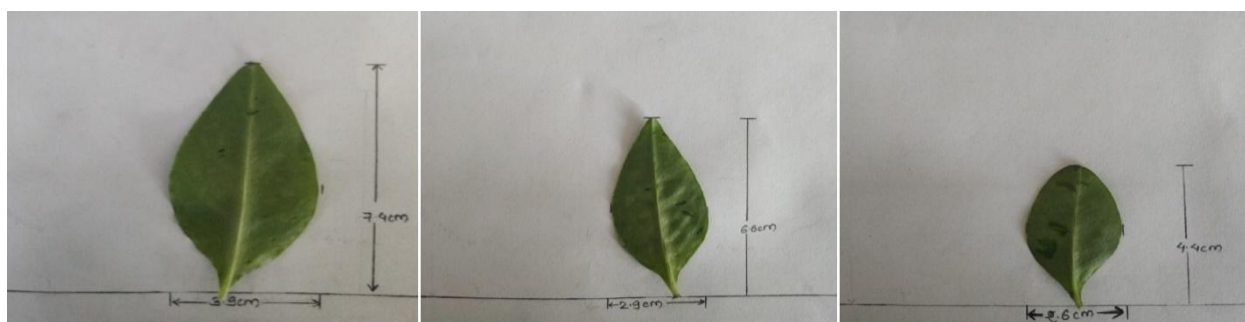
### III. Results & Discussion

#### Macroscopical Features:

- i. **Leaf:** The information obtained from the macroscopical studies of the leaf (like type, base, margin, apex, color, odor, etc.) are summarized in table no 1.



**Fig. i):** Upper Surface of the leaf



**Fig. ii):** Lower Surface of the leaf

	Observation
Condition	Fresh
Type	Simple
Size	Length: 4.4-7.4cm Width: 2.6 - 3.9cm
Shape	Ovate to cordate
Margin	Entire

Apex	Acute
Base	Attenuate
Venation	Unicostate reticulate venation
Phyllotaxy	Alternate
Surface	Glabrous
Color	Upper Surface: Dark Green Lower Surface: Light Green
Odor	Slightly aromatic
Taste	Mucilaginous
Extra Feature	Very thick leaf with cuticle present in it.

**Table No 1:** Macroscopical Features of *E. tithymaloides* leaf

**ii. Stem**

The stem is about 28.3cm long and 1.6-3.1cm thick with branches different in both size/vigor and angle from the main axis (anisotomous branching). It is non-woody, greenish in color and cylindrical in shape which sprouts from woody crown of the root. It has got distinct nodes and internodes (internodal length varies from 1.8cm-3.9cm). The stem when broken exudes a caustic milky sap (latex) which gives mucilaginous taste. Its odor is slightly aromatic.



**Fig. iii):** Stem of *E.tithymaloides*

**iii. Root:**

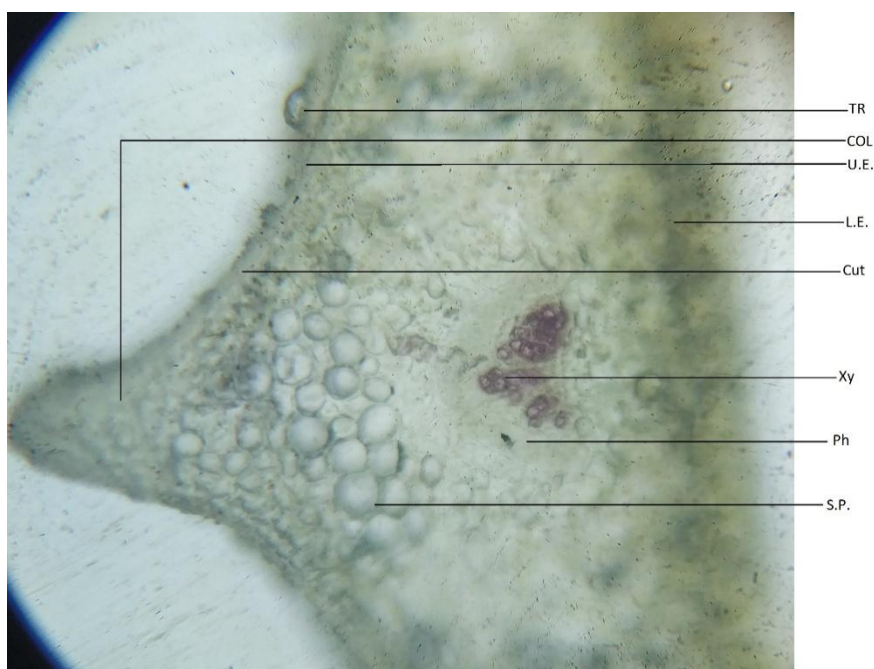
Root present in this plant is a tap root (having length of about 13.1cm) with few branches and numerous small lateral roots that show horizontal downward growth. It is light brown in color. The woody crown present in the top is durable in structure. The root is externally smooth with thin bark (easily gets peeled off exposing pale yellow inner part) which is featured with few cracks and fissures on its surface. It is slightly aromatic in odor but tasteless in taste.



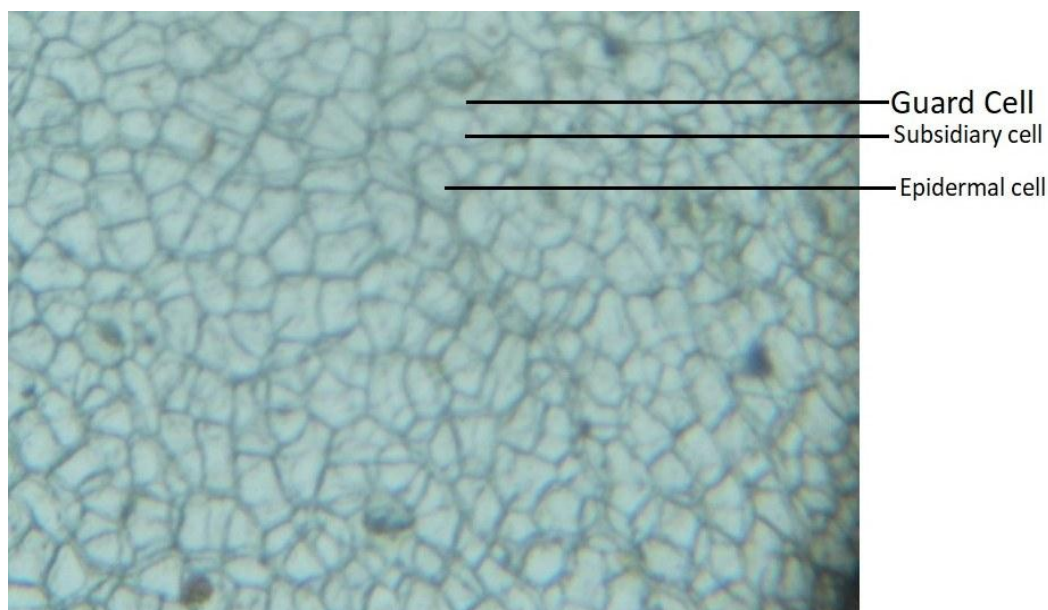
**Fig. iv):** Root of *E. tithymaloides*

**Microscopical Study:** The microscopical studies of the leaf are summarized in the following points.

- i. It consists of single layered upper and lower epidermis followed by 6-8 layers of spongy parenchymatous cells. The cells are closely packed without any intercellular space.
- ii. Presence of a thick cuticle and trichomes (both glandular and covering). 2-3 layers of collenchyma are seen towards the upper epidermis.
- iii. Anomocytic stomata are present in the epidermis of the leaf (Lower surface has comparatively higher no of stomata than upper surface). The epidermal cells surrounding the subsidiary cells are thick walled and irregular with walls straight at some part of the surface.
- iv. Presence of C-shaped, centrally situated, bi-collateral vascular bundle (phloem-xylem-phloem). Metaxylem is seen towards the lower epidermis side while protoxylem can be seen towards the upper epidermis side. Pith is absent in the T.S.



**Fig. v)** T.S. of leaf of *E. tithymaloides*. (TR- Trichome, Col- collenchyma, U.E.- Upper Epidermis, L.E.- Lower Epidermis, Cut-Cuticle, Xy- Xylem Ph- Phloem, S.P. -Spongy Parenchyma)



**Fig. vi)** Surface preparation of leaf (upper surface) of *E. tithymaloides*.



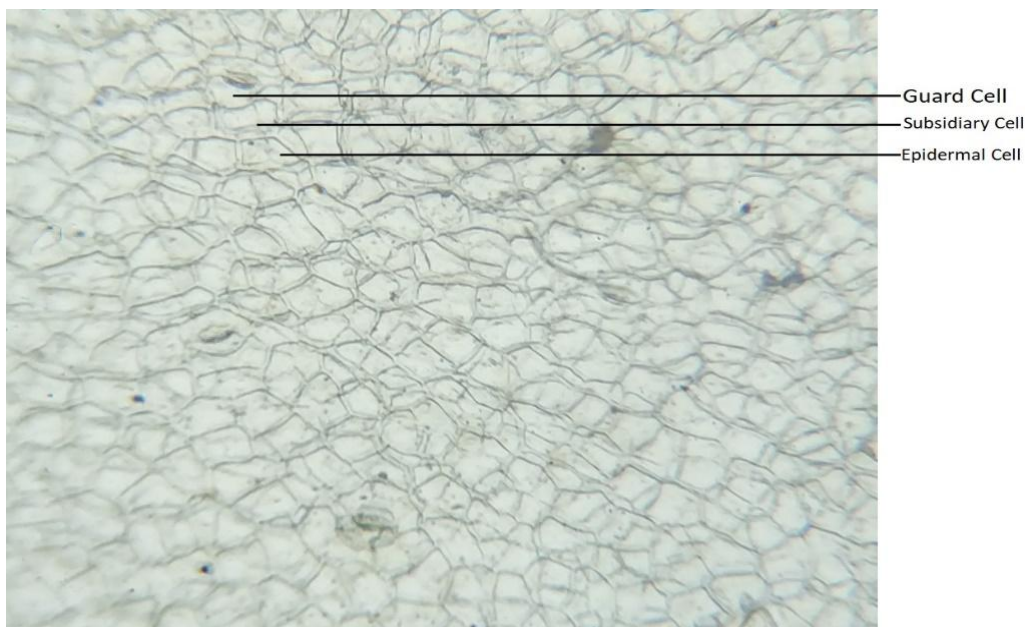


Fig. vii) Surface preparation of leaf (lower surface) of *E. tithymaloides*.

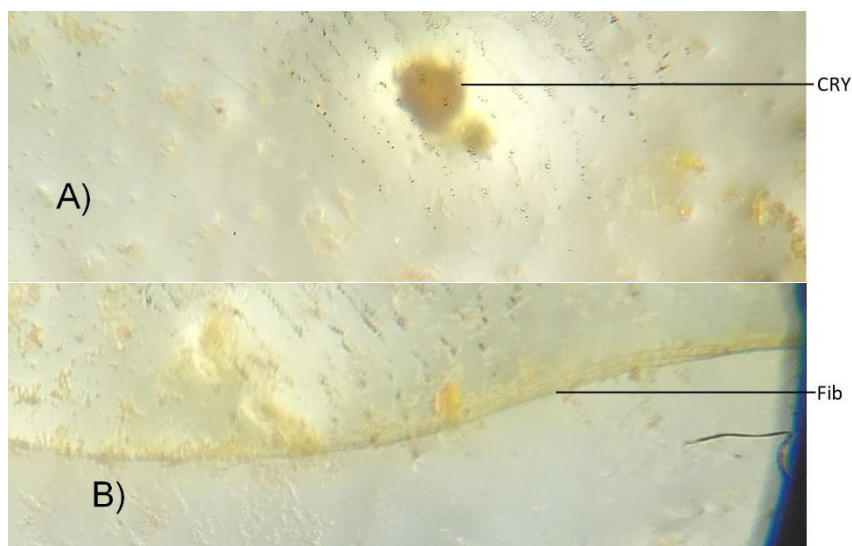
**Quantitative Microscopy of leaf:** The quantitative microscopy of the leaf of *E. tithymaloides* helped us to know the values of the following parameters.

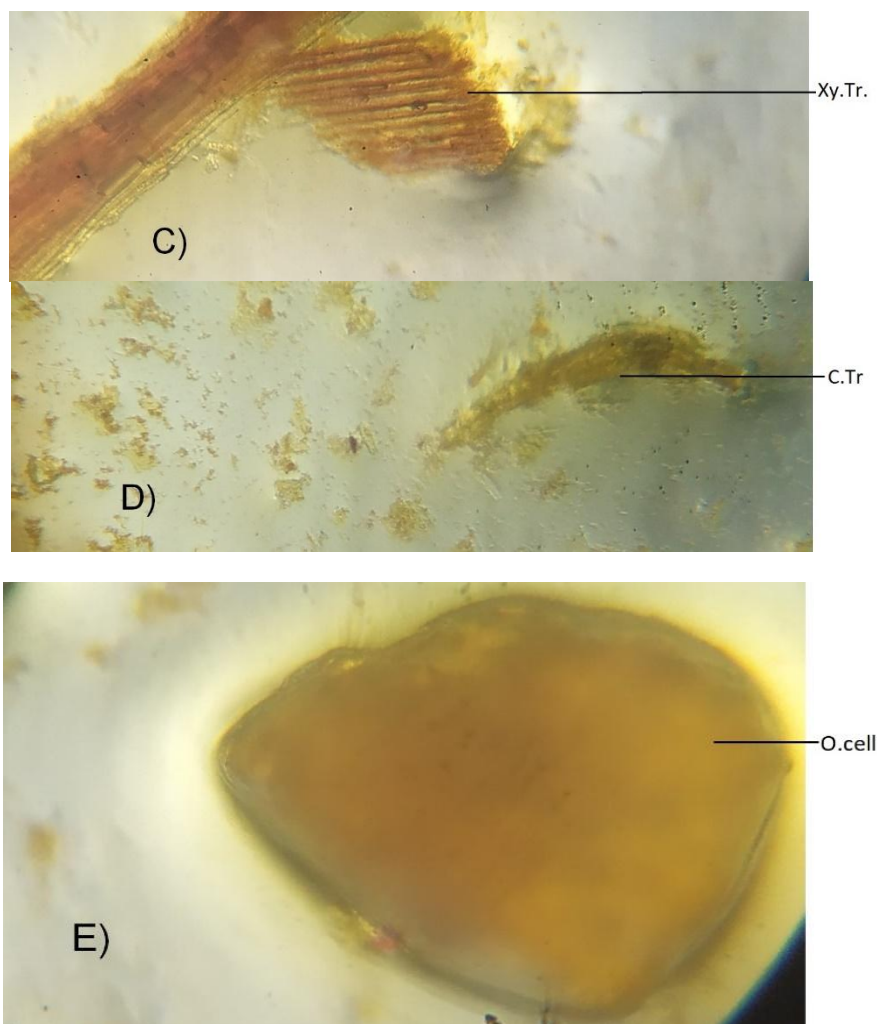
**Table No 2: Leaf Constants for *E. tithymaloides* leaf**

Leaf Constants	Values (Upper Surface)	(Lower Surface)
Stomatal No	11.88	22.5
Stomatal Index	4.17	7.88

**Powder Microscopy of Whole Plant:** Powder of the plant is light brown in color, faint in odor and has got slightly mucilaginous taste. Its microscopy revealed some diagnostic features like

- i. Epidermal cells with numerous spongy parenchymatous cells.
- ii. Numerous prismatic crystals of calcium oxalate (crystals are variable in size with smaller ones often forming small aggregates).
- iii. Very occasional covering trichomes (they are unicellular, thick-walled and conical in shape)
- iv. Long, non-lignified fibres with slit-shaped pit. Some of them occur as associated with vessels. Numerous lignified xylem tracheids (pink) and vessels are also seen.
- v. The oil cells occur singly. They are very large, subspherical to ovoid in shape and have moderately thickened walls. In uncleared mounts, cells are filled with oil and are yellow in color.





**Fig. viii)** Powder microscopy of whole plant of *E. tithymaloides* (A-E: CRY – Crystals of calcium oxalate, Fib- Non-lignified fibre, Xy.Tr- Xylem tracheid along with vessel, C.Tr- Covering Trichome, O.cell- Oil cell)

**Physicochemical Analysis:** Estimation of ash value is a significant parameter for the detection of nature of material, which is added to the drug for the purpose of adulteration, impurities & determination of authenticity, quality & purity of test sample. The higher ash value (11.715%) of this plant indicates that carbonates, phosphates, silicates & silica are present in higher amount.

The water-soluble extractive value (22.867%) indicates the presence of sugar, acids & inorganic compounds & the alcohol-soluble extractive value (11.216%) indicates the presence of polar constituents like phenols, steroids, glycosides & flavonoids. The determination of these values is primarily useful for the identification of exhausted drug. Similarly, % moisture content (4.883%) indicates the percentage loss of volatile substances along with water. Less moisture content is needed for prevention of chemical decomposition & microbial contamination in the natural products.

The following table (Table No 3) shows the values of all the parameters obtained during physicochemical studies of powder of whole plant of *E. tithymaloides*.

Analytical Parameter	Values obtained (%w/w)
Total Ash	11.715%
Acid insoluble ash	4.12%
Water soluble ash	5.02%
Alcohol soluble extractive value	11.216%
Aqueous extractive value	22.867%
% moisture content	4.883%
Foaming Index	Not significant

**TableNo 3:** Physicochemical Constants of Powder of Whole Plant of *E. tithymaloides*

#### IV. Conclusion

Thus, after a thorough investigation about the pharmacognostical and physicochemical profile of *E. tithymaloides*, we were able to determine germane qualitative and quantitative information which helps to serve as the standards to ascertain the identity and to determine the quality and purity of the plant material in future studies. Besides these, these crucial information also serves as a basis for monograph development and as the markers for the development of effective and non-toxic drug. As this plant has shown some promising features regarding therapeutic uses and as there has been a large void in the network of its attainable data, there seems to be a necessity for the involvement of researchers in its exhaustive study.

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