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PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHYSICO-CHEMICAL EVALUATION OF LEAVES OF *TRAGIA INVOLUCRATA* LINN.,

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Keywords:

Physico-chemical, Phytochemical, Powder microscopy, Organoleptic, *Tragia involucrata*

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ABSTRACT: Background: *Tragia involucrata* Linn**.**, leaves are traditionally used for the treatment of liver disorders, eczema, viral infections, headache, respiratory infections, *etc*. There is lack of documentation of safety data and non-availability of quality control standards for the ayurvedic traditional medicines. **Aim & Objective:** The aim of the study was designed to determine the pharmacognostic, phytochemical and physico-chemical standardization of leaves of *Tragia involucrata* Linn. **Materials & Methods:** The Pharmacognostical parameters such as organoleptic evaluation (macroscopy) and microscopy (T.S of Leaf) were done to identify the morphological and anatomical structures. For powder microscopy, coarse powder is treated with routine reagents to identify the diagnostic features of the plant. Preliminary phytochemical screening, physicochemical parameters was determined as per the prescribed guidelines and fluorescent behaviour of coarse leaf powder was also determined. These parameters are useful tools to differentiate the powdered drug material from the adulterated ones. **Results:** The macroscopical characters such as colour, shape, size, odour and surface characteristics were observed. Transverse section of leaf and powder microscopy revealed the presence of spongy parenchyma, paracytic stomata, glandular trichomes, *etc*. Phytochemical screening showed the presence of tannins, volatile oils, flavonoids, saponins, triterpenes and glycosides. Physicochemical parameters such as moisture content, ash value, extractive value, etc and fluorescent behaviour of leaf powder were determined. **Conclusion:** The data collected from the present study will definitely contribute to develop quality standards of *Tragia involucrata* and serve as a baseline for planning future studies.

INTRODUCTION: The significance and recognition of traditional ayurvedic medicines, herbal remedies, and formulations are on the rise globally.

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There is a growing demand for herbal medicines in both developed and developing nations due to their diverse biological properties, increased safety profile, and affordability for all socioeconomic groups.

However, the lack of proper standardization methods and quality control has led to the production of finished products without adherence to good manufacturing practices [1]. In order to change the perspective of inferior quality of drug manufacturing practices and create products that

benefit the public, it is essential to establish quality standards for herbal formulations, natural products, and other related items.

The purpose of this study is to establish quality standards for *Tragia involucrata* leaves and to verify the standardization parameters in accordance with WHO guidelines. The collection of plant material, along with factors such as time, temperature, drying method, moisture content, geographical and seasonal variations, are crucial for ensuring good manufacturing practices in the production of ayurvedic formulations and herbal medicines [2]. Each step in the process of preparing raw drugs requires careful attention. *Tragia involucrata*, a member of the Euphorbiaceae family, has traditionally been utilized for treating liver disorders, eczema, viral infections, headaches, respiratory infections, and more. This study aims to assess the pharmacognostic characteristics, conduct preliminary phytochemical screening, and analyze the physico-chemical properties of *Tragia involucrata* Linn.

MATERIALS & METHODS:

Plant Material Identification and Authentication: *Tragia involucrata* leaves were collected from Kasampatti, Natham in Dindigul district and authenticated (Authentication no: R0138) by Dr. Stephen, Professor in the Department of Botany at American College in Madurai-20. The herbarium of this specimen has been preserved in the department for further reference.

Pharmacognostical Evaluation: The characteristics of leaves, including colour, smell and taste, are examined through organoleptic testing following specific guidelines. Fresh leaves were chosen for microscopic analysis by cutting them in cross-section. In powder microscopy, a coarse powder is created and treated with standard chemicals to identify unique plant features [3].

Physio-chemical Parameters: The powder undergoes analysis to determine its physiochemical characteristics including the presence of foreign organic matter, moisture content, ash content, and extractable components using solvents of varying polarity. The testing method followed the standard prescribed guidelines [4].

Fluorescence Analysis: Behavioural characters of *Tragia involucrata* Linn. (Leaf-Crude Powder) was determined with water and different reagents such as Conc. HCl, Conc. H_2SO_4, Conc. HNO_3, CH_3COOH, aqueous NaOH and aqueous $FeCl_3$[4].

Preparation of the Extract: Approximately 250 g of the dried leaves of *Tragia involcucrata* was coarsely powdered and soaked in the menstruum which consists of 70:30 Ethanol: Water. The solvent/menstruum was selected based on the order of increasing polarity. The solvent was poured on to the drug material until it is completely soaked and kept for 3 days with occasional shaking to achieve complete extraction. Then the supernatant was decanted carefully and evaporated to dryness on a water bath. The concentrated extract residue was found to be 5g. The percentage yield was found to be 3.99%.

Qualitative Analysis: The hydroalcoholic extract of *Tragia involucrata* Linn (Leaf) was analyzed through a series of chemical tests to determine the presence of various compounds [5].

RESULTS & DISCUSSION:

Pharmacognostic Evaluation: The Organoleptic characteristics of was showed in the **Table 1** and the macroscopy of leaves-dorsal and ventral view of *Tragia involucrata* was showed in **Fig. 1**. The cross-section of *Tragia involucrata* leaves revealed distinctive traits, including circular petioles covered by a cuticle and sparse elongated, glandular capitate trichomes. These trichomes featured elongated biseriate stalks and club-shaped secretory bodies. The epidermal layer consisted of small, square-shaped cells.

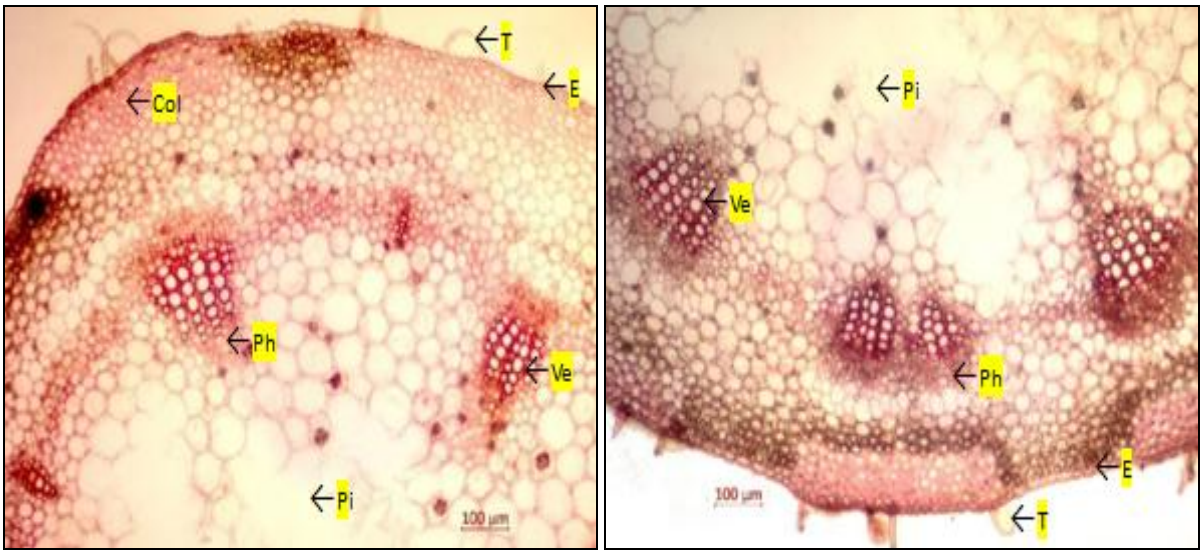
The parenchymatous ground tissue was thin walled, with circular cells that were less compact at the edges and larger, angular cells that were more tightly packed towards the center. Additionally, numerous rosette crystals were observed throughout. The vascular system appeared multi-stranded, with a ring of distinct collateral vascular strands containing a high concentration of xylem and a lower concentration of phloem elements, as shown in **Fig. 2**. These assessment methods assist in distinguishing between pure samples and adulterated ones based on their unique characteristics [6].

TABLE 1: MACROSCOPICAL STUDIES OF *TRAGIA INVOLUCRATA* LINN (LEAF)

S. no.	Parameters	Observation
1	Colour/surface	Dorsal-Darkgreen; Ventral-Lightgreen
2	Odour	Characteristic dour
3	Taste	Characteristic taste
4	LeafType	Simple
5	Shape	Ovateorelliptic
6	Arrangement	Alternate
7	Apex	Acuminate
8	Base	Acuteorrounded
9	Stipules	Present
10	Margin	Serrate
11	Venation	Pinnate
12	Surface	Stinging hairs
13	Length	6cmto 10cm
14	Width	3.5cmto 5cm
15	Petiole length	2.5cm long
16	Flower	Yellow

DORSAL VIEW **VENTRAL V**

FIG. 1: MACROSCOPY OF *TRAGIA INVOLUCRATA* LINN LEAF



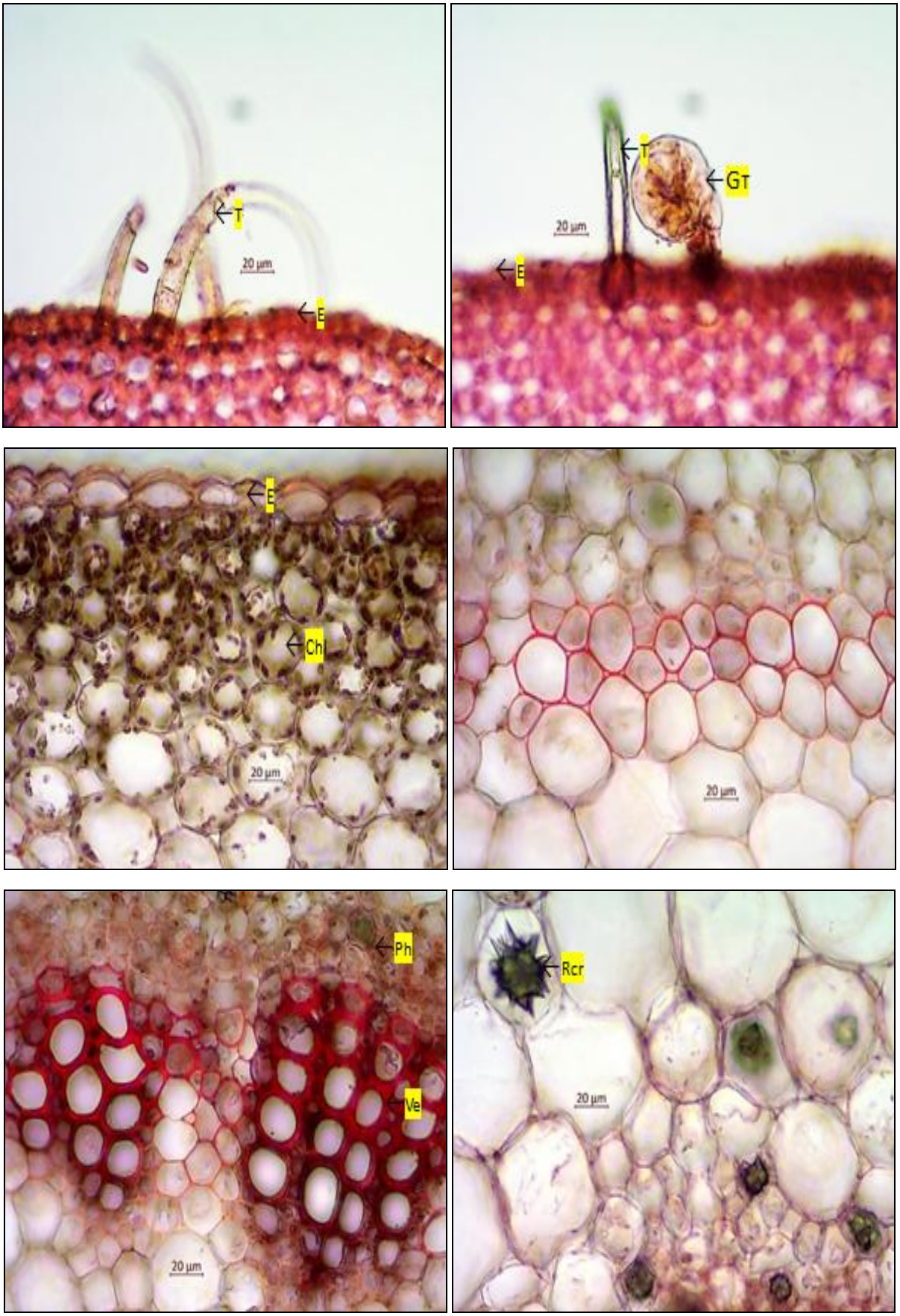


FIG. 2: T.S OF LEAF OF *TRAGIA INVOLUCRATA*. E–Epidermis; Chl–Chlorenchyma; Col–Collenchyma; GT–Glandular Trichome; Ph–Phloem; Pi–Pith; Rcr–Rosettecrystal; T–Trichome;Ve–Vessel.

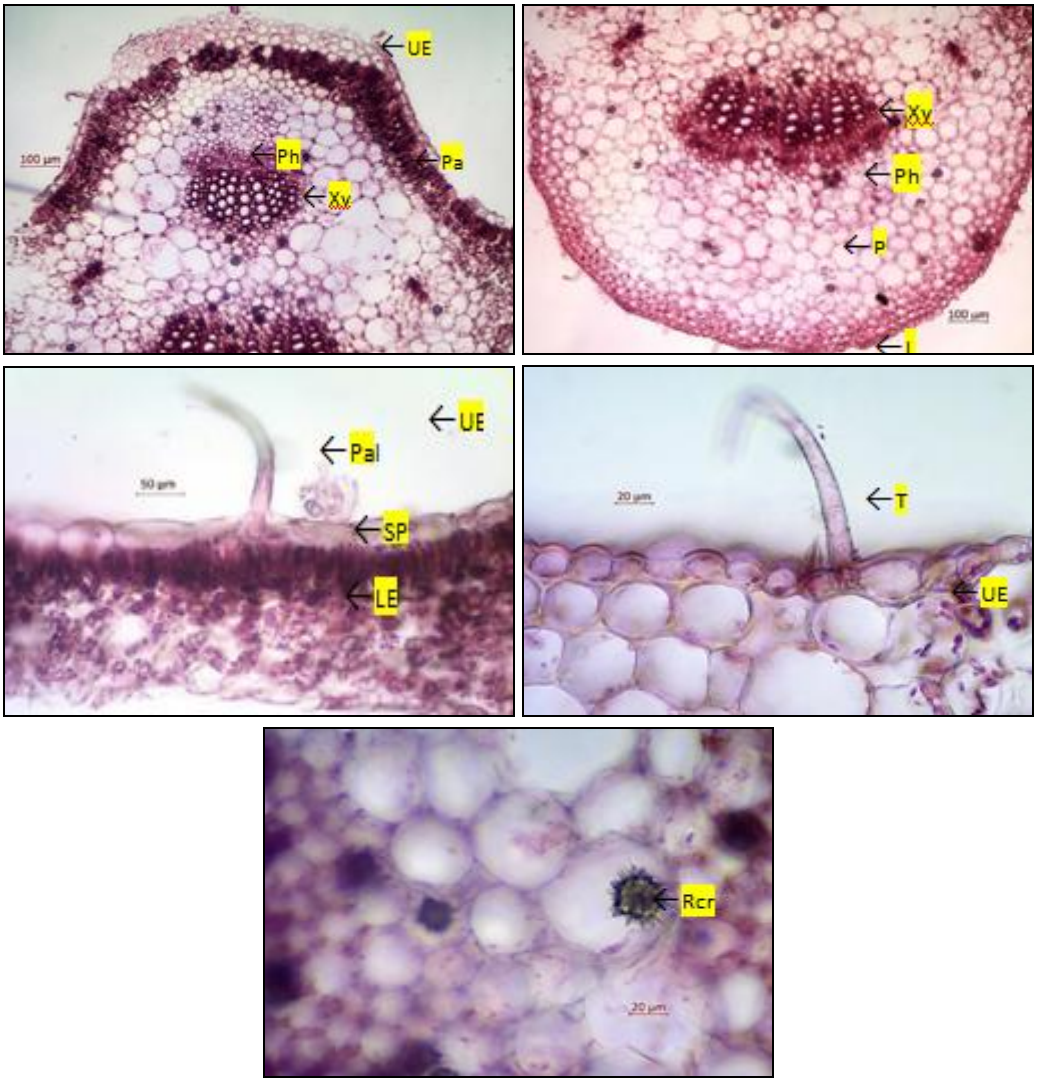


FIG. 3: T. S OF LAMINA THROUGH MIDRIB. LE–Lowerepidermis, Me–Mesophyllcells, Pal–Palisadecells, Ph–Phloem, Rcr–Rosettecrystal, SP–Spongy Parenchyma, VB–Vascular Bundle, UE–Upperepidermis, Xy–Xylem.

Powder Microscopy: The powder microscopy of the plant material is buff in colour, unicellular trichome, glandular trichome, paracytic stomata and showed crystals of calcium oxalate and Xylem vessels as shown in **Fig. 4.**

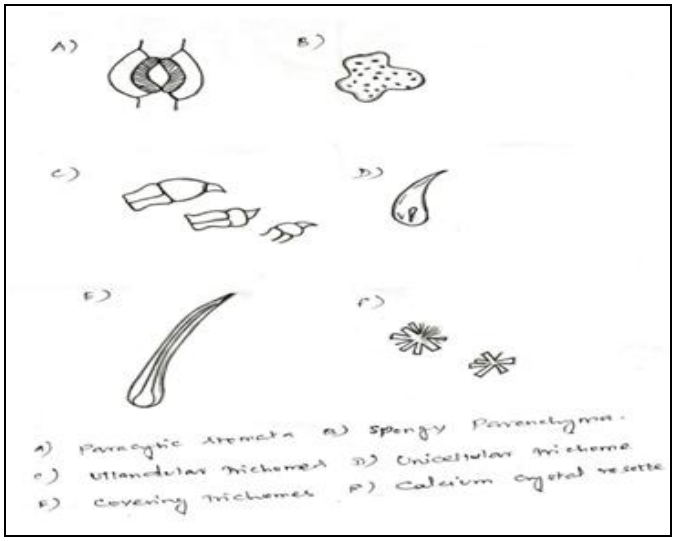


FIG. 4: POWDER MICROSCOPY

Physicochemical Evaluation: The various physicochemical parameters of leaves of *Tragia involucrate* i.e., Loss on drying, Total solids, petroleum ether extractive, Ethyl acetate extractive, chloroform extractive, ethanol extractive, aqueous extractive, Total ash value water soluble ash and acid insoluble ash was showed in **Table 2.**

Total ash is the measure of the total amount of material left after burning and includes ash derived from the part of the plant and adherent material. The total ash value for *Tragia invoucrata* samples was 0.223333±0.017639%w/w, which was in the limit (not more than 15%). The acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and burning the remaining insoluble matter [7]. The process measures the amount of silica present, especially in the form of sand and siliceous earth. The value of acid-insoluble ash was 0.60 ± 0.034749 %w/w, which is lesser than 5% and thus passes the test. Water-soluble extractives are indicative of water-soluble active constituents of crude drugs, such as tannins, sugars, plant acids, mucilage, glycosides etc. The water-soluble extractive value is 9.003333 ± 0.702405 % w/w. Alcohol is an ideal solvent for extraction of various chemicals like tannins, resins etc and the value was found to be 3.966667 ± 0.833818 % w/w. The moisture content of a drug should be minimized in order to prevent decomposition of crude drugs, either due to chemical change or microbial contamination [8]. The percentage of moisture content ranging from 10 - 20% shows an ideal range for minimum bacteria as well as for fungal growth. Ash value is a criterion to judge the identity or purity of crude drugs. The Loss on drying value was found to be 1.98 ± 0.863178 % w/w. The powder did not possess any foreign matter, bitter principle. The powder also exhibits petroleum ether extractive value (1.656667±0.331689%w/w) Ethylacetate extractive value (2.323333±0.689376 % w/w) Chloroform extractive value (1.873333 ± 0.218359 % w/w).

TABLE 2: DETERMINATION OF PHYSICO-CHEMICALPARAMETERS OF *TRAGIA INVOLUCRATA* (LEAF)

S. no.	Physico–Chemical constant	Reports
1	Foreign Matter	NIL
2	Bitterness Value	NIL
3	Loss on Drying	1.98± 0.863178 %w/w
4	Total solids	98.02± 0.704782 %w/w
5	Petroleum ether extractive	1.656667± 0.331689 % w/w
6	Ethyl acetate extractive	2.323333± 0.689376 %w/w
7	Chloroform extractive	1.873333± 0.218359 % w/w
8	Ethanol extractive	3.966667± 0.833818 % w/w
9	Aqueous extractive	9.003333± 0.702405 % w/w
10	Total ash	0.223333± 0.017639 % w/w
11	Water soluble ash	3.46± 0.110591 %w/w
12	Acid in soluble ash	0.60± 0.034749 %w/w

Preliminary Phytochemical Screening: A preliminary analysis was conducted on the hydroalcoholic extract to identify phytochemical compounds. The findings are detailed in **Table 3**. Qualitative screening of the drug was performed to detect alkaloids, flavonoids, saponins, tannins, and proteins.

TABLE 3: PRELIMINARY PHYTOCHEMICAL SCREENING OF HYDRO-ALCOHOLIC EXTRACT OF *TRAGIA INVOLUCRATE* (LEAF)

S. no.	Name of the test	Haeti
1	**Test for carbohydrate**	
	Fehling's test	Negative
	Molisch test	Positive
	Benedict's Test	Positive
2	**Test for proteins and free amino acid**	
	Biuret test	Positive
	Millon's Test	Negative
	Ninhydrin Test	Negative
	Sulphur Containing Amino acid	Negative
3	**Test for Flavonoides**	

	Ferricchloride test	Positive
	Shinoda test	Positive
	Alkaline reagent test	Positive
	Zinc-Hydrochloric acid reduction test	Negative
4	**Test for alkaloids**	
	Mayer's test	Positive
	Wagner's test	Positive
	Dragendorff's test	Positive
	Hager's test	Negative
5	**Test for saponins**	
	Foamtest	Positive
6	**Test for sterols**	
	Salkowaski test	Positive
	Libbermann Burchard test	Positive
7	**Test for triterpenoids**	
	Salkowaski test	Positive
8	**Test for Glycosides**	
	Keller-Killiani test	Positive
	Baljet's test	Positive
	Legal test	Positive
	Raymond's test	Negative
	Bromine water test	Negative
9	**Test for Tannins**	
	Ferric-chloride test	Positive
	Gelatin test	Positive
10	**Test for fats**	
	Solubility test	Negative
	Filter paper test	Negative
11	**Test for volatile oils**	
	Odor test	Positive
12	**Test for Mucilage**	Positive
13	**Test for Coumarin**	Negative
14	**Test for Resins**	Negative
15	**Test for Gum**	Negative
16	**Test for Quinone**	Negative
17	**Test for Anthocyanin**	Negative

Hydroalcoholic extract of *Tragia involucrate* (Leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract for the identification of phytochemicals, secondary metabolites.

Fluorescence Analysis: The alterations in the behavior of powdered drug with distinctive chemical reagents were analyzed under both UV and visible light, as detailed in **Table 4.**

TABLE 4: BEHAVIOURAL CHARACTERS OF THE *TRAGIA INVOLUCRATA*

Crude powder of *Tragia involucrata (*Leaf)	Visible light	UV Light (254nm)	UV Light (365nm)
Powder+Water	Light green	Dark black	Dark green
Powder+Conc.Hcl	Dark green	Black	Green
Powder +Con.H_2SO_4	Black	Dark green	Black
Powder+HNO_3	Black	Green	Dark green
Powder+CH_3COOH	Light black	Brown	Darkgreen
Powder+Con.HCL+Water	Light green	Light black	Dark green
Powder+Con.H_2SO_4+Water	Dark black	Light brown	Light black
Powder+Con.HNO_3+ Water	Light black	Dark brown	Light green
Powder+CH_3COOH+ Water	Light black	Light brown	Dark green
Powder+aqueousNaOH	Light green	Black	Dark green
Powder+aqueous $FeCl_3$	Dark black	Black	Dark green

TABLE 5: QUANTITATIVE MICROSCOPY OF TRAGIA INVOLUCRATA LEAVES

S. no.	Parameters	Upper epidermis	Lower epidermis
1	Epidermal Number	60/mm^2 -65/mm^2-70/mm^2	70/mm^2-72/mm^2 – 75mm^2

2	Stomatal Number	132/mm^2 -133/mm^2-135/mm^2	152/mm^2-155/mm^2 –158/mm^2
3	Stomata Index	65/mm^2-66/mm^2-68/ mm^2	67/mm^2-67/mm^2-68/mm^2
4	Palisade Ratio	10/mm^2-12/mm^2 – 14/mm^2	9/mm^2-10.5/mm^2-12/mm^2
5.	Vein Islets	8/mm^2-9/mm^2 -10/mm^2	
6.	Vein termination	8/mm^2-10/mm^2-12mm^2	

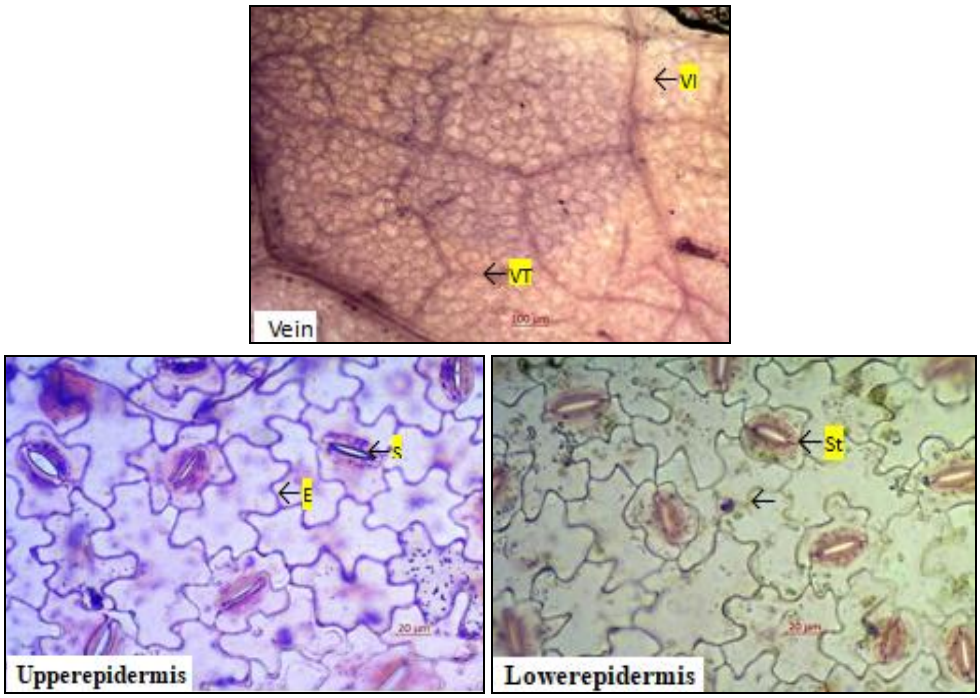


FIG. 5: QUANTITATIVE MICROSCOPY. E–Epidermis; St– Stomata, VI–Veinislet, VT–Veintermination

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CONFLICT OF INTEREST: The Authors have no competing interests to declare.

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