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PHARMACOGNOSTICAL AND PHYTOCHEMICAL INVESTIGATION OF *FLUEGGEA LEUCOPYRUS* LEAVES

Venkata Rathina Kumar T[1]*, Sesha Kumar S S[1], Satheeshkumar K[1], Balasubramaniyam P N[1], Subha Sree B S[1], Abdul Hasan Sathali A[2]

[1]Professor and HOD, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India.
[1]PG Scholar, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India.
[2]Principal, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India.

ABSTRACT
Flueggea leucopyrus belonging to euphorbiacea family. The purpose of this research is to study the pharmacognostical parameters and also to identify the phytoconstituents present in this plant. Organoleptic and microscopical characters and physicochemical constants such as ash values like water soluble ash, acid insoluble ash, extractive value, moisture content was determined. The leaf powder was extracted using hydroalcohol and the preliminary phytochemical analysis revealed the presence of alkaloids, saponins, terpenoids, flavonoids, carbohydrates, sterols, and tannins in this extract. The results of this study will be helpful in the proper identification and authentication of this medicinal plant.

Keywords: *Flueggea leucopyrus*, Pharmacognosy, Phytochemistry

INTRODUCTION

India has rich diversity of medicinal plants and from ancient times these plants were utilized as therapeutic agents. Western Ghats is very rich in its medicinal wealth. The forests and hills of this region is a treasure house of about 700 medicinal plants. Today's research is mainly focused on medicinal plants because the bioactive compounds and therapeutic efficacy mainly depends on phytochemical constituents that have great pharmacological significance. The phytochemical constituents, natural bioactive compounds, nutrients and fibres present in medicinal plants, fruits and vegetables defend us from various ailments.[1] *Flueggea leucopyrus* Wild. (Family: Euphorbiaceae) locally known as 'bush weed'. The plant is found in many parts of Sri Lanka particularly in dry zones as shrubs. It is an erect shrub 1.5-4m tall with branches cylindrical or obtusely angular when young and grey in colour. The plant grows in south eastern Queensland, southern India and Sri Lanka. The leaves of *F. leucopyrus* have been used in the treatment of cancer, boils, external ulcers and sores in traditional medicine in Sri Lanka.[2] *Flueggea leucopyrus* Wild proved as very effective in the treatment of different diseases in Ayurveda medicine, such as cough, hay asthma, bowel complaints, laxatives, diarrhoea, gonorrhoea, constipation, mental illness, kidney stones and cancer.[3] Traditionally the leaves paste of *Securinega leucopyrus* used to destroy sore worms and stomach ache. It was also used as fish poison and anti-cancer treatment.[4] The whole plant was used for the treatment of cancer in the sole of the foot.[5] The main objective of the present study is to evaluate this plant by means of organoleptic, microscopical, physicochemical constants and preliminary phytochemical and quantitative estimation of phytoconstituents.

MATERIALS AND METHODS

Collection of plants

Leaves of Flueggea leucopyrus were collected from kallikudi village, Madurai district and it was authenticated by Dr. D. Stephen, M.Sc., Ph.D., Assistant Professor, Department of Botany, The American College, Madurai -20, Tamilnadu. The herbarium of this specimen was kept in our department for further reference.

Pharmacognostical studies

Preparation of plant materials

The leaves of *Flueggea leucopyrus* were shade dried and powdered in a blender. The coarse powder was sieved (Sieve no: 40) and were stored in a well closed container.

Corresponding Author: **Venkata Rathina Kumar T Email: -** vrkmmcmadurai@gmail.com

Macro and microscopical evaluation

The leaves were investigated for its morphological characters by organoleptic test such as colour, odour, size, shape and taste etc. Sample was preserved in a fixative FAA for more than 48 hr. The preserved specimens were cut into a thin transverse section using sharp blade and the sections were stained with safranin. Transverse sections were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss AxioCam Erc5s digital camera under bright light. Magnifications were indicated by a scale bar. A pinch of powdered sample of *Flueggea leucopyrus* were taken and mounted on a microscopic slide with a drop of 50% glycerol. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss AxioCam Erc5s digital camera under bright light. Photo micrographs of diagnostics characters were captured and documented. The fresh leaves of *Flueggea leucopyrus* were boiled with 0.1% chloral hydrate solution for slide preparation. Vein islets, vein termination, stomatal number, stomatal index, and palisade ratio were determined and calculated by observing the slide with the help of microscopy.

Physicochemical analysis

The powdered drugs were evaluated for loss on drying, foreign matter analysis, ash value and extractive value by using standard procedure [8].

Phytochemical studies

Phytochemistry is a branch of science that studies the chemical and structural composition of secondary metabolites found in plants. The optimal phytochemical composition is vital for scientific validation of current traditional and ethnomedical medicinal plant uses. Modern phytochemistry offers a variety of approaches for confirming secondary metabolite identification, isolation and characterization.

Extract preparation

Previously dried, powdered, sieved and stored leaves of *Flueggea leucopyrus* was taken. It was extracted with hydroalcohol (70%) by cold maceration for about 72 hrs. The extracts were then filtered through whattman filter paper No. 42 (125 mm) to remove all non-extractable matter, including cellular materials and other constituents which are insoluble in the solvent used for extraction. The entire extracts were concentrated to dryness using a buchi rotary evaporator under reduced pressure. The final dried extracts were stored in labelled sterile containers.

Preliminary Phytochemical screening

The various chemical tests were performed on hydroalcoholic extract of *Flueggea leucopyrus* leaves (HAEFLL) for the identification of flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannins, amino acids, and sterols by using the standard procedures [6&7].

Quantitative estimation of Phytoconstituents

Various phytoconstituents like total phenolic content, total flavonoid content and total tannin contents were quantitatively estimated by using standard procedures [9&10].

RESULTS AND DISCUSSION

Macroscopical evaluation

The macroscopical description of the leaves of *Flueggea leucopyrus* **(figure:1)** was studied and the results were recorded in the **table:1**. Macroscopical studies of *Flueggea leucopyrus*. L showing the upper surface is greyish green and lower surface greyish in colour with no characteristic odour and slightly bitter taste. The leaves are simple, obovate shape with emarginate apex and cuneate base. The leaf shows entire margin with thick and slightly curved abaxially. The leaves are of 2.5 cm length x 1.5 cm in width and petiolate.

Microscopical evaluation

Transverse section of Petiole

TS of petiole **(figure 2)** is concave-convex shape with a narrow, distinct layer of epidermis composed of circular, thick walled cells and covered by distinct cuticle. The ground tissue consists of dilated, compact fairly thick-walled collenchyma cells. The cells contain large calcium oxalate crystals; each cell has single crystal filling the entire lumen. They are also seen in abundance in the mesophyll tissue. Starch grains are seen distributed throughout the cells. The vascular strand is single, prominent and semi-circular. It has a several, parallel rows of xylem elements and an arc of phloem.

Transverse section of leaflet

The leaf is dorsiventral with prominent midrib and bilaterally symmetrical lamina.

Midrib

The midrib is slightly raised on the adaxial side and semi-circular on the abaxial side. The epidermal layers of the midrib consist of small thick-walled cells covered by cuticle. A single prominent vascular strand is placed in the centre of midrib. 3 to 4 rows of collenchyma cells are present both below the upper epidermis and above the lower epidermis. The vascular bundle is radial, with short row of thick walled, wide and circular xylem elements and a narrow arc of phloem strands. The lateral veins also have prominent vascular strand.

Lamina

The lamina is dorsiventral. Both upper and lower epidermis covered with cuticle; the upper epidermis is composed of larger hyaline cells when compared to the lower epidermis. The mesophyll tissue is differentiated into upper zone of dense, darkly stained, two to three layers of palisade cells, a median row of dilated, angular hyaline parenchyma cells and a lower zone of small, lobed mostly vertically oriented spongy parenchyma. The leaf margin is thick and slightly curved abaxially.

The TS of lamina, lamina passing through midrib, enlarged lamina, lamina showing the margin were shown in the figure 3,4,5 and 6.

Powder microscopy

The powder microscopical evaluation of *Flueggea leucopyrus* leaves shows the presence of epidermis with paracytic stomata, parenchyma cells, mesophyll cells, fragments of sectional view of lamina, spiral vessel and vessel elements associated with fibres containing crystals. The *Flueggea leucopyrus* leaves are said to be hypostomatic, since it contains only paracytic stomata and the powder microscopy shows the presence of prismatic crystals, which is also confirmed by its presence in the vascular bundle region of midrib of leaves. The characters were figured in **figure 7.**

Table:1 Macroscopical characters of *Flueggea leucopyrus* (Leaf).

S.NO	MORPHOLOGICAL CHARACTERS	OBSERVATION
1	LEAF COLOUR • Fresh leaves • Dried leaves	 • Greyish green • Greyish in colour
2	Odour	No characteristic
3	Taste	Slightly bitter
4	Size	2.5 cm length x 1.5 cm in width
5	Leaf type	Simple
6	Leaf arrangement	Alternate
7	Apex	Emarginate
8	Base	Cuneate
9	Leaf margin	Entire, Thick and Slightly curved abaxially
10	Lamina	Dorsiventral
11	Leaf apices	Obovate

Table:2 Quantitative microscopy of *Flueggea leucopyrus* (Leaf)

PARAMETERS	UPPER EPIDERMIS (/mm^2)	LOWER EPIDERMIS (/mm^2)
Epidermal number	320-360	380 – 400
Stomatal number	-	240 – 260
Stomatal index	-	37-40
Palisade ratio	4-7	
Vein islets number	9	
Vein termination number	31	

TABLE: 3 Physiochemical parameters of *Flueggea leucopyrus*

S.NO	Physio-chemical constant	Results
1	Foreign matter	NIL
2	Loss on drying	14±0.161%w/w
3	Extractive value	
	Petroleum ether extractive	1.06±0.030 %w/w
	Acetone extractive	6.533±0.190 %w/w
	Chloroform extractive	2.32±0.066 %w/w
	Ethanol extractive	10.93±0.315 %w/w
	Aqueous extractive	29.53±0.854 %w/w
	Diethyl ether extractive	1.26±0.036 %w/w
	Hydroalcoholic extractive	35.60±1.02 %w/w
4	Ash value	
	Total ash	5.85 ± 0.06%w/w
	Water soluble ash	3.8± 0.04%w/w
	Acid insoluble ash	1.20 ± 0.01%w/w

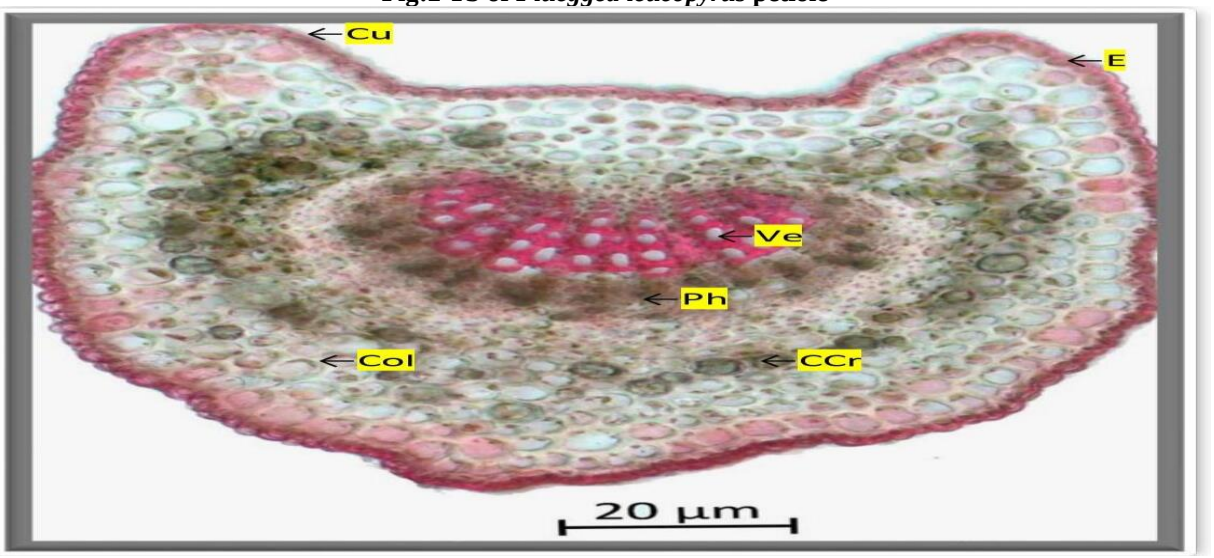
Table:4 Preliminary phytochemical screening of hydroalcoholic extract of *Flueggea leucopyrus* leaves

S.NO	NAME OF THE TEST	OBSERVATION	INFERENCE
1	**Test for alkaloids** Mayer's test	 Cream colour precipitate is formed	 Presence of alkaloids
	Wagner's test	Reddish brown precipitate is formed	Presence of alkaloids
	Hager's test	Formation of yellow precipitate	Presence of alkaloids
	Dragendroff's test	Orange brown precipitate is formed	Presence of alkaloids

2	**Test for carbohydrates** Molish's test	Appearance of purple colour	Presence of carbohydrates
	Fehling's test	Formation of reddish-brown precipitate	Presence of reducing sugar
	Benedict's test	Formation of red precipitate	Presence of reducing sugar
3	**Test for glycosides-Anthraquinone** Borntrager's test	No pink colour is formed in the ammoniacal layer	Absence of anthraquinone glycoside
	Modified borntrager's test	No pink colour in ammoniacal layer	Absence of anthraquinone glycoside
	Test for cardiac glycosides (for deoxysugar) Keller Killiani test	No Reddish brown colour ring at the junction	Absence of cardiac glycoside
	Legal's test	No Blood red colour	Absence of cardiac glycoside
	Test for cyanogenetic glycosides	No brick red colour on paper	Absence of Cyanogenetic glycosides
4	**Test for sterols** Salkowski's test	Appearance of red colour in chloroform layer	Presence of sterols
	Liberman burchard's test	Brown ring at the junction of two layers and the appearance of green colour in the upper layer	Presence of sterols
5	**Test for flavonoids** Shinoda's test	Appearance of red colour	Presence of flavonoids
	Alkali test	Appearance of yellow-orange colour	Presence of flavonoids
	Lead acetate test	Formation of white precipitate	Presence of flavonoids
	Acid test	Appearance of yellow-orange colour	Presence of flavonoids
6	**Test for proteins** Millon's Test	No red colour is formed on heating	Absence of protein
	Biuret Test	Violet colour is not formed	Absence of protein
7	**Test for aminoacids** Ninhydrin test	Violet colour is not formed	Absence of aminoacids
	With nitric acid	Yellow colour is not formed	Absence of aminoacids
8	**Test for terpenoids** Noller's test	Pink colour solution appeared	Presence of terpenoids
	Test for triterpenoids	Reddish violet colour is formed	Presence of triterpenoids
9	**Test for saponins** Foam test	Frothing occurs	Presence of saponins
10	**Test for tannins** Ferric chloride test	Appearance of bluish black colour	Presence of tannins
11	**Test for fixed oil and fats**	Soap is formed	Presence of fixed oil and fat
12	**Test for coumarins**	No yellow colour is formed	Absence of coumarins

Fig:1 Macroscopy of *Flueggea leucopyrus* Willd. Leaves

Fig:2 TS of *Flueggea leucopyrus* petiole



Cu - cuticle, CCr - cluster crystal; Col - collenchyma, E - epidermis, Ph - Phloem; SG - starch grains; Ve – vessel

Fig:3 TS of lamina

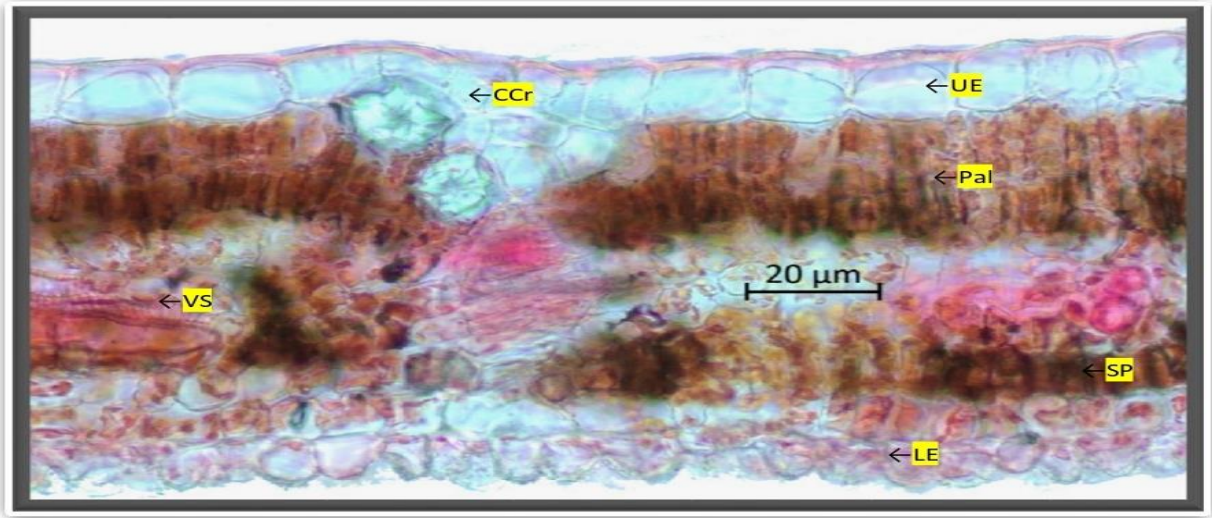


Fig:4 TS of lamina passing through midrib

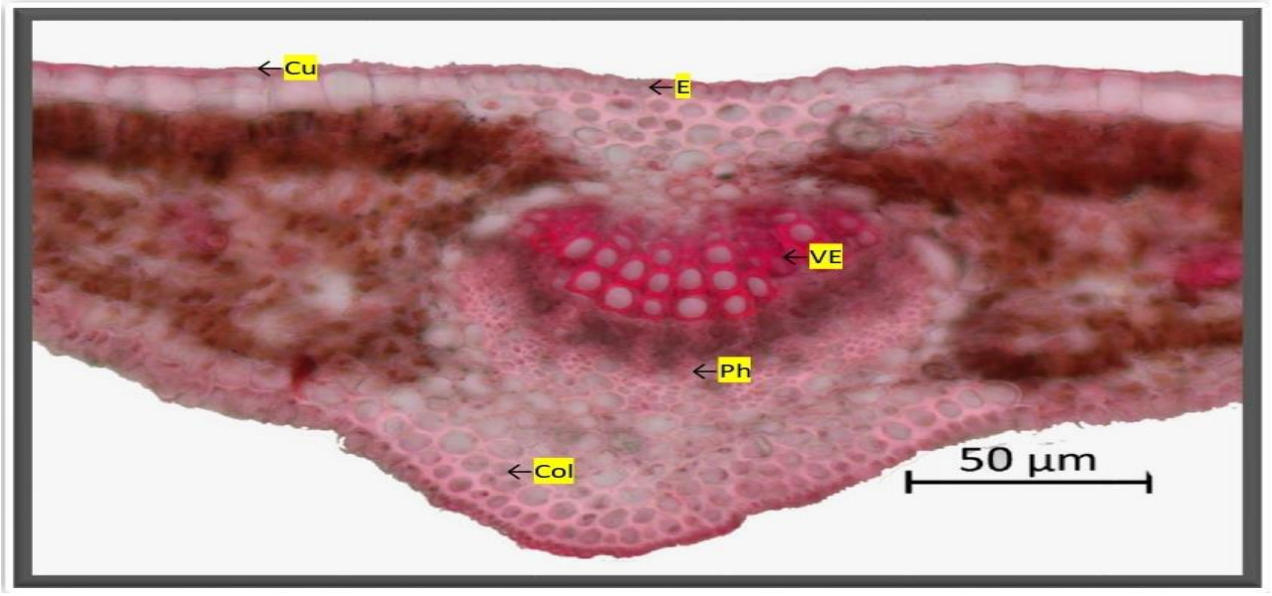
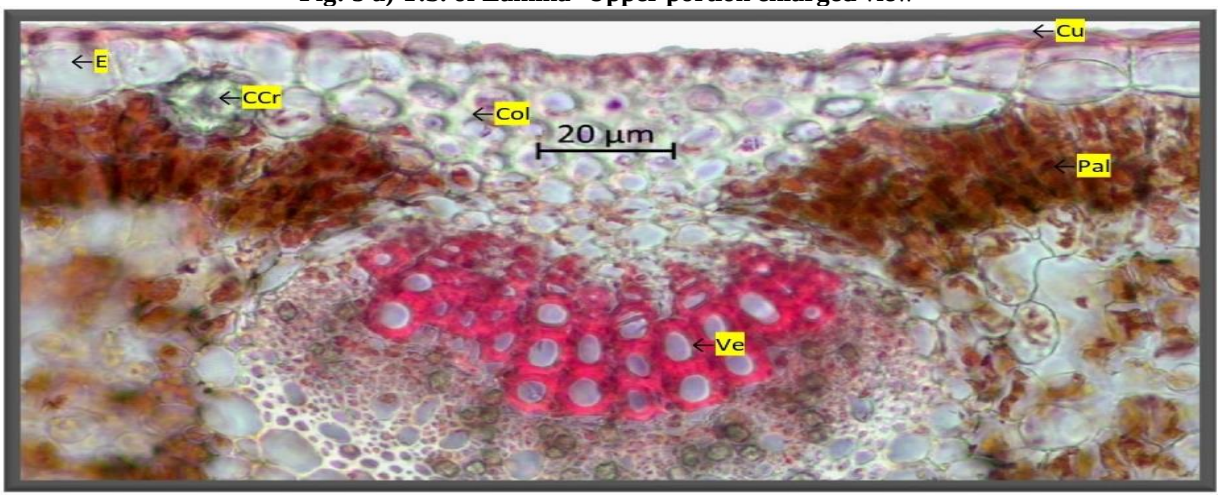


Fig: 5 a) T.S. of Lamina- Upper portion enlarged view



b) T.S. of Leaf - Lower portion enlarged view

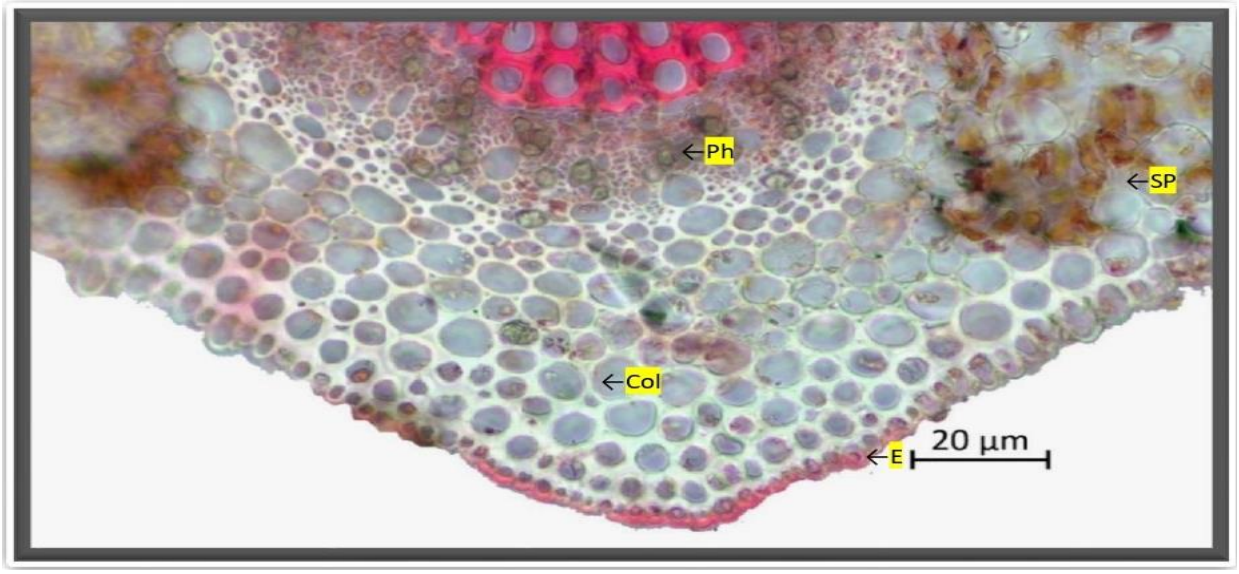
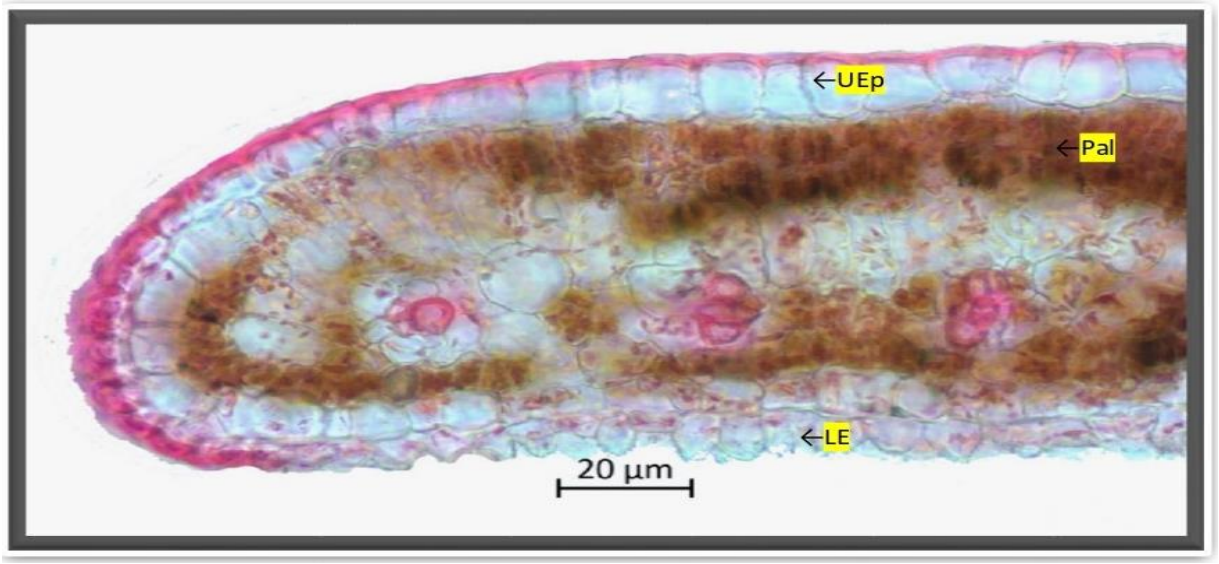


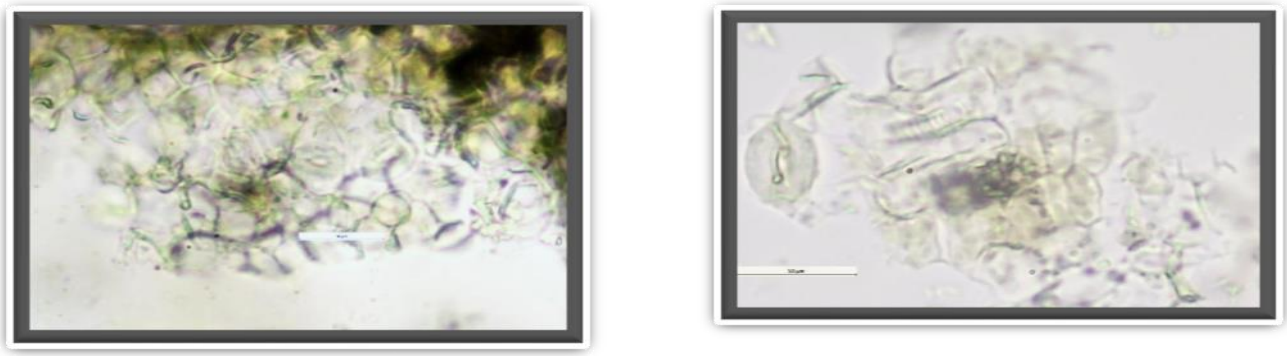
Fig:6 TS of lamina showing the margin



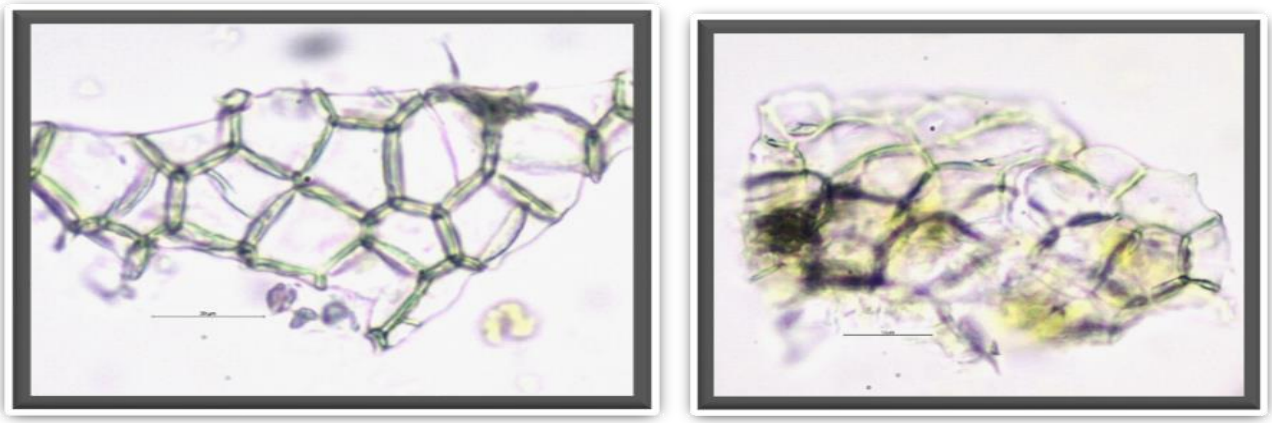
Cu - cuticle, CCr - cluster crystal; Col - collenchyma, E - epidermis, LE - lower epidermis; Mes - mesophyll; Pal - palisade; UE - upper epidermis; Ph-Phloem; Ve - vessel, VS - vascular strand

Fig 7 Powder microscopy of *Flueggea leucopyrus* leaf

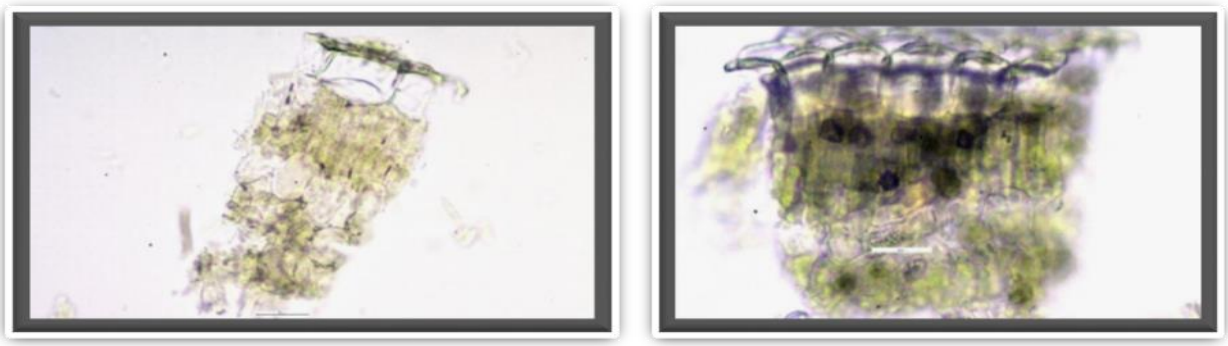
A) Epidermal fragment showing paracytic stomata



(B)Parenchyma



(C)Fragment of lamina



(D) Vessel **(E) Vessel associated with fibre**

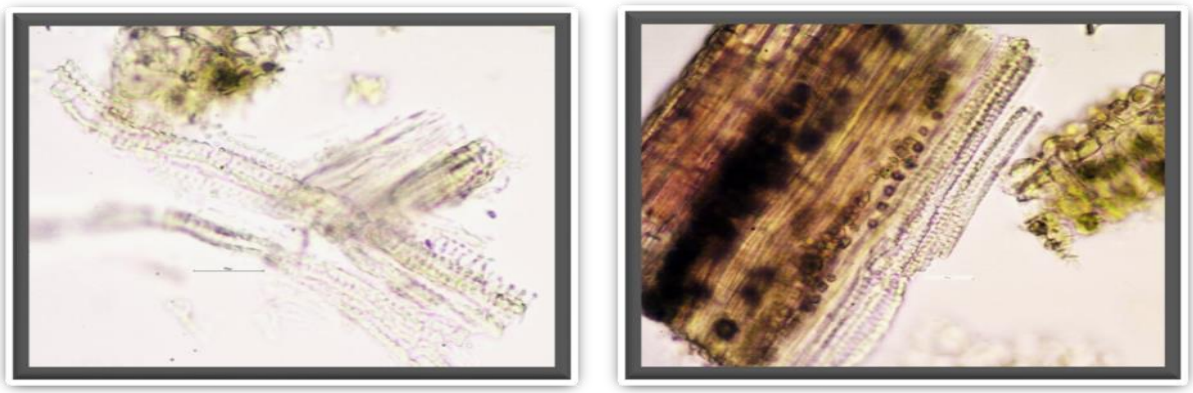


Fig:8 Upper epidermis

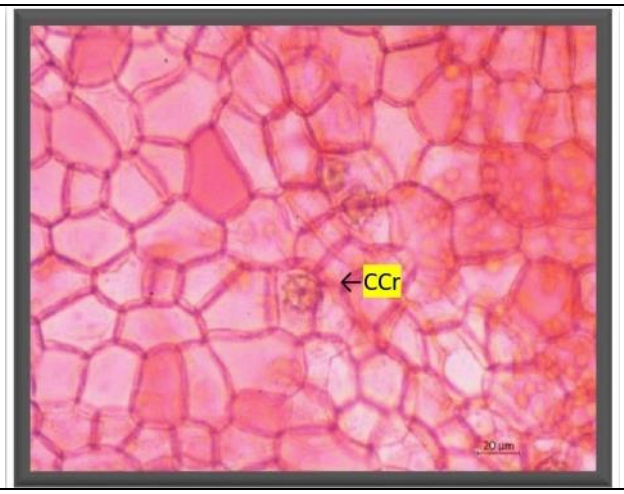


Fig:9 Lower epidermis
CCr - cluster crystals; St - stomata

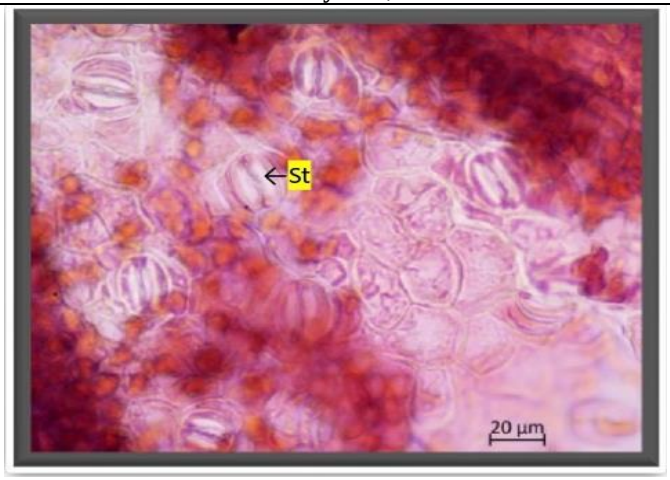


Fig:10 Vein islet and terminations
VI - vein islet; VT - vein termination

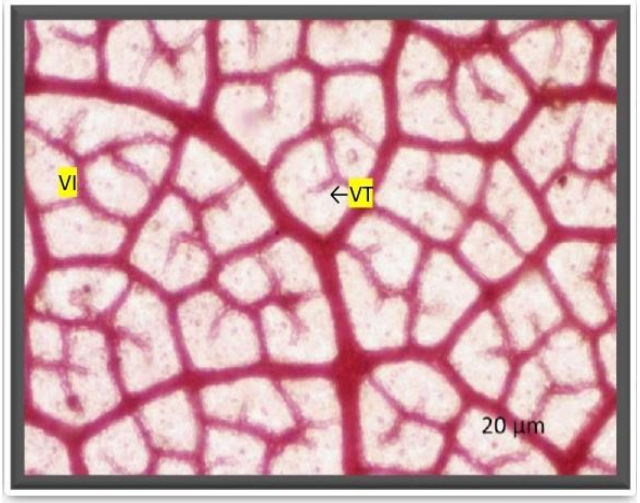


Fig:11 Calibration curve of standard quercetin

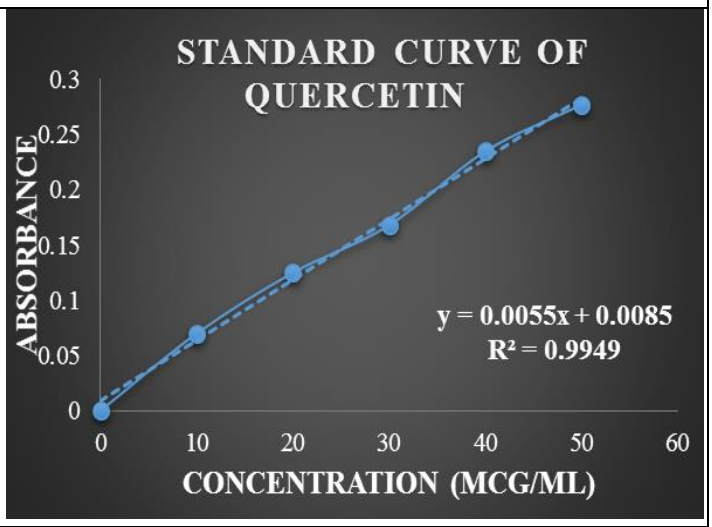


Fig: 12 Calibration curve of standard gallic acid

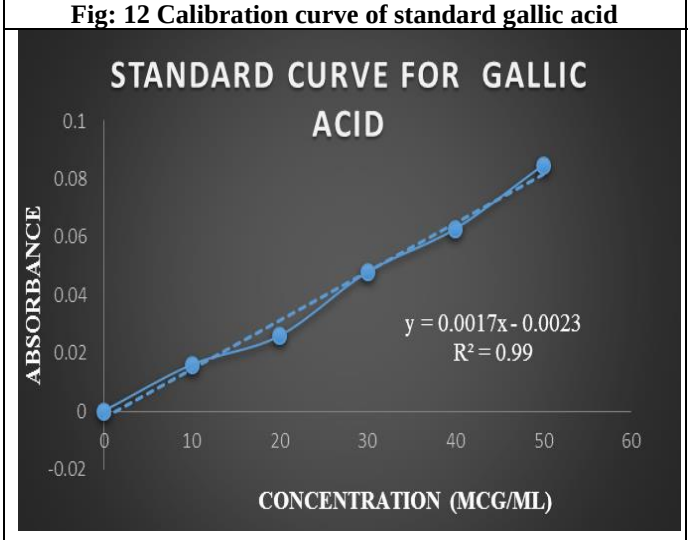
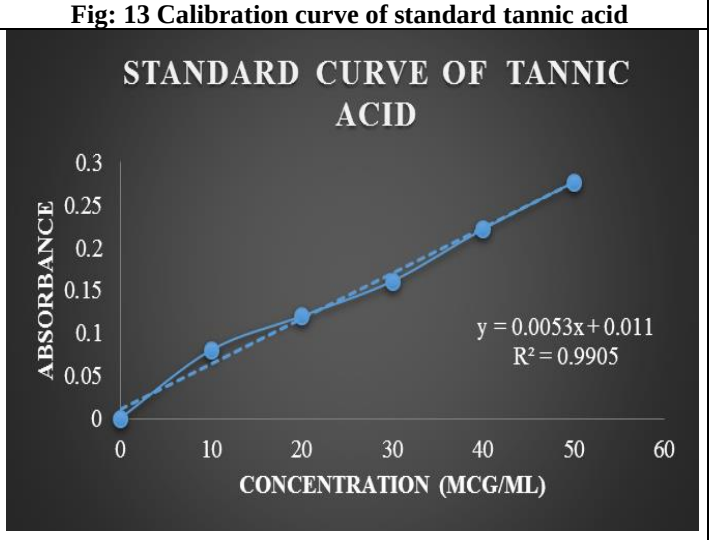


Fig: 13 Calibration curve of standard tannic acid



Quantitative microscopy

The values obtained with respect to quantitative analysis were tabulated in the **table:2**. It reveals that the leaves showed hypostomatous (single kind of stomata only) with numerous paracytic stomata distributed throughout the lower epidermis; few cluster crystals are observed in the upper epidermis **(figure 8&9)**. The vein islet and vein termination which were shown in the **figure 10**. The epidermal number of adaxial and abaxial surface are 320-360/mm^2 and 380 – 400/mm^2. The stomatal number and

stomatal index of abaxial surface are 240 – 260/mm^2 and 37-40/mm^2 respectively. The palisade ratio of the leaves was found to be in the range 4-7 /mm^2. The leaf vein islet and vein termination number were found to be 9 and 31 respectively.

Physicochemical constants

The powdered drugs were evaluated for loss on drying, foreign organic matter, ash value and extractive value. The physiochemical constants of leaves of *F.leucopyrus* were analyzed and tabulated in the **table:3**.

Phytochemical studies

Preliminary phytochemical tests were observed and tabulated in **table:4.** From the above accentuation, different chemical compounds such as alkaloids, carbohydrates, flavonoids, terpenoids, tannins etc., were detected in hydroalcoholic extract of *Flueggea leucopyrus*.

Quantitative estimation of phytoconstituents

The quantitative estimation was performed by using UV spectroscopy to detect the total flavonoid, phenolic and tannins content which were plotted in **figure 11,12&13**. The amount of total flavonoid content present in the extract in terms of mg quercetin/g of extract was found to be 31.81 mg/g by using linear equation. The amount of total phenolic and tannin content present in the extract in terms of mg gallic acid and tannic acid/g of extract was found to be 95.6 mg/g and 54.32mg/g respectively by using linear equation.

CONCLUSION

Macroscopical, microscopical and physicochemical parameters were determined which will be useful for the standardization of this plant material. Phytochemical screening revealed the presence of various primary and secondary metabolites. This study will be useful to the researchers, physicians and traders for the identification of this plant in future and also to procure genuine plant materials.

Acknowledgement

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