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Pharmacognostical studies of *Eriolaena lushingtonii* Dunn. Leaf and Stem bark: A Vulnerable medicinal tree taxon of Eastern Ghats

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Abstract
The present study highlights the pharmacognostical parameters such as macroscopic, microscopic characters, physic-chemical evaluation of leaf and stem bark of *Eriolaena lushingtonii*. The physico-chemical analysis revealed that ash, moisture content is more in stem bark. Powder microscopic studies of leaf revealed the presence of characteristic anisocytic stomata, stellate glandular trichomes and pitted and spiral vessels. Stem bark possess blunt end fibres, stone cells and prismatic crystals. Flourescence studies of leaf and stem bark powder exhibited characteristic colours in visible light, UV-short and long wavelengths. Histochemical studies confirmed the presence of phenols, alkaloids, starch grains, oil globules and mucilage in the tissues of leaf and stem bark. The finding of this study will help in the pharmacognostical identification and standardization of the drug in the crude form and also distinguish the drug from its adulteration.

Keywords: Physico-chemical, histochemistry, Extractive values, Moisture content, Flourescence analysis

Introduction
Medicinal plants are being employed in modern as well as in traditional medicine to treat illness due to their curative properties [1]. Throughout the world there is a resurgence in naturally derived drugs particularly the plant based medicine. Consumption of natural drugs is considered as safe to health. The advantage of natural drugs is their easy availability, economic and less or no side effects when compared to chemical ones. The disadvantage of using natural drug is that they are the subjected to adulteration. The more effective the natural drug more is its demand and the chances of non-availability increases. To meet the growing demand, the natural drug is easily adulterated with low grade material [2].
Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. Pharmacognostic studies ensures right identification, standardization and authentication of medicines from natural sources such as plants. These studies also help to prevent adulteration of herbal medicine, which increase safety and efficacy of natural drug.
Eriolaena lushingtonii Dunn, belongs to the family Sterculiaceae, which is a moderate-sized deciduous tree that grows in open forest areas at elevations ranging from 700 to 850 meters. This plant is endemic to the southern peninsular region of India and is currently classified as a vulnerable species due to habitat loss and limited population distribution. Ethanobotanical studies reveal that the plant has been traditionally used by local communities as an antidote for snake bites and scorpion stings [3]. Though the plant has been using for medicinal purpose its validation is not taken so far. Hence, the present study is undertaken to identify the pharmacognostical characters of leaf and stem bark to prevent adulteration.

Materials and Methods: Leaf and stem bark of *Eriolaena lushingtonii* are collected during field visit to the Lankamalla forest area, YSR Kadapa district, Andhra Pradesh. Authentication of the plant specimen was one by using the flora and the herbarium specimens of SVU herbarium, Department of Botany, Sri Venkateswara University, Tirupati. The voucher specimen (No. 647, SVU Herbarium) was prepared and preserved in the herbarium of Department of Botany, S.V.University, Tirupati as per the standard method [4].
Leaves separated from the twigs and stem bark made into small pieces, shade dried for 15 days, coarsely powered using a mechanical blender and used for the study. The rest of leaf

sample was preserved in the solution of F.A.A (70% Ethyl alcohol: Glacial acetic acid: Formalin in the ratio of 90:5:5) to study the histological profile [5].

Morphological studies: Morphology of the plant and its parts were examined using a simple microscope and described using taxonomic description with the help of floras [6,7].

Macroscopic and Microscopic studies: Organoleptic evaluation was carried out for the appreciation of texture such as size, color, odour, and taste. Free hand transverse sections of FAA fixed leaves and stem bark were taken and examined under the microscope. Surface preparation was done and both the surface of leaves were observed. The powder microscopy of dried leaves and stem bark was also carried out. The microphotographs were taken using the Olympus trinocular microscope and photographed using microscope attached with Industrial digital camera.

Physico-chemical studies: The physico-chemical parameters such as loss on drying, total ash content, pH, and extractive values, (water-soluble and alcohol soluble) were determined. Physico-chemical parameters were analysed as per the methods of Anonymous [8] Khandelwal [9], Evans and Trease [10].

Total ash

About 3 g of each powder wass accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air-dried powder.

Acid insoluble ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Water soluble ash

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min. and weighed.

The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried parts respectively.

Sulfated ash: A silica crucible was heated to red for 10 min. and was allowed to cool in desiccators and weighed. A gram of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1 ml of concentrated sulfuric acid, heated gently until white fumes are no longer evolved and ignited at 800ºC ± 25ºC until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool. A few drops of concentrated sulphuric acid were added and heated ignited as before and was allowed to cool and weighed.

Moisture content: Air dried material of 10g was dried in an oven at 105 °C. The loss of weight was calculated and values were tabulated.

Extractive values: Coarsely powdered air-dried plant material 20 g was placed in a glass stopper conical flask with 200 ml of solvents shaking frequently, and then allowing it to stand for 18 hours. Filtered it rapidly through Whatmann No. 1 filter paper, taking care not to lose any solvent. Transfered the 25 ml filtrate to flat- bottom dish and evaporated it on a water bath. Dry at 105ºC for 6 hours, cooled in a desiccators for 30 minutes and weigh it immediately. Calculated the content of extractable matter in% of air-dried material by the standard method mentioned in Kokate [11].

Powder drug Microscopy: The powders of *E.lushingtonii* leaf and stem bark were evaluated for microscopic structures, each of them were separately stained with the reagents such as phloroglucinol 1% and conc. HCl (for lignified structures), H_2SO_4 (for calcium oxalate crystals), iodine solution (for starch granules), sudan red G (for cuticular cell walls) and sudan red G in acetic acid and ethanol (for essential oils, resins, fats and fatty oils) were used on bleached powders. All samples were observed under Olympus trinocular microscope and photographed using microscope attached with Industrial digital camera.

Flourescence Analysis: A small quantity (1 gram) of leaf and stem bark powders were treated with acids, alkaline solutions and different solvents. The drug powders were treated with acids (Conc. HCl, 50% HCl, Conc. H2SO4, 50%, Conc. HNO3 and 50% HNO3), alkaline solutions (10% NaoH, alcoholic NaOH, 5% KOH, alcoholic KOH) other chemicals 5% FeCl3 water and acetic acid) and distilled water. They were subjected to study the fluorescence analysis in visible light, in short UV-light (245nm) and long UV-light (360nm).

Microscopic - Anatomical studies: Transverse sections of leaf and stembark were taken and stained with saffranin, observed the distinguished characters of the tissue systems. Transverse sections were photographed using Olympus trinocular microscope attached with Industrial digital camera.

Histochemistry studies: The leaf and stem bark samples were preserved in fixative FAA for anatomy section, the specimen were cut into thin transverse section using a sharp blade and the sections were stained with different reagents like Wagners reagent,(2 grams of iodine and 6 grams of Potassium iodide in 100 ml of water) Sudan-IV, Sudan red-III (Dissolve 0.01 g of sudan red III in 5 ml of ethyl alcohol (90 per cent) and 5 ml of pure glycerine), phloroglucinol (1g of phloroglucinol in 100ml of 90% ethyl alcohol), Alcoholic ferric chloride (1% of $FeCl_2$), ruthenium red (Dissolve 0.008 g of ruthenium red in 10 ml of a 10 per cent solution of lead acetate), Iodine [12].

Results

Morphological studies: It is a Moderate sized tree; bark greyish; young branches are stellately pubescent. Leaves up 6-9×5-10 cm, orbicular, cordate at base, acute, sometimes

younger leaves slightly lobed towards apex, irregularly shortly dentate, stellate hairy above, softly tomentose beneath, 7-nerved from the base; petioles 3-5 cm long. Flowers ca 2 cm long, in slender 2-3-flowered racemes, near the ends of the branches, peduncles longer than leaves, pubescent; flowering buds oblong, constricted at the middle; pedicels jointed above middle; involucral bracts 2-3, 2-4 mm long, linear, little distant from the flower, minute, multisect, caducous. Flowers yellow. Sepals 4 or 5, linear, wider near the base and apex, very acute, 1.5-2 × 0.2-0.3 cm long, pubescent within and tomentose without, glandular at the base inside. Petals 4 or 5, obovate, equaling the sepals, white or yellow; glabrous except narrowed densely pubescent base, undulate near apex. Staminal column ca 2 cm long; anthers 10-25, linear to oblong, basifixed, irregularly arranged near apex; staminodes absent. Ovary pubescent; style as long as staminal column, densely stellate hairy below, sparsely towards apex; stigma 4-7 lobed, sparsely hairy, the lobes revolute. Capsules woody, ovoid, ca. 4 cm long, 4-7 celled, 5-10 valved; valves smooth, more or less pubescent, usually silky villous at the inner angles. Seeds numerous, winged on one side at top.

Fig 1: A.*Eriloaena lushingtonii*-Habitat B.Leaves C. Flowering twig, D. Stem and E-Pieces of stem bark

Table 1: Physicochemical Analysis

Parameter	Leaf	Stem bark
Total ash	15.5% w/w	19.7% w/w
Acid insoluble ash	7.6% w/w	9.3% w/w
Water soluble ash	3.9% w/w	4.8% w/w
Sulphated ash	2.5% w/w	3.2% w/w
Moisture Content	21% w/w	37% w/w

Table 2: Extractive Values (mg)

Solvent	Leaf	Stembark
Methanol	2.85	2.18
Ethylacetate	2.14	1.72
Acetone	1.86	1.58
Petroleum ether	1.15	0.92
Hexane	0.68	0.59

Macroscopic studies (Organoleptic evaluation)

Fig 3: A. *E.lushingtonii*-Leaves B. Stem bark

Table 3: Organoleptic/ Physical evaluation

S. No.	Organoleptic Characteristics	Observation	
		Leaf	Stembark
1	Size and Shape	Ovate shaped, cordate shape with irregular dentates with acuminate apex. Up to 10 cm in length and upto 7 cm in width.	Cut pieces of stem, up to 2cm in width, up to 5 cm in length and up to 0.5 cm in thick ness. Single quill of curved cut pieces inwards and rarely outwards.
2	Colour	Upper surface: Green to slight yellowish green Lower surface: whitish green	Outer region: creamish brown to reddish brown. Inner region: Pale reddish orange to brownish orange
3	Surface characteristics, texture	Upper surface rough, lower surface pubescent and leathery. Brittle in nature	Outer surface is rough with longitudinal wrinkles. Inner region: rough with fibrous appearance. Short fracture at outer surface and fibrous fracture on the inner surface
4	Odour	Not Characteristic	Not characteristic
5	Taste	Slightly astringent	Astringent and bitter

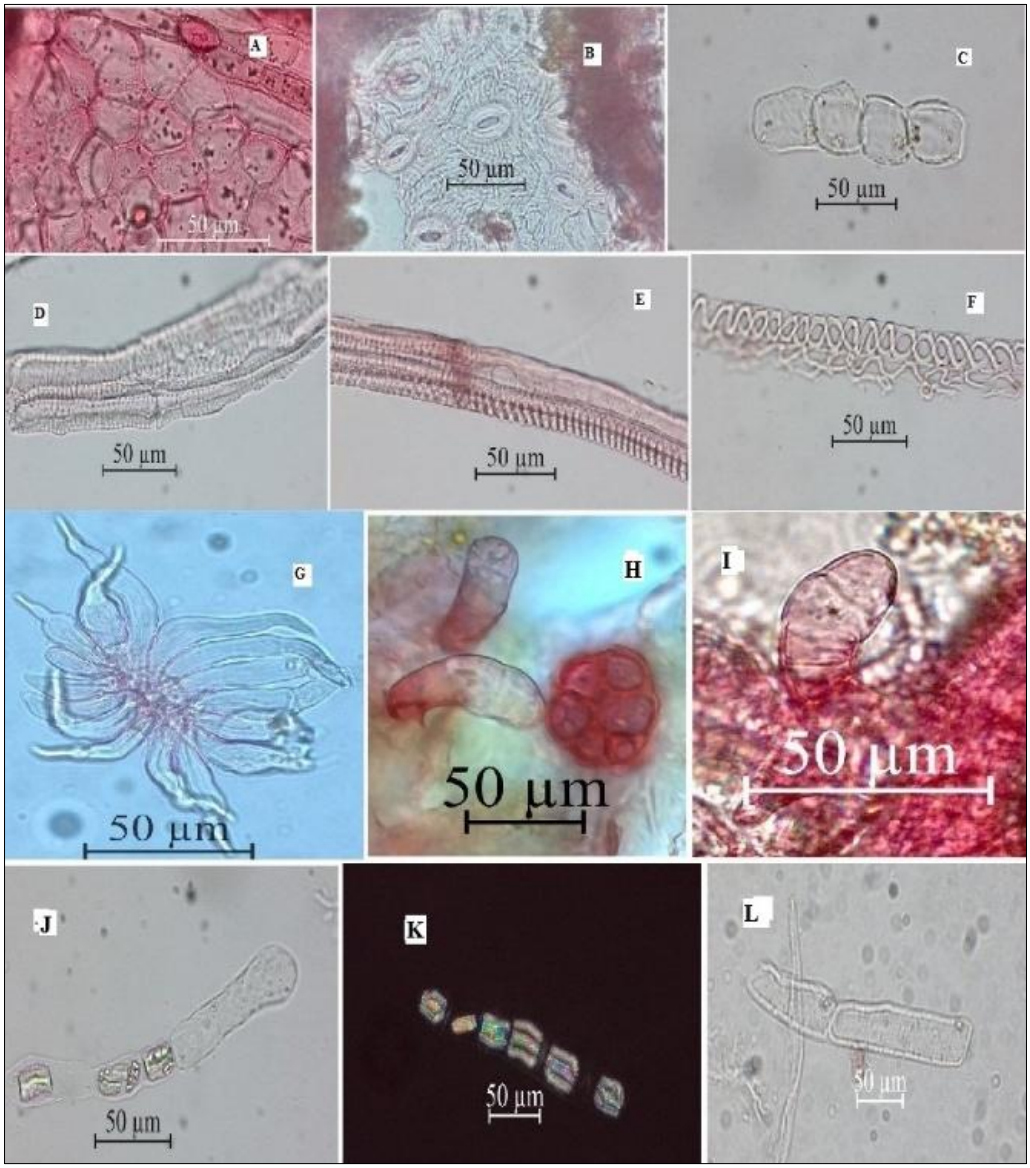

Fig 4: Powder microscopy of *Eriolaena lushingtonii* Dunn. - Dried Leaf: A. Upper epidermal layer B. Lower epidermal layer with anisocytic stomata C. parenchyma cells D. Sclereids, E. pitted vessels F. spiral vessels G. stellate trichome H. multiheaded glandular trichome and I. secretory trichome, J. Parenchyma cells embedded with prismatic crystals, K. Fibre crystals of calcium oxalate, L. pitted parenchyma cells

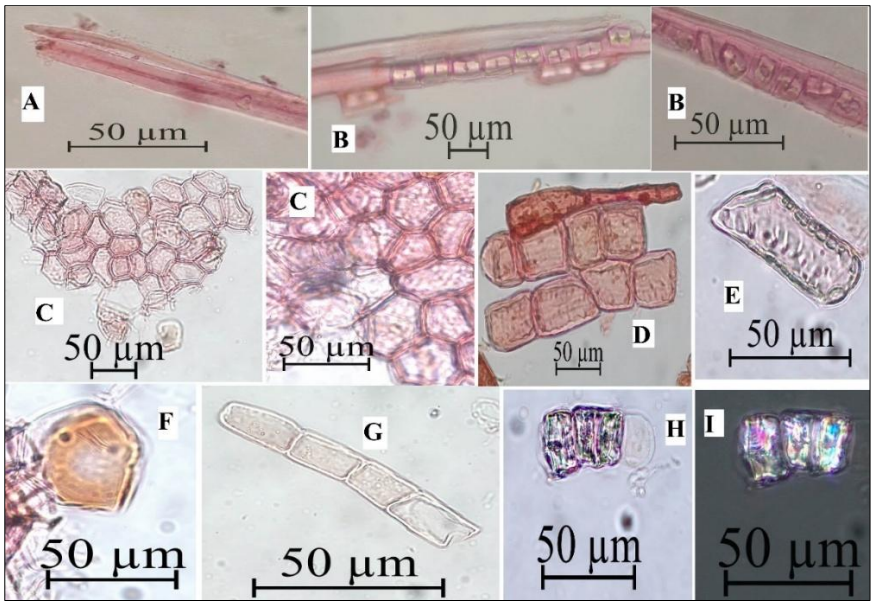

Fig 5: Powder microscopy of *Eriolaena lushingtonii* Dunn. - Stem bark: A. fiber with blunt end, B. fiber crystals, C. cork cells, D. group of stone cells, E. pitted stone cell with wide lumen, F. brownish orange content, G. parenchyma cells, H. prismatic crystals and I. Brownish prismatic crystals under polarizer.

Table 4: Flourescence Analysis

Chemical treatment	Visible Light		250-270nm		360-390nm	
	L	Sb	L	Sb	L	Sb
Powder as such	Gr	Br	L. Gr	L. Br	B	Bl
Powder+water	T. Gr	T.R.Br	T. Gr	Bl. Br	B	Bl
Powder+Conc.HCl	Bl. Gr	Bl	T. Gr	Bl	B	Bl
Powder+50% HCl	T. Gr	R.Br	Bl. Gr	L.Y	B	Bl
Powder+Conc.HNO_3	T. Gr	Br	Y. Gr	R.Br	Br	Bl. Br
Powder+Conc.H_2SO_4	Bl. G	T.R.Br	Bl.Gr	Bl	B	Bl
Powder+50% H_2SO_4	R. Gr	L.R.Br	Y. Gr	T.R.Br	B	Bl
Powder+NaoH	T. Gr	Bl.Br	T. Gr	Bl. Br	Rb	Bl
Powder+ Alc.NaoH	T. Gr	T.R.Br	L. Gr	L. Br	Lb	Bl. Br
Powder+KOH	Br. Gr	L. Br	T. Gr	Bl. Br	Rb	Bl
Powder+Alc.KOH	T. Br. Gr	T.R.Br	T. Gr	L.Br	Lb	Bl
Powder+Ammonia	Y. Gr	T.Br	R.Br	T. Gr	TG	Bl.Br
Powder+$FeCl_3$	T. Gr	Bl.Br	Bl. Gr	L.Br	BG	Bl. Br
Powder+ Acetic acid	L. Gr	L.R. Br	Br. Gr	R.B	Br. Gr	Bl.Br

L- Leaf, Sb-Stem bark

Gr: Green, L. Gr: Light Green, T. Gr: Thick Green, Y. Gr: Yellowish Green Bl. Gr: Blackish Green R. Gr: Reddish Green, Br: Brown, Br. Gr: Brownish Green, T. Br. Gr: Thick Brownish Green, R.Br: Reddish Brown, L.R.Br: Light Reddish Brown, T.R.Br: Thick Reddish Brown, Bl: Black, Bl.Br: Blackish Brown, L.Y: Light yellow.

Table 5: Histochemistry

Test for	Reagents used	Nature of change	Leaf	Stembark
Lignins	Phloroglucinol	Pink-cherry red	Pericycle, pitted parenchyma from pith regions and throughout the section vessels and fibres wall	Phloem fibre wall
Mucilage	Ruthenium red	Pink	Ground tissue cavities	throughout the phloem regions
Starch grains	Iodine	Blue	adjacent to the vascular region	Very few in phloem tissue
Alkaloids	Wagners reagent	Orange-red	Around the vascular bundle and few cells of parenchymatous ground tissue	Cells of phloem region
Phenols	Dil.$FeCl_2$	Black	Midrib, petiole, ground tissue adjacent to the fibre and cavities, lamina mesophyll region	Cortex and adjacent cavities
Calcium oxalate crystals	Conc.HCl	Bright effervesc	Ground tissue	Secondary cortex and phloem
Cutin/suberin	Sudan red III	Orange-red	Cutin on cellwall	Suberin on cellwalls of cork tissue

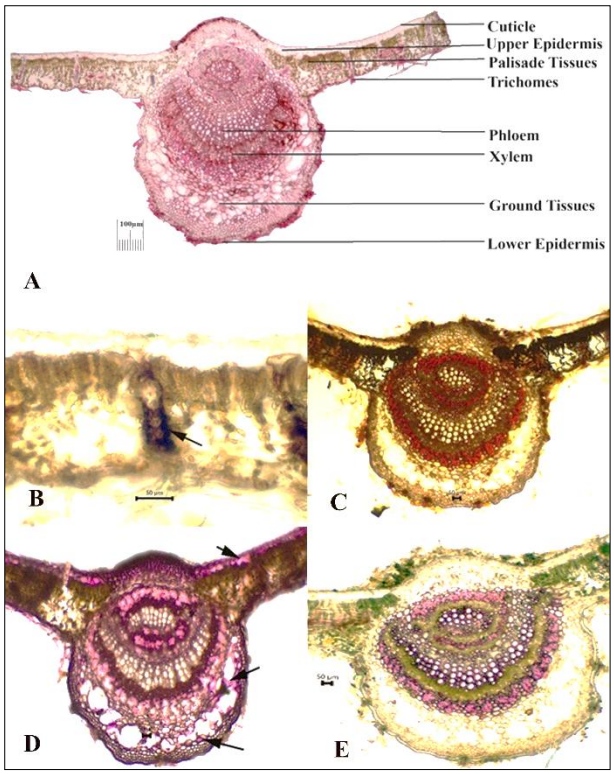

Fig 6: Histochemical studies of *E.lushingtonii* leaf; A.Transverse section of Leaf B. Phenols- lamina region C. Alkaloids in the ground tissue D. Mucilage around midrib, ground tissue and epidermis E. Lignin present in pericycle and xylem vessels

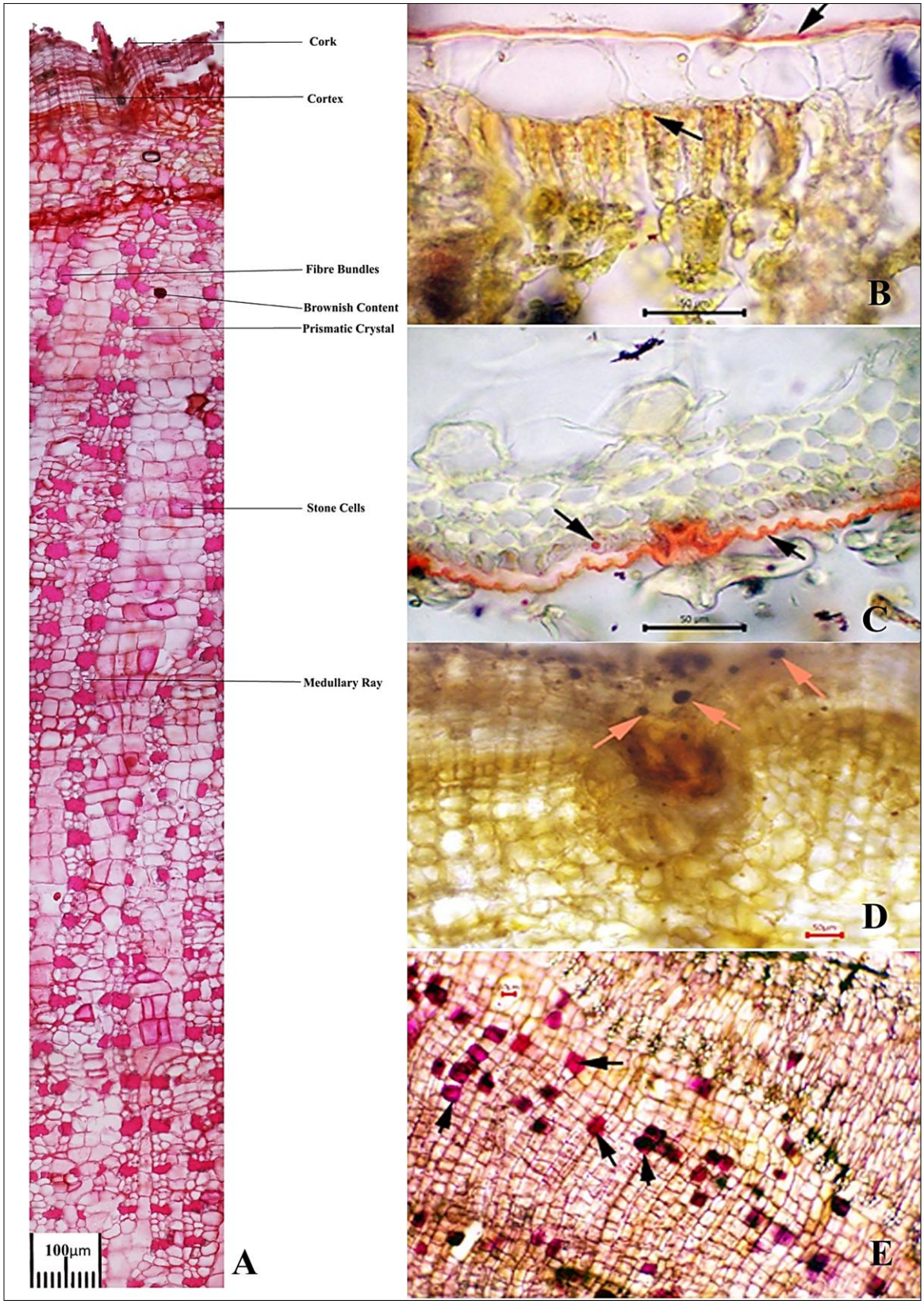

Fig 7: a. Histochemical studies of *E.lushingtonii* Stem bark. A. Transverse section of Stem bark B. Cutin present in the epidermal region C. Cutin and oil globule present in the midrib lower epidermal region D.Phenols present in cork and adjacent the phloem fibre region E. Diffused Mucilage cells

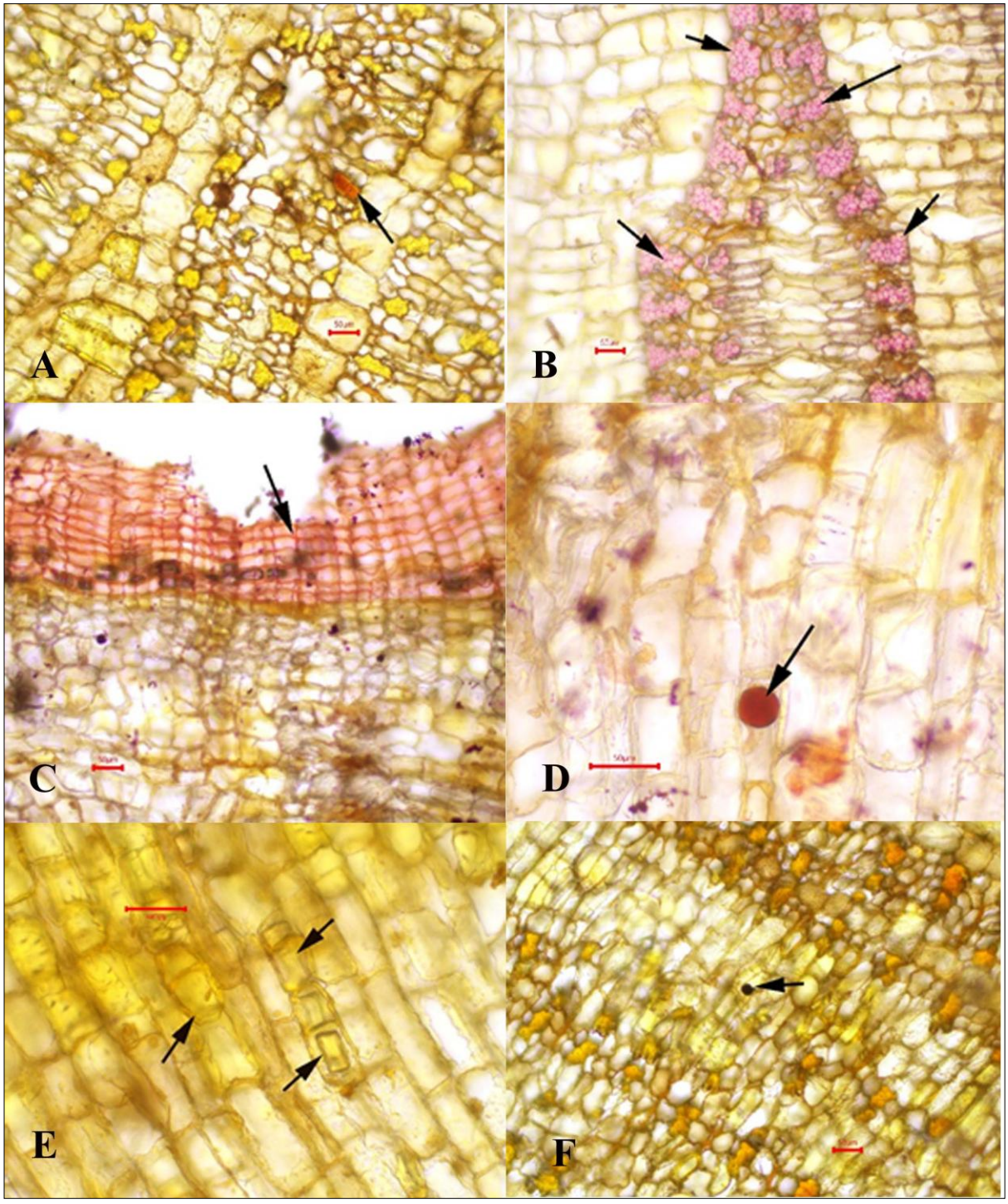

Fig 7: b. A. Alkaloid present in phloem regions B. Lignin present in phloem fibre C. Suberin in cork tissue D.Oil globule in cortex and phloem regions E. Prismatic crystals in the cortex region F.Starch grains in the Phloem

Physico-chemical analysis: Leaf consists total ash 15.5%, acid insoluble ash 7.6%, water soluble ash 3.9% and sulfated ash 2.5%. In stembark total ash 19.7%, acid insoluble ash 9.3%, water soluble ash 4.8% and sulfated ash 3.2%. Stem bark contains 37% of moisture content where as in leaf the moisture content is 21%.

Microscopic (Anatomical) studies: Leaf T.S consists of a single layer of upper epidermis covered with cuticle, 10 layers of palisade tissues, embedded with vascular cells in-between; Two patches of vascular bundles are present, oval and cordate shape, up to 10 layers of phloem region followed by 5-8 layers of xylem region containing patches of sclereids covering the xylem; 15-20 layers of ground tissue ending containing prismatic crystals embedded in them single layer of lower epidermis embedded with stellate trichomes.
Stembark T.S consists of 5-6 layers of cork tissue. Cork tissue followed by 15-18 layer of cortex made up of collapsed parenchyma cells filled with brownish contents, starch grains and prismatic crystals. Uniseriate medullary rays embedded with scattered bundles of fibres, prismatic crystals of calcium oxalate and stone cells.

Powder drug microscopic studies: Leaf powder analysis resulted the characteristic features anisocytic stomata, stellate trichomes, multiheaded glandular and secreory trichomes, vessels pitted and spiral shaped, calcium oxalate prismatic crystals, parenchyma cells pitted with prismatic crystals. Stem

bark powder contains fiber with blunt end, cork cells, stone cells, prismatic crystals, pitted stone cells with wide lumen.

Flourescence Analysis: Leaf and stembark powder treated with various chemicals and observed for the colour under visible light, short wavelength (250-270nm) and long wavelength (360-390nm). The colour observations are mentioned in the table-4. It is an important parameter of pharmacognostical evaluation as it can be often used to assess some crude drugs qualitatively.The usage of chemical reagents may convert some of the substances that are not fluorescent in nature into fluorescent derivatives [13, 14].

Histochemistry (Table-5): Transverese sections of leaf and stem bark of *E.lushingtonii* were tested with various reagents to find the accumulation of phytochemical constituents. Alkaloids, phenols, mucilage, lignins, starch grains, calcium oxalate crystals, cutin and suberin are located in the tissue systems of leaf and stembark.

Discussion

Quality control and the standardization of the herbal medicines is also necessary to assure the quality of the drug because substitute or counterfeit herbal materials are often found in the market. This analysis will help to ensure the identity, quality, purity and safety of drug for the human use. Quantitative analysis of different pharmacognostic characters are helpful to establish quality standards of the plant drug.

Physico-chemical evaluation total ash, acid soluble, acid insoluble, sulphated ash and moisture content are the characteristic for the plant powders. Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage.

Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used.

Powder microscopy of leaf reveals the presence of stellate hairs, anisocytic stomata, multiheaded glandular trichome, parenchyma cells embedded with prismatic structures, Fibre crystals of calcium oxalate, spiral shaped vessels. Stem bark powder contains fiber with blunt end, cork cells, stone cells, prismatic crystals, pitted stone cells with wide lumen.

Fluorescence analysis is an important tool for the screening of those compounds which have the property of exhibiting different colors under UV light. Some compounds are not fluorescent themselves but when they are treated with solvents are converted into fluorescent derivatives. Leaf and stem bark powders treated with chemicals yielded characteristic colours in the visible light and UV- short wavelength when compared to UV-long wavelengths. Hence the fluorescence analysis is an important parameter for qualitative analysis of crude drugs.

Histochemical studies of Leaf and stem bark revealed the presence of phenols, alkaloids, starch grains, lignins, mucilage, cutin in leaf and suberin in stem bark. Similar findings on histochemistry of leaf and stem bark in *Shorea tumbaggia* (Ankanna and Savithramma, 2012) [15], and *Cochlospermum religiosum* (Patrakar *et al*.,2021) [16].

Conclusion

From the present study, it can be concluded that leaves and stem bark of *E.lushingtonii* show the characteristic colours in the fluorescence analysis, powder microscopy the cells and tissues, histochemistry-the localization of bioactive compounds which might be ideal for the identification and standardization of crude drug. The polarity of solvent plays a major role in extraction of phytoconstituents. Among the solvents used for extraction, methanol extracts of leaf and stem bark. Were found to be the superior compared to ethyl acetate, acetone, and petroleum ether and acetone extracts.

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