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Research Article

PHARMACOGNOSTIC EVALUATION AND PHYTOCHEMICAL SCREENING OF *LEUCAS CEPHALOTES*

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ABSTRACT

Leucas cephalotes rainy season weed and has been traditionally used as mild stimulant, diaphoretic and used in fever and liver disorder. The present study was carried out to investigate pharmacognostical and phytochemical screening of whole plant. Morphological studies of leaves, stem, root, flower and seed showed the presence of various diagnostic characters. In the microscopical studies, leaves showed the presence of vascular bundle, sessile glandular trichomes, palisade and anomocytic stomata. Prismatic calcium oxalate, pericycle, bicollateral vascular bundle with non distinct cambium was found in stem. Secondary xylem was abundant in root. Ash value and extractive value was determined for quality standard of drugs. Phytochemical investigation shows the presence of flavanoids, phenol, phytosterol and tannins.

Keyword: *Leucas cephalotes*, Pharmacognostical evaluation, Phytochemical screening.

1. INTRODUCTION

Plants are one of the most important sources of medicines. Today the large number of drugs in use is derived from plants, like morphine from Papaver somniferum, Aswagandha from Withania somnifera, Ephedrine from Ephedra vulgaris, Atropine from Atropa belladonna, Reserpine from Roulphia serpentina etc. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice.[1]

Leucas Cephalotes (Labiatae or Lamiaceae) rainy season weed mainly found in North India. It is commonly known as 'Kubo or Kubi' in traditional medicine of Gujarat. The plant is also known as "Dronapushpi in Sanskrit. The genus *Leucas* includes about 100 Asiatic and African species. It is a valuable drug for snake bite. The plant is useful in bronchitis, inflammation, asthma, dyspepsia, paralysis and leucoma. The leaves are useful in fever and urinary discharge.[2]

According to Ayurveda, the plant is mild stimulant, diaphoretic and used in fever and also used in liver disorder. Flowers mixed in honey are used as domestic remedy of cough and colds.[3] It is valuable homoeopathic drug and as such is used for the treatment of chronic malaria and asthma.[4] Dry leaves along with tobacco (1:3) are smoked to treat bleeding as well as itching piles.[5] The plant was evaluated for *in vitro* antifilarial activity[6] and antidiabetic activity.[7] The plant was found to contain triterpenes, oleanolic acid, sterols and flavones.[8]

2. EXPERIMENTAL

2.1. Collection and identification of the plant material

The whole plant of *Leucas Cephalotes* was collected from the Dist.Ambikapur (C.G.) in the month of December 2010 and authenticated at Safia college of Science Bhopal, Madhya Pradesh. The voucher specimen **(235/BOT/SAFIA/11)** was deposited in Department of Pharmacognosy, T I T College of Pharmacy, Bhopal, and Madhya Pradesh.

2.2. Preparation of Leucas Cephalotes powder

The whole plants of Leucas Cephalotes were dried in shade and then powder with a mechanical grinder. The powder was passed through sieve no. 40 and stored in a labeled air tight container for further studies.

2.3 Macroscopic studies

The various parts of fresh herb was subjected to macroscopic studies which comprised of organoleptic characters of the drugs viz., color, odour, appearance, taste, smell, texture, fracture, etc.[13]

2.4 Microscopic studies

Qualitative microscopic evaluation was carried out by taking transverse sections of fresh leaves, root and stem of *Leucas cephalotes.* Free hand sections of the fresh roots were boiled with chloral hydrate to remove all the coloring matter and then carefully stained with phloroglucinol and HCl (1:1). The sections were transferred to mounted (glycerin) on a slide and a cover slip was placed over it. Powder characteristics of whole herb powder were also studied using reported methods.[13, 14]

2.5 Powder study

Plants are oven dried at 60°C for 4-6 hrs to make it moisture free and grounded using electric grinder and powder was passed through sieve no. 60. Powder characteristics were studied by standard methods. Photomicrographs of microscopy were made at different magnifications depending upon the anatomical details to be studied. Photomicrography was carried out using Olympus microscope and attached with Magnus MIPS camera.[13, 14]

2.6. Physicochemical studies

Physicochemical studies include ash value and extractive value to determine the quality and purity of the powder of plant of Leucas Cephalotes (Indian Pharmacopoeia, 1996).

2.6.1. Ash values

*2.6.1.1. Total ash value***.** Accurately weighed 2 g of air dried sample were taken in a tarred silica dish and incinerated at a temperature not exceeding 450°C until free from carbon.

Then cooled and weighed. When a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water, residue was collected in an ash less filter paper, incinerated the residue along with the filter paper until the ash was white or nearly so, filtrate was added, evaporated to dryness and ignited at a temperature not exceeding 450°C. Percentage of ash value was calculated with reference to the crude air-dried drug.[9]

2.6.1.2. Acid insoluble ash. Ash was boiled with 25 ml of 2 M HCl for 5 min, insoluble matter was collected in a Gooch crucible in an ash less filter paper, washed with hot water, ignited, cooled in a desiccators and weighed. Percentage of acid insoluble ash was calculated with reference to the air dried drug.[9]

2.6.1.3. Water soluble ash. Ash was boiled for 5 min with 25 ml of water, insoluble matter was collected in a Gooch crucible in an ash less filter paper, washed with hot water and ignited for 15 min at a temperature not exceeding 450°C. Weight of insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water-soluble ash. Percentage of water soluble ash was calculated with reference to the air dried drug.[9]

2.6.2. Extractive Values

2.6.2.1. Water soluble extractives. 4 g of air dried plant material was macerated with 100 ml of water in a closed flask, shaking frequently during the first 6 h and allowed to stand for 18 h. Thereafter it was filtered rapidly taking precaution against loss of water. 25 ml of filtrate was evaporated to dryness in a tarred flat bottom shallow dish dried at 105°C and weighed. Percentage water soluble extractive was calculated with reference to the crude air dried plant material.[10]

2.6.2.2. Alcohol soluble extractives. 4 g of air dried plant material was macerated with 100 ml of methanol in a closed flask, shaking frequently during the first 6 h and allowed to stand for 18 h. Thereafter it was filtered rapidly taking precaution against loss of methanol. 25 ml of filtrate was evaporated to dryness in a tarred flat bottom shallow dish dried at 105°C and weighed. Percentage methanol soluble extractive was calculated with reference to the crude air dried plant material.[10]

2.7. Preliminary phytochemical screening

The preliminary phytochemical screenings of various extracts of Leucas Cephalotes was subjected to determine the presence of phytoconstituents. Screening was carried out on all the leucas cephalotes extracts to determine the active principles or secondary plant constituents. Two (2) milliliters of each extract was measured into a test tube for each of the tests and concentrated by evaporating the extract in a trough. Tests were carried out for carbohydrates, reducing sugars, tannins, polyphenols, lipids, flavonoids, ketones, alkaloids, steroids and triterpenes.

2.7.1. Alkaloids

2.7.1.1. Mayer's test. Alkaloids give cream colour precipitate with Mayer's reagent (potassium mercuric iodide solution).

2.7.1.2. Dragandroff's test. Alkaloids give reddish brown precipitate with Dragandroff's reagent (potassium bismuth iodide solution).

2.7.1.3. Wagner's test. Alkaloids give a reddish brown precipitate with Wagner's reagent (Solution of iodine in potassium iodide).

2.7.1.4. Hager's test. Alkaloids give yellow color precipitate with Hager's reagent (saturated solution of picric acid). [11, 12.]

2.7.2. Glycosides

General test for the presence of glycosides:

Part A: extracted 200 mg of the drug by warming in a test tube with 5 ml of dilute (10%) sulphuric acid on a water bath at 100°C for 2 min, centrifuge or filter, pipette off supernatant or filtrate. Neutralize the acid extract with 5% solution of Sodium hydroxide (noting the volume of NaOH added). Added 0.l ml of Fehling's solution A and then B until alkaline (test with pH paper) and heat on a water bath for 2 min. Noted the quantity of red precipitate formed and compare with that formed in Part - B.

Part B: extracted 200 mg of the drug using 5 ml of water instead of sulphuric acid. After boiling add volume of water equal to the volume of NaOH used in the above test. Add 0.l ml of Fehling's solution A and B until alkaline (test with pH paper) and heat on water bath for 2 min. noted the quantity of red precipitate formed.

Compared the quantity of precipitate formed in Part-B with that of formed in Part-A. If the precipitate in Part-A was greater than in Part-B then Glycoside may be present. Since Part-B represents the amount of free reducing sugar already present in the crude drug. Whereas Part-A represents free reducing sugar plus those related on acid hydrolysis of any sides in the crude drug.[11, 12.]

2.7.2.1. Saponin glycosides

Froth test: Placed 1 ml solution of drug in water in a semi micro tube shake well and note the stable froth.[11, 12.]

2.7.2.2. Anthraquinone glycosides

Borntrager's test: Boiled test material with 1.0 ml of dil. sulphuric acid in a test tube for 5 min (anthracene glycosides are hydrolyzed to aglycone and sugars by boiling with acids) centrifuge or filter while hot (if centrifuged hot, the plant material can be removed while anthracene aglycones are still sufficiently soluble in hot water, they are however insoluble in cold water), pipette out the supernatant or filtrate, cool and shake with an equal volume of dichloromethane (the aglycones will dissolve preferably in dichloromethane) separate the lower dichloromethane layer and shake with half its volume with dilute ammonia. A rose pink to red colour is produced in the ammonical layer (aglycones based on anthroquinones give red colour in the presence of alkali).[11]

Modified borntrager's test: Boiled 200 mg of the test material with 2 ml of dilute sulphuric acid, 2 ml of 5% aqueous ferric chloride solution for 5 min and continued the test as above. As some plant contain anthracene aglycone in a reduced form, if ferric chloride was used during the extraction, oxidation to anthroquinones took place, which showed response to the Borntrager's test.[12.]

2.7.2.3. Cardiac glycosides

Keller killiani test (Test for deoxy sugars): Extracted the drug with chloroform and

evaporated it to dryness. Added 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride. Transfered to a small test tube; added carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube, blue colour appears in the acetic acid layer if cardiac glycoside was present.[11.]

2.7.3. Tannins and phenolic compounds
2.7.3.1. Gelatin test. Extract with 1% gelatin solution containing 10% sodium chloride gives white precipitate.
2.7.3.2. Ferric chloride test. Test solution gives blue green color with ferric chloride.
2.7.3.3. Vanillin hydrochloride test. Test solution when treated with few drops of vanillin hydrochloride reagent gives purplish red color.
2.7.3.4. Heavy metal test. Tannins get precipitated in the solution when treated with heavy metals.
2.7.3.5. Alkaline reagent test. Test solution with sodium hydroxide solution gives yellow to red precipitate within short time.
2.7.3.6. Mitchell's test. With iron and ammonium citrate or iron and sodium tartarate. Tannins give a water soluble iron tannin complex, which is insoluble in solution of ammonium acetate.[11, 12]

2.7.4. Flavonoids
2.7.4.1. Shinoda test: (magnesium hydrochloride reduction test). To the test solution add few fragments of magnesium ribbon and add conc. hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few min.
2.7.4.2. Zinc hydrochloride reduction test. To the test solution add a mixture of zinc dust and conc. hydrochloric acid. It gives red color after few minutes.
2.7.4.3. Alkaline reagent test. To the test solution add few drops of sodium hydroxide solution; formation of an intense yellow colour, which turns to colourless on addition of few drops of dil. acid, indicates presence of flavonoids.[12]

2.7.5. Proteins and amino acids
2.7.5.1. Millons test. Test solution with 2 ml of Millons reagent (mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appears, which turns red upon gentle heating.
2.7.5.2. Ninhydrin test. Amino acids and proteins when boiled with 0.2% solution of ninhydrin (Indane 1, 2, 3 trione hydrate), violet colour appears.[12]

3. RESULT AND DISCUSSION

The plant of *Leucs cephalotes* is an indigenous herb which was chosen for this study. The plant belongs to the family Lamiaceae. The scanty availability of information on this plant facilitates the study on it since ages various part of this part is being used for their medicinal use. The attempt is made to study the pharmacognostical activity of the plant of Leucas cephalotes. The study was divided in two parts:-

- Pharmacognostical studies
- Phytochemical screening

3.1. Pharmacognostical Studies

3.1.1. Macroscopic evaluation

The macroscopic character was useful in quick identification of plant material and also serves as an important standardization parameter. Organoleptic evaluations of different parts of *Leucas cephalotes* Spreng herb were reported in table-1. Leaves are Simple, lanceolate with pubescent surface, subacute apex and serrate margin. Stems are quadrangular, rough and hairy surface with prominent furrow and distinct nodes and internodes. Fruits are schizocarpic carcerule, nutlets and oblong in shape as shown in Figure1. Roots are tortuous, fibrous and many lateral root shown in Figure 2.

3.1.2. Microscopic evaluation

T.S. of leaf passing through midrib shows single layered upper epidermis interrupted by abundant glandular and few non glandular trichomes followed by single layered palisade cells in lamina portion. Midrib shows arc shaped vascular bundles, bicollateral, 2 to 4; present in centre, lower portion of the midrib is occupied by collenchymatous cells. Both simple and glandular trichomes are present, former being plenty on lower surface, uniseriate 2 to 3 celled with pointed apex, latter, stalked with 2 to 4 celled and unicellular head, the sessile glandular trichomes with 2 to 6 cell head. Stomata present only on lower side and are anomocytic.

T.S. of stem are quadrangular and ridge in outline, covered with thick cuticle, epidermis, contain prismatic crystal of calcium oxalate, trichomes, and sessile glandular trichomes with broad base and 3 to 4 celled head, cortex parenchyma forms 3 to 7 rows, with thick walled cells, endodermis distinct, pericycle is characterized by a row or two of big parenchymatous cells, vascular bundles bicollateral, a large vascular bundle found underneath the ridge and 3 to 4 small vascular bundle in between the ridges.

3.1.3. Ash Values

The physicochemical analysis of plants powder was carried out. In this study ash values (total ash, acid insoluble ash and water soluble ash water soluble ash) were determined. The results are shown in table no. 1.

3.1.4. Extractive values

Extractive values of *Leucas cephalotes* is determine in Methanol, Water, and Petroleum Ether and the results are shown in table no. 2.

3.2. Phytochemical screening

Quantitative phytochemical analysis was performed in aqueous and methanolic extracts and the results showed the presence and absence of certain phytochemicals in the drugs. Phytochemical tests revealed the presence of tannins, saponins, flavanoids, carbohydrates and steroids and results are given in table no.3.

CONCLUSION

Preliminary phytochemical as well as various aspects of the plant were studied and described along with physico chemical studies in authentification adulteration for quality control of raw drugs. The plant of Leucas cephalotes exhibits a set of diagonistic characters which will help to identify the drug in dried condition. It has been concluded from this study that estimation is

highly essential for raw drugs or plant parts used for the preparation of compound formulation drug. The periodic assessment is essential for quality assurance and safer use of herbal drugs.

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Table no. 1: Macroscopic evaluation of different parts of *Leacus Cephalotes*

S. No.	Characteristic	Leaves	Stem	Root	Flower
1.	Color	Greenish yellow	Yellowish green	Light brown	White
2.	Odour	Odourless	Odourless	Odourless	Odourless
3.	Taste	Bitter	Slight bitter	Slight bitter	

Table no. 2: Ash values of Leucas Cephalotes.

S.NO.	Ash value	% W/W
1	Total ash	15
2	Acid insoluble ash	5
3	Water soluble ash	2.5

Table no. 3: Extractive values of Leucas cephalotes in following solvents

S.NO.	Physicochemical parameters	% w/w
1	Methanol soluble extractive	20
2	Water soluble extractive	15
3	Petroleum ether soluble extractive	10

Table no. 4: Phytochemicals tests in Methanolic, and Aqueous extract of whole plant of Leucas cephalotes.

S.No.	Experiment	Aqueous Extract	Methanolic Extract
1	Alkaloids	-	-
2	Glycosides	-	-
3	Tannins & Phenolic comp.	+	+
4	Saponins	-	-
5	Flavanoids	+	+
6	Carbohydrates	+	+
7	Steroids	-	-
8	Amino acids	-	-

(+)Present (–) Absent

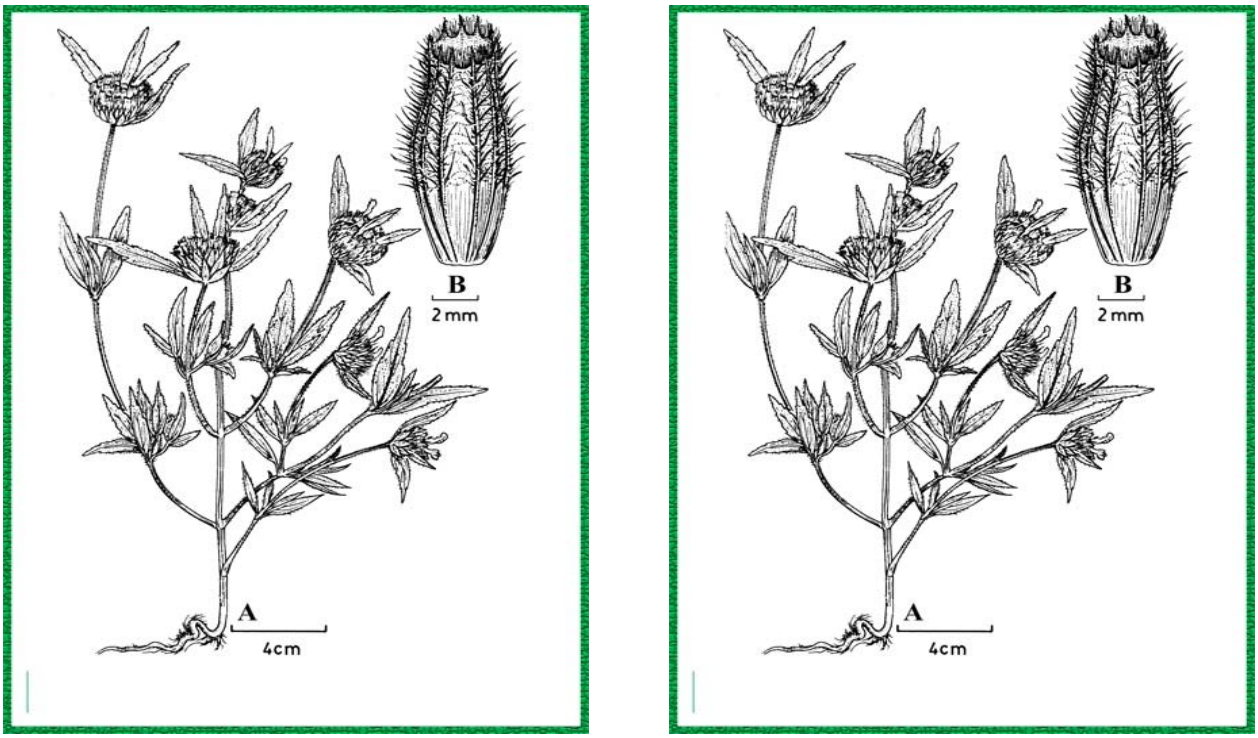


Figure no. 1: Herb of *Leucas Cephalotes*

Figure No. 2: Roots of *Leacus cephalotes.*

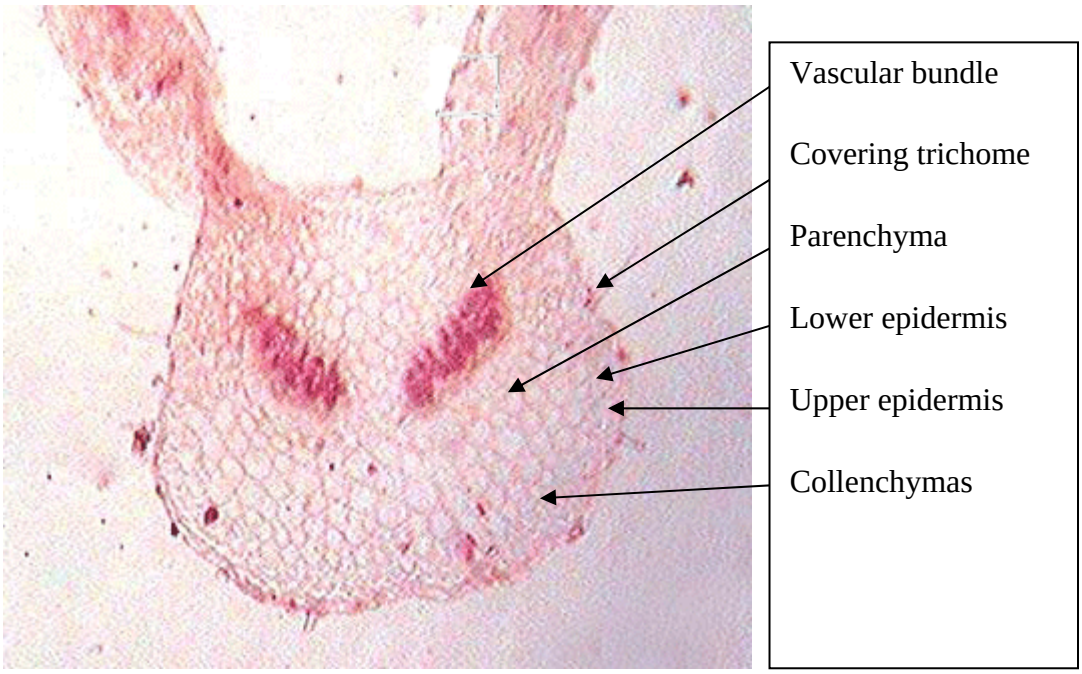


Figure no. 3: T.S of leaf of *Leacus Cephalotes.*

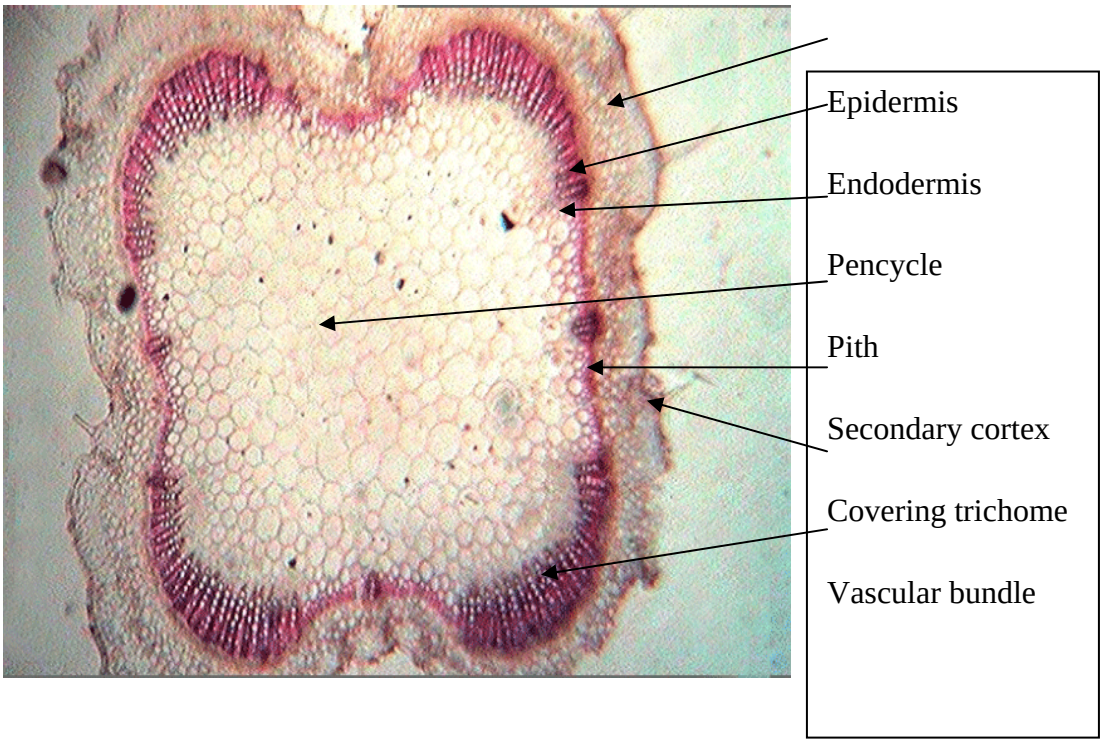
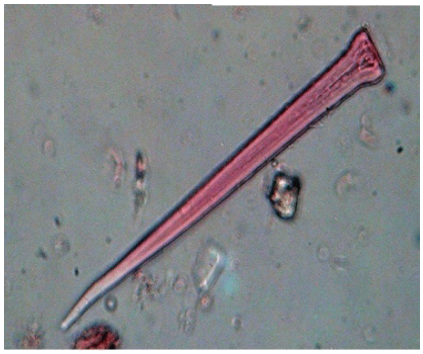
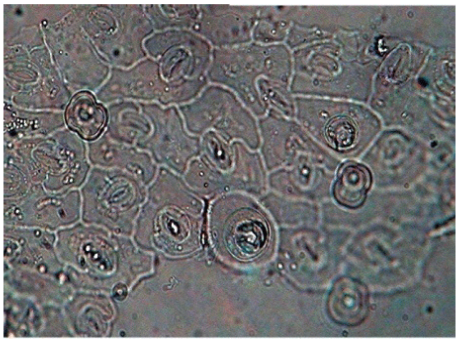


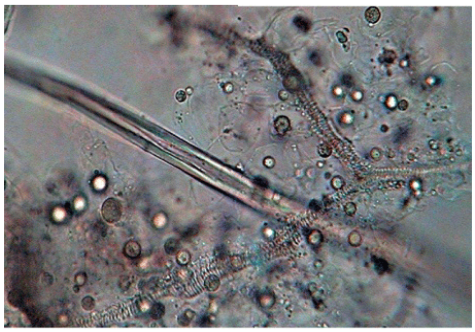
Figure no. 4: T.S. of stem of *Leacus Cephalotes.*



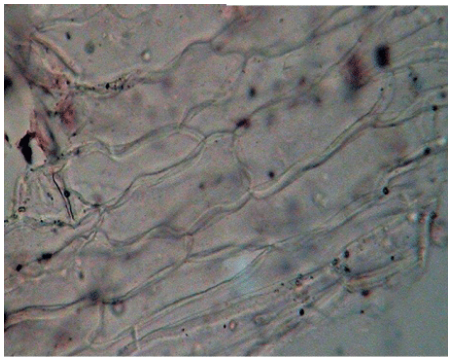
Unicellular trichomes



Anomocytic stomata



Oil Globules



Epithelial cell

Figure no. 5: Powder microscopy of *Leucas Cephalotes.*