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PHARMACOGNOSTIC EVALUATION OF CYNANCHUM TUNICATUM (RETZ.) ALST. -A RARE MEDICINAL PLANT

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ABSTRACT

Medicinal plants play an important role for primary health care as a preventive and curative measure in different ailments. Ethnic peoples are the raw resources for innovation of new drugs for Indian system of medicine as well as a western system of medicine. Present study is on *Cynanchum tunicatum* (Retz.) Alst, belonging to the family Asclepiadaceae, a rare medicinal plant used to treat the maggot infected wounds by few ethnic peoples of Tumkur district of Karnataka. During literature survey found scanty scientific information about this plant. Therefore, attempts were made for pharmacognostic standardization of medicinally potential plant as a natural drug. The pharmacognostic approach towards the leaf constituent's parameters such as stomatal index, palisade ratio; vein islets numbers. Anatomy of officinal parts, powder microscopy and phytochemical analysis was conducted. Stomatal index of upper surface is 13.51 No/sq.mm and lower surface is 18.13 No/sq.mm, vein islets number is 5.76/mm^2, average palisade ratio is 1:6.5 No/unit areas. Pollinia are multicelluar with cellular caudicles and corpuscules. The anatomical structure of stem of this plant showed unique character, two broad vessels attached to the pericycle and lie opposite to each other. The powder microscopy reveals presence of reticulate vessels, latex cells and scleroids. These are the important characters for identification and authentication of drug. Presence of alkaloids, terpenes, steroids and other phytochemicals in the plants may be the potential source for healing chronic wound. These quantitative and qualitative pharmacognostic data are first step towards the establishing the identity and purity of botanical, to which are used as a natural drugs. So these parameters can be used as a quality control standard of this potential medicinal plant.

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INTRODUCTION

Medicinal plants are the source of the drugs for indigenous and western system of medicine. It is reported that about 80% of the world population is partially or wholly dependent on plant based drugs [1]. Due to side effects, economic point of view and resistance of pathogens to synthetic drugs, encourages the scientists to search for new natural drug sources like medicinal plants. Indiscriminate harvesting of plants as raw materials for preparation of medicine and extraction of active principles in commercial scale leads to the stage of rare state. Rarity of botanicals is sometimes due to natural barrier like, lack of pollinator, incompatibility in the development of androecium and gynoecium. During the field survey at Rajapura of Mavinakere, Tumkur district of Karnataka, found that the shepherds using a one botanical to heal the maggot infected wounds of their domestic animals. They called this plant as *Kodumurukana balli.* This botanical specimen was collected and identified as a *Cynanchum tunicatum* (Retz.) Alst, by referring local and regional floras [2, 3]. It belonging to the Asclepiadaceae family, it is a twinning herb; leaves are opposite, simple, ovate, acuminate. Inflorescence is an axillary corymbose cyme; bracts minute, caduceus, Calyx-5, free to more than half way down, glandular within at base, green, angular, acute. Corolla rotate, white, 5-partite; lobes overlapping to right; corona membranous, adnate to the base of staminal column, annular lobed at top. Stamens 5, adnate to the base of the corolla tube; filaments fused to form a column; anther with hard appendages; pollinia two in each cell; pollen carriers horny, ovary of two free carpels; style free at base, 5-angled at top, rounded. Fruits are pair of follicles. Seeds are Plano convex, margined, and hairy at apex.

Family Asclepiadaceae which comprises about 2980 species in 315 genera [4]. This family has many medicinal plants with a wide range of therapeutic activities [5]. The *Cynanchum* genus comprises about 200 species [4], reported for their use in folk medicine as antifebrile, anti-tumour, antitussive, diuretic, expectorant, anticonvulsant, anodyne, tonic and effective against chronic hepatitis [6]. More than 300 compounds have been isolated from *Cynanchum* species, including steroids, alkaloids, terpenes, flavonoids, polysaccharides and steroidal glycosides as the major constituents [7]. C-21 steroids are the typical phytoconstituents of *Cynanchum* species [8].

During literature survey, it was found that there is a meagre research work on species of genus *Cynanchum* such as C. *stauntonii, C. sarcomedium, C. wilfordii, C. auriculatum, C. acutum* and *C. tunicatum*. Three glycoside steroids namely stauntoside L, M and N were reported from *C. stauntonii*, it is a significant source of steroidal glycosides [9]. *C. sarcomedium* exhibits antigen toxic activities on aberration assay [10]. Conduritol was demonstrated to be contained only in *C. wilfordii.* Therefore, it may be useful as a chemical marker to identify the two species *C. wilfordii* and *C. auriculatum* [11]. Research work on *C. acutum* revealed the presence of several bioactive metabolites including β-sitosterol, lupeol-amycin [12]. Methanolic extract of *C. acutum* showed antioxidant and antimicrobial activities [13]. The alcoholic extract of leaves of *C. acutum* could be used as anti-inflammatory, analgesic, antipyretic and also in the treatment of cardiac arrhythmia, intestinal colic as hypotensive and for improving respiration in asthma [14-16].

But inadequate scientific research on *Cynanchum tunicatum,* the aqueous leaves extract of this plant was showed a non-antibacterial activity against *Staphylococcus aureus, Pseudomonos aeruginosa, Bascillus subtilis and Escherischia coli* [17]. This is only the one research work as on today on *C. tunicatum* as per our knowledge. Therefore *Cynanchum tunicatum* has been selected to be the subject of the present research. This plant is an endemic to India and Sri Lanka and status is under red list [18]. Till now no pharmacognostic research have been done on *C.tunicatum*, so current study was undertaken to standardise this herbal drug. Pharmacognostic parameters such as leaf constituent's parameters, pollen study, microscopy of root and aerial parts, Organoleptic study, powder microscopy and Preliminary phytochemical analysis of whole plant powder was done. The result obtained on stomatal index; vein islets numbers and palisade ratio are significant quantitative data and can be used for herbal drug standardisation. The structure of pollinial-apparatus is a finger print for a particular species of plant. Anatomy of stem showed unique arrangement of vessels. Presence of latex cells, scleroids, reticulate vessels etc., in powder microscopy and phytochemical analysis are the qualitative data. Both quantitative and qualitative data can be used to standardise this lesser known, rare and potential medicinal plant. It is the first study on Pharmacognosy of *Cynanchum tunicatum* as per the literature survey.

MATERIALS AND METHODS

Fresh plant materials along with its roots were collected from the field and voucher specimen was deposited in the department. The sufficient quantity of plant material was collected and part of collected specimens was preserved in FAA preservative for microscopical studies. Whole plant material was shade dried and powdered by using pulveriser and sieved the powder. Coarse and fine powder was stored in air tight container. Fresh leaf sample was used for stomatal index, a thin transparent layer was peeled from upper and lower epidermis of leaf and these peelings were observed under microscope and calculate stomatal index by using the formula SI= S/S+E X 100 and expressed the stomatal index in No/sq.mm [19]. Fresh leaf was used for vein islets study, pieces of leaf blade between margin and midrib from the basal, middle and apical part were selected and processed by using 90% alcohol and saturated solution of chloral hydrate. Processed lamina were mounted in chloral hydrate solution and observed under microscope. Vein islets were calculated by observing lower epidermis. Area of microscopic field of vision and the number of vein islets /mm^2 was calculated [19]. Fresh leaf was used for palisade ratio, leaf blade pieces between margin and midrib from the apical, middle and basal part were selected and boiled in distilled water for few minutes and in 95% ethyl alcohol on water bath until to remove chlorophyll pigment and boiled in saturated solution of chloral hydrate till the lamina become completely transparent. These transparent pieces were mounted on clean glass side. Palisade cells were observed from upper surface of the leaf. The numbers of four contiguous epidermal cells as well as palisade cells were counted by changing the focus. Four reading were taken and the palisade ratio was determined [19] and express in No/Unit area. Pollens were collected from anther of fresh flower bud and was pre-treated, processed and mounted on glass side, for studying the external morphology by adapting standard procedure [20, 21]. Preserved plant sample was used for free hand sectioning of petiole, leaf, stem and root for anatomical studies, safranin and haematoxylin was used to stain the sections and mounted on a glass slide with glycerine [22, 23].

Organoleptic study was conducted by observing physical sensory, colour, odour and taste of the drug powder [21, 23]. Powder microscopy was carried out by following standard pharmacognostical method [23]. Preliminary phytochemical analysis was done by extracting 100 Gms of shade dried whole plant powder. Distilled water was used as a solvent in the soxhlet apparatus. Extract was filtered through Whatman filter paper. Collected filtrate was distilled off to reduce the volume of solvent to $1/10^{th}$ and remaining solvent was removed by evaporation on a water bath. The dried extract was stored in a sterile container and used for analysis [23].

RESULTS

Leaf constituent's parameters were determined and presented in the tables. Leaf having paracytic type of stomata [Fig.I.A&B], the stomatal index of upper surface is 13.51No/sq.mm [Tab. I] and stomatal index of lower surface is 18.13No/sq.mm [Tab. II]. Vein islets number is 5.76 /mm^2 [Tab. III]. Palisade ratio of apical part of lamina is 1:5.5No/Unit areas. [Tab. IV], middle part of lamina is 1:8.25No/Unit areas [Tab.V and Fig. I. D-E] and basal part of lamina is 1:5.75 No/Unit areas [Tab.VI]. Average palisade ratio of leaf is 1:6.5No/Unit area. Pollen is typical Asclepidaceaeous type. Pollinial-apparatus consists of multi-cellular pollinia, cellular caudicles and corpuscules.

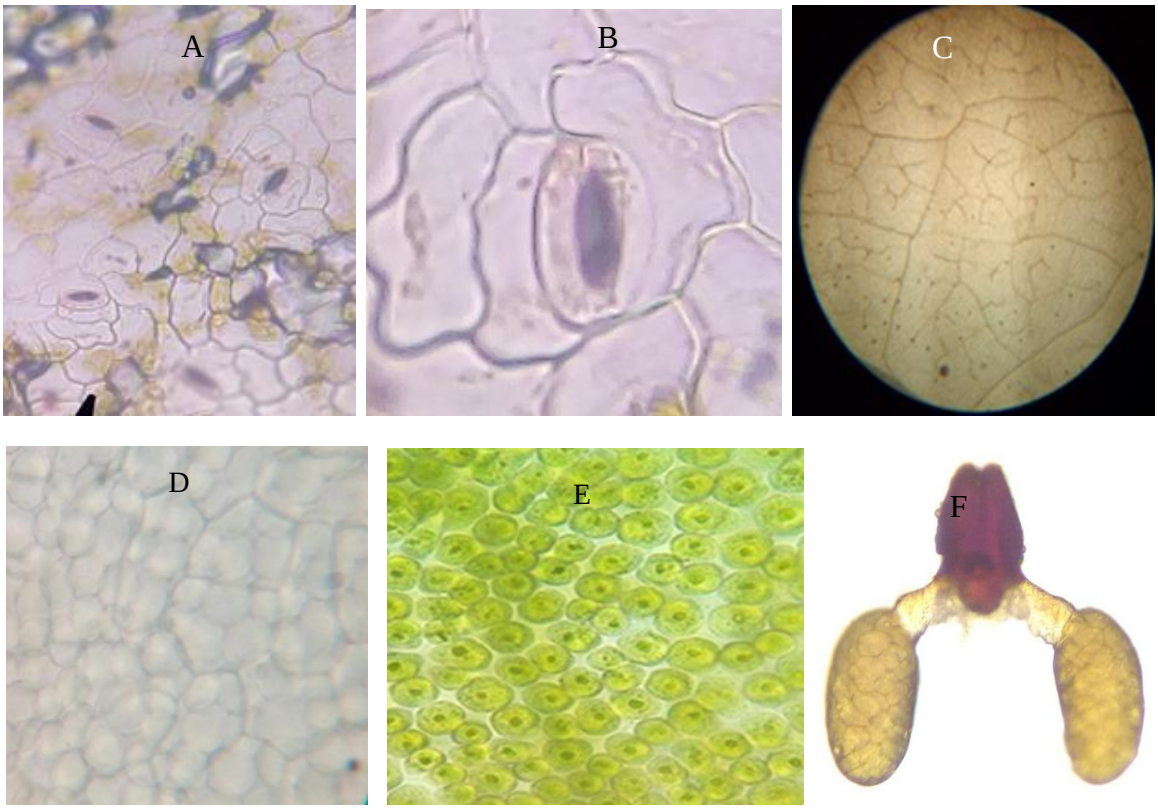


Figure I: Stomata, Chlorophyll, Palisade cells, Vein islets and Pollens.

A &B. Stomata, C. Vein islets, D. Palisade cells in epidermal cells, E. Palisade cells, F. Pollinia

Table: I. Stomata index of upper surface.

No. observation	Total No. of stomata/Microscopic field of vision	Total No. of epidermal cells/microscopic field of vision	Mean total No. of stomata/Microscopic field of vision	Mean Total No. of epidermal cells/ microscopic field of vision	Stomatal index of upper surface
1	16	72			
2	13	82	15	96	13.51
3	16	134			

Table: II. Stomatal index of lower surface

No. observation	Total No. of stomata/Microscopic field of vision	Total No. of epidermal cells/microscopic field of vision	Mean total No. of stomata/Microscopic field of vision	Mean Total No. of epidermal cells/ microscopic field of vision	Stomatal index of lower surface
1	22	76			
2	20	94	22	99.33	18.13
3	24	128			

Table: III. Study of vein islets number

No. observation	No. Vein islets/ Microscopic field of Vision	Average vein islets/ microscopic field of vision	App. Vein islets
1	22		
2	18	21.33	5.76
3	24		

Table: IV. Palisade ratio of apical part of leaf.

No. observation	No. of epidermal cell	No. of palisade cells	Total no. of epidermal cells	No. of palisade cells	Palisade ratio
1	1	5			
2	1	4			
3	1	6	4	22	1:5.5
4	1	7			

Table: V. Palisade ratio of middle part of leaf.

No. observation	No. of epidermal cell	No. of palisade cells	Total no. of epidermal cells	No. of palisade cells	Palisade ratio
1	1	10			
2	1	6	4	33	1:8.25
3	1	9			
4	1	8			

Table: VI. Palisade ratio of basal part of leaf.

No. observation	No. of epidermal cell	No. of palisade cells	Total no. of epidermal cells	No. of palisade cells	Palisade ratio
1	1	6			
2	1	4	4	23	1:5.75
3	1	6			
4	1	7			

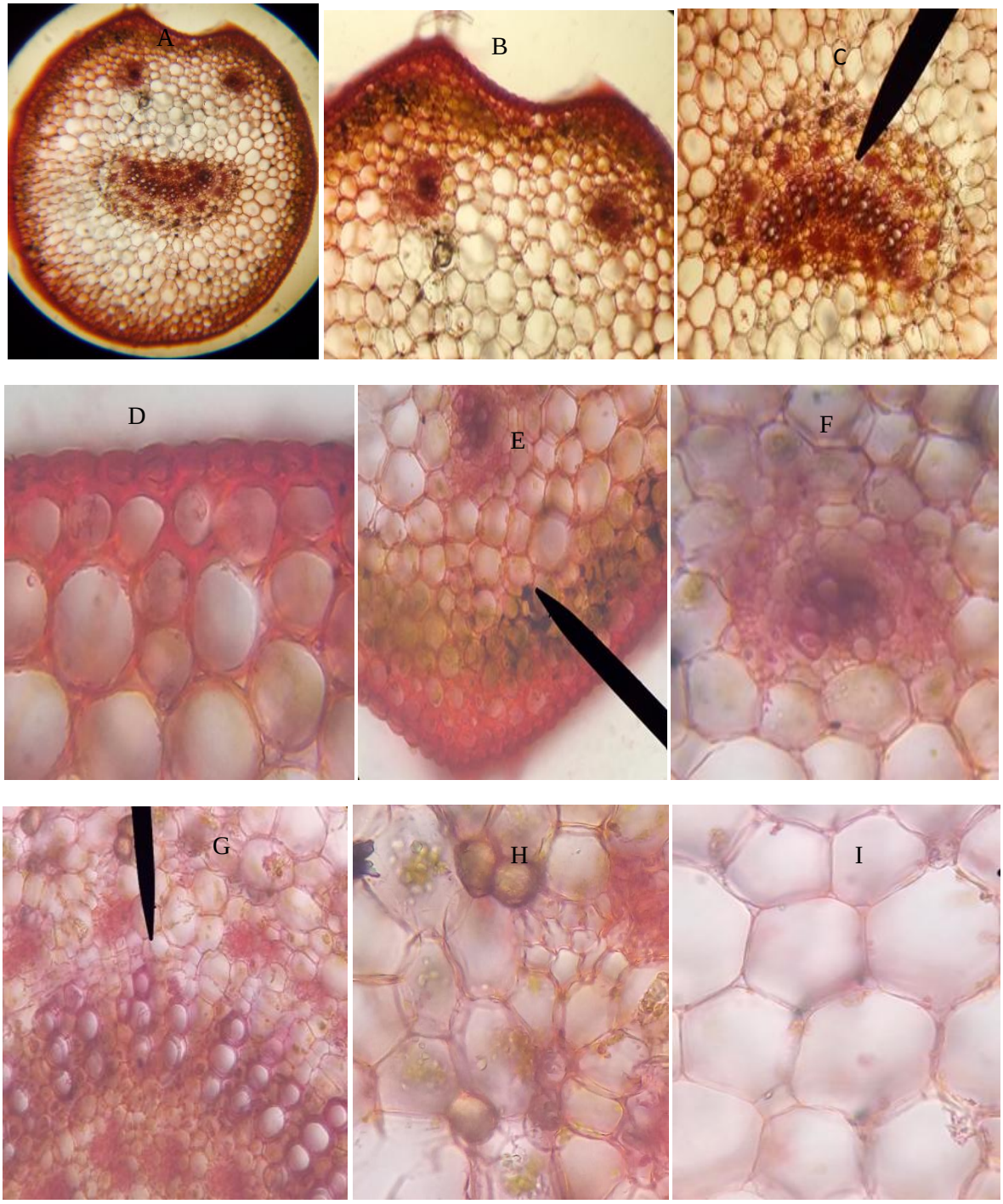


Figure: II. Transverse section of petiole

A.TS of Petiole. B. Two smaller steles, C. Central stele, D. Epidermis, E. Hypodermis, F. smaller vascular bundle, G. Central vascular bundle enlarged H. Starch, latex cell & calcium oxalate crystal, I. Parenchyma cell.

T.S. of petiole: Outline of cross section is somewhat circular [Fig. II. A] with groove on upper surface with blunt edges [Fig. II. B]. Cortex is vast with parenchyma cells, within the cortex two smaller vascular bundles and one larger vascular bundle is present at the centre of the section [Fig. II. B & C]. Outer surface is surrounded by single layer of ovular shaped epidermal cells covered by a thick cuticle [Fig.II.D]. Hypodermis is made up of 2-3 layers of collenchyma cells followed by 3-4 layers of chlorenchyma cells [Fig. II.E]. Vascular elements arrangement is unique with central xylem and surrounded by a phloem [Fig.II. F&C].Cortex cells contain starch grains, latex cells and calcium oxalate crystals [Fig. II.H]. Cortex is well developed with circular to polygonal parenchyma with intercellular space [Fig. II. I].

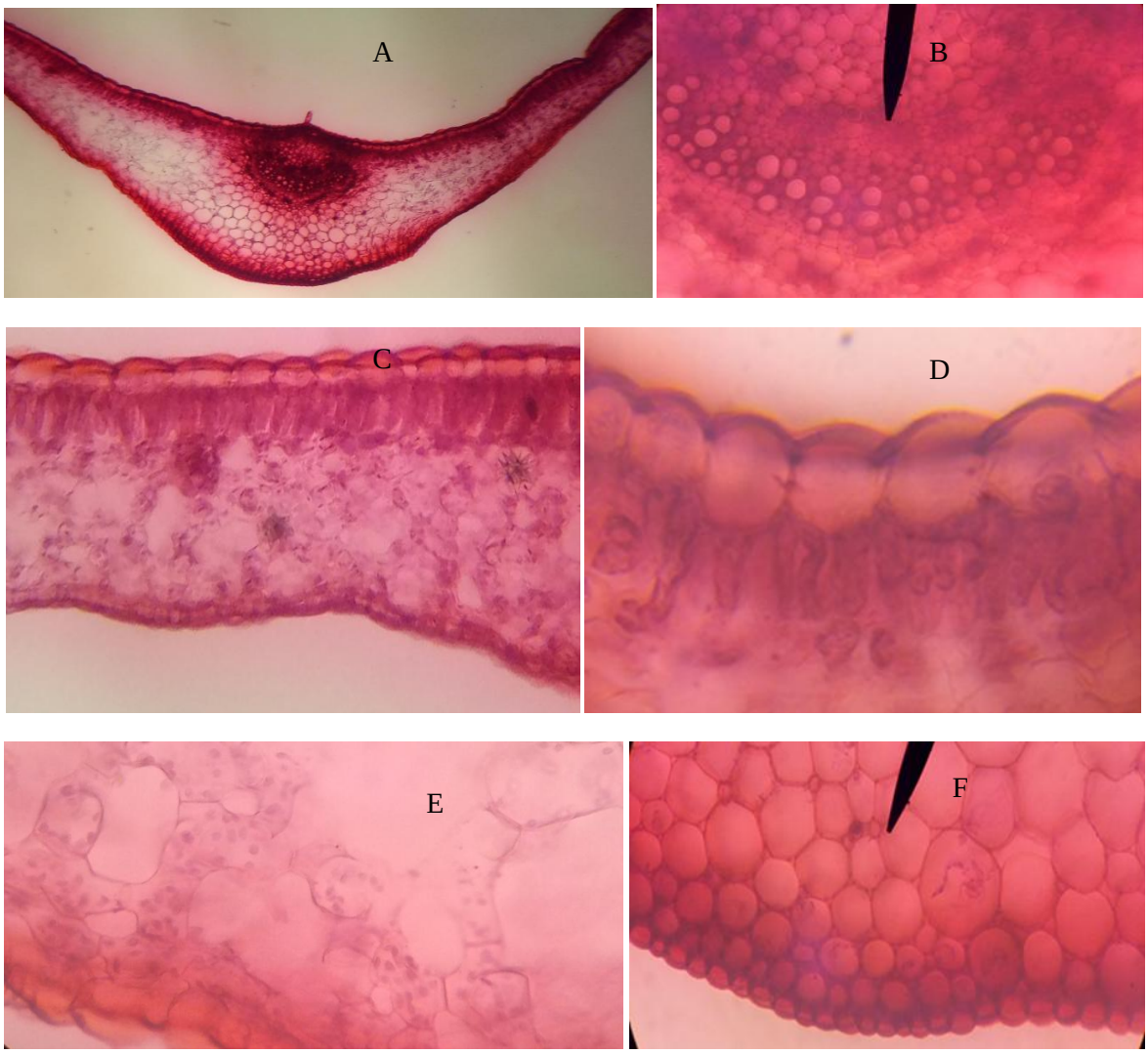


Figure: III. Vertical transverse section of leaf

A. Vertical transverse section of leaf, B. Midrib portion enlarged, C. VTS of Lamina, D. Upper epidermis, E Lower epidermis, F. Ventral part of midrib.

VTS of Leaf: VTS of leaf at mid rib showed that, the adaxial side at mid rib region is convex shaped and a central arc shaped vascular bundle bounded by a sheath [Fig. III.A]. Vascular elements were prominent. On both the sides of adaxial and abaxial, there is 2-3 layers of collenchyma cells. [Fig.III.B]. Mesophyll tissues is bounded by upper and lower epidermal cells. Upper epidermal cells were elongated and covered by cuticle [Fig. III.C] and below the upper epidermis, there is a two layer of palisade cells which were elongated with prominent chlorophyll pigment followed by spongy parenchyma cells [Fig. III.D]. Calcium oxalate crystals are prominent which are in the form of druses crystal. Lower epidermal cells were not so elongated compare to the upper epidermal cells and spongy parenchyma cells were loosely arranged and they were prominent in the section [Fig. III. e]. Latex cell were found between the parenchyma cells which present around the central vascular bundle [Fig. III.F].

T.S of Stem: TS of stem under compound microscope showed circular outline [Fig. IV. A], there are three zones which are outer cortex, middle vascular and inner pith [Fig. IV.B]. The outer most layer is epidermis made up of single layer of ovular shaped cells covered by a thick cuticle. The cuticle is prominent. Below the epidermis 2-4 layers of hypodermis made up of collenchyma cells. Few cells present towards epidermis contain chlorophyll pigments. There is a well-developed parenchymatous cortex which is interspersed with prominent sclerenchymatous patch made up of 10-30 cells in a group [Fig. IV.C]. Starch grains and calcium oxalate crystals are found in cortex. Cortex towards inner side of the pith there is two numbers of well-developed vessels made up of one or two cells laying in an opposite side to each other which are directly attached to the pericycle [Fig. IV.D]. Pericycle is a prominent continuous ring of sclerenchymatous cells made up of 3-4 layers [Fig. IV.E].Vascular bundles are conjoint, collateral but cambium is not prominent. Pith is well developed with parenchymatous cells and contains few latex cells [Fig. IV.F].

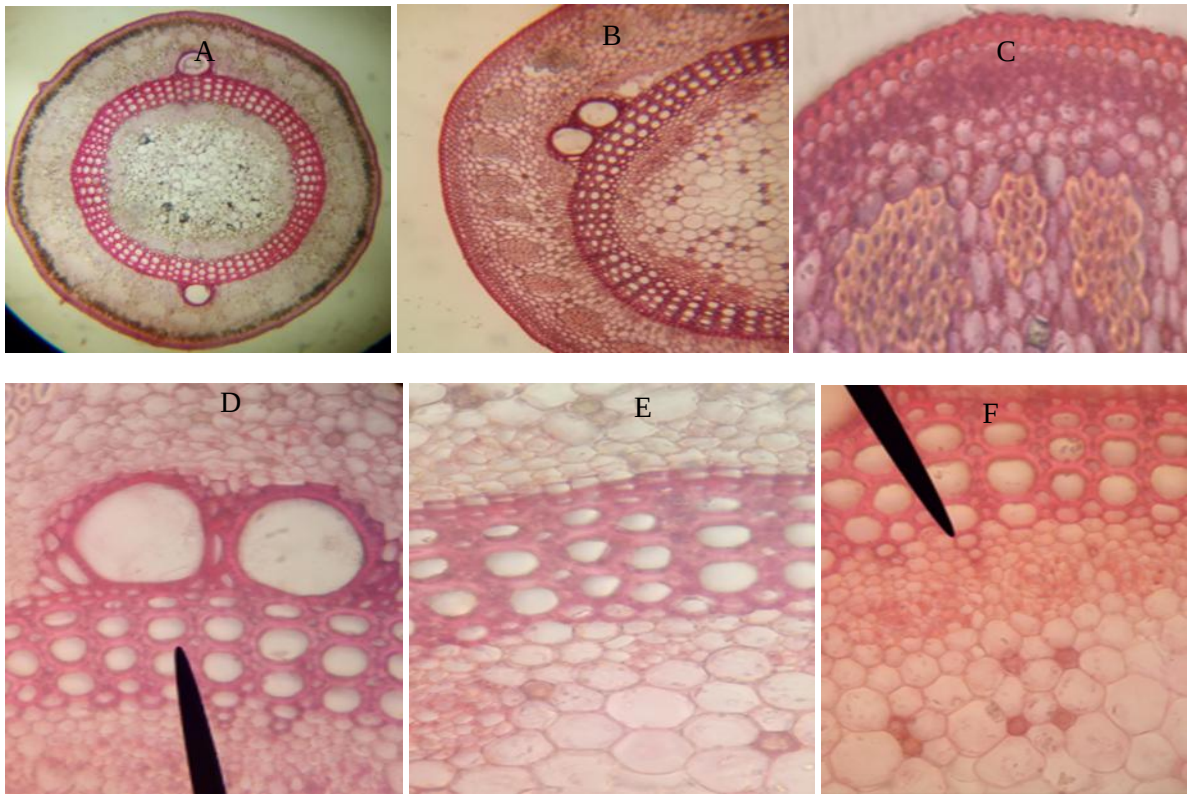


Figure: IV. Transverse section of Stem

A.TS of stem, B.TS of Stem portion enlarged, C. Cortex, D. Pericycle, E. Pericycle enlarged, F. Vascular bundle.

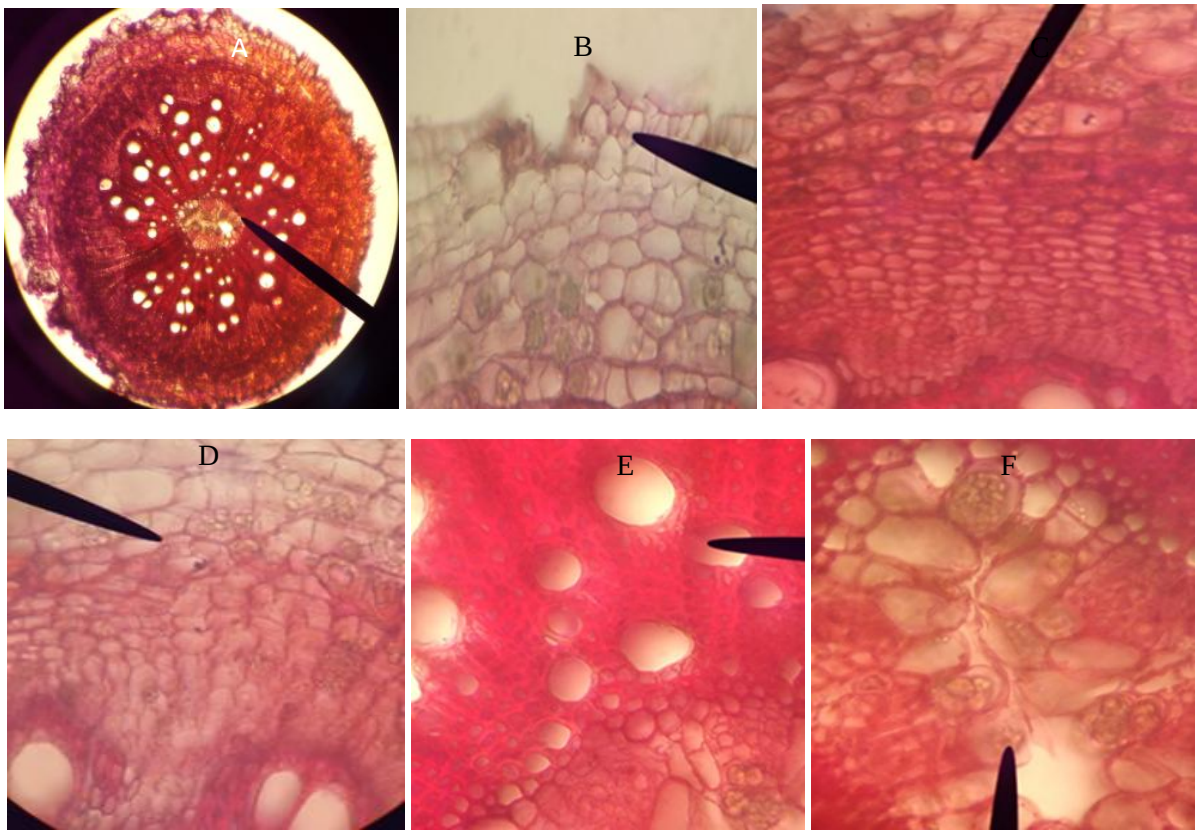


Figure: V. Transverse section of root

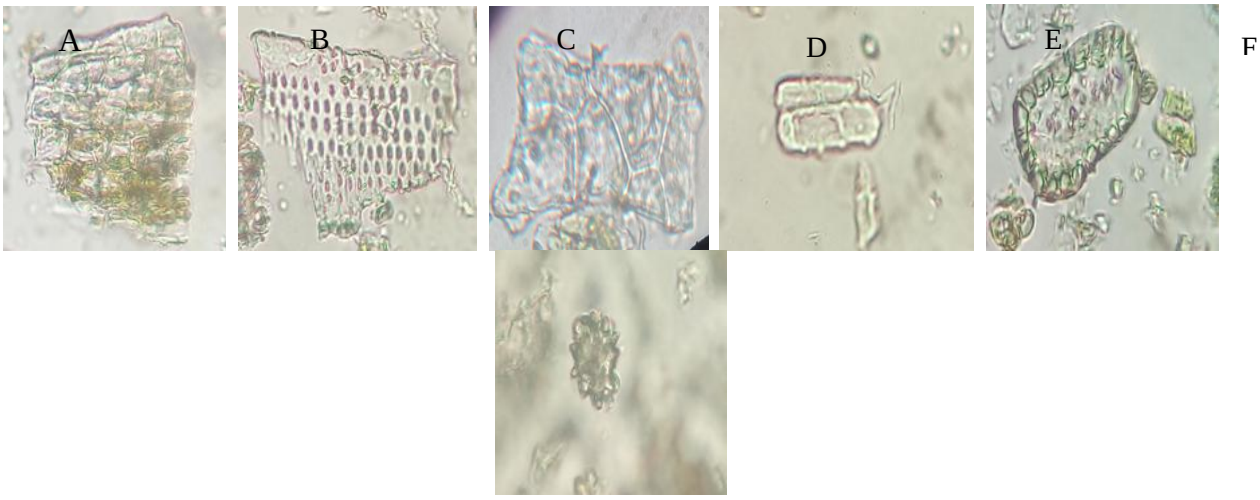
A. TS of root, B. Cork region, C. Cortex, D. Cortex enlarged, E. Vascular region, F. Central core.

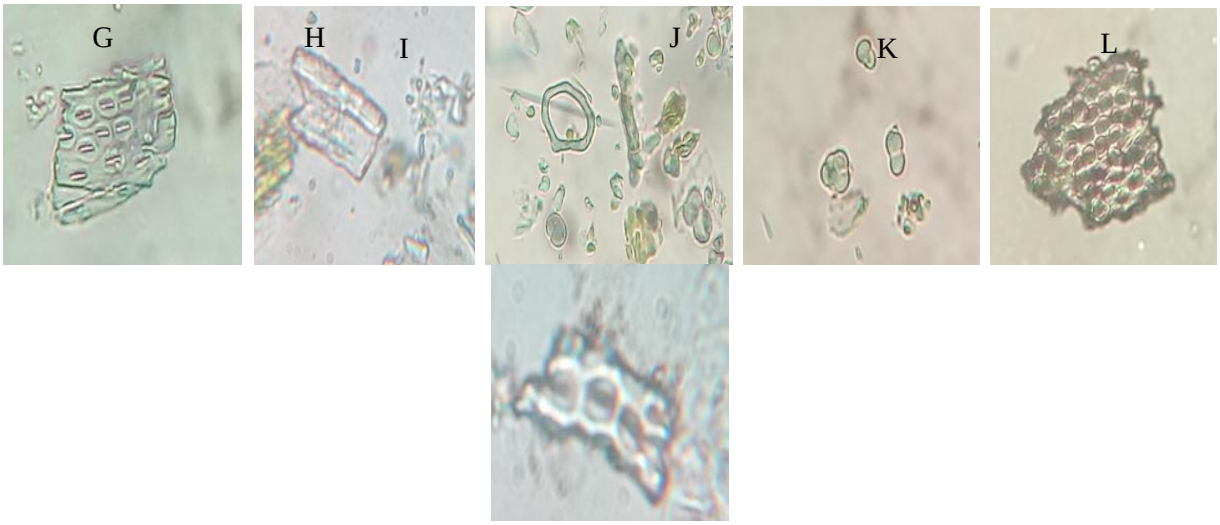
TS of root: T S of root showed circular outline with uneven surface due to the development of cork layer [Fig. V. A], broadly there were three region, outer most cork -secondary cortex, vascular zone and central core. Cork is well developed, the outer most 4-5 layers of cells were lose their content and take different shapes and also ruptured [Fig. V. B]. Cork cambium is prominent; the inner cells of the cork contain starch, calcium oxalate crystals in the form of druses and stone cells in the form of scleroids [Fig. V. C]. Secondary cortex is prominent with parenchyma cells, it also contains starch, scleroids and latex cells and it is interspersed with multiserrated medullary rays [Fig.V. D]. Vascular zone is well developed; the xylems have prominent metaxylem and protoxylem [Fig.V. E]. Inner core is start occupied by xylem elements but it showed presence of loosely arranged parenchyma cells with plenty of starch in few parenchyma cells [Fig. V. F].

Organoleptic characters of the powder of whole plant showed coarse texture, light green colour, characteristic light odour and light sweet taste [Tab.7]. Preliminary phytochemical analysis of aqueous extract of whole plant powder reveals the presence of alkaloids, phytosterols, carbohydrates, saponnins, flavonoids, tannins and absence of proteins and fixed oil [Tab.8]. Cell constituents study for powder is presented [Fig.6].

Table: VII. Organoleptic study.

Sl. No	Features	Observation
01	Nature of powder	Coarse
02	Colour	Light green
03	Odour	Characteristic light odour
04	Taste	Light sweet taste





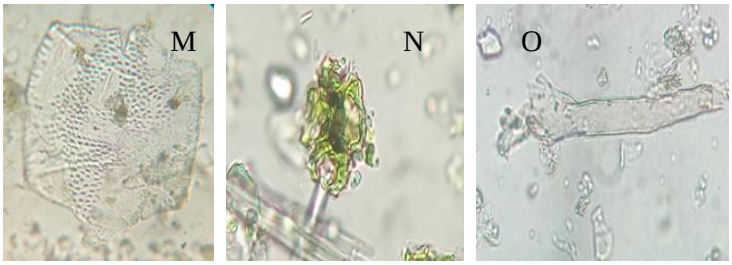


Figure: VI. Powder microscopy:

A.Cortex cells, B.Fragment of trachea, C.Epidermal cells, D.Fragment of epidermal cell, E.Scleroids, F.Druse crystal, G. Fragment of trachea, H.Fragment of epidermal cell, I. Latex cell, J. Starch, K. Collenchyma cells, L.Sclerenchyma cells, M. Xylem vessel, N. Chlorenchyma cell, O. Laticiferous tube.

Table: VIII. Preliminary phytochemical analysis of aqueous extract

Sl. No	Phyto- constituents	Test	Result
01	Alkaloid	Mayer's test	+
		Wagner's test	+
02	Phytosterol	Salkowski test	+
		Liebermann-Burchard	+
03	Carbohydrates	Benedicts test	+
		Molisch's test	+
04	Proteins	Biuret test	-
		Ninhydrin test	-
05	Fixed oil	Stain test	-
06	Saponin	Froth's test	+
07	Flavonoid	Lead acetate test	+
08	Tannins	Gelatine test	+

+ Positive, - Negative.

DISCUSSION

Stomata is rubiaceous type; the stomata has two subsidiary cells, the long axis of which are parallel to that of the stomata, it is one of the supportive point for species identification. Somatal index of upper surface is 13.51No/sq.mm and lower surface if 18.13No/sq.mm. Abaxial surface had more number of stomata than the adaxial surface. So stomatal index represent the number is fairly constant for any species and can be used as a specific character, which has proved useful for distinguishing the leaf of same genus. Vein islets numbers is 5.76No/mm^2. The number of vein-islets per square millimetre and this number are independent of the size of the leaf and do not alter with the age of the plant. Palisade ratio of middle part is 1:8.25 No/Unit area, which is the highest ratio compare to basal part and apical part. The average palisade ratio is 1:6.5No/Unit area. Certain extent palisade ratio also important parameters for drug identification, this ratio has been shown to be constant to serve as a diagnostic character of species belonging to the same genus in certain instances [25].

Pollens are the finger print of botanical species. In *C. tunicatum,* pollinia in a flower are oriented in pendulous form. There is no extra pollinial appendage. The corpuscules [Fig.I.F] is dark brown in colour and harder consistency than that of the pollinium and caudicles. It is a stunted unit of two halves. The suture is open throughout on the ventral surface. The caudicle is obliquely oriented downwards and attaches with the pollinium.

Anatomical study of petiole [Fig.II A], showed that the presence of large central vascular bundle and two small vascular bundles at the adaxial surface is a unique character for identification and authentication of this botanical. VTS of leaf reveals the presence of arc shaped vascular bundle which is present towards the adaxial side. Presence of plenty of calcium oxalate crystals in the form of druses [Fig.III.C] is also one of the identification characters. TS of stem in the [Fig.IV.A] showed a unique characteristic feature, a continuous ring of sclerenchymatous pericycle. Two numbers of one or a group of two, broad vessels are directly attached to the pericycle which is opposite to each other. Presence of well-developed patches of sclerenchyma in the cortex, is the another characters to evaluate the drug. TS of root showed well developed secondary xylem, presence of scleroids in the secondary cortex and starch granules [Fig.V.D] are the added identification features.

Organoleptic characters and powder microscopy are important for rapid identification of drugs. The ordinary diagnostic features of the drugs are disappeared in the powdered form. The modified characters have become prominent. Reticulate vessels

[Fig.VI.B], scleroids [Fig.VI.E] and Latex cells [Fig.VI.I] are the prominent characters. Phytochemical analysis proved the presence of alkaloids, phytosterol, carbohydrates, saponnins, flavonoid and Tannins. These secondary metabolites have remarkable pharmacological actions.

CONCLUSION

According to the WHO guidelines herbal drugs are characterised according to macroscopic, microscopic and organoleptic characters. These characters are first step towards the establishing the identity and the degree of purity of botanicals, which are used as a drug. The quantitative determinations of pharamacognostical parameters are useful for setting standard for crude drugs of botanical origin. These data can be used as a quality control standard. Further active principle identification, isolation and characterisation are recommended before clinical study.

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CONFLICT OF INTEREST

The authors declared no conflict of interest

ABBREVIATIONS

FAA : Formaldehyde Alcohol Acetic Acid
SI : Stomatal Index
S : Stomata
E : Epidermal cell
Sq : Square
Mm : Mili-meter
VTS : Vertical transverse section
TS : Transverse section

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