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PHARMACOGNOSTICAL EVALUATION OF AERIAL PARTS OF *CORALLOCARPUS EPIGAEUS*

J. Venkata Suresh[*1], G. Nagarjuna Reddy[1], V. Ramanarayana Reddy[1]

[1*]Department of Pharmacognosy and Phytopharmaceuticals, KLR Pharmacy College, Palvancha, T.S, India.

ABSTRACT

This study was carried out to evaluate the pharmacognostical parameters for the aerial parts (leaves and stem) of the plant *Corallocarpus epigaeus* (*Cucurbitaceae*). Traditionally the plant is used as bitter, emetic, cures inflammations (Ayurveda). Root tuber for snake bite, anaemia, leprosy, eczema, dysentery, arthitis, rheumatism, chronic mucous enteritis, diabetes. Stem for filariasis, wounds, emetic, goiter and diabetes. An attempt has been made for proper identification of this folk herb for obtaining its complete therapeutic effects. With this view the morphoanatomy of the leaves and stem, along with its quantitative microscopy, microscopic linear measurements, WHO recommended physico-chemical determinations and authentic phytochemical procedures are the important diagnostic characters have been carried out to aid the complete pharmacognostical evaluation of the plant. The parameters reported in this paper may be proposed as the referential standards to establish the authenticity of *Corallocarpus epigaeus*. This study also helps in differentiation of this drug from its other species.

KEYWORDS

Corallocarpus epigaeus, Cucurbitaceae, Pharmacognostical and Leaf and stem.

Author for Correspondence:

Venkata Suresh J,

Department of Pharmacognosy and Phytopharmaceuticals,

KLR Pharmacy College,

Palvancha, Khammam, T.S, India.

Email: vsjilakara@yahoo.co.in

INTRODUCTON

The early man explored is immediate natural surroundings, tried many things like plants, animals and minerals and developed a variety of therapeutic agents[1]. Search for eternal health and longevity and to seek remedy to relieve discomfort prompted man to develop diverse ways and means of health care. Plants are also appreciated in pharmaceutical research as a major resource for new medicine and a growing body of medical literature supports the clinical efficacy of herbal treatments[2]. In almost all the traditional medicines, the medicinal plants play a major role and constitute the back bone of the traditional medicine. Standardization of natural

products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof[3,4]. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential.

The first step towards ensuring quality of starting material is authentication. Despite the modern techniques, identification of plant drugs by pharmacognostical studies is more reliable. According to the world health organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plants is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken[5,6.]

Corallocarpus epigaeus[7-9] (Syn: *Broyonia epigaea)* belongs to the family C*ucurbitaceae*. Known locally as Nagadonda and Akasagaruda. It is distributed in Punjab, Sind, Gujarat, Rajputana, Andhra Pradesh and Ceylon. In the AP state, the plant is available at lower hill slopes, especially on hedges, Nagapatla reserve forest and Talakona hills of Tirumala. Traditionally the plant is used as bitter, emetic, cures inflammations (Ayurveda). Root tuber for snake bite, anaemia, leprosy, eczema, dysentery, arthitis, rheumatism, chronic mucous enteritis, diabetes. Stem for filariasis, wounds, emetic, goiter and diabetes[7-9]. Though the plant has several uses, no scientific data is available to identify the genuine sample. The present investigation was therefore, take up to establish identity of aerial parts morphologically, microscopically and physicochemically for the standardization of the drug.

EXPERIMENTAL

MATERIAL AND METHODS

Collection and authentication of plant material

The selected herb *Corallocarpus epigaeus* pertained to the study was collected from their natural habitates at Tirumala hills, Chittoor District, AP, India, i.e., from Nagapatla reserve forest and Talakona hills of Tirumala. It was identified by Prof. P. Jayaraman, Taxonomist and Director, Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu. The Voucher specimens for *Corallocarpus epigaeus* (PARC/2007/182) have been deposited at the college of pharmaceutical sciences, AU, Visakhapatnam. The specimens (Leaf and stem) were used for the study for macroscopical and microscopical characters and quantitative microscopy. The dried powdered material was used for the determination of ash values, extractive values, qualitative chemical examination and the phytochemical constituents present in the selected herbs.

Instruments and chemicals

Rotary microtome, compound microscope, watch glass, glass slides, cover slips and other glassware were the basic apparatus and instruments used for the study. Microphotographs were taken using a Nikon Labphoto 2 Microscopic unit. Solvents viz. petroleum ether, chloroform, ethanol (95%) and reagents viz. toluidine blue, phloroglucinol, glycerin, Hcl, chloral hydrate and sodium hydroxide. The reagents utilized were of analytical grade supplied by Sigma Chemicals Co, St. Louis, USA or Ranbaxy Fine Chemical Ltd, Mumbai, India.

Macroscopic and microscopic analysis

The macroscopy and microscopy of the leaves were studied according to the method of Brain and Turner[10]. For microscopical studies, cross sections were prepared and stained as per the procedure of Johansen[11].

Physico-chemical analysis

Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed Indian Pharmacopoeia[12] and WHO guidelines on quality control methods for medicinal plant materials WHO/QCMMPM guidelines[13].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedures described by Kokate[14] and Harborne[15].

RESULTS AND DISCUSSION

Macroscopical characters

It is a prostrate or climbing, monoecious herb. Roots tuberous, large, turnip-shaped. Stem slender,

grooved, zigzag, glabrous. Tendrils simple, slender, glabrous. Leaves suborbicular in outline, 2-7.5 cm long, usually a little broader than long, light green above, paler beneath, hairy on both surfaces, deeply cordate at the base, angled or more or less deeply 3-5 lobed, the lobes usually lobulate, sometimes apiculate, more or less irregularly dentate on the margins. Petioles 2-3.8 cm long, glabrous. Male flowers small, 5-15 at the apex of a straight stiff glabrous, peduncle 3.8-6.3 cm long. Female flowers usually solitary; peduncles short, stout and glabrous. Pedicels filiform, 1-2 mm long. Calyx slightly hairy tube 1.5 mm long, slightly rounded at the base; teeth minute, erect, distant and subulate. Corolla greenish yellow, segments 1 mm long. Anthers yellow; connective green, bifid. Fruit stalked, 1.3-2.5 cm long, ellipsoid or ovoid, suddenly contracted into a slende beak 6 mm long, scarlet in the middle, the base and beak green, circumscissilely dehiscent at the junction of the green and red portions near the base. Seeds 6-9, in orange-coloured pulp, pyriform, 3-4 by 2-2.5 mm, turgid, brown, with a whitish corded margin. Flowers and fruit November - April.

Microscopic characters of *Corallocarpus epigaeus*

Microscopy of the *C. epigaeus* leaf

Dorsiventral midrib and the lateral veins are prominent. The lamina is bilateral with fairly dense trichomes (Figure No.1). The major view (midrib) is plano-convex in the sectional view with convex and semi-circular abaxial side (Figure No.2). It is 200 µm thick and 250 µm wide. The adaxial epidermis of the midrib has thin walled, rectangular cells; the abaxial epidermal cells are small and squarish. The abaxial part has parenchymatous ground tissue of thin walled compact cells. The adaxial part has transcurrent palisade zone. The vascular strand is a single, small, collateral and has bundle sheath. It has a small cluster of fairly thick walled xylem elements and a few small groups of phloem (Figure No.3).

Lamina (Figure No 4)

The lamina is even surfaced on both the sides, epidermal trichomes are seen or both sides of the lamina. The adaxial epidermal cells are cylindrical with thick cuticle (Figure No. 4). The cells are 20 µm thick. The abaxial epidermal cells are narrow, rectangular or squarish. The mesophyll tissue consists of an adaxial band of single row of thin, cylindrical palisade cells and four or five layers of lobed, loosely arranged spongy parenchyma.

Epidermal trichomes (Figure No.5)

Multicellular, uniseriate, unbranched, non-glandular trichomes are distributed all over the lamina. The trichome arises from a rosette of rectangular epidermal cells. Two or three basal cells are wide, thick walled and become gradually narrow towards the apex, terminating to a uniseriate pointed tip. The basal cell is 40 µm wide; the terminal cell is 10 µm wide. The trichomes are 50-100 µm in height.

Epidermal cells and stomata (Figure No. 6 and 7)

The epidermal cells are lobed and amoeboid in outline. The anticlinal walls are fairly thick and smooth. Some of the epidermal cells are modified into lithocysts which contain calcium carbonate bodies called cystoliths (Figure No.7). The lithocyst may be solitary or grouped into 2 to many, forming regular rosettes or circles. The lithocyst will contain only one crystal which is triangular in outline. A group of four lithocysts is 60 µm in diameter.

Stomata (Figure No.6)

Both abaxial and adaxial stomata are either anomocytic or anisocytic type. A stoma is surrounded by 3 – 5 epidermal cells which are not much different from neighbouring epidermal cells. The guard cells are elliptical and measure 15 x 25 µm in size.

Venation pattern (Figure No. 8, 9, 10)

The leaf shows dense reticulate venation system. The tertiary veins and their branches are uniformly thin, rigid and straight. The thicker veins are also straight and rigid. The vein-islets (aereoles) are well defined and distinct. The islets are wide and vary in shape; they may be broadly rectangular, polygonal or squarish (Figure No.8). The vein-islets have distinct vein-terminations which are ultimate ends of veinlets found within the boundary of vein-islets (Figure No.9). In variably all the islets have vein-terminations. The vein-terminations are long and consist of single row, of xylem elements. They are mostly forked or simple. The ultimate ends of vein-

terminals have thick and short trachids. These terminal tracheids have spiral thickenings (Figure No.10).

Microscopy of the *C. epigaeus* petiole (Figure No.11, 12)

The distal part of the petiole is elliptical with even and smooth outline (Figure No.11). It is 650 µm and 1.2 mm in size. The epidermal layer is narrow with small squarish cells. The ground tissue consists of two or three outer zone of collenchyma, the remaining part being parenchymatous. There are about seven discrete vascular bundles arranged in a ring. The bundles are wedge shaped and bicollateral.

The proximal part of the petiole is almost circular with even outline. The epidermal layer has small spindle shaped cells. It is followed by two layers of small, compact thick walled cells, two or three layers of chlorenchyma cells and three or four layers of thin walled narrow fibres. Remaining portion is parenchymatous, thin walled, wide and angular.

There are 6 or 7 vascular bundles forming a ring; the bundles are variable in size. They are bicollateral and have wide, circular xylem elements. Phloem occurs in thick mass both on the inner and the outer parts of the xylem (Figure No.12). Distributed in the ground tissue, there are secretory cavities which are narrow, circular and contain dark contents; they are surrounded by rosette of parenchyma cells (Figure No.13).

Quantitative microscopy

The vital quantitative microscopic leaf constants like stomatal index, palisade ratio, vein-islet and vein termination number were carried out according to the standard method Wallis[16-19] and the results are tabulated in Table No.1.

Physico-chemical constants

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid-insoluble ash, water-soluble ash, and sulphated ash values of the leaf powder were done as per the WHO guide lines[20], Indian Pharmacopoeia[21] and the results are tabulated in Table No 2.

Extractive values

The leaf powder was subjected to successive solvent extraction with petroleum ether, chloroform, ethanol, and water as solvents by the reported method kokate[14] and Harborne[15] . Percentages of the extractive values were calculated with reference to air dried drug and the values are reported in Table No.3.

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of triterpenes, terpenoidal saponins, glycosides and bitters (1:200 dilutions). The results are shown in the Table No.4.

Table No.1: Quantitative microscopy (leaf constants) of *Corallocarpus epigaeus*

S.No	Parameter →	Stomatal Number and Stomatal Index per sq. mm							
1	Epidermis →	Upper (40X)				Lower (40X)			
2	Trial No. →	I	II	III	IV	I	II	III	IV
3	No. of Stomata per sq. mm (S)	2	1	1	1	4	4	5	5
4	No. of epidermal cells / sq. mm (E)	28	19	19	18	24	24	25	33
5	Stomatal Index S I= (S/E+S)x100	6.6	5	5	5.26	14.28	14.28	16.66	13.15
6	Average Stomatal No.	1.25 per sq. mm				4.5 per sq. mm			
7	Average Stomatal Index	5.46 per sq. mm				14.59 per sq. mm			
8	Parameter →	Palisade Ratio							
9	Trial No. →	I		II		III		IV	
10	No. of epidermal cells (E)	4		4		4		4	
11	No. of Palisade cells/sq.mm (P)	23		23		20		16	
12	Palisade ratio	5.75		5.75		5		4	
13	Average Palisade Ratio	5.12 per sq. mm							
14	Parameter →	Vein-Islet and Veinlet-Termination Number per sq. mm							
15	No. of Vein-Islet per 4 sq.mm	56		60		64		80	
16	No. of Vein-Islet per sq.mm	14		15		16		20	
17	Average Vein-Islet No.	16.25							
18	No. of Veinlet-Terminations per 4 sq. mm	28		28		36		40	
19	No. of Veinlet-Terminations per sq. mm	7		7		9		10	
20	Average Veinlet-Termination No.	8.25 per sq. mm							

Table No.2: Quantitative determinations (ash and extractive values) of *C. epigaeus*

Parameter →	Ash values (% w/w)
Parts used →	Aerial parts
Total ash	8.33
Water soluble ash	3.00
Acid insoluble ash	2.00
Sulphated ash	11.33
Parameter →	Extractive values (% w/w)
Ether soluble	2.21
Alcoholic soluble	4.15
Water soluble	5.95

Table No.3: Physical characteristics of extracts of *C. epigaeus*

Physical characteristics of aerial parts extracts				
S.No		**Nature**	**Color**	**%yield (w/w) g**
1	Petroleum ether	Greasy	D. g	2.21
2	Chloroform	Greasy	B .g	2.18
3	Alcoholic	Viscous	D. b g	4.15
4	Aqueous	Sticky	Brown	5.95

Table No.4: Qualitative chemical tests for phytoconstituents of *C. epigaeus*

Part used →	**Aerial parts**				**Part used →**	**Aerial parts**			
Plant constituents and Chemical tests↓	**Pet. Ext**	**Chl. Ext**	**Alc. Ext**	**Aq. Ext**		**Pet. Ext**	**Chl. Ext**	**Alc. Ext**	**Aq. Ext**
Tests for Steroids (a) Salkowski test	–	–	–	–	(d) Hager's test	–	–	–	–
(b) Liberman Burchards test	–	–	–	–	Carbohydrates (a) Molisch's test	–	–	+	+
Triterpenes (a) Salkowski test	+	+	+	+	(b) Fehling's test	–	–	+	+
					(c) Benedict's test	–	–	+	+
(b) Liberman Burchards test	+	+	+	+	(d) Barfoed's test	–	–	+	+
(c) Tschugajeu test	+	+	+	+	Tests for Flavanoids (a) Shinoda test	–	–	–	–
(d) Briekorn and Brinars test	+	+	+	+					
Tests for saponins (a) Foam test	–	–	+	+	(b) Ferric chloride	–	–	–	–
					(c) Lead acetate	–	–	–	–
(b) Haemolysis test	–	–	+	+	(d) ZnCl/HCl reduction test	–	–	–	–
Steroidal saponins (a) Salkowski test	–	–	–	–	Tests for Tannins (a) Ferric chloride	–	–	–	–
(b) Haemolysis test	–	–	–	–					
Triterpenoidal saponins (a) Salkowski test	+	+	+	+	(b) Gelatin test	–	–	–	–
(b) Liberman Burchard test	+	+	+	+	Testsfor Glycosides (a) Baljet's test	+	+	+	+
(c) Tschugajeu test	+	+	+	+	(b) Legal's test	+	+	+	+
(d) Briekorn and Brinars test	+	+	+	+	(c) Keller-Killiani	+	+	+	+
Tests for alkaloids (a) Mayer's test	–	–	–	–	Tests for bitters (a) vanillin Sluphuric acid	+	+	+	+
(b) Dragendorff's	–	–	–	–					
(c) Wagner's test	–	–	–	–	(b) serial dilutions	MB	MB	MB	MB

Note: "+": Present, "-": Absent, Pet. Ext: Petroleum ether extract, Chl. Ext: Chloroform extract, Alc Ext: Alcoholic extract and Aq Ext: Aqueous extract, MB: Moderately bitter in taste.

Anotomy of th leaft c. *C. epigaeus*

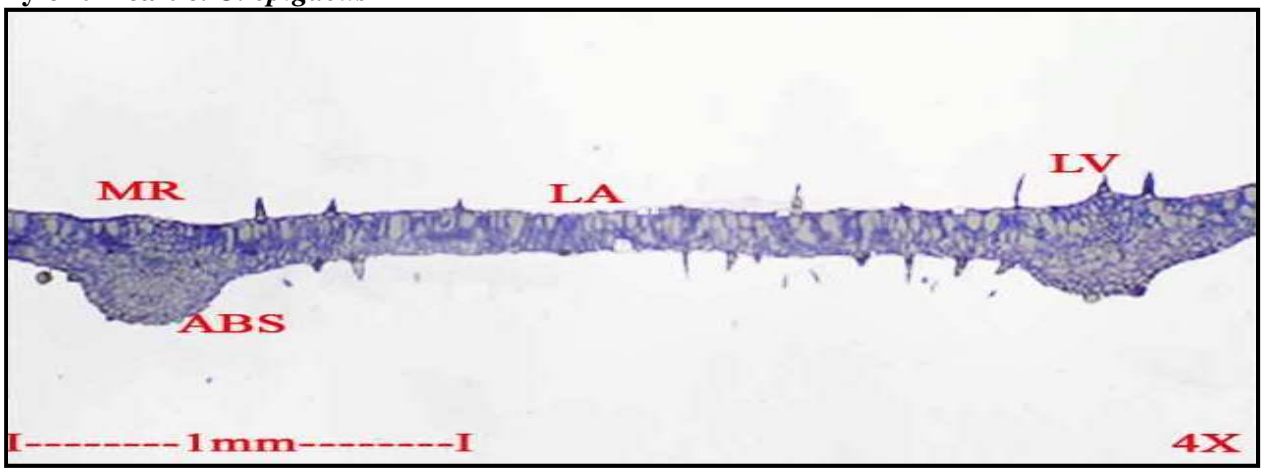

Figure No.1: T.S of leaf through midrib with lamina

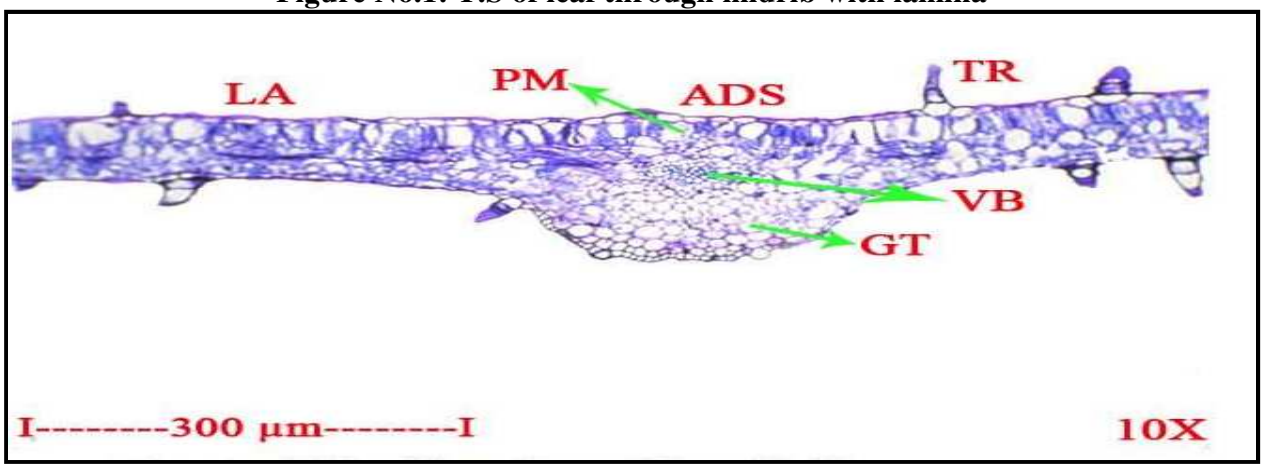

Figure No.2: T.S of lamina with midrib

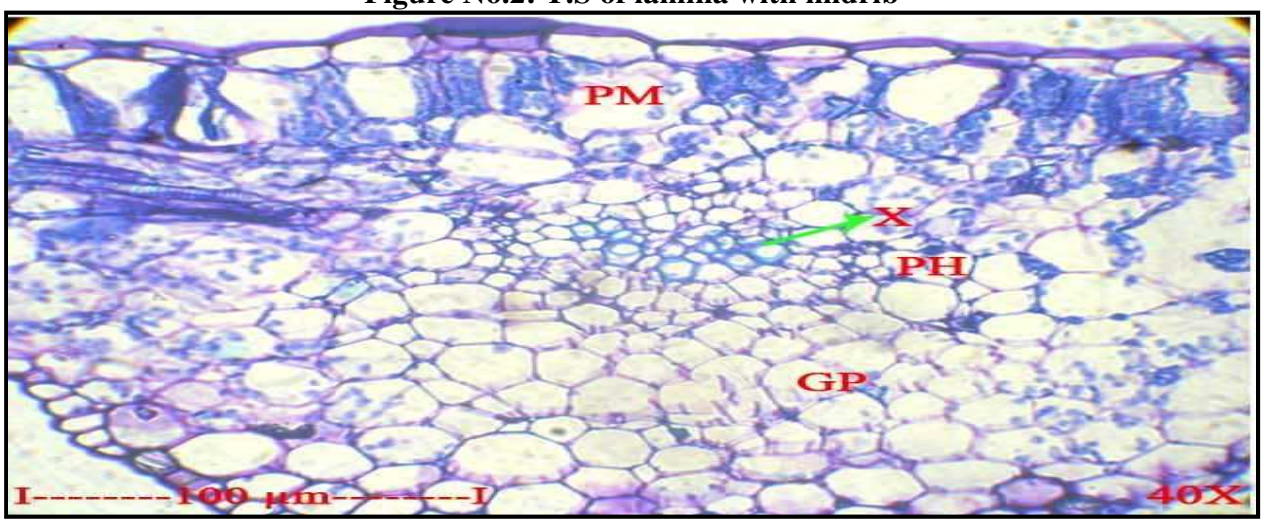

Figure No.3: T.S of midrib Vascular bundle enlarged

ABS – Abaxial side; Ads-Adaxial side; GP-Ground Parenchyma; GT-Ground tissue; LA-Lamina; LV-Lateral vein; MR-Midrib; PH-Phloem; PM-Palisade Mesophyll; TR-Trichome; VB-Vascular bundle; X-Xylem

Anotomy of th lamina *C. epigaeus*

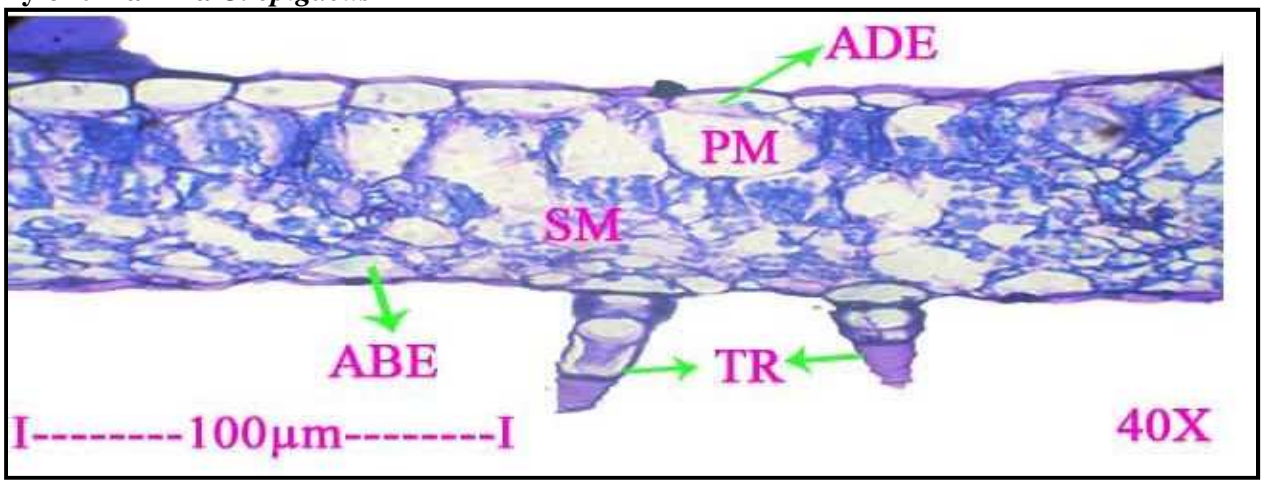

Figure No.4: T.S of lamina

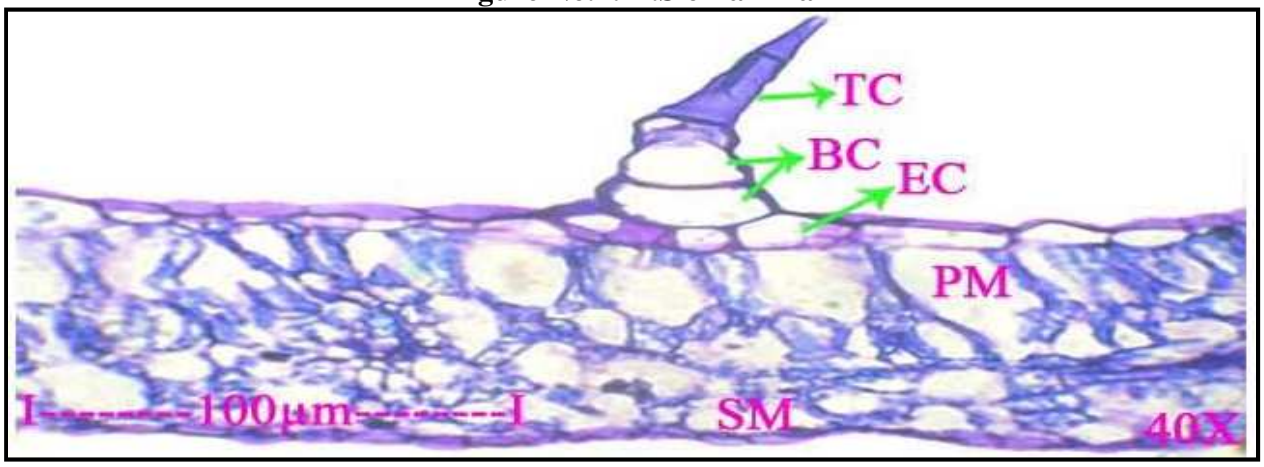

Figure No.5: T.S of lamina showing a non-glandular tricome on the adaxial side
ABE-Abaxia epidermis, ADE-Adaxial epidermis, BC-Basal cell of the trichome, EC-Epidermal cell, PM-Palisade mesophyll, SM-Spongy mesophyll, TC-Terminal cell of the trichome, TR-Trichome

Epidermal morpohogy of *C. epigaeus*

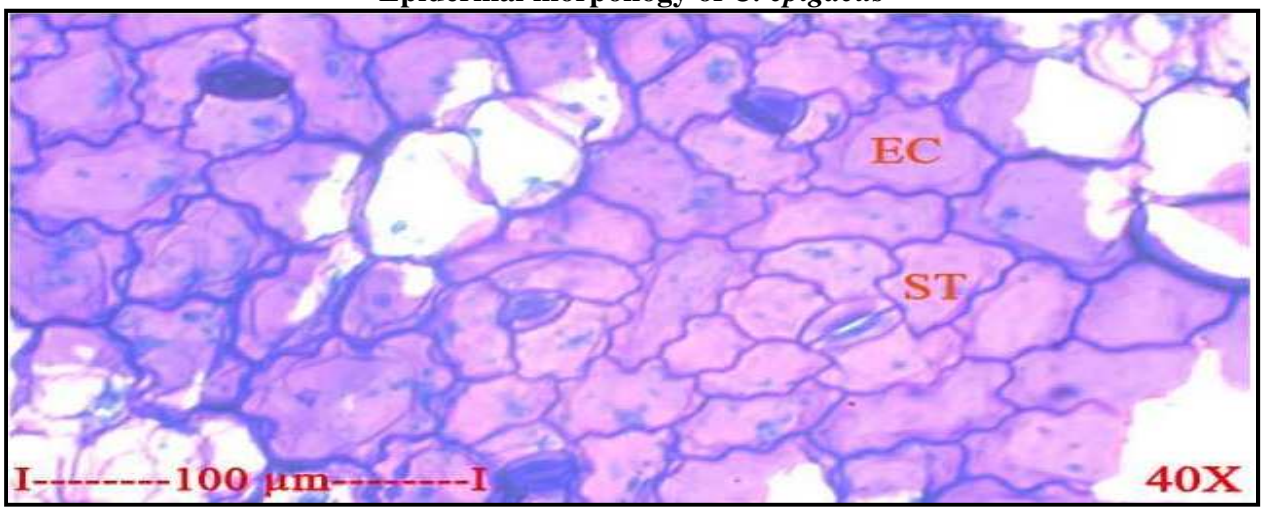

Figure No.6: Abaxial epidermis with stomata

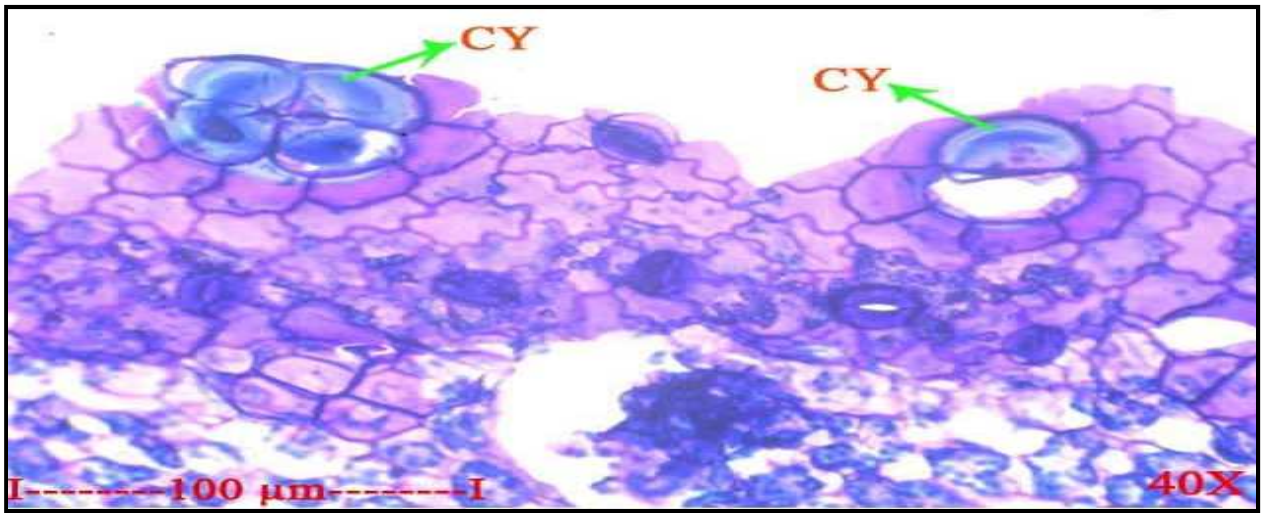

Figure No.7: Abaxial epidermis with double and rosette custolith
Cy-Cystolith; EC-Epidermal cells; ST-Stomata

Venation Pattern of *C. epigaeus*

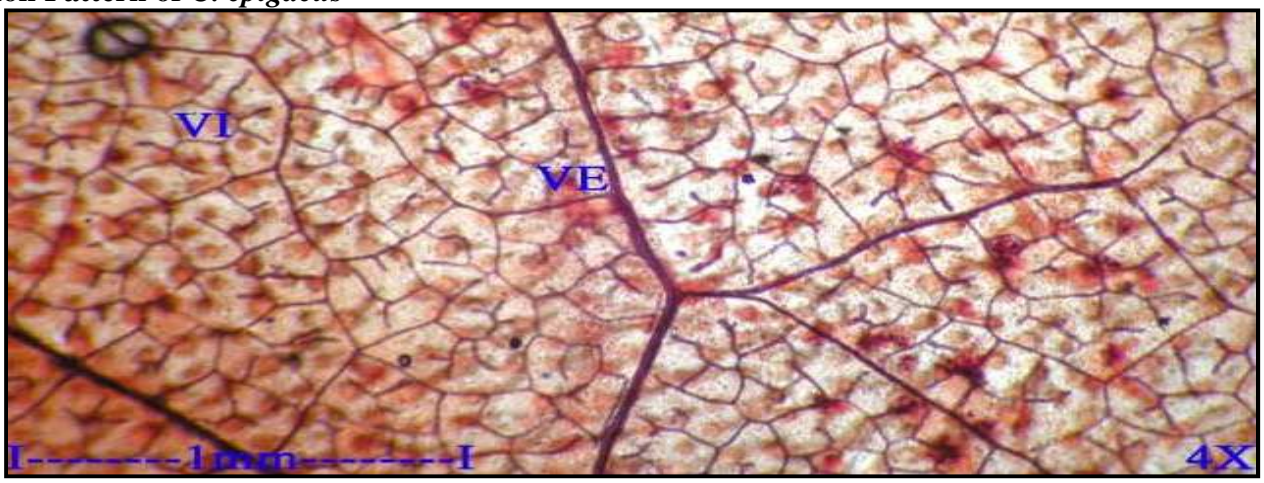

Figure No.8: Cleared leaf showing vein-islets and vein-termination

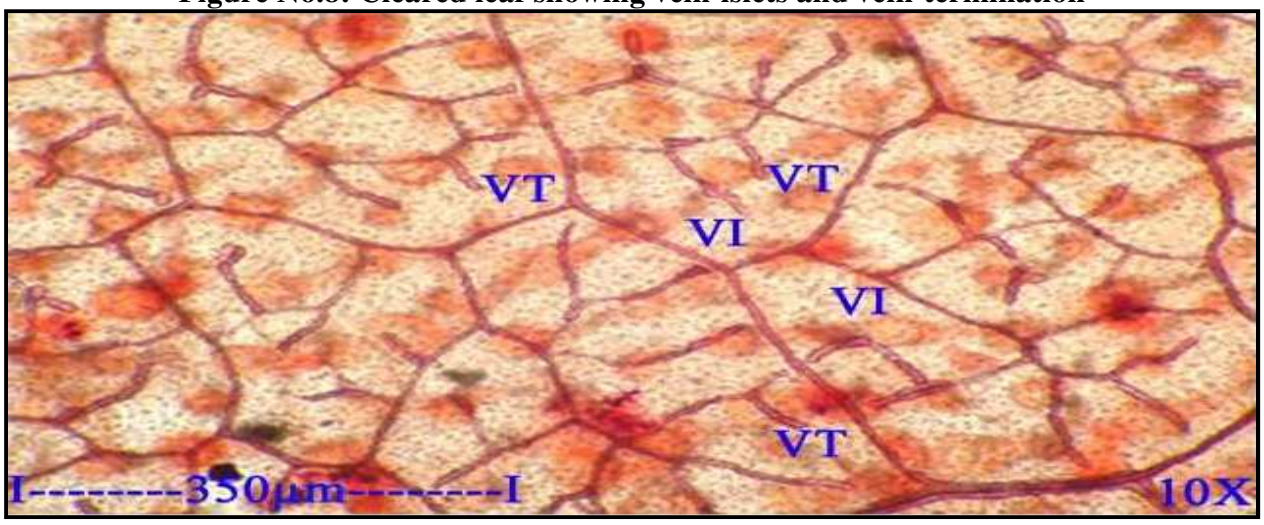

Figure No.9: Enlarged view of cleared leaf showing vein-islets and vein-termination

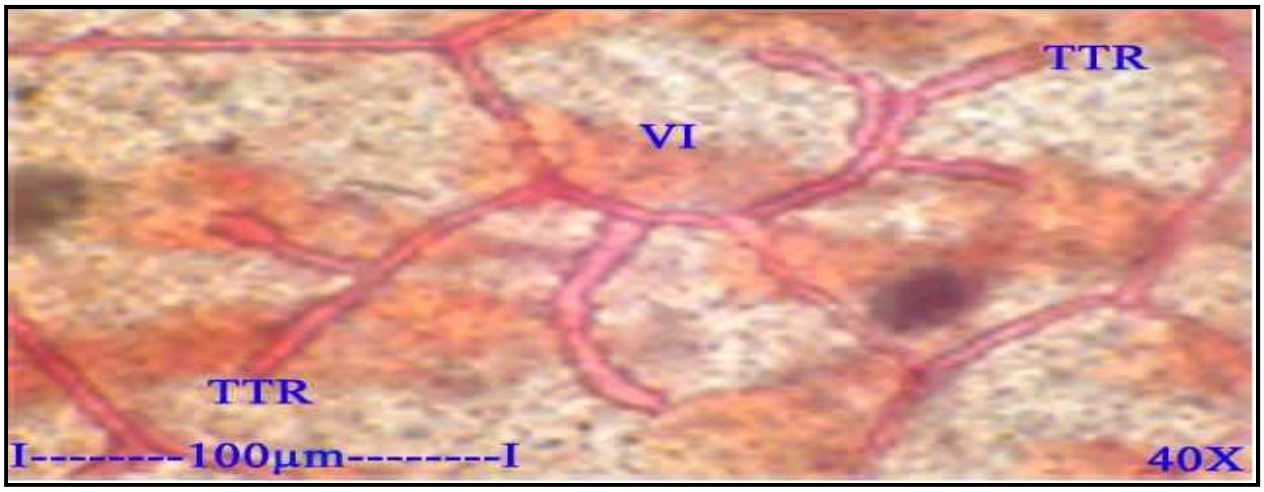

Figure No.10: Enlarged view of terminal tracheids

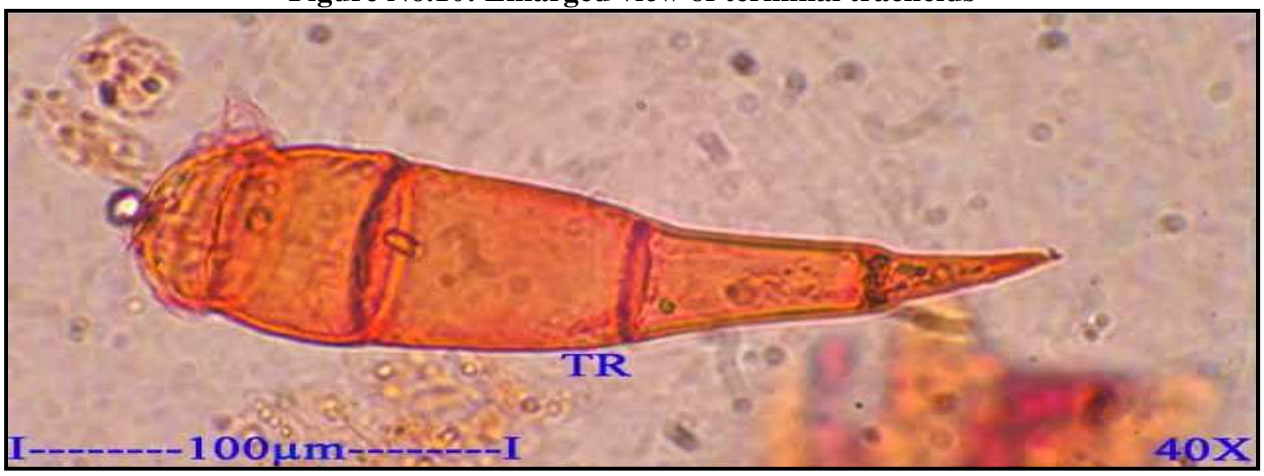

Figure No.11: Non-glandular epidermal trichome
TR-Trichome, TTR-Terminal tracheids, VE-Vein; VI-Vein-islets, VT-Vein-termination

Anatomy of the petiole *C. epigaeus*

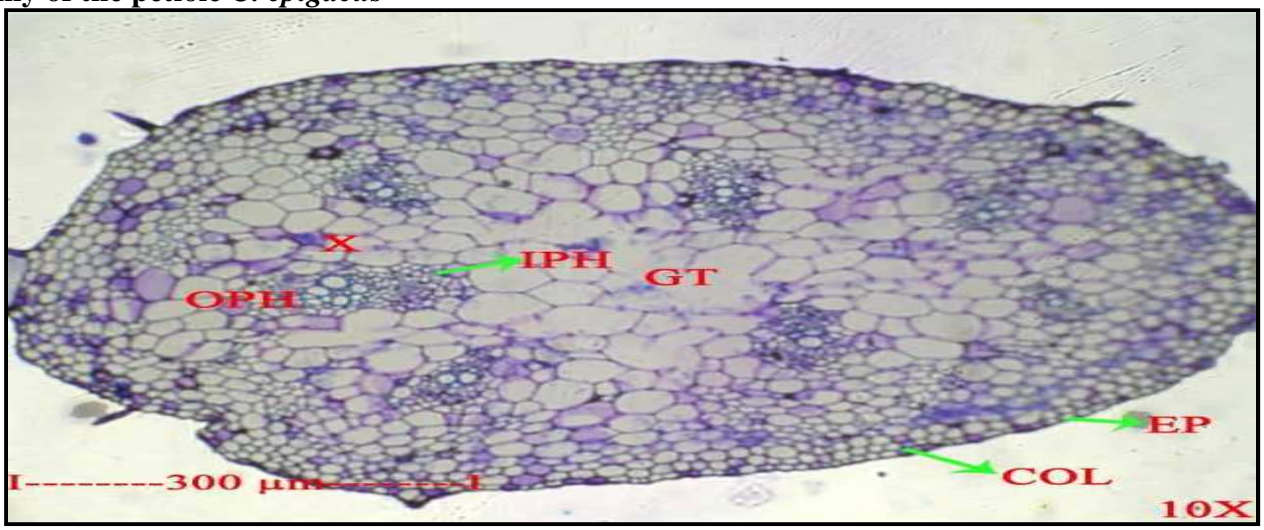

Figure No.12: T.S of petiol ground plan [Disital-part]

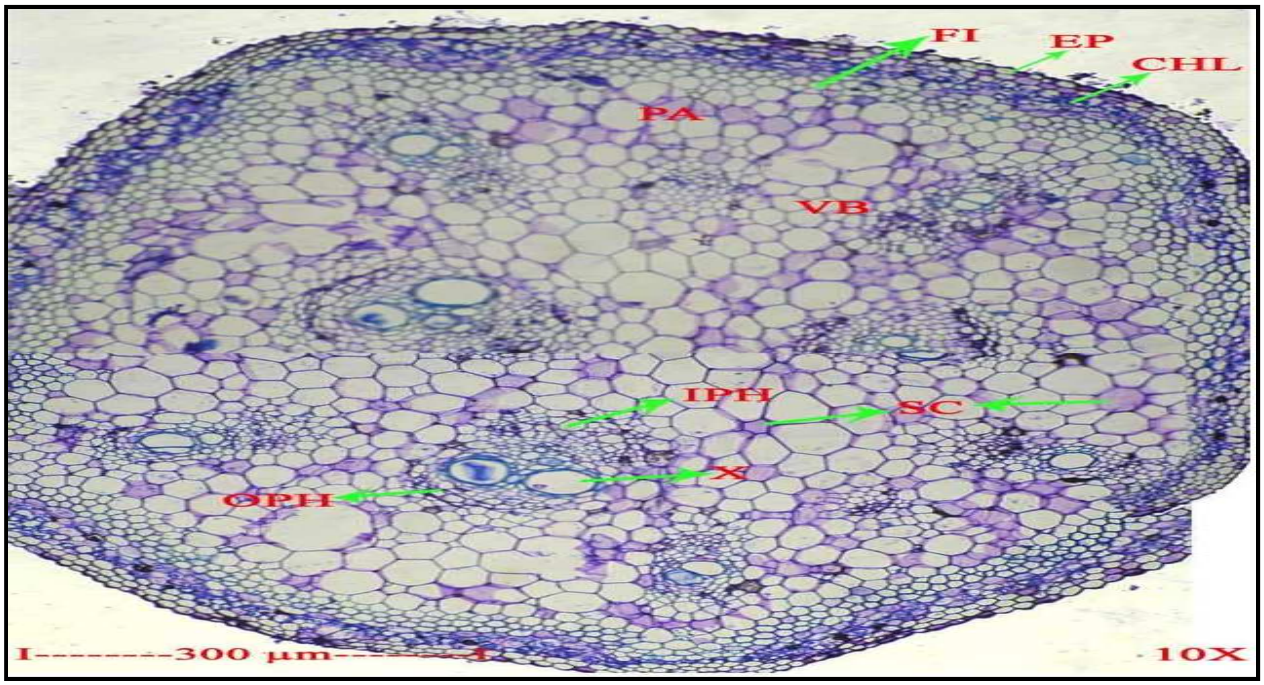

Figure No.13: T.S of petiole proximal region enlarged
CHL-Chlorenchyma, COL-Collenchyma, EP-Epidermis, FI-Fibre, GT-Ground tissue, IPH-Inner phloem, OPH-Outer phloem, PA-Parenchyma cells, SC-Secretory cavity, VB-Vascular bundle, X-Xylem

CONCLUSION

In conclusion, the present study on pharmacognostical evaluation of *Corallocarpus epigaeus* will be providing useful information in regard to its correct identity and help to differentiate from the other closely related species. The other parameters observed may be useful for the future identification of the plant.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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