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PHARMACOGNOSTICAL EVALUATION OF TUBEROUS ROOTS OF *CORALLOCARPUS EPIGAEUS*

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ABSTRACT

In this study the pharmacognostical parameters for the tuberous roots of the plant *Corallocarpus epigaeus* (*Cucurbitaceae*) were evaluated. Traditionally the plant is used as bitter, emetic, cures inflammations (Ayurveda). Root tuber for snake bite, anaemia, leprosy, eczema, dysentery, arthritis, 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. In this context the morphoanatomy of tuberous roots along with, 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 Tuberous roots.

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INTRODUCTION

The new area of herbal drug standardization include pharmacognostical, chemical, biological, biopharmaceutical and molecular approaches of drug development. According to the World Health Organization guidelines for quality control methods of medicinal plants/plant materials¹, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity. The degree of purity of such plant materials should be carried out by physico-chemical determinations and authentic phytochemical procedures were conducted before any tests are

undertaken^{2,3}. The identification of drugs will remain the prime difficulty until the prominent characteristics of each drug are well established.

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^{4,5}. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential.

*Corallocarpus epigaeus*⁶⁻⁸ (Syn: *Broyonia epigaea*) belongs to the family *Cucurbitaceae*. 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, arthritis, rheumatism, chronic mucous enteritis, diabetes. Stem for filariasis, wounds, emetic, goiter and diabetes⁶⁻⁸. Though the plant has several uses, no scientific data is available to identify the genuine sample. The present investigation was under taken to establish identity of tuberous roots 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 (tuberous roots) were used for the study for macroscopical and microscopical characters and microscopic linear

measurements. 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 tuberous roots were studied according to the method of Brain and Turner⁹. For microscopical studies, cross sections were prepared and stained as per the procedure of Johansen¹⁰.

Physico-chemical analysis

Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed Indian Pharmacopoeia¹¹ and WHO guidelines on quality control methods for medicinal plant materials WHO/QCMMPM guidelines¹².

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedures described by Kokate¹³ and Harborne¹⁴.

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 slender 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* root tuber

The tap-root becomes highly swollen forming spherical or subspherical, soft and heavy tuber with smooth brownish surface. Thin lateral roots which are non storage are also seen on the surface of the tuber. A young root-tuber consists of the following regions: (Figure, 1- 28)

Periderm: (Figure No.5)

It is superficial, broad and continuous periderm all around. The periderm has outer zone of phellem and equally thick inner zone of phelloderm. The periderm is 300 μm wide. The cells are narrowly rectangular, thin walled and are arranged in regular radial files.

Cortex: (Figure No.5)

Inner to the periderm is a wide parenchymatous cortex in which narrow, angular thin walled cells are randomly oriented.

Vascular cylinder

It is unusual (anomalous) in structure. It has a central core of parenchymatous ground tissue where several circular vascular nodules are embedded. The nodule has one or two excentric xylem

elements and several concentric rectangular cells and small nest of sieve elements (Figure No.3, 4).

Outer to the central group of vascular nodules, is a wide cylinder of secondary xylem and secondary phloem (Figure No.7). Due to dilatation of the vascular rays, xylem-phloem cylinder is cleaved into several thin radial segments. The segments have one or two rows of wide, circular, thick walled solitary vessels and the xylem segments have xylem fibres which ensheathes the vessels. The radial xylem segments are wavy and widely separated from each by the intervening parenchymatous tissue (Figure No.7, 8). The xylem sclerenchyma (fibres) are thick walled and lignified. The vessels have tyloses within the cell lumen.

Microscopy of the *C. epigaeus* old tuber

The thicker old tuber has basically similar structure as the thin tubers. However, the old tuber has more dimensional values of the tissues and cells (Figure No.9, 10). It consists of shallow fissured, continuous superficial periderm, wide cortex and several radiating arms of vascular tissues and central circle of vascular nodules.

Periderm

It is 250-300 μm wide. It consists of a wide phellem of tabular suberised cells and equally wide phelloderm cells of rectangular thin walled cells (Figure No.9). The phelloderm gradually merges with the cortical parenchyma.

Cortical tissue is parenchymatous, the cells being thin walled, compact and random in orientation. The cells are densely filled with starch grains.

The vascular cylinder has a central ring of about vascular nodules and equal number of thin, gradually expanding radial bands of vessels ensheathed by the xylem fibres (Figure No.10). The vessels are circular, thin walled and solitary. In between the radial band of xylem occur wide, rectangular, starch filled starch grains. The vessels are up to 100 μm in diameter.

Microscopy of the *C. epigaeus* thin root (Figure No.11, 22)

The root has central tetrarch primary xylem strands and deeply lobed, fan shaped radial segments of xylem and phloem. The vascular segments are

separated by dilated vascular rays which are wide and consist of radial panel of parenchyma cells. The xylem segments consist of solitary and small clusters of wide, thin walled vessels ensheathed by fibres (Figure No.12). The peripheral vessels are wider than the interior vessels. The diameter of inner vessels is 30 µm and the dimension gradually increases up to 100 µm towards the periphery wide, semicircular masses of phloem are seen at the outer boundary of the xylem segments. Outer most phloem tissue and a few layers of inner cortex are crushed due to radial compression; such crushed cells are seen as a thin dark tangential network (Figure No.11).

The periderm is superficial and wide. It is 150 µm thick and consisting of several layers of thin walled tabular cells. Some 5 or 6 outer layers are phellem cells and the remaining inner layers represent the phelloderm (Figure No.11). A narrow zone of cortex remains intact in between the periderm and secondary phloem.

Starch grains: (Figure No.13, 14)

Starch grains are the major inclusions; they occur mostly in the xylem and phloem dilated rays and in the cortex. The grains include both simple and compound types. They are circular and concentric with central hilum and X-shaped polarimark (Figure No.14). The simple grains are 10-15 µm in diameter. The compound grains are 20-25 µm wide.

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 tuberous root powder were done as per the WHO guide lines¹⁵, Indian Pharmacopoeia¹⁶ and the results are tabulated in Table No.1.

Extractive values

The tuberous root powder was subjected to successive solvent extraction with petroleum ether, chloroform, ethanol, and water as solvents by the reported method kokate¹³ and Harborne¹⁴. Percentages of the extractive values were calculated with reference to air dried drug and the values are reported in Table No.2.

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of steroids, steroidal saponins, glycosides, carbohydrates and bitters (1:500). The results are shown in the Table No.3.

Table No.1: Quantitative determinations (ash and extractive values) of C. epigaeus

Parameter →	Ash values (% w/w)
Parts used →	Tuberous roots
Total ash	3.66
Water soluble ash	1.66
Acid insoluble ash	1.00
Sulphated ash	8.00
Parameter →	Extractive values (% w/w)
Ether soluble	0.74
Alcoholic soluble	3.02
Water soluble	5.92

Table No.2: Physical characteristics of extracts of *C. epigaeus*

S.No	Physical characteristics of Tuberous roots extracts			
		Nature	Color	% yield (w/w) g
1	Petroleum ether	Waxy	Yellow	0.74
2	Chloroform	S. p	R. b	0.57
3	Alcoholic	T .p	Brown	3.02
4	Aqueous	Sticky	D. b	5.92

Table No.3: Qualitative chemical tests for phytoconstituents of *C. epigaeus*

S.No	Part used →	Tuberous roots				Part used →	Tuberous roots			
		Pet. Ext	Chl. Ext	Alc. Ext	Aq. Ext		Pet. Ext	Chl. Ext	Alc. Ext	Aq. Ext
1	Plant constituents and Chemical tests↓									
2	Tests for Steroids (a) Salkowski test	+	+	+	+	(d) Hager's test	-	-	-	-
						Carbohydrates (a) Molisch's test	-	-	+	+
3	(b) Liberman Burchards test	+	+	+	+					
4	Triterpenes (a) Salkowski test	-	-	-	-	(b) Fehling's test	-	-	+	+
						(c) Benedict's test	-	-	+	+
5	(b) Liberman Burchards test	-	-	-	-	(d) Barfoed's test	-	-	+	+
6	(c) Tschugajeu test	-	-	-	-	Tests for Flavanoids (a) Shinoda test	-	-	-	-
7	(d) Briekorn and Brinars test	-	-	-	-					
8	Tests for saponins (a) Foam test	-	-	+	+	(b) Ferric chloride	-	-	-	-
						(c) Lead acetate	-	-	-	-
9	(b) Haemolysis test	-	-	+	+	(d) ZnCl/HCl reduction test	-	-	-	-
10	Steroidal saponins (a) Salkowski test	+	+	+	+	Tests for Tannins (a) Ferric chloride	-	-	-	-
						(b) Haemolysis test	+	+	+	+
12	Triterpenoidal saponins (a) Salkowski test	-	-	-	-	(b) Gelatin test	-	-	-	-
						Testsfor Glycosides (a) Baljet's test	+	+	+	+
13	(b) Liberman Burchard test	-	-	-	-					
14	(c) Tschugajeu test	-	-	-	-	(b) Legal's test	+	+	+	+
15	(d) Briekorn and Brinars test	-	-	-	-	(c) Keller-Killiani	+	+	+	+
16	Tests for alkaloids (a) Mayer's test	-	-	-	-	Tests for bitters (a) vanillin Sluphuric acid	+	+	+	+
17	(b) Dragendorff's	-	-	-	-					
18	(c) Wagner's test	-	-	-	-	(b) serial dilutions	IB	IB	IB	IB

Note: “+”: Present, “-”: Absent, Pet. Ext: Petroleum ether extract, Chl. Ext: Chloroform extract, Alc Ext: Alcoholic extract and Aq Ext: Aqueous extract, IB: Intensely bitter in taste

Anatomy of the thin-tuber *C. epigaeus*

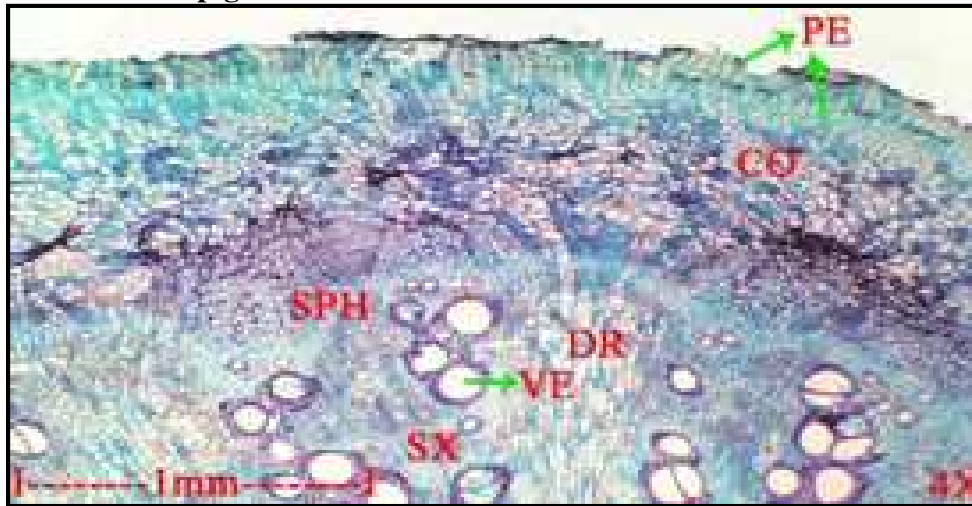


Figure No.1: T.S of tuber outer sector

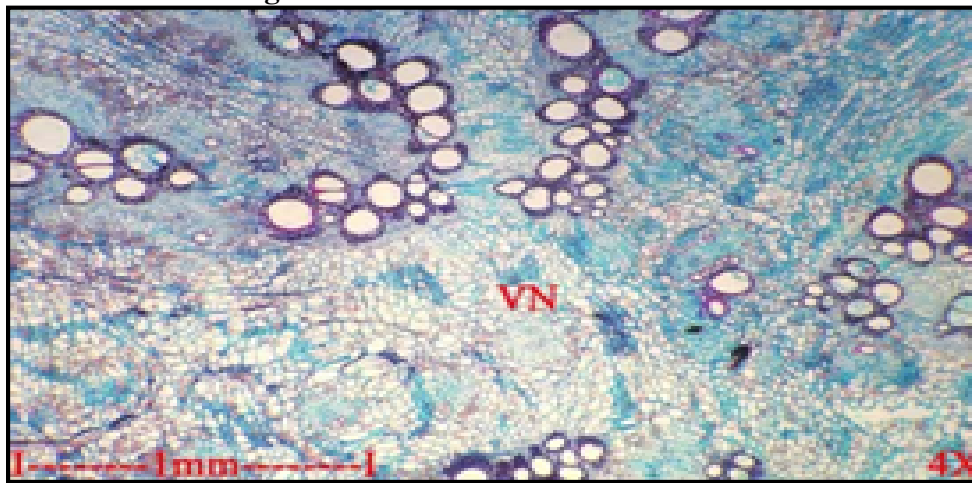


Figure No.2: T.S of tuber inner sector

CO-Cortex, DR-Dilated Ray, PE-Periderm, SPH- Secondary Phloem, SX- Secondary xylem, VE-Vessel, VN-Vascular Nodule

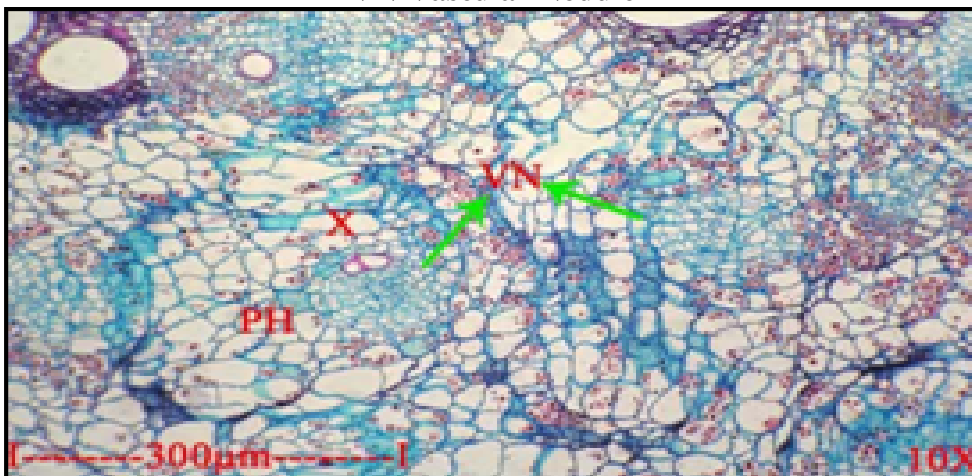


Figure No.3: Central Nodules

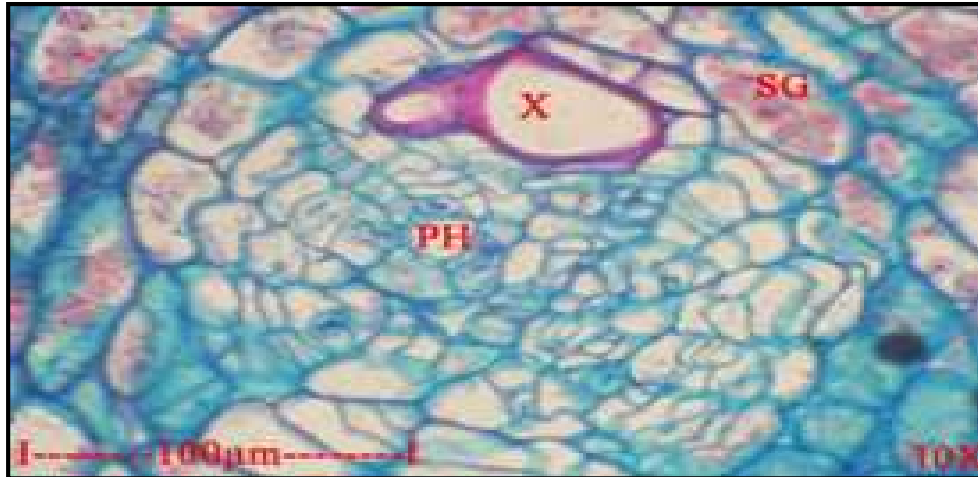


Figure No.4: One nodule enlarged

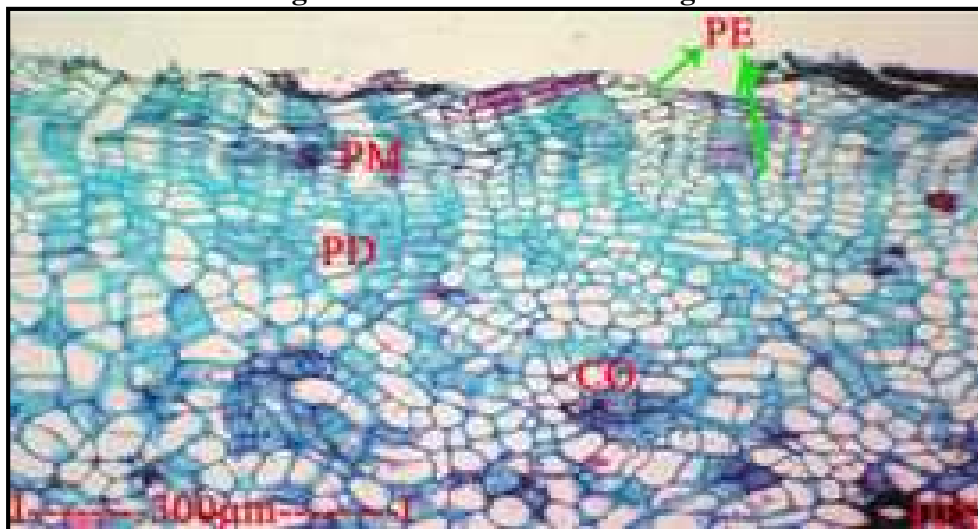


Figure No.5: Periderm and cortex enlarged

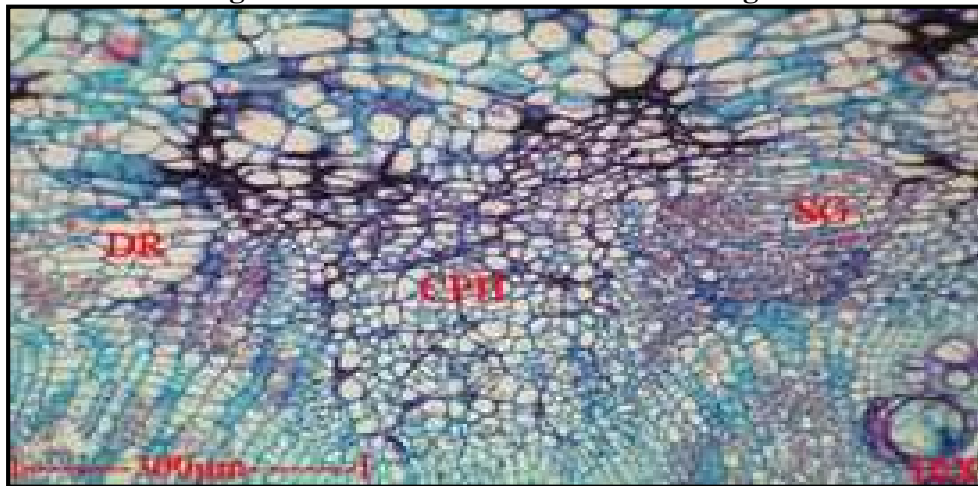


Figure No.6: Secondary Phloem enlarged

CO-Cortex, CPH-Collapsed Phloem, DR-Dilated Ray, PD-Phelloderm, PE-Periderm, PM-Phellem, SG-Starch Grains

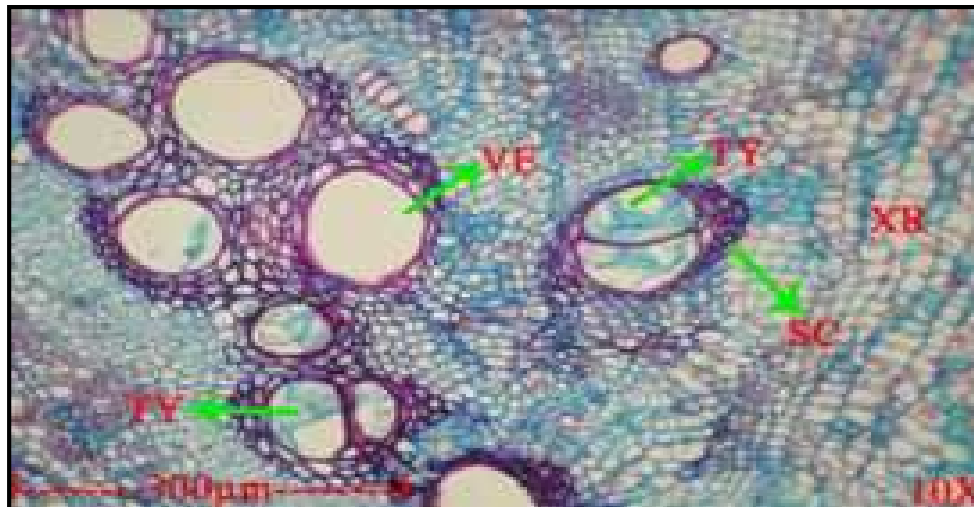


Figure No.7: Xylem segments enlarged

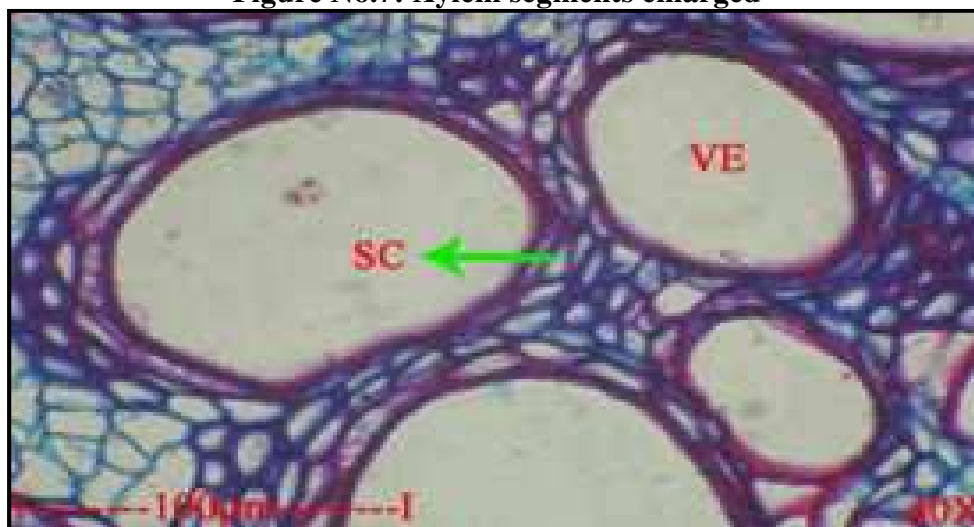


Figure No.8: Vessel with Fibre sheath enlarged

Anatomy of the thick-tuber *C. epigaeus*

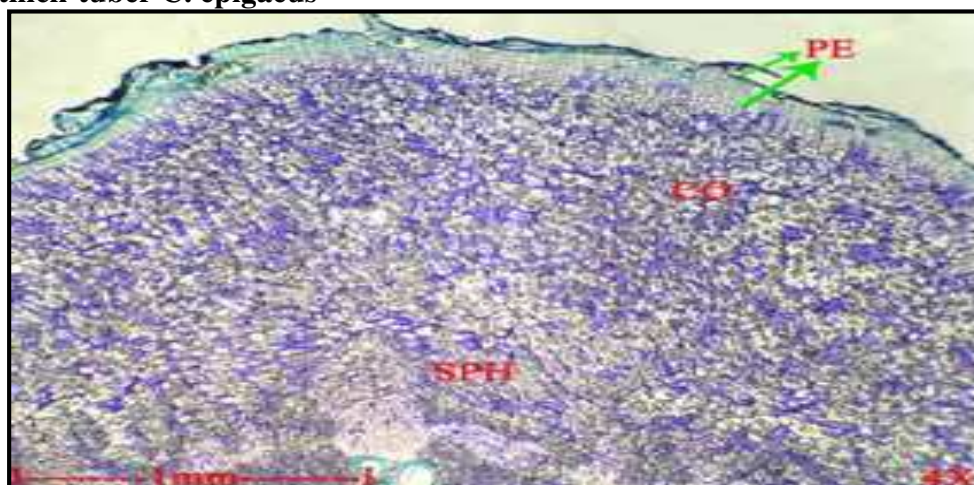


Figure No.9: Periderm, cortex and secondary phloem

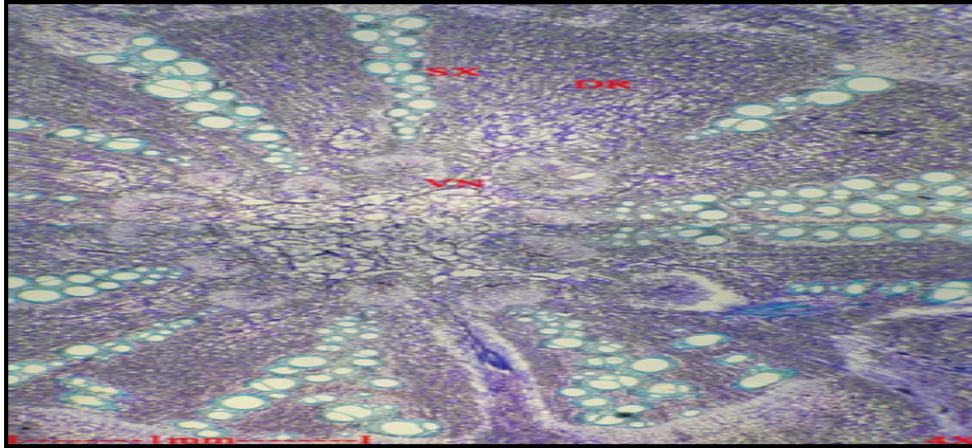


Figure No.10: Central, vascular nodules and radiating arms of vascular segments
CO-Cortex, DR-Dilated Rays, PE-Periderm, SPH-Secondary Phloem, SX-Secondary Xylem, VN-Vascular Nodule
Anatomy of the root *C. epigaeus*

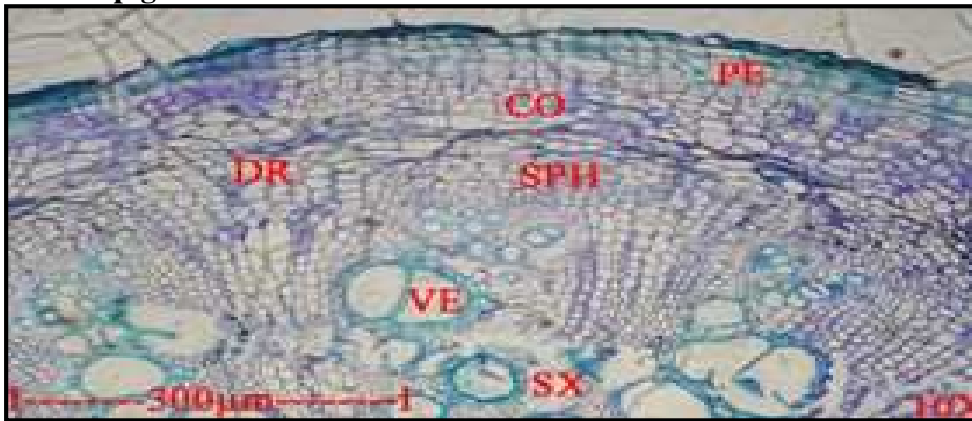


Figure No.11: T.S of root outer sector

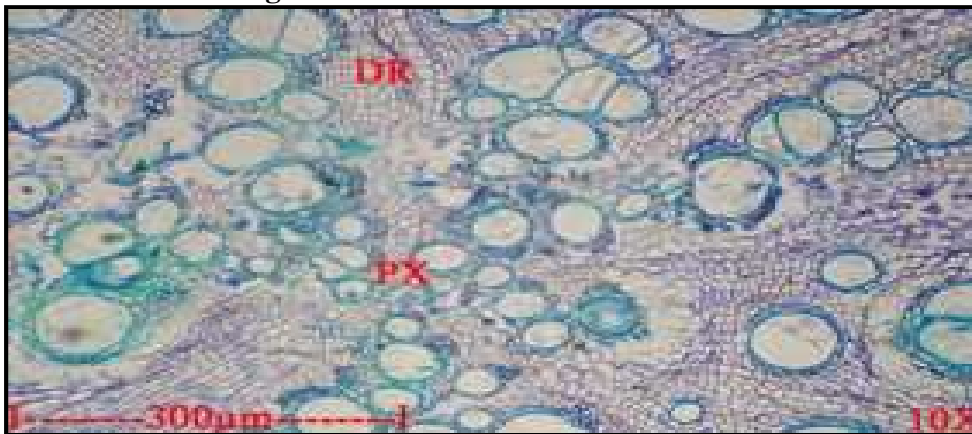


Figure No.12: T.S of root inner sector

CO-Cortex, DR-Dilated Ray, PE-Periderm, PX-Primary Xylem, SPH-Secondary Phloem, SX-Secondary Xylem, VE-Vessel
Distribution of starch grains in the rhizome
(under polarized light microscope)

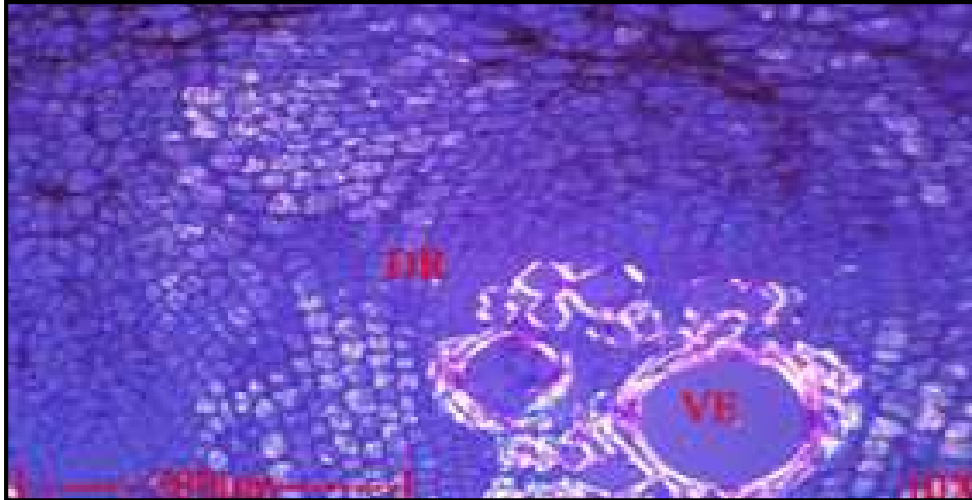


Figure No.13: T.S of rhizome showing starch grains in the dilated ray

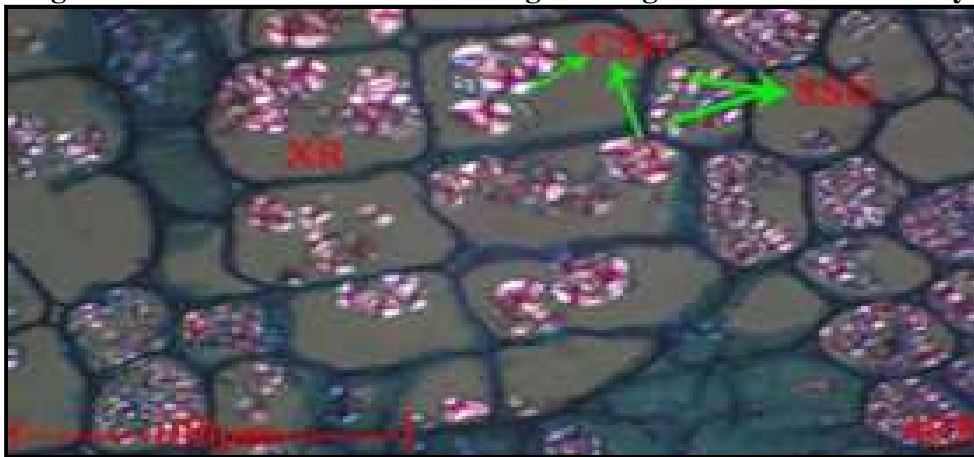


Figure No.14: Starch grains enlarged

CSG-Compound starch grain, DR-Dilated Ray, SSG- Simple Starch Grains, VE-Vessel, XR-Xylem Ray

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.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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