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PHARMACOGNOSTICAL EVALUATION OF TUBEROUS ROOTS OF *CYANOTIS TUBEROSA*

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ABSTRACT

The present study carried out to find out the pharmacognostical parameters for the tuberous roots of the plant *Cyanotis tuberosa* (*Commelinaceae*). Traditionally the root is used in long-continued fevers and also worms in cattle and diabetes[12-15]. 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 *Cyanotis tuberosa*. This study also helps in differentiation of this drug from its other species.

KEYWORDS

Cyanotis tuberosa, Commelinaceae, Pharmacognostical, Tuberous roots.

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INTRODUCTON

Anatomical studies of phytodrugs form a major aspect of pharmacognosy. The relevance of micro morphological protocols of the phytodrugs is often ignored (or) depreciated earlier. Pioneer luminaries in Pharmacognosy like Wallis[1], Evans[2] and Claus[3] have put forth paradigm of tenets as rationale for the micro morphological spectrum of herbal drugs. Their works have been the basis for the pharmacognostical studies all over the world.

So the author has developed considerable interest to bring out systematic investigation of pharmacognostical standards for the selected

tuberous herbs on modern scientific lines. Pharmacognostical evaluation primarily includes a comprehensive study on macroscopic and microscopic descriptions, useful for establishing the identity, followed with physico-chemical parameters and phytochemical investigation.

Cyanotis tuberosa[4-7] (Syn: *Tradescantia tuberosa Roxb*) belongs to the family *Commelinaceae*. It is distributed throughout India, W. Peninsula, Ceylon. In Andhra Pradesh, it is common in wet places as forest undergrowth, Talakona, Gundalakona, Bathinayunikona of Tirumala region. Traditionally the root is used in long-continued fevers and also worms in cattle and diabetes[4-7]. 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

Materials and Methods

Collection and authentication of plant material

The selected herb *Cyanotis tuberosa* pertained to the study was collected from their natural habitates at Talakona, Gundalakona, Bathinayunikona of Tirumala region, 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 *Cyanotis tuberosa* (PARC/2008/299) have been deposited at the college of pharmaceutical sciences, AU, Visakhapatnam. The specimen (tuberous roots) was 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 Turner[8]. For microscopical studies, cross sections were prepared and stained as per the procedure of Johansen[9].

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[10] and WHO guidelines on quality control methods for medicinal plant materials WHO/QCMMPM guidelines[11].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedures described by Kokate[12] and Harborne[13].

RESULTS AND DISCUSSION

Macroscopical characters

It is an erect or spreading diffuse herb; branchlets softly villous. Root fusiform tubers. Stem 15-90 cm long, swollen and very hirsute at the very base, suberect or prostrate and creeping below, densely villous or almost glabrous. Leaves sessile, the radical and lower cauline 15-25 by 0.8-2.5 cm, often purple beneath, linear or ensiform, villous, the upper cauline leaves much shorter; sheaths of radical leaves 2.5cm long, glabrous, those of the cauline leaves shortly silky. Flowers cymes villous or densely hirsute, 1.3-2.5 cm long, usually pedunculate, in the axils of short ovate acute leaves (bracts) which are shorter than the cyme, strongly falcately decurved bracteoles imbricate in 2 series, usually many, 8-17 mm long. Sepals 6 by 1.6 mm linear-lanceolate, acute, densely villous and ciliate. Corolla 8 mm long, bluish purple; lobes 2.5-3 mm long, ovate, subacute. Filaments spirally twisted, fusiform towards the tips. Anthers 1.25 mm long, yellow. Style thickened at the tip, with a tuft of

hairs near the apex. Capsules 4 by 2.5 mm, ellipsoid, the upper half hairy, the lower half glabrous. Seeds 1.6 mm long and broad, truncate at the base, shortly conic at the apex, obscurely rugose, brown. Flowers and fruit September – December.

Microscopic characters of *Cyanotis tuberosa*

Microscopy of the *C. tuberosa* root (Figure No. 1-7)

The root is circular with rough surface. It is 1.6 mm thick. It has multiple epidermis, hypodermis, cortex and a wide stele (Figure No.1).

Epidermis (Rhizodermis): is multiple and consists of three layers of dark, thick walled polygonal cells. The multiple epidermis is 70 µm thick (Figure No.1, 2).

Hypodermis: Inner to the epidermis is a layer of radially oblong, rectangular, thin walled cells. The hypodermal layer is 40 µm wide (Figure No.1,2).

Cortex: Is 400 µm wide. The outer zone is about five layers of cells of the cortex are circular and thick walled. Remaining part of the cortex has small, angular, compact parenchyma cells (Figure No.2).

Stele: Is 500 µm in diameter. It has a distinct endodermis with u-shaped thickening of the inner tangential walls and radial walls. The pericycle is fairly distnict. It consists of a single layer of small elliptical thin walled cells. These are about 12 extarch xylem strands alternating with 12 phloem strands (Radial arrangement of xylem and phloem). The metaxylem elements are wide angular and thin walled. They are 50 µm wide. The pith tissue is sclerenchymatous. The cells are heavily thick walled, and lignified with narrow lumen. These may a few thin walled cells in the center of the pith (Figure No.2).

The root tuber is thick, fleshy and cylindrical. The epidermal layer is broken and replaced by a narrow zone of collapsed parenchyma cells. The cortex of the root-tuber is wide, homogeneous and parenchymatous. The cortex is 1.3 mm wide. The cortical cells are square shaped and are arranged regular circles (Figure No.3). The cortical cells are heavily loaded with starch grains. The starch grains are large, circular, simple type with central hilum (Figure No.4). The grains are 15-20 µm wide.

Stele (Figure No.3-5): The stele circular measuring 900 µm in diameter. It has distinct endo-dermoid layer and the cells lack the characteristic thickenings. Pericyclic layer is fairly distinct; it consists of a thin layer of elliptical parenchyma cells.

There are 15-20 exarch xylem elements and alternating with the same number of phloem elements in a circle (Figure No.4). The metaxylem elements are wide, angular thick walled and 50-80 µm in diameter (Figure No.5).

Pith: Is wide and parenchymatous. The cells are angular, thin walled and compact. The starch grains are not evident in the pith cells (Figure No.6).

Starch-grains (Figure No.7): The starch grains are simple type; they are prominent and vary in shape and size. The shape varies from spherical, ovoid to elliptical. When viewed under the polarized light microscope, the starch grains exhibit "+" shaped, x-shaped and x-shaped dark lines (Figure No.7). When stained with IKI (Potassium-Iodine-Iodide) the starch grains appear dark purple colored. The size of the starch grains vary from 40 x 40 µm to 40 x 100 µm in breadth and length.

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 lines[14], Indian Pharmacopoeia[15] 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 kokate[12] and Harborne[13]. 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 for steroids, glycosides, alkaloid, carbohydrates and bitters (1:50 dilutions). The results are shown in the Table No.3.

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

S.No	Parameter →	Ash values (% w/w)
1	Parts used →	**Tuberous roots**
2	Total ash	9.50
3	Water soluble ash	3.50
4	Acid insoluble ash	2.00
5	Sulphated ash	9.00
6	Parameter →	**Extractive values (% w/w)**
7	Ether soluble	1.40
8	Alcoholic soluble	6.84
9	Water soluble	7.90

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

S.No	Physical characteristics of Tuberous roots extracts			
		Nature	**Color**	**%yield (w/w) g**
1	Petroleum ether	Greasy	D. g	1.30
2	Chloroform	Greasy	B .g	2.66
3	Alcoholic	Viscous	D. b g	5.24
4	Aqueous	Sticky	Brown	5.31

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

Part used →	**Tuberous roots**				**Part used →**	**Tuberous roots**			
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	–	–	–	–
					Testsfor Glycosides (a) Baljet's test	+	+	+	+
(b) Liberman Burchard 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	SB	SB	SB	SB

Note: "+": Present, "-": Absent, Pet. Ext: Petroleum ether extract, Chl. Ext: Chloroform extract, Alc Ext: Alcoholic extract & Aq Ext: Aqueous extract, SB: Slightly bitter in taste.

Anatomy of the root *C. tuberosa*

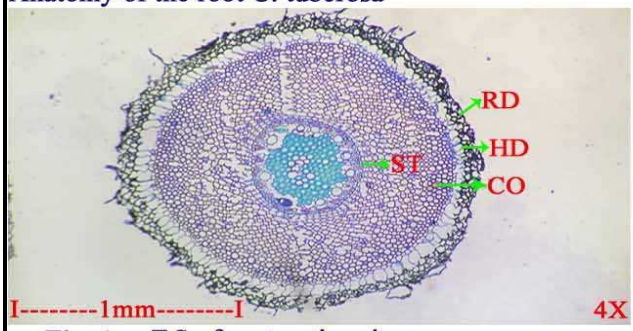


Fig. 1 : T.S of root entire view

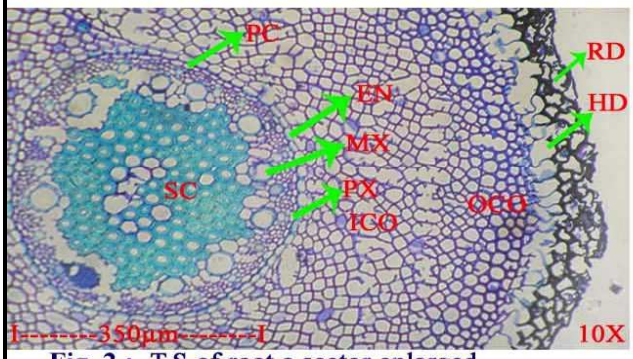
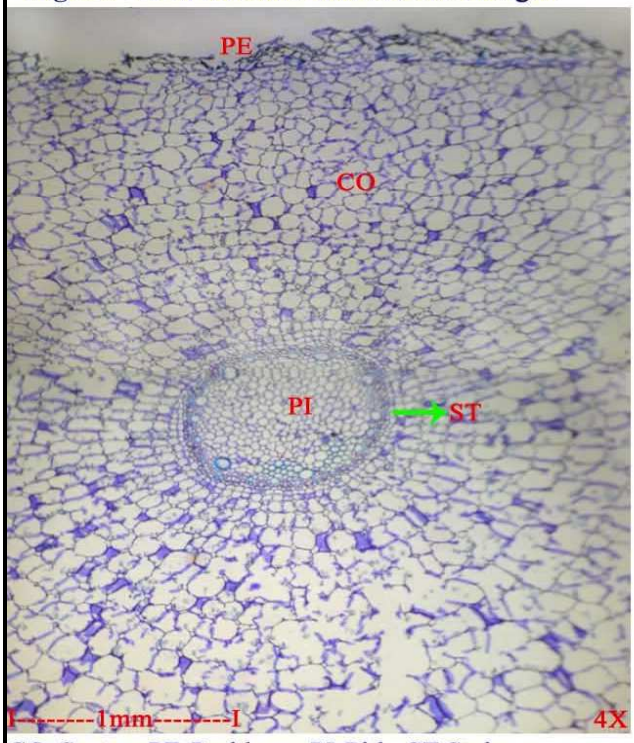


Fig. 2 : T.S of root a sector enlarged

CO-Cortex; EN-Endodermis; HD-Hypodermis; ICO-Inner cortex; MX-Meta xylem; PC-Pericycle; OCO-Outer cortex; PX-Proto xylem; ST-Stele; RD-Rhizodermis; SC-Sclereinchyma

Fig. 3 : T.S of tuberous root-a sector enlarged



CO-Cortex; PE-Periderm; PI-Pith; ST-Stele

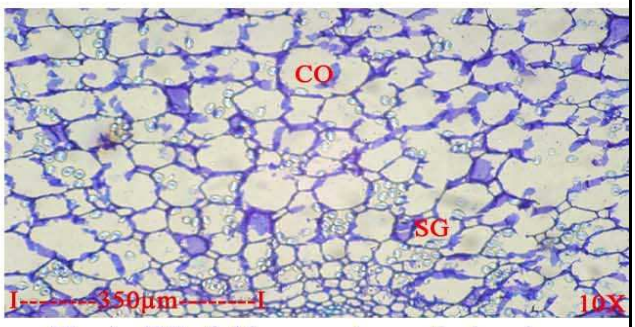


Fig. 4 : T.S of rhizome outer cortical region

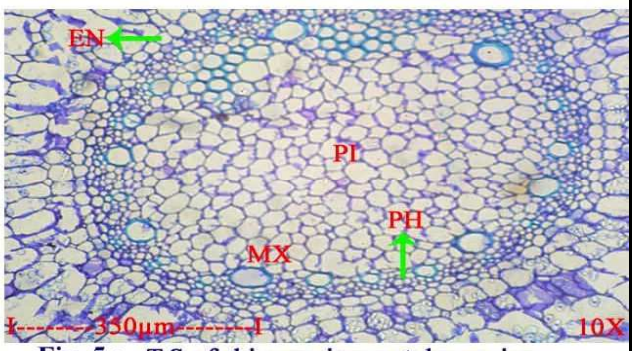


Fig. 5 : T.S of rhizome inner stelar region

CO-Cortex; EN-Endodermis; MX-Meta xylem; PH-Phloem; PI-Pith; SG-Starch grains

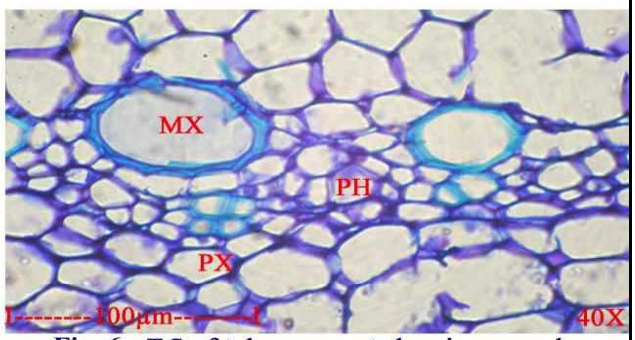


Fig. 6 : T.S of tuberous root showing vascular elements

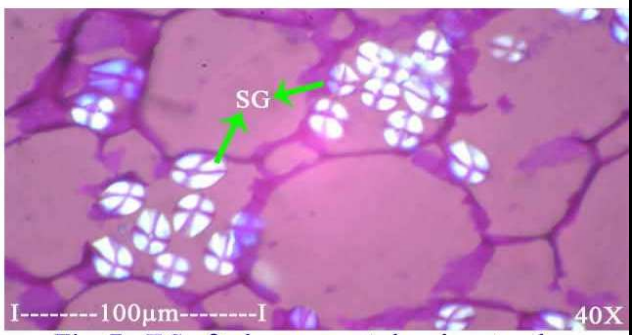


Fig. 7 : T.S of tuberous root showing starch grains in the cortical region

MX-Meta xylem; PH-Phloem; PX-Protoxylem; SG-Starch grains

CONCLUSION

In conclusion, the present study on pharmacognostical evaluation of *Cyanotis tuberosa* 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.

ACKWOLEDGEMENT

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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