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Pharmacognostical and phytochemical evaluation of stem of *Calotropis gigantea* Linn

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Abstract
The objective of present studies deals with the macroscopical and microscopical studies of stems of Calotropis gigantea Linn. Some distinct and different characters were observed with section of young thin stems. The microscoy shows the cork, cortex, vascular bundle, medullary rays xylem element are few and phloem elements. Physiochemical parameter and Preliminary phytochemical studies of the stems powder were also carried out. The present study on Pharmacognostical investigation of stems of Calotropis gigantea Linn. Might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

Keywords: pharmacognostical, physiochemical, *calotropis gigantea* linn, stems, methanolic extract

Introduction
A medicinal plant is any plant which, in one or more of it contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. The plants Posses therapeutic properties or exert Beneficial Pharmacological effects on the animal body are generally designated as "Medicinal Plant". In current scenario of medical and pharmaceutical advancement, microbes involve in the change of their metabolism and genetic structure to acquire resistant against the drugs used in the treatment of common infection disease [1-2]. The continued emergence or persistence of drug resistant organisms and the increasing evolutionary adaptation by pathogenic organisms to commonly used antimicrobials have reduced the efficacy of antimicrobial agent currently in use [3]. Plant have the capacity to produce a large number of organic chemicals called as phytochemicals. The accumulation of phytochemicals in the plant cell cultures had been studied for than thirty years and the generated knowledge had helped in realization of using cell culture for the production of desired phytochemicals [4].
Calotropis belong to Asclepidaceae family. It is also known as Akada, Aak, Mandar, Aakh etc. It has two species procera and gigantea .here we study about Calotropis Gigantea [5]. The roots and leaves of calotropis gigantea are used traditionally for treatment of abdominal, tumours boils, skin diseases, wound, insect bites. A literature review showed that Calotropis Gigantea contained cardenolide, glucosides, a non-protein, amino acid, flavonoids and steroids. Calotropis gigantea in small dose are also useful in the treatment of cold, cough, asthma inflammatory diseases and loss of digestive and analgesic property of Calotropis Gigantea [6-7].

Material and Method
Plant material
The plant *Calotropis gigantea* Linn. is widely found throughout India. For my work the plant was collected from in the deep forest of Satpuda hills with the help of forest officers of Chopda Tahsil, Dist. Jalgaon, Maharshtra (India) and authenticated by Prof. (Dr.) C. R. Jadhav, scientist, BSI (Botanical Survey of India), Pune (M.S.). (Specimen no. 01). The stems of the plant were dried under shade and then coarsely powdered with help of mechanical grinder. The powder was passed through sieve no. 40 and stored in an airtight container for further studies. Extraction was carried out by continuous soxhlet extraction process for 72 hr.
The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin – 5 ml + acetic acid – 5ml + 70% Ethyl alcohol – 90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol as per method [8]. Infiltration of the specimens were carried out by gradual addition of paraffin wax (melting point 58-60^{0}C) until TBA solution attained super-saturation. The specimens were casted into paraffin blocks.

Sectioning
The paraffin embedded specimens were sectioned with the help of rotary Microtome. The thickness of the sections were 10-12 µm. Dewaxing of the sections were done by customary procedure [9]. The sections were stained with Toluidine blue as per the method [10]. Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some Cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc.

Photomicrographs
Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Lab photo 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale – bars [11].

Physicochemical Parameters

Physicochemical parameter of stems of *Calotropis gigantea* Linn. Were determined such as Total ash, Acid insoluble ash, Water soluble ash, Sulphated ash, Moisture content (Table 1) [12-13].

Preliminary Phytochemical Parameters

Preliminary phytochemical test of *Calotropis gigantea* Linn. Were performed and the chemical constituents were detected (Table 2) [14-15].

Results and Discussion

Morphology of *Calotropis gigantea* Linn

Large shrub or small tree up to 4–10 m tall, much-branched at base, stems erect, up to 20 cm in diameter; bark pale grey, longitudinally cracked; young shoots woolly hairy; latex in all parts. The leaf is simple, decussate and sessile. Leaf blade is ovate to obovate in shape with entire margin without any stipules. The leaf is 10-20 cm. long. Flower consists of five pointed petals each 3-5 cm in diameter, purplish or white in umbellate lateral cymes and a small "crown" rising from the center which holds the stamens, having clusters of waxy flowers that are either white or lavender in colour. The fruits having are boat-shaped, 9-10 cm long, small, thick green with seeds in fleshy follicles covered with white woolly pubescence and a pair of follicles, with tapering at both ends. Ovoid shape, 5-6 mm long with 2–3cm long coma at one end.

Fig 1: *Calotropis gigantea* Linn. (Photo)

Anatomy of the stem (Microscopy)

The microscopic studies of stem showed following tissue systems

Cork: The cork comprises of an outer zone of thick walled brownish compressed cells and an inner zone of thin walled colourless, tangentially arranged cells. The cork tissue is broken at some places due to lenticels.

Cortex: Cortex is wide. The outer zone of cortex consists of 3 to 5 rows of irregularly arranged tangentially elongated chlorenchymatous cells and the cells situated towards the inner side are polygonal in shape filled with abundant starch grains. The starch grains are simple, ovoid; several secretory cells found scattered in the cortex. Pericyclic fibres lignified are associated with a large number of crystal fibres containing a single prism in each chamber.

Vascular bundle: Vascular zone is composed of discrete vascular strands with 10 to 12 or more wedge shaped strips of xylem, externally surrounded by semicircular strips of phloem alternating with wide medullary rays; phloem parenchyma contain calcium oxalate crystals; cambium is of 1-2 layers; xylem consists of vessel elements, tracheids, parenchyma and fibres. Vessel elements cylindrical in shape bearing bordered pits.

Outer layer of epidermis of loosely arranged cells are present. Cells are oval in shape. Central large portion of stem are occupied by pith, outside of it hypodermis is present. Barrel shaped cells of pith store large amount of food. Vascular bundles are arranged in parallel rays.

The presence of discrete vascular strands in the mature stem of *Calotropis gigantea* is one of the anomalous secondary structures found Figure 2 and 3.

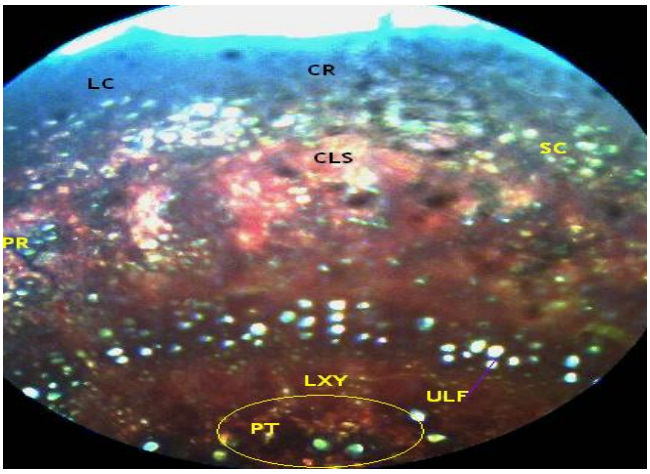

Fig 2: T.S. of stem of *Calotropis gigantea* Linn. (Stained with Toludine blue, A polychromatic stain)

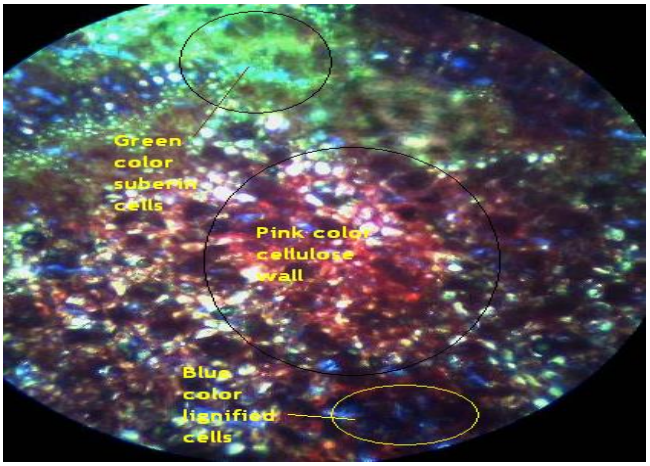

Fig 3: Central portion (Pith) of stem of *Calotropis gigantea* Linn. CR= Cortex LC= Lignified cellsa CLS- Cellulose wall SC= Suberin cell ULF= Unlignified fibres LXY= Lignified xylems PT= Oith PR= Protien

Powder Microscopy

Microscopy study of powder showed the presence of fibres which are lignified, long with blunt ends. Tracheids with bordered pits and horizontal perforations. Pericylic fibres are longer than tracheids. Xylem vessels cylindrical and bear bordered pits. Starch grains are present in parenchymatous cells (Figure 4 and 5).

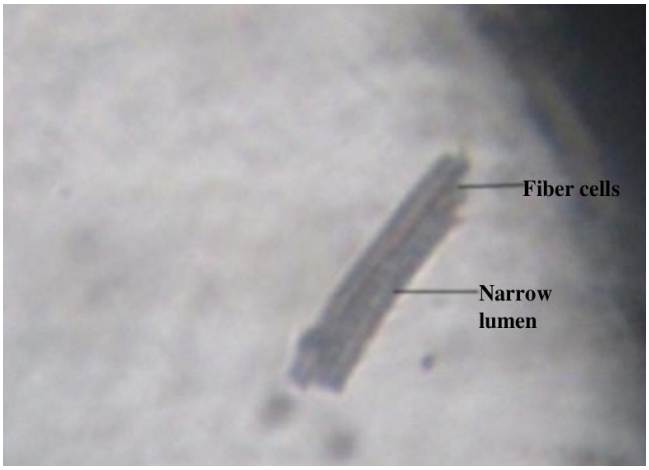

Fig 4: Powder microscopy of stem of *Calotropis gigantea* (fibers)

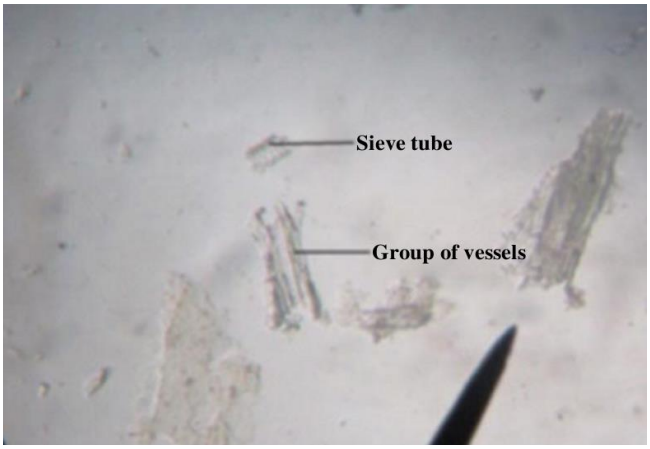

Fig 5: Powder microscopy of stem of *Calotropis gigantean* (Components of xylem and phloem)

Physicochemical Parameters

Table 1: Determination of quantitative standards of dry powder of stem of *Calotropis gigantean*

Sr. No.	Parameter	Values obtained in air dried basis (% w/w)
1.	Ash value	
	Total ash	12.26 % w/w
	Acid soluble ash	6.42 % w/w
	Water soluble ash	6.80 % w/w
2.	Extractive value	
	Alcohol soluble extractive	12.98 % w/w
	Water soluble extractive	19.14 % w/w
3.	Moisture content	10.62 %

Table 2: Preliminary phytochemical screening of various extracts of stem of *Calotropis gigantean*

Sr. No.	Constituents	Tests	Methanol	Aqueous
1.	Alkaloids	Mayer's test	-	-
		Dragordraff's tes	-	-
		Hager's test	-	-
		Wagner's test	-	-
2.	Sterols	Libermann's Burchard test	+	+
		Salkowski's	+	+
3.	Carbohydrates and Glycoisdes	Molisch's test	+	+
		Fehling's test	+	+
		Benedict's test	+	+
		Borntrager's test	+	+
4.	Fixed oils and fats	Spot test	-	-
		Saponification test	-	-
5.	Phenolic compound	$FeCl_3$ test	-	+
6.	Protein and aminoacids	Biuret test	-	-
		Ninhydrin test	-	-
		Xanthoprotein test	-	-
		Millon's test	-	-
7.	Triterpinoid and saponins	Foam test	+	+
		Haemolysis test	+	+
8.	Tannins	Gelatin test	-	-
		$FeCl_3$ test	-	-
9.	Gums and mucilage	Precipitation with 90% alcohol	-	-
10.	Flavonoids	Aqueous NaOH	+	+
		Conc. H_2SO_4	+	+

Conclusion

The present Pharmacognostical studies of stem of Calotropis gigantean Linn. Might be useful to supplement assumed significantly in the way of acceptability of herbal drugs in present scenario that lacks regulatory laws to control quality of herbal drugs.

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