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Pharmacognostical standardization of *Borassus Flabellifer* root

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ABSTRACT

The Borassus flabellifer is a tall, erect palm, easy to recognize by its large, fan-shaped leaves which are quite unlike the pinnate leaves of other palms. The different parts of the plant are used for ailments like secondary syphilis, antiperiodic, heart burns, liver and spleen enlargement. The chemical constituents of Borassus flabellifer include gums, albuminoids, fats, steroidal glycosides and carbohydrates like sucrose. The fresh pulp is reportedly rich in vitamins A and C. The fresh sap is reportedly a good source f vitamin B-complex. The male inflorescence constitutes borassosides and dioscin, spirostane-type steroid saponins. Though the plant has pharmacological potentials no standardization has been done from pharmacognostically, hence form the basis for performing the work. The transverse section showed the characters of typical monocot root with rhizodermis and 18-20 pairs of vascular bundles. The powder microscopy showed the presence of cork. The proximate analysis, fluorescence analysis and preliminary chemical tests were performed and reported. These data's would help in the development of a root profile for the plant.

Key words: Borassus flabellifer, borassosides, rhizodermis, pericycle, physico chemical, flourescence analysis

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INTRODUCTION

The *Borassus flabellifer* is a tall and erect palm, with large, fan-shaped leaves which are quite unlike the pinnate leaves of other palms. *Borassus* is from a Greek word describing the leathery covering of the fruit and *flabellifer* means "fan-bearer". Synonyms of the plant include jaggery palm, Palmyra palm, toddy ALM, toddy palm, wine palm. The Palmyra tree is the official tree of Tamil Nadu, highly respected in Tamil culture; it is called "karpaha" or celestial tree because all its parts without exception have a use. It is a natural symbol tree of Cambodia. [1]. This species is globally distributed from Africa to Australia. Within India, it is found throughout tropical regions, especially along the peninsular coast and in West Bengal and Bihar. It is often cultivated. The Palmyra palm has long been one of the most important trees of Cambodia and

India, where it is used over 800 different ways. The uses to which various parts of the tree are put are innumerable. Hindus and Buddhists both venerate this tree because sacred writings were inscribed on its leaves in olden times. The Palmyra is one of the most valuable and important Indian trees. It is not indigenous to this country but is extensively cultivated as it readily propagates itself in regions where it is abundant; it is also found growing wild. The uses to which various parts of the tree are put are innumerable. The hard outer wood is universally employed for posts, rafters and domestic purposes, but it is of no great strength and iron nails rust rapidly in it. The jelly like pulp of the fruit and the soft kernels of young fruit are pleasant to eat, while the germinated nuts, with their enlarged, fleshy embryos are cooked and eaten as vegetables. The mid-ribs of the leaves and the fibers from their stalks are used in brush-making and the web-like substance at the base of young leaf stalks is used for straining the toddy and for making into torches. The chief product of the Palmyra however is arrack or toddy the intoxicating drink of the country. Before fermentation it is a saccharine juice which, when freshly drawn before sunrise, makes a tasty and health giving drink and, taken in large morning doses, has a laxative effect. Hot, sunny and well drained conditions are suitable for the plant to grow. It is drought tolerant but cold sensitive. Propagation is usually done by means of seeds, especially when the seeds are its final position. The flowering and fruiting time of the plant is usually from February-April [2]. The different parts of the plant is used for the various ailments like secondary syphilis, antiperiodic, heart burns, liver and spleen enlargement etc. Other than these pharmacological uses the juice of the plant is used in preparation of health drinks, jellies etc. The leaves are use to make baskets, hats and many other useful items. *Borassus flabellifer* contains gums, albuminoids, fats and the fresh pulp is reportedly rich in vitamins A and C [3]. The fresh sap is reportedly a good source f vitamin B-complex [4].Male inflorescence constitutes spirostane-type steroid saponins like borassosides and dioscin. It also contains 20 known steroidal glycosides [5] and carbohydrates like sucrose [6]. It also contains bitter compound called flabelliferrins, these are steroidal saponins.

MATERIALS AND METHODS

The plant species *Borassus flabellifer* were collected in regions of Nalgonda district. The plant material is collected in the months of November to December. The plant material is authenticated by Mr. A. Lakshma Reddy, Retired Professor, Dept. of Botany, Nagarjuna Govt. College (Autonomous) Nalgonda. The plant was identified as Borassus flabellifer and was certified under Voucher No: NCOP-NLG/ph'cog/2009-10/004.

Instruments used

Micro senior precision rotary microtome (latest Spencer 820 type), Sisco muffle furnace (3003137), Rotary vacuum evaporator, Stage micrometer, Eye piece micrometer.

Chemicals and reagents

All the chemicals and reagents like chloral hydrate, phloroglucinol, hydrochloric acid, nitric acid, potassium hydroxide, picric acid, lead acetate etc used were of analytical grade.

Microscopical studies

Transverse section of root

Microtome sectioning was done for fresh root to obtain a thin section. Phuloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope. A thin transverse section of *Borassus flabellifer* root was taken and studied [7, 10].The descriptions are given as per standard anatomical references [11, 12].

Powder microscopy
Shade dried roots were powdered with the help of an electric grinder till a fine powder was obtained .This fine powder was subjected to powder microscopy, as per standard procedures mentioned [7, 10].

Measurement of cell structure and content [10, 13]
The length and width of phloem fibres and diameter of the starch grains were measured using stage micrometer and the eyepiece micrometer by standard methods [7].

Determination of physico chemical properties [7, 13]
Total ash, acid insoluble ash and water soluble ash of *Borassus flabellifer* root was determined by standard method and the results are tabulated in table. The crude fibre content, moisture content, alcohol soluble extractive value, water soluble extractive value, chloroform soluble extractive value and petroleum ether soluble extractive values of *Borassus flabellifer* root were determined by standard method and the results obtained are tabulated in table.

Determination of Fluorescence analysis [8, 9, 13]
Powdered root was subjected to analysis under ultra violet light after treatment with various chemical and organic reagents.

Extraction
The collected roots were washed and dried under the shade. It was then coarsely powder using an electric grinder.50 g of the coarsely powdered root was packed in a soxhlet apparatus and extracted with ethanol after defatting with petroleum ether. The extract obtained was concentrated under vacuum using rotary vacuum evaporator.

Preliminary chemical screening [7, 14, 15]
The extract obtained was subjected to various chemical tests as per the procedure mentioned in the standard reference books.

RESULT AND DISCUSSION

Microscopical studies
Transverse section of root
The root was divided into three zones, epidermis or rhizodermis, cortex, stele (Fig 1).
Epidermis or rhizodermis: The outer most layer was found tbe the rhizodermis.It was made up of thin walled, rectangular parenchymatous cells which were arranged compactly without intercellular spaces (Fig 2).

Cortex was divided into three sub zones namely exodermis, general cortex and endodermis.
Exodermis was found beneath the epidermis and composed of two to three layers of sclerified parenchyma (Fig 4).

General cortex was found next to the exodermis. The cortex was wide, extensive and made up of several rows of thin walled parenchyma showing intercellular spaces. Beneath this layer aerenchyma ells with large inter cellular space was found (Fig 3). The aerenchyma cells formed structures like bridges between the external and internal region of the cortex.
Endodermis formed the inner most layer of cortex. It was well developed and clearly demarcated as a single layer of barrel shaped cells arranged compactly without leaving any intercellular spaces. The cells found in this region were non lignified.

Stele showed three specific regions: pericycle, vascular bundles and medulla or pith. Pericycle consist of 3-4 layer of cells beneath the endodermis .The cells were thin walled ,parenchymatous , rectangular and completely arranged without intercellular spaces.

Vascular bundles contained radial polyarch vascular bundles with 18-20 pairs of xylem and phloem cells. The conjunctive tissue was found between the xylem and phloem strands (Fig 6).

Medulla or pith was found as wide central part of the stele. It was made up of thin walled parenchyma cells. Certain parts of medulla were composed of thick walled lignified cells (Fig 5).

Powder microscopy

Sclerified parenchyma was found scattered in the powder (Fig 7, 8).
Xylem vessel were found to be lignified, pitted walls and with spiral arrangements (Fig 11, 12, 13)

Fibers of phloem were found to lignified with lumen in it (Fig 9, 10).
Calcium oxalates were present in abundance and were of prismatic and rectangular in shape (Fig 15).

Starch grains present were circular to oval in shape (Fig 16).
Polygonally shaped parenchyma cells were found through out the powder (Fig 14)

Measurement of cell structure and content

This helps in identification of adulteration. The results obtained are tabulated in Table 2.

Determination of physico chemical properties

The physico chemical properties will help to estimate the amount of impurities like soil and particle present in the drug. It also helps to assess the calculi salts present in the drug sample. The results obtained for the ash values, extractive values, moisture content and crude fibre content are tabulated in Table 1.

Determination of Fluorescence analysis

Fluorescence analysis is a tool to determine the kind of chemical nature of the drug. The fluorescence obtained in short wavelength, long wave length and day light after treatment with different chemicals and reagents are tabulated in Table 3.

Preliminary chemical screening

Chemical test helps in the confirmation of the chemical nature of the active principles present in the plant extract. The results of the chemical tests are tabulated in Table 4.

Acknowledgement

The authors are highly grateful to the Management of Nalanda College of Pharmacy for providing necessary help and laboratory facilities in performing this experiment. They express their gratitude to Mr.Laxma Reddy for authenticating the plant specimen**.**

Table1 Physico chemical properties

Parameter	Results in %w/w
Total ash	5
Acid insoluble ash	2.5
Water soluble ash	1.5
Moisture content	10.2
Crude fiber content	51
Alcohol soluble extract value	4.8
Water soluble extract value	14.4
Chloroform extract value	0.8
Petroleum ether extract value	1.6

Table 2Measurements of cell structure and content

Parameter	Results
Width of fibre	13.04 µm -32.33 µm-52.16 µm
Length of fibre	104.32µm-294.94µm-782.4µm
Diameter of starch	13.04 µm -21.19µm -78.24µm

Table 3 Fluorescence analysis

Reagent used	Day light	U.V. Light	
		Short wave length 0	Long wave length 0
Powder+50%H_2SO_4	Light brown	Light green	Light yellow
Powder + 50%HNO_3	Reddish brown	Green	Yellow
Powder + 5%NaoH	Dark brown	Pale green	Green
Powder + Me NaoH	Dark Brown	Brownish black	Green
Powder + 1N KoH	Brown	Light green	Green
Powder + 5%KoH	Brown	Green	Green
Powder + 5%$Fecl_3$	Brown	Green	Green
Powder + Methanol	Brown	Brown	Green
Powder + con.Hcl	Brown	Pale green	Brown
Powder + con.H_2So_4	Brownish black	Greenish black	Greenish black
Powder + Ammonia	Brown	Light green	Green
Powder+con.HNo_3	Reddish brown	Green	Black

Table 4 Qualitative Preliminary phytochemical studies

Phyto Constituents	Petroleum ether extract	Benzene extract	n-butanol extract	Ethanol extract
Alkaloids	Absent	Absent	Absent	Absent
Glycosides	Absent	Absent	Absent	Absent
Carbohydrates	Present	Present	Present	Absent
Tannins &Phenolic compounds	Absent	Absent	Present	Absent
Amino acids	Absent	Absent	Absent	Absent
Proteins	Absent	Absent	Absent	Absent
Steroids	Present	Absent	Absent	Absent
Flavonoids	Absent	Absent	Absent	Present
Saponins	Absent	Absent	Present	Present

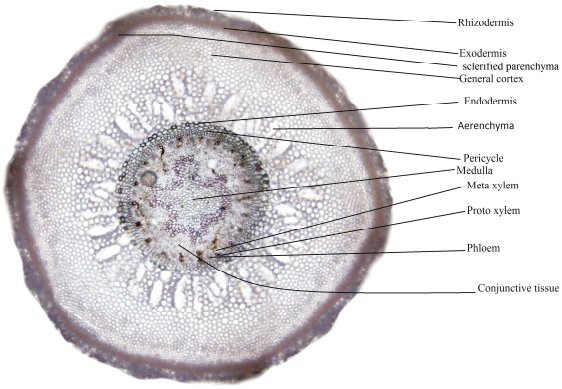

Fig 1 Transverse section of the root.

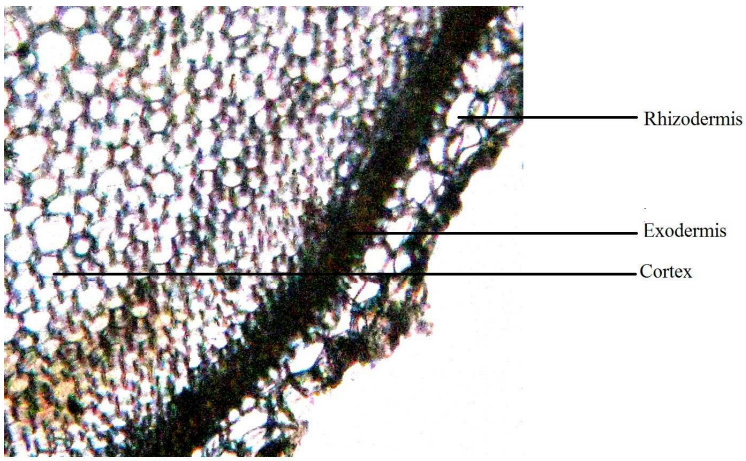

Fig 2 Portion showing the rhizodermis, exodermis and cortex.

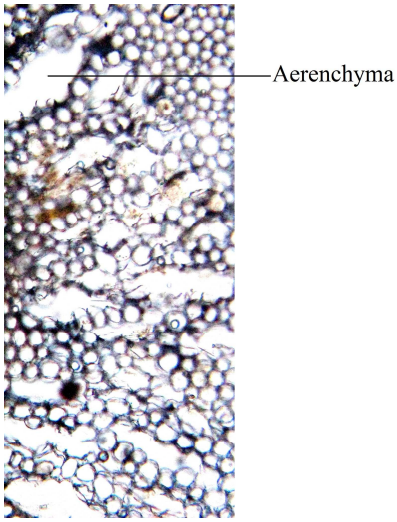

Fig 3 Aerenchyma

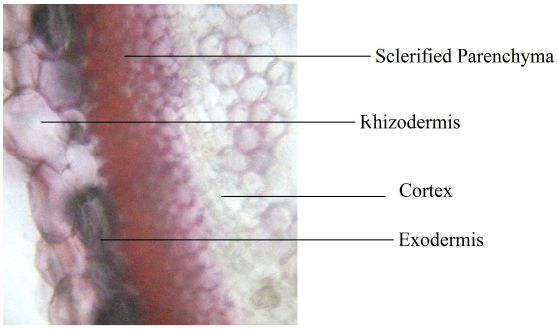

Fig 4 sclerified parenchyma

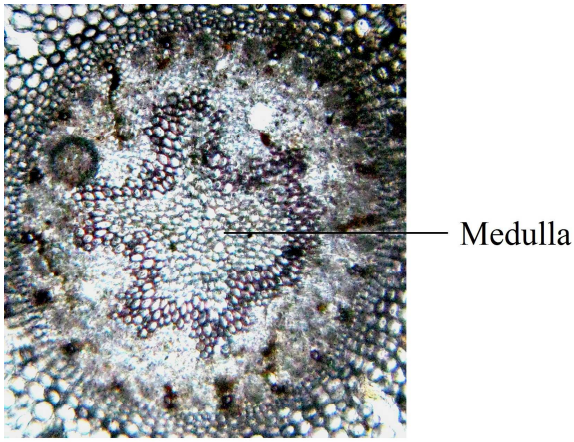

Fig 5 Pith or medulla

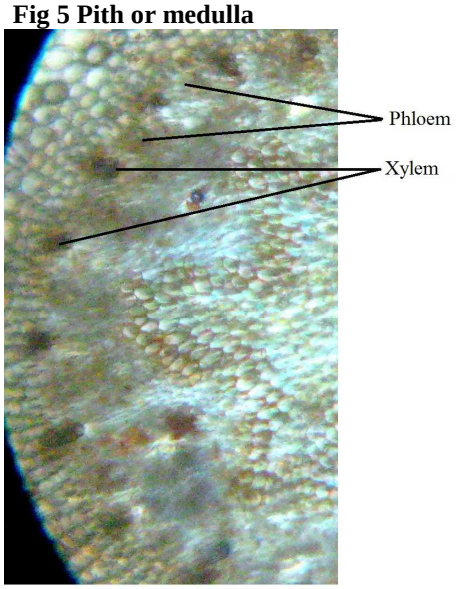

Fig 6 Vascular bundle

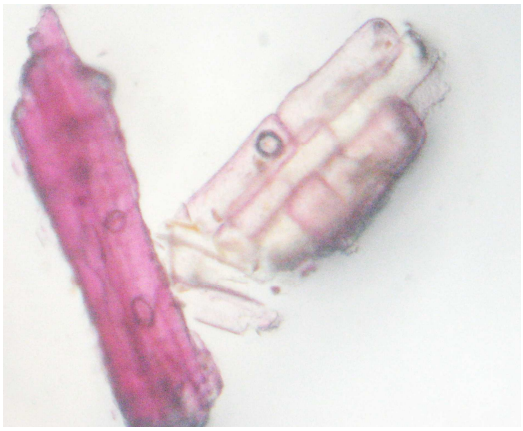

Fig 7 Fibre and sclerified parenchyma

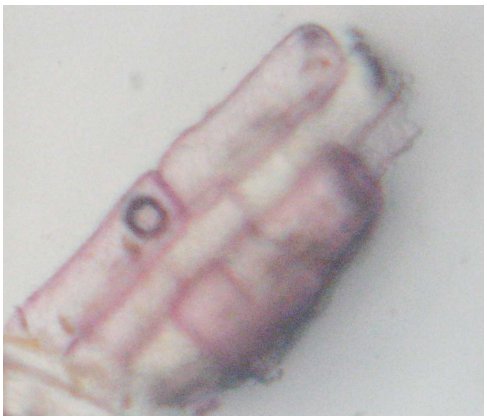

Fig 8 Sclerified parenchyma

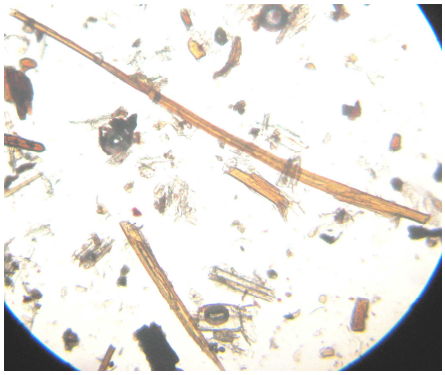

Fig 9 Phloem fibres

Fig 10 Phloem fibre with lumen

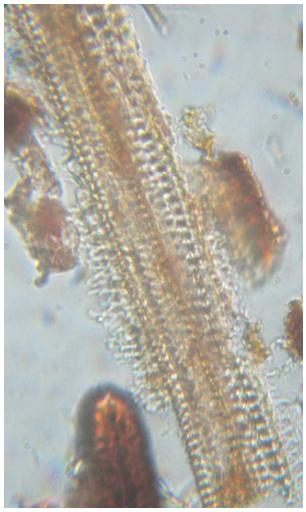

fig 11 Vessel with spiral arrangement

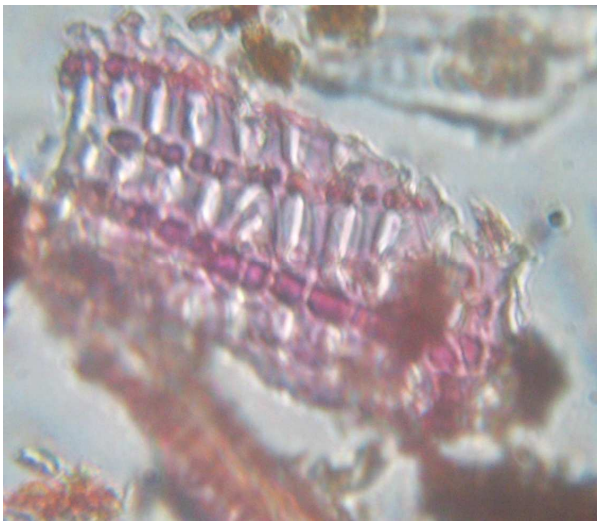

Fig 12 Xylem vessel

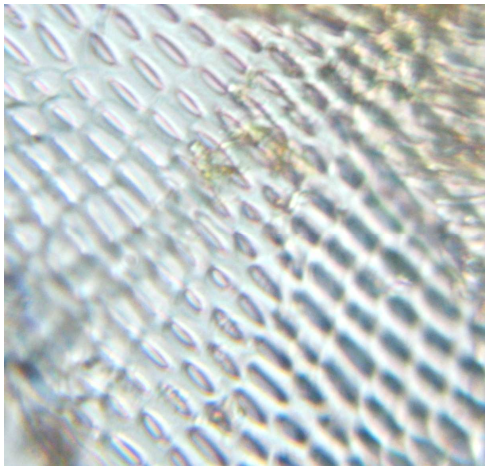

Fig 13 Vessel with pitted lateral walls

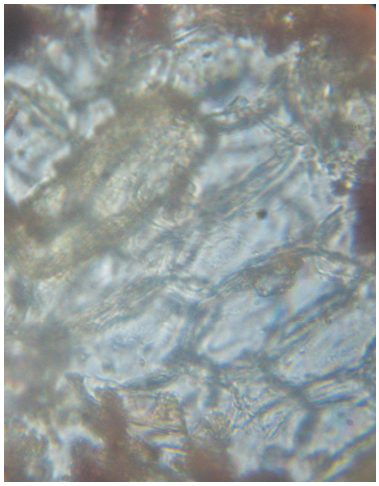

Fig 14 Parenchyma cells

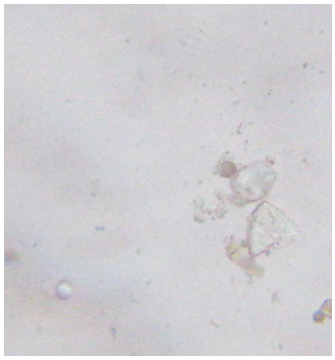

Fig 15 Calcium oxalate crystals

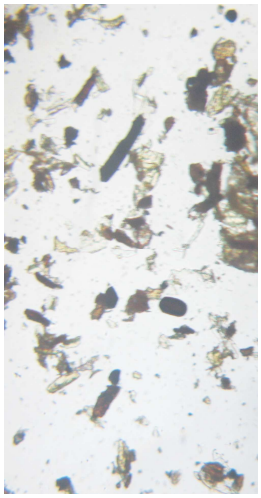

Fig 16 Starch grains

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