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Pharmacognostic and Preliminary Phytochemical Standardization of *Talanguli* (Male inflorescence of *Borassus flabellifer* Linn.)

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Abstract

Introduction: *Borassus flabellifer* Linn. (Palmyrah palm) belongs to the Arecaceae family and is identified as *Tala* in Ayurveda classics. The male inflorescence called *Talanguli* in Sanskrit and *panaviral* in Malayalam is traditionally used to treat liver disorders, ascites and rheumatic arthritis **Aim:** The present study reports the pharmacognostic and phytochemical standardization of male inflorescence of *Borassus flabellifer* Linn. **Materials and methods:** The male inflorescence of *Borassus flabellifer* Linn. was subjected to pharmacognostic, physico-phytochemical and HPTLC analysis as per standard procedure. **Results:** The external surface of the male inflorescence *Borassus flabellifer* Linn is rough due to scales and is yellowish green when fresh. The transverse surface of the staminate spadix has a central axis surrounded by 10-12 spikelets. The microscopic analysis revealed numerous vascular bundles that are conjoint collateral. Sclereids, starch grains, needle-like crystals, pollen grains, fragments of vessels and fibers were observed in the powder microscopy. The alcoholic-soluble extractives are more than the water-soluble extractives. The total sugar was 8.57 %. High-performance thin-layer chromatography exhibited 10 and 8 peaks at 254 and 366 nm respectively. **Conclusion:** Alkaloids, steroids, saponin, flavonoids, tannins and phenols are present in the male inflorescence of *Borassus flabellifer* Linn. The pharmacognostic, physicochemical and HPTLC fingerprinting of the male inflorescence of *Borassus flabellifer* Linn. serves as a reference standard for the authentication.

Introduction

Borassus flabellifer Linn. is a dioecious tree widely distributed and cultivated in India. It has ecological, economic and medicinal benefits. Palm jaggery, toddy, palm sugar, palm candy, fibers, etc. are some of its by-products. In India, Palmyrah palm is found in Tamil Nadu, Maharashtra, Odisha, Bihar, Andhra Pradesh, West Bengal and Kerala. The Ayurvedic Pharmacopoeia of India (API) identifies *Tala* as *Borassus flabellifer* Linn. *Talanguli*- the male inflorescence is its official part[1]. *Tala* is included in the *salasaradi gana* and many formulations like *Panavirladi bhasma*, *Avilttoladi bhasma, Chandanadya taila* and *Mahamayura ghrita* which underscores its medicinal value[2]. *Talapushpa* has been described as a remedy for

hepato-biliary diseases[3]. The male inflorescence has been reported to have antidiabetic, antioxidant, antimicrobial, anti-inflammatory, analgesic and antipyretic activity [4]. *Borassus flabellifer* is a tree with palmate leaves and the male spadix are interfoliar, branched and enclosed in spathes. Around a central axis,10-12 spikelets are found in a cavity formed by the wedge-shaped bracts. Staminate flowers are yellowish about 1-1.5 cm with three sepals and six stamens[5]. The chemical constituents are steroidal glycosides, flabelliferins, saponin like dioscin, borossoside, coumarin, flavonoids, gallic acid, quercetin.[6,7] The flowering is from November to August or April to December depending on different regions[8]. Over the past two decades, there has been an increase in demand for herbal medications, highlighting the importance of guaranteeing their quality, safety, and efficacy. Even though the male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*) is traditionally used, it is an unexplored area. The pharmacological action of drugs is due to their constituents such as tannins, flavonoids, alkaloids and other secondary metabolites, reflecting the importance of preliminary phytochemical analysis. HPTLC can be used to identify and authenticate the quality of herbal medications[9]. High-performance thin-layer chromatography, or planar chromatography outperforms standard TLC regarding separation power and reproducibility[10]. So, the male inflorescence (*Talanguli*) was studied to gain insight into its pharmacognostic properties and phytochemical ingredients, including a high-performance thin-layer chromatography (HPTLC) profile.

Taxonomy [11]

Kingdom:	Plantae
Sub Kingdom:	Tracheobionta
Division:	Magnoliophyta
Class:	Liliopsida
Subclass:	Arecidae
Order:	Arecales
Family:	Arecaceae
Genus:	*Borassus*
Species:	*flabellifer*

Materials and methods

Collection and preservation of the sample

The male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*) was collected from Kaliyakkavilai, Thiruvananthapuram, Kerala in the month of May. It was authenticated. It was then washed, dried and stored under proper conditions.

Chemical and reagents

All the chemicals and reagents used in the study were as per the standard analytical grade

Pharmacognostical study

Macroscopic study

The exterior and sensory properties of dried specimens, such as color, odour, size, shape, taste, touch, fracture, texture and so on, were observed were as per API.

Microscopic evaluation

A thin hand slice of a freshly collected male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*) was cut, dyed with safranin and mounted with glycerin. The photographs were documented using an Olympus biological microscope at 4x, 10x and 40x magnification.

Powder microscopy

Powder microscopy was performed using coarse powder of dried male inflorescence of *Borassus flabellifer* Linn, which was put on a glass slide mounted using glycerin and photographed at 40x and 100x.

Physicochemical parameters and qualitative analysis

The Ayurvedic pharmacopeia of India was followed to analyze the physicochemical properties of the male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*) including pH, loss on drying, total-ash value, water-soluble extractive value and alcohol-soluble extractive value.[12] Secondary metabolites were analyzed qualitatively using conventional techniques with water and alcohol extracts.[13]

Preliminary Phytochemical Evaluation

Preliminary phytochemical analysis was done with the methanolic extract of the male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*) to detect the presence of various secondary metabolites, including alkaloids, flavonoids, phenols, saponins, steroids and tannins.

Qualitative Analysis for the Presence of Phytochemicals

Test for Alkaloids

HCl was added to the methanolic extract of male inflorescence and filtered. Then Dragendorff's reagent was added and if alkaloids were present orange-brown precipitate will be formed.[14]

Test for Flavonoids

The methanolic extract of male inflorescence was dissolved in alcohol followed by the addition of magnesium ribbon and conc. HCl. A reddish brown colour indicates flavonoids.[14]

Test for Phenols

Evaporate the methanolic extract of the male inflorescence of *Borassus flabellifer* Linn and the residue was dissolved in alcohol and FeCl3 was added. A violet colour confirms the presence of phenols.[14]

Test for Saponins

To the methanolic extract, sodium bicarbonate solution was added and shaken. A frothy, honeycomb-like appearance indicates saponins.[14]

Test for Steroids

The methanolic extract was evaporated and then acetic anhydride and concentrated H_2SO4 were added to the residue through the sides of the test tube. The colour change from yellow to brown indicates the presence of steroids. [14]

Test for Tannins

100 ml of distilled water and 10g of the dried male inflorescence were refluxed for 1 hour. It was filtered to a flask and adjusted to 100ml. 2ml of lead acetate solution was added and the formation of precipitate indicates tannins.[14]

High-performance thin-layer chromatography

HPTLC fingerprinting was performed on an aluminum sheet of silica gel 60 F254 using various solvent system using trial and error method. The densitometric scanning was performed at the wavelengths 254 and 366nm**.**

A Hamilton syringe and the CAMAG LINOMAT 5 applicator were used to apply an 8mm band length of the methanolic extract of the male inflorescence to a TLC plate. It was then placed in the developing chamber (100 x 100mm) with a mobile phase measuring up to 70mm. The plate was then inserted into the TLC Visualizer 2 for the photo documentation. The plate was placed in the CAMAG TLC SCANNER 4 with deuterium, tungsten and mercury lamps for detection and was scanned at UV 254nm and UV 366nm. The derivatization of the plate was done with vanillin sulphuric acid and heating at 105°C and then visualized under white light. The peaks table and Rf values were then documented using the Server DESKTOP-60R112G, version 3.2.23095.1 software.

Results and Discussion

Macroscopy of the male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*) [Fig 2]

Organoleptic characters

- The transverse surface reveals a core axis surrounded by 10-12 male spikelets.
- Flowers are yellowish and measure 1-1.5mm long.
- Flowers are sessile, actinomorphic and unisexual.
- The perianth comprises six free, valvate sepals and six stamens.
- Filaments are free, while anthers are basifixed.

Microscopic evaluation of the male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*) [Fig 3]

The transverse section of the male inflorescence represents two parts the central axis and the partition wall that separates the cavities that contain the spikelets. The microscopic features of the male inflorescence of *Borassus flabellifer* Linn. include:

Table 1: Macroscopic evaluation of the male inflorescence of *Borassus flabellifer* Linn.

Characters	Fresh male inflorescence of *Borassus flabellifer* Linn	Dried male inflorescence of *Borassus flabellifer* Linn
Colour	Yellowish grey	Brownish-black
Appearance	Scaly	Scaly
Smell	Characteristic odour	No characteristic smell
Touch	Rough	Rough
Taste	No taste	No taste

- The central axis of the male inflorescence has multiple vascular bundles distributed across a parenchymatous ground tissue.
- Conjoint collateral vascular bundles are present. The phloem is on the outer side, while the xylem is on the inner side.
- The vascular bundles are surrounded by parenchymatous cells.
- The ground tissue has cells with thick walls.
- The ground tissue contained patches of stone cells (sclereids).
- Pigmented cells are seen in ground tissue.
- Oblique vascular zones can be detected in the partition wall.

Powder microscopy of the male inflorescence of *Borassus flabellifer* Linn. [Fig 4]

The powder microscopy of male inflorescence of *Borassus flabellifer* Linn. revealed the presence of sclereid, starch grains, parenchyma cells, fragments of vessels, needle-like crystals, rosette crystals of calcium oxalate, prismatic crystals, pollen grains and fragments of fibers.

Table 2: Results of Preliminary Physico-chemical analysis of the male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*)

Parameters	Male inflorescence of *Borassus flabellifer* Linn.
Foreign matter	Nil
Loss on drying	1.5%
Water soluble extractive	3.6%
Alcohol soluble extractive	4.2%
Total ash	7.2%
Acid insoluble ash	1.4%
Total sugar	8.57%
Reducing sugar	6.3%

Table 3: Results of Preliminary phytochemical analysis of the male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*)

Constituents	Observations
	Male inflorescence of *Borassus flabellifer* Linn.
Alkaloids	**+**
Flavonoids	**+**
Phenol	**+**
Saponin	**+**
Steroid	**+**
Tannin	**+**

High-performance thin-layer chromatography [Fig 5 & 6]

HPTLC analysis of male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*) was done with the solvent system: Methanol: Chloroform: Acetic acid (0.5:9:0.5) at 254 nm and 366 nm. Tracks 1 & 2 represent the male inflorescences of *Borassus flabellifer* Linn at different doses (1.5 µl & 2 µl respectively)

At 254 nm

- The chromatogram scanned at 254 nm for track 1(1.5µl) represents 10 peaks with Rf values ranging from 0.013 to 0.976 with areas 17.99 % and 29.30 %.
- The predominant Rf values are 0.976 and 0.013 with a maximum height of 29.69 % and 27.79 % respectively.
- The chromatogram scanned at 254 nm for track 2 (2µl) represents 10 peaks with Rf values ranging from 0.016 to 0.965 with areas of 15.56 % and 31.14 %.
- The predominant Rf values are 0.965 and 0.016 with a height of 27.07% and 24.03% respectively.
- The bands are dark blue.

At 366 nm

- The chromatogram scanned at 366nm for track 1(1.5µl) represents 8 peaks with Rf values ranging from 0.015 to 0.973 with areas 35.26 % and 13.61 %.
- The predominant Rf values are 0.015 and 0.092 with 55.01 % and 12.81 % respectively maximum height.
- The chromatogram for track 2(2µl) represents 8 peaks with Rf values ranging from 0.016 to 0.956 with areas 36.27 % and 10.12 %.
- The predominant Rf values are 0.016 and 0.802 with a maximum height of 46.48 % and 15.24 % respectively.
- The spots are dominated by navy blue color bands.

Discussion

The microscopic analysis of male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*) can serve as a reference standard. The preliminary phytochemical analysis of the male inflorescence of *Borassus flabellifer* Linn. (*Talanguli*)detected the presence of steroids, alkaloids, saponins, flavonoids, tannins and phenols. The phytochemicals like alkaloids and flavonoids have free radical scavenging activity[15]. Alkaloids possess anti-inflammatory, anti-bacterial and anti-viral properties[16]. Phenols can block enzymes that cause inflammation and most of the anti-oxidant activity is exhibited by gallic acid[17]. Saponins have been found to have anti-inflammatory and hepatoprotective effects by the antioxidant mechanisms while tannins have free radical scavenging, anti-inflammatory and hepatoprotective actions[18] HPTLC fingerprinting is a powerful tool for determining a plant's identity is based on its chemical composition[19]. The HPTLC fingerprinting of male inflorescences of *Borassus flabellifer* Linn. (*Talanguli*) was demonstrated utilizing the solvent system methanol: chloroform: acetic acid (0.5:9:0.5). This solvent system is used as high-polarity solvents for the separation of phytoconstituents. When exposed to short-wave UV light of 254 nm, UV-active compounds will appear as dark spots on a bright background and those that absorb 366 nm UV light will appear as bright spots on a dark background[20]. The densitogram exhibited 10 peaks at 254 nm and 8 peaks at 366 nm where the number of peaks reflects phytoconstituents. The presence of numerous phytochemicals may contribute to its pharmaceutical actions which are helpful in new drug development.

Conclusion

It can be concluded that the male inflorescence of *Borassus flabellifer* Linn. contains a significant number of secondary metabolites, some of which can be turned into pharmacotherapeutic agents in the future to treat various diseases. Pharmacognostic and phytochemical standardization set forth in this study can be considered quality standards. The fingerprints created in this work are anticipated to help with quality control and standardization of Talanguli by serving as the reference values

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