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Jeenu Joseph
Professor, Department of Pharmacognosy, Pushpagiri College of Pharmacy, Thiruvalla – 689107, Kerala, India

Santhosh M Mathews
Principal, Pushpagiri College of Pharmacy, Thiruvalla – 689107, Kerala, India

Correspondence:
Dr. Jeenu Joseph
Medicity, Pushpagiri College of Pharmacy, Perumthuruthy, Thiruvalla – 689107, Kerala, India
Email: jeenukuruvilla@gmail.com

Physicochemical, phytochemical and pharmacognostic study of *Phaleria macrocarpa*

Jeenu Joseph, Santhosh M Mathews

ABSTRACT

Background: *Phaleria macrocarpa* (Thymelaeaceae family) commonly called as God's crown, Buah mahkota dewa or Pau, is an evergreen tree geographically distributed around Indonesia. Traditionally the plant is exercised to treat diarrhea, cholesterol, diabetes, blood pressure, tumor problems and also to reduce pain. Despite the increasing interest in the medicinal values of the plant, no pharmacognostical study was conducted on this plant. **Objective:** The present study aims a thorough macroscopic, organoleptic, microscopic, physicochemical and phytochemical evaluation of the leaves and fruits of *P. macrocarpa.* **Materials and Methods:** The physicochemical analysis was performed as per WHO guidelines which include loss on drying, determination of ash values, swelling index, foaming index and extractive values. Various phytoconstituents are determined using preliminary phytochemical screening. **Results:** Morphological studies revealed organoleptic and surface characteristics of leaves and fruits. Histological study, powder microscopy, physicochemical parameters and leaf constants revealed valuable data that enables to set up standards for the plant. Preliminary phytochemical screening revealed the presence of carbohydrates, alkaloids, tannins, phenolics, mucilage, flavonoids. Microscopical study revealed the presence of unicellular and multicellular covering trichomes, rosette shaped calcium oxalate crystals, anomocytic stomata, straight walled epidermis, bicollateral vascular bundle and pitted xylem vessels. **Conclusion**: The present pharmacognostic study furnishes valuable referential information that enable the identification, authetication and standardization of *P. macrocarpa*. The study also facilitates the monograph preparation of *P. macrocarpa*, ensuring the safety and efficacy of the crude drug.

Keywords: Mahkota Dewa, Microscopic Study, Pharmacognostic Study, Phytochemical Screening, Fluorescence Analysis, Physicochemical Analysis.

INTRODUCTION

Medicinal plants have proven as worldwide cornerstone of traditional medicine systems for centuries. They offer a huge array of natural compounds with potential therapeutic benefits for treating as well as managing various health conditions and diseases [1]. *P. macrocarpa*, also known as Buah mahkota dewa or "God's crown fruit, of the family Thymelaeaceae holds significant importance in traditional medicine across Malaysia and Indonesia. Its various parts, including the leaves, fruits, seeds, stem boast a wide array of medicinal properties that have been utilized for generations [2].

It is an evergreen shrub or tree that attains a height of 1m to 18m, with a productive age ranging from 10 to 20 years and grows well above 10 to 1200 m above sea level. Unripe fruits are green in colour and become maroon on ripening [3]. It is ethno medicinally significant drug in Asian countries, especially Malaysia and Indonesia. The fruits of *P. macrocarpa* are traditionally employed in treating ailments such as flu, rheumatism, heart diseases and even cancer. Moreover, the eggshells of these seeds are utilized in countering a range of serious illnesses including lung disease, liver ailments, breast cancer, cervix cancer and heart diseases.

Meanwhile, the leaves are utilized for addressing conditions like dysentery, blood diseases, allergies, tumors, diabetes and impotence. Additionally, the stems are valued for their potential in the treatment of bone cancer [4]. Various parts of *P. macrocarpa* contain phytoconstituents such as palmitic acid, dodecanoic acid, mahkoside A, flavicordin A, flavicordin D, des- acetyl flavicordin- A, flavicordin- A

glucoside, ethyl stearate, alkaloids, saponins, lignans and sucrose [5].

In spite of its enormous medicinal attributes, no prior reports on pharmcognostical and physicochemical profile of *P. macrocarpa* are available. Taking this into account, the present investigation was taken over to establish the pharmacognostic, physicochemical and phytochemical screening profile of *P. macrocarpa* as per WHO guidelines, which could be useful in preparing monograph of the plant and prevent intentional or unintentional adulteration.

MATERIAL AND METHODS

Collection, Authentication and Preparation of Plant Material

Fresh aerial parts and fruits of *P. macrocarpa* were collected from Idukki District, Kerala and authenticated by Dr. Jacob Thomas, Herbariun Curator (PG & Research Dept. of Botany), Mar Thoma College, Thiruvalla. The voucher specimen (No. MTCHT 24/111) was deposited in the herbarium of PG & Research Dept. of. Botany, Mar Thoma College. The leaves and fruits were dried under shade, mechanically reduced to moderate coarse powder and stored for further studies. Fresh leaves were utilized for the macroscopical and microscopical evaluation whereas coarse powder was employed for determination of physicochemical parameters such as moisture content, Ash values, swelling index, foaming index, fluorescence analysis and preliminary phytochemical screening.

Chemicals, reagents and solvents

All chemicals, reagents and solvents utilized were of analytical grade.

Physicochemical parameters

Coarsely powdered air-dried leaves and fruits were subjected to physicochemical analysis. Evaluation of physicochemical characters is important parameter to detect improper handling or adulteration of crude drugs. Physicochemical parameters such as ash values, extractive values, moisture content, swelling index and foaming index of the leaves and fruits were determined [6,7].

Fluorescence analysis

Fluorescence analysis of the leaf and fruit powder was conducted. Small quantity of the powder was added with freshly prepared acidic, neutral and basic solvents for few minutes. There after it was kept inside a UV chamber and exposed to day light, short wavelength (254 nm) and long wavelength (365 nm) UV light [8,9].

Preliminary phytochemical screening

Air dried leaves and fruits were extracted with ethanol in a soxhlet apparatus by continuous hot percolation method [10,11]. Preliminary phytochemical screening of ethanolic extract of the leaves and fruits were conducted to detect the presence of various phytoconstituents such as alkaloids, glycosides, carbohydrates, flavonoids, proteins, saponins, tannins and mucilage.

Pharmacognostic studies [12,13]

Morphological evaluation

Organoleptic evaluation of plant material such as colour, odour, taste, shape, texture was performed. The exterior characteristics of *P. macrocarpa* fresh leaves were evaluated according to conventional procedure.

Microscopic studies

For microscopic studies, transverse sections of *P. macrocarpa's* fresh leaves were taken with the aid of sharp blades, thin sections were chosen, pigmentbs were removed by treating with 5% KOH solution, stained with phloroglucinol and conc. HCl. Then the sections were mounted, examined them under a microscope and photographed.

Powder Microscopy

Dried leaf powder was placed in a test tube, cleared with a 5% KOH solution, stained with phloroglucinol and conc. HCl, and then mounted the glass slide under microscope.

RESULTS

Physicochemical parameters

Various physicochemical evaluation parameters of *P. macrocarpa* leaves and fruit are given in the Table 1. Loss of drying of *P. macrocarpa* leaves and fruits was 6.5% w/w and 8.1% w/w respectively. Total ash value for the leaves was 17.00% w/w and fruits were 11.6% w/w. Swelling index of the leaves and fruits was 14.6 and 14.9 respectively and foaming index for both was less than 100.

Fluorescence analysis

The fluorescence characteristics of crude powdered drugs and various extracts were determined under UV radiation of long and short wavelengths and ordinary visible light. When the powdered drug and extracts were treated with different reagents and observed under UV and ordinary light, they emitted various colour radiations. The colour changes for crude powder and individual extract were distinctive and reproducible revealing the solvent properties of the phytoconstituents. Data is presented in the Table 2.

Preliminary phytochemical screening

Preliminary phytochemical screening of the ethanolic extracts of *P. macrocarpa* leaves and fruits is given in Table 3. Crude powder of *P. macrocarpa* leaves and fruits contain carbohydrate, mucilage, proteins, flavonoids, alkaloids, tannins, phenolic compounds and saponins. Alkaloids are found to be maximum in both leaves and fruits whereas tannins and phenolic compounds are found to be maximum in fruits.

Pharmacognostic studies

Morphological evaluation

P. macrocarpa unripe fruits are green colour which turns maroon on ripening. Shape of fruit is round or elliptical and becomes 3 to 5 cm broad on maturing. Leaves are simple, dark green and oppositely arranged. Size is 6-15 cm long, 3.5-6.5 cm broad with petiole of 0.5 cm long. Characteristic taste and odour were found.

Microscopic characters of *P. macrocarpa* leaf

Transverse section of P. macrocarpa leaf

Transverse section of the leaf is dorsiventral and shown in figure 2. It reveals upper and lower epidermis, covered with thick cuticle and numerous anomocytic stomata. Lignified multicellular cork like cells are seen below the lower epidermis along midrib region. Upper epidermis sparingly consists of unicellular covering trichomes. Midrib is elevated on upper and lower side and contains single meristele at the centre surrownded by schlerenchymatous band of pericycle. Vascular bundle is characteristic shaped which is bicollateral, closed and conjoint. Rosette shaped crystals of calcium oxalate are abundantly scattered throughout the spongy parenchyma. Lamina consists of mesophyll that is differentiated in to palisade cells and spongy parenchyma. Palisade cells consist of compactly arranged columnar cells with chloroplast.

Transverse section of Petiole

Transverse section of petiole is round lunette flattened on the upper surface with two winged projections. Outer region is wavy with multicellular lignified epidermal cells. Below the epidermis consists of large parenchymatous cells with abundant rosette shaped calcium oxalate crystals. Vascular bundle at the centre is arc shaped and srrownded by pericyclic layer.

Powder microscopy P. macrocarpa leaves

P. macrocapa leaf powder is light green and consist of rosette shaped crystals of calcium oxalate**,** anomocytic stomata with straight walled epidermal cells, unlignified long slender unicellular covering trichomes with pointed apex, short uniseriate multicellular covering trichomes and pitted xylem vessels.

Table 1: Physiochemical Parameters of *P. macrocarpa*

S. No.	Parameters	Leaves (%w/w)	Fruits (%w/w)
1	Total Ash value	17.0	11.6
2	Acid insoluble ash value	3.66	11.42
3	Water soluble ash value	13.00	12.5
4	Loss on drying	6.50	8.10
5	Swelling index	14.6	14.9
6	Foaming index	Less than 100	Less than 100

Table 2: Fluorescence analysis of *P. macrocarpa* Leaves and Fruits

S. No.	Treatment	Leaves		Fruits	
		UV 254 nm	UV 366 nm	UV 254 nm	UV 366 nm
1	Powder drug +ethanol	Light green	Da rk green	Yellowish brown	Light yellow
2	Powder drug +diethyl ether	Light green	Brownish yellow	Yellowish green	Light green
3	Powder drug+20%NaOH	Dark green	Black	Yellowish Brown	Fluorescent cream
4	Powder drug +50%HNO_3	Yellowish green	Black	Brownish green	Black
5	Powder drug +$FeCl_3$	Greenish Brown	Black	Dark green	Black
6	Powder drug+$CHCl_3$	Dark green	Black	Yellowish green	Brown
7	Powder drug +picric acid	Yellowish green	Black	Light green	Black
8	Powder drug + petroleum ether	Dark green	Yellowish brown	Greenish Brown	Fluorescent green
9	Powder drug + HCl	Light green	Black	Light green	Black

Table 3: Preliminary Phytochemical Screening of ethanolic extracts of *P. macrocarpa* Leaves and Fruits

S. No.	CHEMICAL TEST	Leaves	Fruits
1	**Test for reducing sugar**		
	Fehling's test	+	+
	Benedict's test	+	++
2	**Test for non-reducing sugar**		
	Iodine test	-	-
3	**Test for mucilage**		
	Powder drug swells in water or aq. KOH	+	++
4	**Test for proteins**		
	Biuret test	-	++
	Million's test	+	
5	**Test for amino acids**		
	Ninhydrin test	-	-
6	**Test for flavonoids**		
	Shinoda test	+	+
7	**Test for alkaloids**		
	Dragendorff's test	+++	++
	Mayer's test	+	++
	Wagner's test	+	++
8	**Test for tannins and phenolic compounds**		
	Lead acetate solution	++	+++
	Acetic acid solution	+	+
9	**Test for cardiac glycosides**		
	Legal's test	_	_
	Keller-killiani test	_	_
10	**Test for saponin glycosides**		
	Foam test	+	+

+++: Highly positive, ++: Moderately positive, +: Slightly positive, -: Negative

Table 4: Organoleptic features of *P. macrocarpa*

Characteristics	Observation	
Part	**Leaves**	**Fruits**
Arrangement	Opposite	-
Size	6-15 cm long 3.5-6.5 cm broad	3 to 5 cm broad
Shape	Elliptic to oblong lanceolate	Round / elliptical
Colour	Green	Green (unripe), Maroon (ripe)
Odour	Characteristic	Characteristic
Taste	Slightly sour and bitter	Bitter
Margin	Entire	-
Apex	Acuminate	-
Base	Symmetrical	-
Petiole	Petiolate	-
Texture	Glabrous	Rough
Venation	Reticulate	-
Outer surface	Smooth	Rough with vertical ridges

Figure 1: A: Aerial part of *P. macrocarpa* tree, B: Upper surface of *P. macrocarpa* leaf, C: Lower surface of *P. macrocarpa* leaf, D: Fruit of *P. macrocarpa*

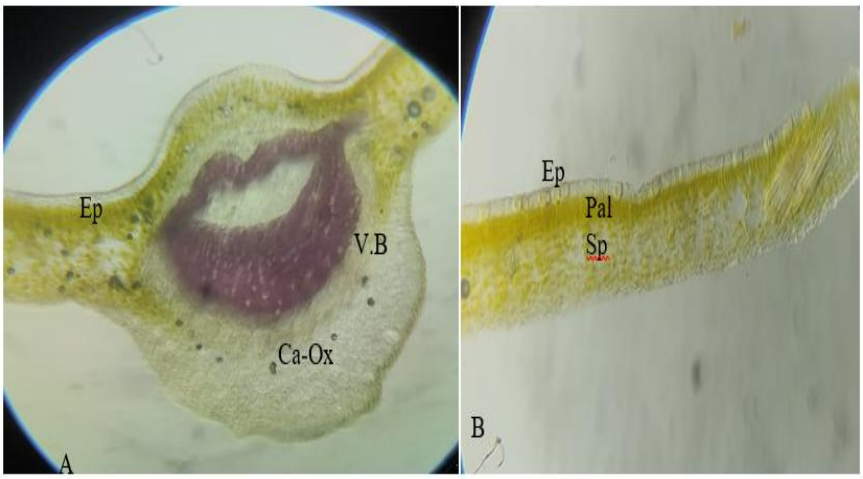

Figure 2: Transverse section of *P. macrocarpa* leaf
A: Transverse section of *P. macrocarpa* leaf, B: Transverse section of *P. macrocarpa* leaf lamina
Ep-Epidermis, V.B-Vascular bundle, Ca-Ox- Calcium oxalate, Pal-palisade cells, Sp-spongy parenchyma

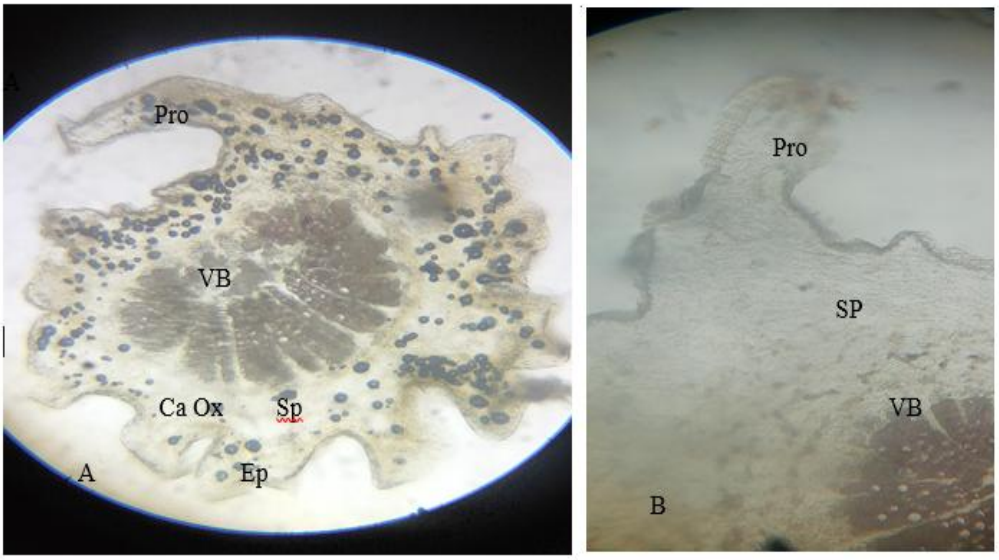

Figure 3: Transverse section of *P. macrocarpa* petiole
A: Transverse section of *P. macrocarpa* petiole, B: T.S of *P. macrocarpa* petiole showing protuberance
Ep-Epidermis, V.B-Vascular bundle, Ca-Ox- Calcium oxalate, Sp-spongy parenchyma

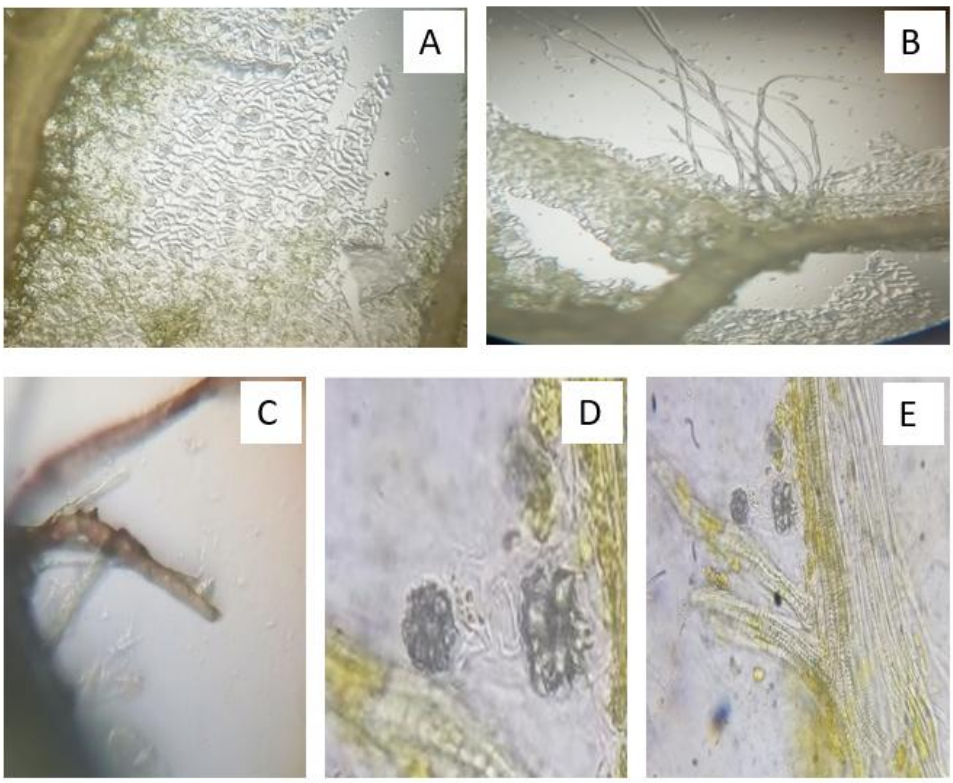

Figure 4: Powder microscopy *P. macrocarpa* leaves
A: anomocytic stomata with straight walled epidermal cells, B: unlignified unicellular covering trichomes,
C: multicellular covering trichomes, D: rosette shaped ca-oxalate crystals, E: pitted xylem vessels

DISCUSSION

Medicinal plants serve a critical role as vital source of drug discovery. Standardization of crude drugs plays a pivotal role to maintain efficacy of crude drugs. Various standardization parameters such as physicochemical analysis, phytochemical screening, fluorescence analysis, macroscopic, microscopic study and powder analysis of *P.macrocapa* was included. All these evaluation parameters will ascertain and sustain purity, quality, efficacy of crude drugs. It will also avert adulteration and intentional or unintentional substitution of herbal drugs.

Evaluation of physicochemical parameters enable the identification of adulterants as well as improper handling of plant material [14]. The physicochemical parameters such as loss on drying, total ash, water soluble ash, acid insoluble ash, swelling index and foaming index were determined. Loss on drying of leaves was 6.5% and fruits was 8% that indicate efficient drying process of the plant material. Loss on drying is a salient parameter to measure drying efficiency of the plant material indicating the stability of the crude drug and effectiveness of the phyto constituents during storage. Proper drying inhibits the outgrowth of decay causing microorganisms [15].

The leaf and fruit extract was found to be rich in alkaloids, tannins and phenolics which indicate its biological activities. Alkaloids are reported to have antimicrobial [16], antiprotozoal [17] and antimicrobial activity [18]. Saponins are reported to possess antioxidant [19], antidiabetic [20], antimicrobial [21] and cytotoxic activity [22]. Flavonoids are reported to possess anticancer [23], antioxidant [24], antimicrobial [25] and anti-inflammatory activity [26].

Fluorescence analysis is a requisite parameter for standardization of crude drugs. Short ultraviolet wavelength rich light actively produces fluorescence so that natural substances which do not produce fluorescence in daylight may produce fluorescence in short ultraviolet wavelength. Thus, it serves as an important parameter for pharmacognostic evaluation [27]. The microscopic evaluation showed leaf midrib with a central bicollateral, conjoint,closed vascular bundle and lamina with palisade cells and spongy parenchyma. The leaf powder analysis revealed the presence of unicellular and multicellular covering trichomes, anomocytic stomata, rosette shaped calcium oxalate crystals and pitted xylem vessels.

Hence the data enlisted from present study may supplement information that aid in the identification, authentication and standardization of *P. macrocarpa*.

CONCLUSION

Physicochemical analysis, phytochemical screening, fluorescence analysis, macroscopic, microscopic study and powder analysis of *P. macrocapa* provided salient diagnostic features that will aid authentication and identification of *P. macrocapa* plant. The above parameters will serve as reference standard for the plant that help in monograph preparation. The studies also enable to maintain the quality and efficacy of formulations using *P. macrocapa*.

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Conflict of interest

The authors declared no conflict of interest.

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ORCID ID

Jeenu Joseph: https://orcid.org/0009-0005-7403-3109

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