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MORPHO-ANATOMICAL AND PHYSICOCHEMICAL EVALUATION OF *GARCINIA PEDUNCULATA* ROXB. EX. BUCH.-HAM.

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ABSTRACT: *Garcinia pedunculata* Roxb. ex. Buch.-Ham. (Family: Clusiaceae), is an evergreen potent medicinal plant with fluted trunk commonly known as Amlavethasa. It is used by various ethnic communities of North-East India to treat various ailments such as asthma, cough, jaundice, fever, dysentery and as a cardiotonic. The plant is considered to be containing much medicinal value and mature fruit, and tender leaves are also eaten raw and as vegetables by the local people. The plant has not been explored scientifically for its pharmacognostical details. Therefore, the study of morpho-anatomical characters, physicochemical analysis of *G. pedunculata* was undertaken to establish the pharmacognostic and phytochemical details about the plant. Macro and microscopical studies of leaf showed the presence of simple, petiolate leaf generally obovate-oblong, some elliptic and oblong with obtuse and sub-acute tip and paracytic stomata. Bicollateral vascular bundle covered with sclerenchymatous fibers in leaf and 8-10 layer of collenchyma, scattered pericyclic fiber, the arrangement of phloem in the ring form in stem are some diagnostic features noted from the anatomical study of the plant. Powder microscopy revealed the presence of palisade parenchyma with the epidermis, parenchyma fibers, and scalariform vessels. HPTLC fingerprinting of plant tried with solvent system chloroform and methanol (8:2) confirmed the presence of 09 spots with different R_f value under UV light 366λ.The phytochemical evaluation revealed the presence of carbohydrate, glycoside, saponins, and phenolic compound.

INTRODUCTION: *Garcinia pedunculata* Roxb. ex. Buch.-Ham (family: Clusiaceae) commonly known as "Amlavethasa" in India and "Taikor" in Bangladesh, is an evergreen tree with a fluted trunk and short spreading branches. It is available particularly in Assam, Arunachal Pradesh, and West Bengal regions of India. Traditionally, the plant has been used for many ailments such as chronic catarrh, asthma, cough, bronchitis, fever, dysentery and as a cardiotonic [1, 2]. The fruits of the plant have been using by the people of Assam as medicine to treat different types of stomach related diseases [3]. The pericarps of the fruits are extensively used in diet across the North Eastern states of India. Fruits are also used as a garnish for curry and in some of the folklore medicine in India and contain 2 - 3% garcinol [4].

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The plant is reported to possess various biological activities like antioxidant [5], anti-inflammatory [6], cardioprotective [7], hepatoprotective [8,] and anti-diabetic activity [9]. The phytochemical studies have shown the dried fruit rinds, and pericarp of *G. pedunculata* contains benzophenones, pedenculol, garcinol and cambogin [10]. However, available literature revealed that no detailed anatomical and physicochemical studies had been carried out on *G. pedunculata*; hence, the present investigation was under taken. The object of the present study is to evaluate pharmacognostical and physicochemical parameters of *G. pedunculata*; which will assist in standardization, and it can also be used to prepare a monograph for the proper identification of the plant.

MATERIALS AND METHODS:

Plant Material: *Garcinia pedunculata* plants were collected from homestead garden of Bamfar village, Kamrup (M) District, Assam, India in April 2017. The specimen was identified by Taxonomist, TERI - Northeastern Regional Centre, Guwahati and later specimen was confirmed in BSI, Shillong and voucher specimen was deposited in herbarium section of TERI -Guwahati for future reference.

Morpho-anatomical Evaluation: Fresh plant of *Garcinia pedunculata* **Fig. 1** was taken for morphological and anatomical study. Various organoleptic and morphological characters of *G. pedunculata* leaves like color, shape, size, apex, margin, *etc*. were studied. For the anatomical studies, freehand transverse sections (T. S.) of the leaves, petiole, and stem were prepared using a razor blade. Lignified, cellulosic and other identifying features were studied by staining the sections with 0.1% w/v phloroglucinol followed by concentrated hydrochloric acid [11, 12]. The stained sections were observed under the microscope. Photomicrographs of all the sections in different magnifications were taken with Olympus digital microscope assisted with 1/3" CCD Sony camera.

Physicochemical Analysis: In this study, shade dried plant material was used for the quantitative determination of physicochemical parameters such as foreign matter, loss on drying, total ash, acid insoluble ash, water soluble ash, extractive values were determined according to the well established official method and recommended procedures [13, 14].

Powder Microscopy: The dried aerial part of *G. pedunculata* was powdered and studied under the microscope. The powder was macerated in chloral hydrate reagent. The macerated powder was then stained with phloroglucinol, iodine reagents separately. Small quantities of the various stained powders were mounted on a slide with glycerin and examined under microscope [14]. Photomicrographs of the different cellular structures and inclusions were taken.

Preliminary Phytochemical Screening: Preliminary screenings of methanol extract of *Garcinia pedunculata* were carried out using the standard procedure [14].

Fluorescence Analysis: Fluorescence study of leaf and fruit powder was performed as per reported standard procedure [15]. A small quantity of each powder was placed on a grease-free clean microscopic slide, and 1-2 drops of the freshly prepared reagent solution were added, mixed by gentle tilting the slide and waited for 1-2 min. Then the slide was placed inside the UV chamber and observed in visible light, short ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded.

HPTLC Profile: For proper meaningful utilization it is important to have quality standards of material, and for this quality standardization high-performance thin layer chromatography (HPTLC) fingerprint profile of methanolic extract of *G. Pedunculata* (10 µl of 1 mg/ml) was developed. The HPTLC analysis was carried out on precoated Silica gel on a 60-F254 plate (Merck, India) with the help of Camag Linomat -IV applicator. The plate was eluted with chloroform: methanol (80:20) as mobile phase. After development, the plate was dried and densitometrically scanned on a TLC scanner III at 366 nm using Wincat software (CAMAG, Switzerland) and peak area was recorded.

RESULTS:

Macroscopic Characters: Macroscopically, the fresh leaf of *G. pedunculata* is 20 to 23.5 cm long, 9.5 to 12.5 cm width and petiole 1.8 to 2.6 cm long, some variation occur in shape of leaf, *i.e.* generally obovate, some elliptic and oblong in shape with

obtuse and sub-acute leaf tip, cuneate at base and green in color. Midrib stout, prominent and lateral veins are distinct **Fig. 1**. The fruit is globose, yellow when ripe and 4 - 8 seeded **Fig. 2**.

FIG. 1: MACROSCOPIC CHARACTERISTICS OF G. *PEDUNCULATA* ROXB. LEAF

FIG. 2: *G. PEDUNCULATA* FRUIT BEARING PLANT

Microscopic Characters:

Leaf Microscopy: Transverse section (T.S.) of leaf passing through midrib region shows dorsiventral in shape. The upper surface of the leaf consist of rounded to ploygonal thin walled two celled epidermis covered with thin cuticle without any trichomes. Lower epidermis is single-celled but the cells are more spherical compared to the upper epidermis. Mesophyll composed of palisade and spongy parenchyma. Single layered palisade cells are small, elongated and compact in the laminar region followed by several layer of spongy parenchyma. Numerous secretary cells and few vascular strands are seen. Collenchyma appears below the upper epidermis and above the lower epidermis. Midrib region show large bicollateral vascular bundle arranged in fetal shaped covered with patches of sclerenchymatous fibres. Xylem is surrounded by phloem and consists of vessels, tracheids, fibers and xylem parenchyma **Fig. 3**.

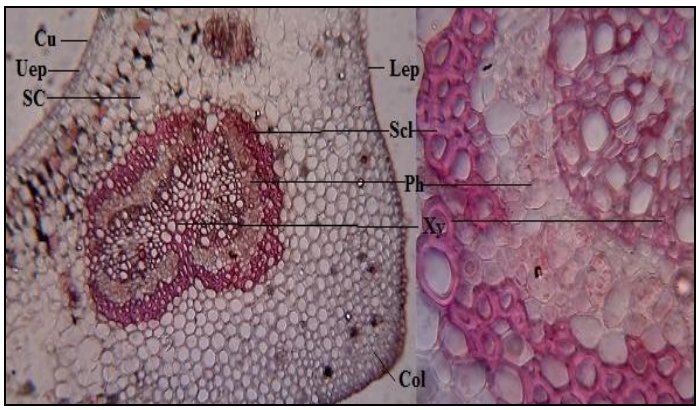


FIG. 3: T. S. OF *G. PEDUNCULATA* LEAF. (Uep: Upper epidermis; Lep: Lower epidermis; Cu: Cuticle; Col: collenchymas; SC: secretary cells; Scl: sclerenchymatous fibers; Xy: Xylem; Ph: Phloem).

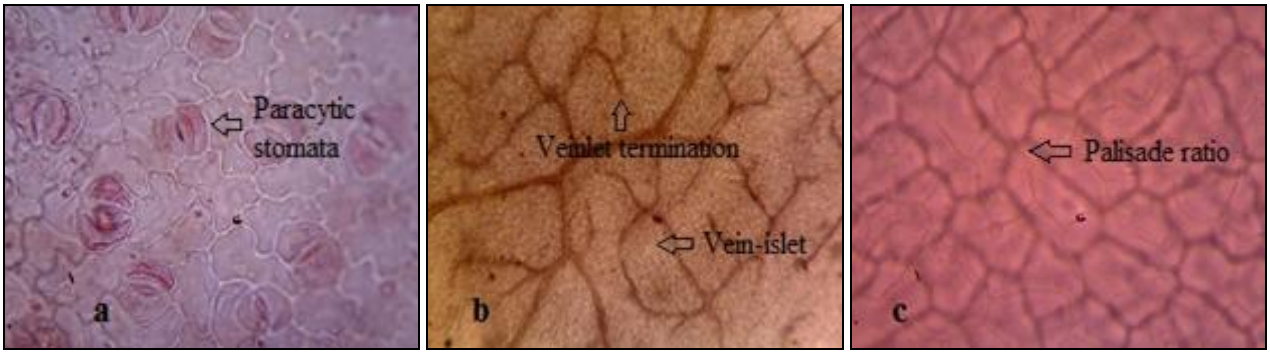


FIG. 4: LEAF SURFACE OF *G. PEDUNCULATA*. a: Paracytic stomata; b: Vein-islet and Veinlet termination; c: Palisade cells

Lower leaf surface of *G. lanceifolia* shows paracytic stomata **Fig. 4a**, while stomata absent in upper leaf surface. Leaf surface also show the presence of veins, vein islets, vein terminations **Fig. 4b** and palisade cell **Fig. 4c**. Leaf constant such as stomata number, stomatal index, vein islets number, vein termination number were measured. The results are shown in **Table 1**.

TABLE 1: LEAF CONSTANTS OF *GARCINIA PEDUNCULATA* (AT 100X)

S. no.	Parameters	Value (in 1 mm^2 area)
1	Stomata number, Upper surface	Nil
2	Stomata number, Lower surface	46
3	Stomatal index, Upper surface	Nil
4	Stomatal index, Lower surface	19.82
5	Vein-islet number	2.0-3.0
6	Veinlet termination number	5.0-6.0
7	Palisade ratio	4.0-6.0 (per cell)

Petiole Microscopy: T.S. of the petiole is somewhat circular; single layered epidermal cells covered with thick cuticle; ground tissue collenchymatous. Vascular tissue is arranged in a continuous ring form in the center of the petiole. Group of pericyclic fibers is seen in the vascular bundle region. Vascular bundle composed of metaxylem, protoxylem, and phloem **Fig. 5**.

Stem Microscopy: T.S. of the stem is almost circular in outline. Single layered and thin-walled epidermis covered with thin cuticle; hypodermis collen-chymatous followed by 8-10 layer of collenchyma; cortex parenchymatous and pericyclic fibers are present in scattered form. Phloem well developed in the form of ring and consist of sieve tubes, companion cells, and phloem parenchyma.

Xylem is present in continuous form ring and consists of vessels, tracheids, fibers, and xylem parenchyma; vessels are in radial rows. Medullary rays are distinct; center portion occupied by collenchymatous pith. The secretory cells are concentrated below the collenchymas **Fig. 6**.

Physicochemical Parameter: The results of physicochemical parameters of leaf and fruit such as foreign matter, moisture content, ash values, and extractive values are presented in **Fig. 7**.

Powder Microscopic Characters: The powder plant material is greenish; showing fragments of palisade and spongy parenchyma with epidermis **Fig. 8a** parenchyma fibers **Fig. 8b** vessels are having scalariform thickening **Fig. 8c**.

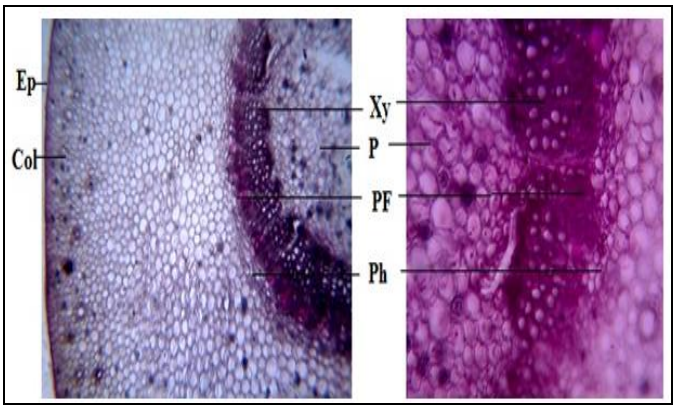


FIG. 5: T. S. OF *G. PEDUNCULATA* PETIOLE
Ep: Epidermis; Col: collenchymas; PF: Pericyclic Fibre; P: Pith; Xy: Xylem; Ph: Phloem

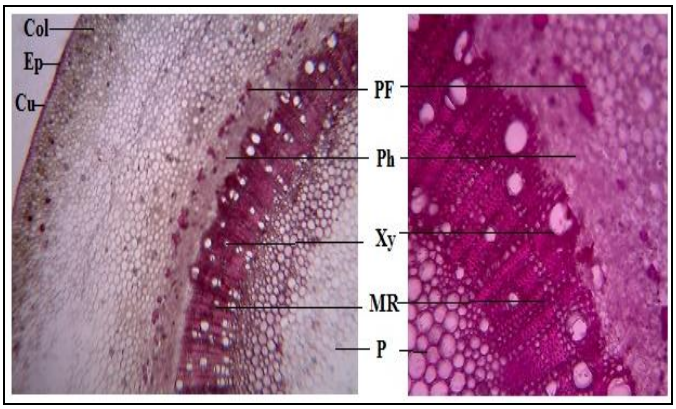


FIG. 6: T. S. OF *G. PEDUNCULATA* STEM
Cu: Cuticle; Ep: Epidermis; Col: collenchymas; PF: Pericyclic Fibre; MR: Medullary rays; Xy: Xylem; Ph: Phloem; P: Pith

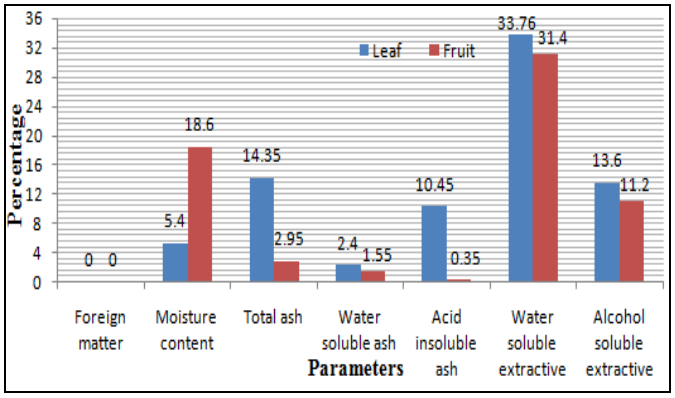


FIG. 7: RESULTS OF PHYSICOCHEMICAL PARAMETERS OF *G. PEDUNCULATA*

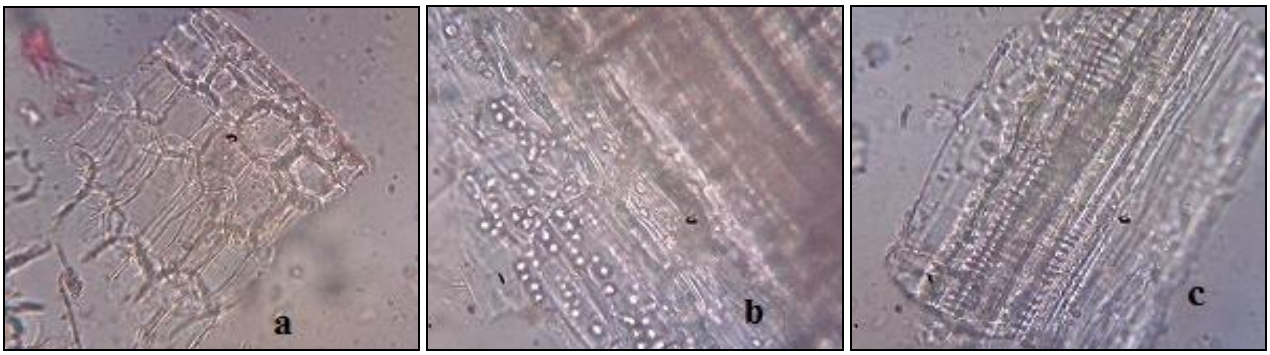


FIG. 8: POWDER CHARACTERISTICS OF *G. PEDUNCULATA* LEAF

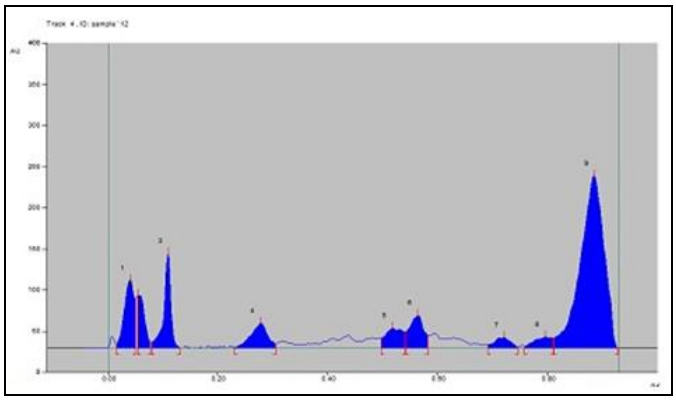
Preliminary Phytochemical Screening: Preliminary phytochemical screening mainly revealed the presence of carbohydrate, glycoside, saponins, phenolic compound, and fixed oil and fat.

Fluorescence Analysis: The fluorescence characteristics of the leaf and fruit powder with different chemical reagents are summarized in **Table 2**.

TABLE 2: FLUORESCENCE ANALYSIS OF *G. PEDUNCULATA*

Treatment	Observation under daylight		Observation under U.V. light (254 nm)	
	Leaf	Fruit	Leaf	Fruit
Powder as such	Pale green	Light brown	Olive green	Olive
Powder + 1N NaOH (aq.)	Mustard	Brown	Green	Dark green
Powder + 1N NaOH (alc.)	Mustard	Brown	Green	Green
Powder + 1N HCl	Light mustard	Brown	Green	Green
Powder + HNO_3 (1:1)	Yellowish brown	Brown	Green	Greenish brown
Powder + H_2SO_4 (1:1)	Mustard	Brown	Green	Greenish brown

HPTLC Profile: A densitometric HPTLC analysis was performed for the development of specific fingerprint profile which may be used as a marker for quality evaluation and standardization of the drug.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.02 Rf	4.5 AU	0.05 Rf	81.5 AU	3.87 %	0.06 Rf	0.0 AU	253.6 AU	9.27 %	unknown *
2	0.06 Rf	3.5 AU	0.06 Rf	63.8 AU	0.86 %	0.09 Rf	7.0 AU	692.3 AU	5.12 %	unknown *
3	0.09 Rf	7.1 AU	0.13 Rf	115.2 AU	9.59 %	0.15 Rf	0.7 AU	201.8 AU	8.89 %	unknown *
4	0.27 Rf	0.8 AU	0.32 Rf	30.0 AU	5.11 %	0.35 Rf	4.8 AU	752.2 AU	5.56 %	unknown *
5	0.58 Rf	2.1 AU	0.60 Rf	23.6 AU	4.01 %	0.63 Rf	9.6 AU	629.9 AU	4.66 %	unknown *
6	0.63 Rf	9.6 AU	0.65 Rf	39.5 AU	6.72 %	0.68 Rf	5.4 AU	841.7 AU	6.22 %	unknown *
7	0.80 Rf	4.1 AU	0.84 Rf	12.3 AU	2.10 %	0.87 Rf	1.1 AU	307.1 AU	2.27 %	unknown *
8	0.88 Rf	2.5 AU	0.92 Rf	13.2 AU	2.24 %	0.94 Rf	2.0 AU	357.8 AU	2.65 %	unknown *
9	0.94 Rf	2.2 AU	1.03 Rf	108.7 AU	15.51 %	1.08 Rf	0.2 AU	487.8 AU	5.37 %	unknown *

FIG. 9: HPTLC PROFILE OF *G. PEDUNCULATA*. Solvent system: Chloroform: Methanol (8:2), Detection: Under UV light λ 366 nm

The preliminary HPTLC studies revealed that the solvent system chloroform: methanol (8:2) was ideal for the alcoholic extract and gave well-resolved peaks of crude extract of *G. lanceifolia* **Fig. 9**.

DISCUSSION: Proper identification plant species is a major concern for users and industry for reasons of safety and efficacy. Therefore, authentication of medicinal plants is of utmost importance. Morphological, anatomical, physico-chemical, and chromatographic fingerprinting solves the problem by differentiating the genuine material from the adulterants, substitutes, and spurious drugs. Therefore, the present study reports the morpho-anatomical characters, physiochemical, and chromatographic profile of *G. xanthochymus*. The presence of paracytic stomata, secretory cells, the arrangement of bicollateral vascular bundle in fetal shape in leaf and presence of xylem and phloem in the form of a ring in stem are the some of the diagnostic features noted from the anatomical study of the plant.

Ash values and extractive values can be used as a reliable aid for detecting adulteration. These studies help in the identification of the plant materials. Extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in the estimation of specific constituents soluble in particular solvents [16], [17]. The extractive values obtained revealed that the majority of the chemical constituents were water-soluble, not alcohol soluble. The moisture content of a drug should be minimized to prevent decomposition of crude drugs either due to chemical change or microbial contamination [18].

The result of moisture content indicates the presence of an appreciable quantity of water in *G. pedunculata* fruit. Preliminary phytochemical analysis indicated the presence of glycoside, saponins, phenolic compound, fixed oil, and fat. The fluorescent analysis under daylight and UV light by treatment with different chemical reagents showed a different color. This analysis suggests that leaves and fruit extract of *G. pedunculata* probably contain active agent(s) and this provides the basis for their folkloric use as a cure for some human ailments. HPTLC fingerprint profile along with their R_f values was recorded, which would serve as a reference standard for the scientist engaged in research on the medicinal properties of the plant.

CONCLUSION: In conclusion, the data generated from this study would help in the development of pharmacopoeial standards and prevent adulteration *G. pedunculata* leaves and fruit. Further, this investigation will provide valuable information to the researchers to establish the pharmacological activities supported by the possible mode of action.

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CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest.

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