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Pharmacognostic study and establishment of quality parameters of *Garcinia xanthochymus* (Gamboge)

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Abstract
Garcinia xanthochymus Hook. f. ex. T. Anderson (Clusiaceae) commonly known as gamboge, is a fruit yielding perennial medicinal plant native to South East Asia. It is used in treatment of various disorders by folk healers. The plant has been scientifically evaluated for various biological activities like antimicrobial, cytotoxic, anti-inflammatory, antioxidant, antidiabetic and nerve growth factor potentiating activity. The present investigation deals with morpho-anatomical evaluation and establishment of its quality parameters, including physicochemical and chromatographic profile of *G. xanthochymus*. Macroscopically, leaf is simple, petiolate, linear-oblong in shape with acute apex and cuneate base. The presence of paracytic stomata, arc shaped xylem in leaf, crescent shape bicollateral vascular bundle in petiole and arrangement of xylem in ring form in stem are some of the diagnostic features noted from anatomical study of the plant. Powder microscopy of leaf revealed the presence of palisade cells, sclerenchymatous fibres and vessels with annular and reticulate thickenings. Phytochemical screening mainly revealed the presence of carbohydrate, glycoside, favonoid and tannins. HPTLC finger printing of plant with solvent system toluene: ethyl acetate: formic acid (7:5:1) confirmed the presence of 06 spots with different R_f value under UV light 366λ

Keywords: *Garcinia xanthochymus;* morphoanatomical; pharmacognosy; physicochemical; standardization

1. Introduction
Garcinia xanthochymus Hook. f. ex. T. Anderson (Clusiaceae) commonly known as gamboge, is a perennial medicinal plant native to South East Asia. Plant is widely used as a traditional folk medicine for bilious condition, diarrhea, dysentery, anthelmintic, cardiotonic and as a tonic to improve appetite [1, 2, 3, 4]. In traditional Chinese Dai medicine, it is used for expelling worms and removing food toxins [5]. Ripe fruits of plant are extensively used in making jams, sherbet, curries beverages and flavoring in other foods [6, 7]. The sap is used as a watercolors and yellow fabric dye [8]. Plants are rich sources of xanthones, biflavonoids and benzophenones [9, 10]. These constituents have been reported to possess several biological activities, such as antimicrobial, antimalarial, cytotoxic, anti-inflammatory, antioxidant, antidiabetic and nerve growth factor potentiating activity [4]. Owing to its ethnopharmacological importance, the present investigation has been undertaken with an objective to establish morphoanatomical, powder microscopical, physicochemical and chromatographic characteristics of *G. xanthochymus* so that authentic plant material could be explored for its therapeutic claim.

2. Materials and Methods
Chemicals
All the chemicals and solvents used for the study were of analytical grade and procured from SD Fine-Chem Ltd, Mumbai, India. For HPTLC, precoated TLC plates were purchased from Merck, India.

Collection of plant material
Fresh aerial parts of the *G. xanthochymus* were collected from homestead garden of Balipukhuri village, Sonitpur district, Assam, India in the month of May 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 *G. xanthochymus* was taken for morphological and anatomical study. Various organoleptic and morphological characters of *G. xanthochymus* leaves like colour, shape, size, apex, margin etc. were studied. For the anatomical studies, free hand transverse sections (T.S.) of the leaf, petiole and stem were prepared using razor blade. The thin sections were stained with phloroglucinol followed by hydrochloric acid in the ratio of 1:1. The stained sections were observed under microscope [11, 12]. 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, air dried plant material was used for 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. xanthochymus* 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 [12]. Photomicrographs of the different cellular structures and inclusions were taken.

Preliminary phytochemical screening

Ethanolic extract of *G. xanthochymus* were subjected to various chemical test as per standard method to determine the nature of chemical constituents present in the plant [12].

HPTLC profile

For proper meaningful utilization it is important to have quality standards of materials and for this quality standardization, high performance thin layer chromatography (HPTLC) finger print profile of methanol extract of *G. xanthochymus* (10 µl of 1mg/ml) was developed. The HPTLC analysis was carried out on percoated Silica gel on 60-F_{254} plate (Merck, India) with the help of Camag Linomat -IV applicator. The plate was eluted with toluene: ethyl acetate: formic acid (7:5:1) 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.

3. Results

Macroscopic characters

Macroscopically, the fresh leaf of *G. xanthochymus* is green, simple, petiolate, linear-oblong in shape with acute apex and cuneate base (Fig. 1). Leaf is 20 to 28 cm long, 5 to 7 cm width and petiole 1.5 to 2.5 cm long. Fruit is subglobose, yellow in colour when ripe and 1-4 seeded.

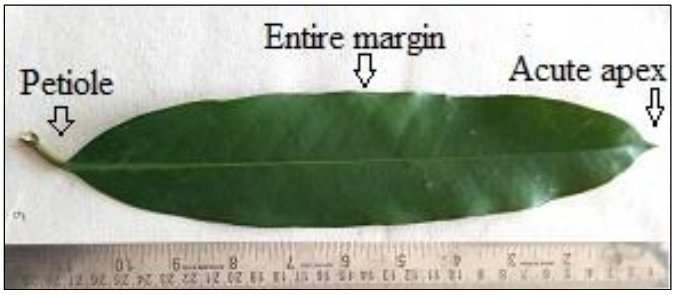


Fig 1: Macroscopic characterstics of *G. xanthochymus* leaf

Fig 2: *G. xanthochymus* fruit bearing plant

Microscopic characters

Leaf microscopy

T.S. passing through midrib region shows dorsiventral in shape. Upper and lower surface of the leaf consist of single layer small more or less rectangular thin walled epidermis covered with smooth cuticle. Trichomes are absent. Palisade is made up of single layer beneath upper epidermis and contains compact elongated cells. Spongy mesophyll is 6-7 layered loosely arranged with intracellular spaces. Midrib shows presence of collenchyma below the upper epidermis and above the lower epidermis. Large vascular bundle is covered with pericyclic fibres. Xylem is arranged in arc shaped and surrounded by phloem. Lower leaf surface shows paracytic stomata. Few cystolith seen in parenchyma region (Fig. 3).

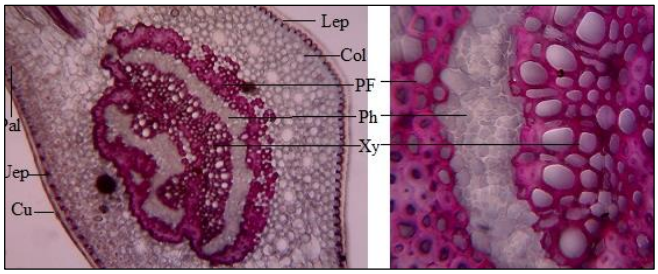


Fig 3: T.S. of *G. xanthochymus* leaf

(Uep: Upper epidermis; Lep: Lower epidermis; Cu: Cuticle; Pal: Palisade cells; Col: Collenchymas; PF: Pericyclic fibres; Xy: Xylem; Ph: Phloem)

Petiole microscopy

T.S. of petiole is circular in shape. Single layered wavy epidermal cell is covered with thick cuticle. Wide region of cortex is occupied by parenchymatous cells. Bicollateral vacular bundle is arranged in crescent form in the center of petiole. Vascular bundle composed of metaxylem, protoxylem and phloem (Fig. 4).

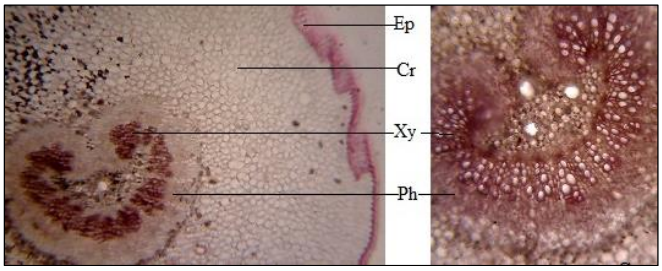


Fig 4: T.S. of *G. xanthochymus* petiole (Ep: Epidermis; Cr: Cortex; Xy: Xylem; Ph: Phloem)

Stem microscopy

T.S. of stem is almost circular with wavy outline. Single layered and thick walled epidermis covered with thick cuticle. Hypodermis is collenchymatous, which is arranged in 5-6 layer provide additional protection and support. The cortex is wide composed of parenchymatous cells with numerous secretary canals. Endodermis is not distinguishable clearly. The phloem is broad, well developed and continuous around the xylem circumference. The outer boundary of the phloem has a layer of sclerenchyma elements. Xylem is present in form continuous ring and consists of vessels, fibers and xylem parenchyma; vessels are in radial rows. Medullary rays are distinct, single seriated; centre portion occupied by collenchymatous pith (Fig. 5).

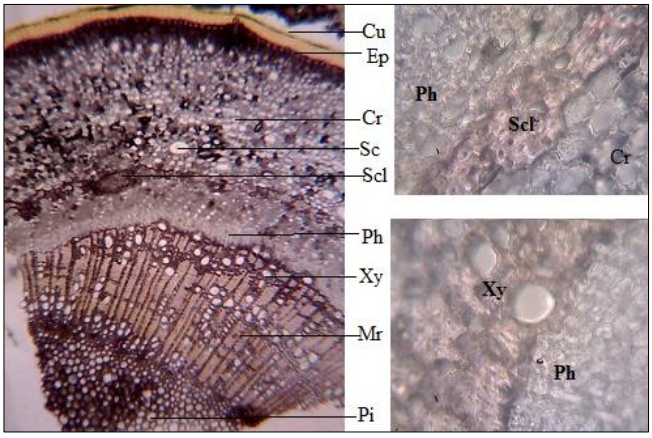


Fig 5: T.S. of *G. xanthocymus* stem

(Cu: Cuticle; Ep: Epidermis; Cr: Cortex; Sc: Secretary canals; Scl; Sclerenchymatous fibre; Mr: Medullary rays; Xy: Xylem; Ph: Phloem; Pi: Pith)

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. 6.

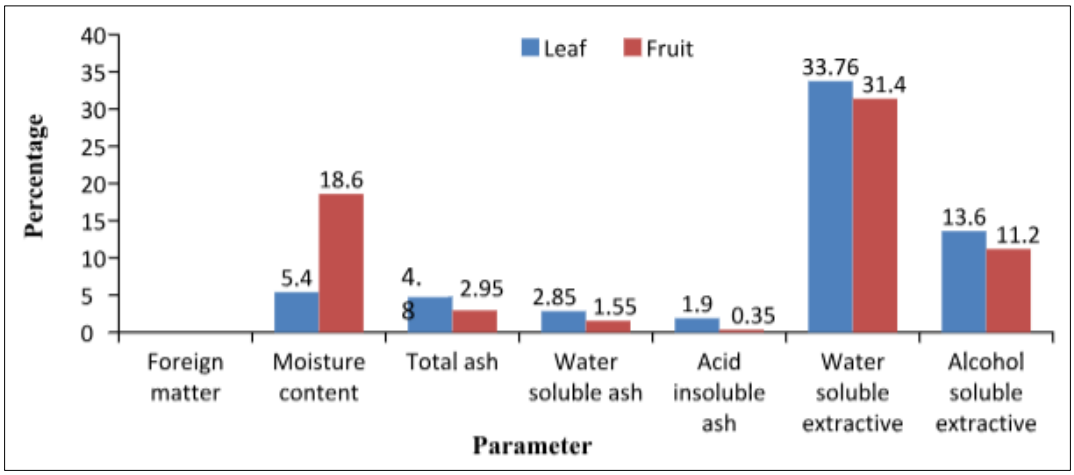


Fig 6: Results of physicochemical parameters of *G. pendunculata*

Powder microscopic characters

The powder plant material is greenish in color; showing paracytic stomata (Fig. 7a), fragments of epidermal cells-irregularly beaded walls seen in surface view (Fig. 7b), layer of palisade cells followed by loosely bound spongy cells (Fig. 7c), vessels with annular and reticulate thickenings (Fig. 7d), parenchymatous cells (Fig. 7e) and sclerenchymatous fibres (Fig. 7f).

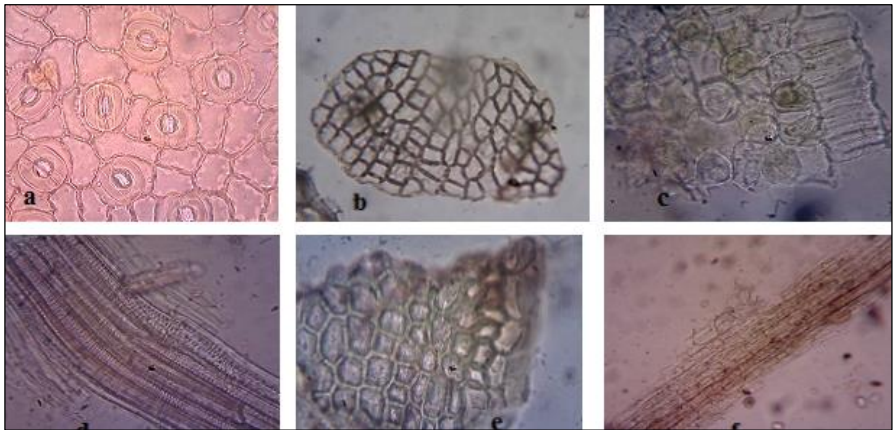


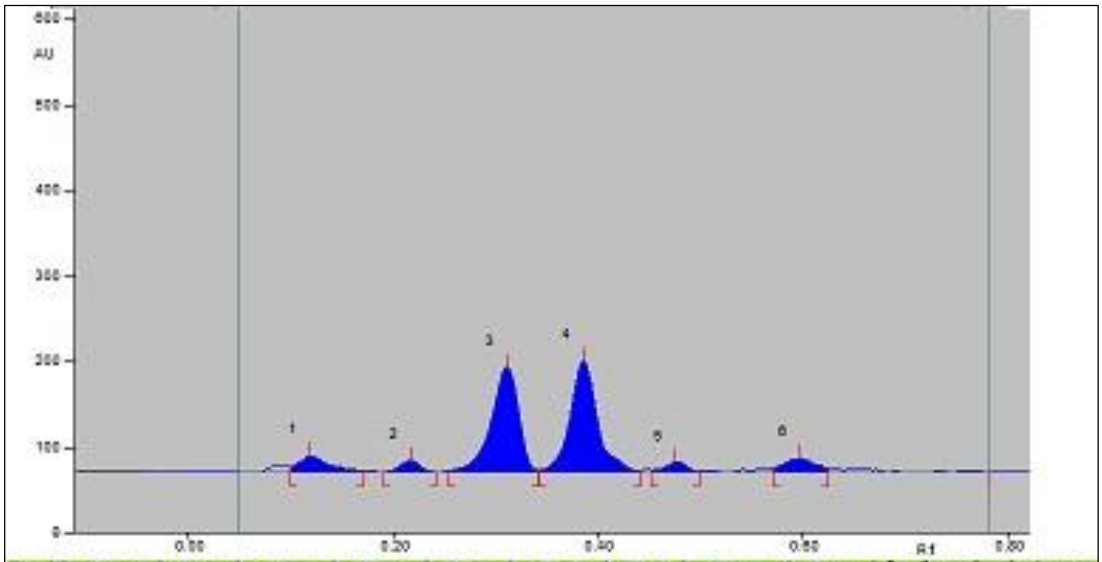
Fig 7: Powder characteristics of *G. xanthochymus* leaf

Preliminary phytochemical screening

Preliminary phytochemical screening mainly revealed the presence of carbohydrate, glycoside, favonoid, tannins and triterpenoid.

HPTLC profile

A densitometric HPTLC analysis was performed for the development of specific finger print profile which may be used as marker for quality evaluation and standardization of the drug. The preliminary HPTLC studies revealed that the solvent system toluene: ethyl acetate: formic acid (7:5:1) was ideal for the methanolic extract and gave well resolved peaks of crude extract of *G. xanthochymus* leaves (Fig. 9).



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.10 Rf	6.2 AU	0.12 Rf	19.1 AU	6.11 %	0.17 Rf	1.6 AU	467.8 AU	6.70 %	unknown *
2	0.19 Rf	0.1 AU	0.22 Rf	13.1 AU	4.21 %	0.24 Rf	0.2 AU	217.5 AU	3.12 %	unknown *
3	0.25 Rf	2.1 AU	0.31 Rf	23.0 AU	9.39 %	0.34 Rf	2.6 AU	763.9 AU	19.59 %	unknown *
4	0.34 Rf	2.8 AU	0.39 Rf	30.1 AU	11.64 %	0.44 Rf	1.1 AU	916.9 AU	11.78 %	unknown *
5	0.45 Rf	2.3 AU	0.48 Rf	11.3 AU	3.61 %	0.50 Rf	1.3 AU	211.1 AU	3.02 %	unknown *
6	0.57 Rf	4.7 AU	0.60 Rf	15.8 AU	5.05 %	0.63 Rf	4.2 AU	403.8 AU	5.78 %	unknown *

Fig. 9 HPTLC profile and densitometric scanning of methanolic extract of *G. xanthochymus* Solvent system: Toluene: Ethyl acetate: Formic acid (7:5:1), Detection: Under UV light λ 366 nm

4. Discussion

Owing to the wide use of leaves and fruit of *G. xanthochymus* in traditional medicines, standardization becomes an important measure for ensuring quality, purity and authenticity of the plant materials. First step in this regard is the authentication of plant species. For this purpose, morpho-anatomical study is one of the simplest steps for establishing the correct identification of the plant materials [15, 16]. As there is no detailed anatomical work reported on this medicinally potent plant, so the present study reports the morpho-anatomical characters of leaf, petiole and stem of *G. xanthochymus*. The presence of paracytic stomata, arc shaped xylem surrounded by phloem capped with pericyclic fibers in leaf, crescent shape bicollateral vascular bundle in petiole and presence of xylem in form of continuous ring in stem are the some of the diagnostic features noted from anatomical study of plant. Physicochemical parameters of plant like moisture content, ash and extractive values acts as reliable tool for detecting adulteration [17]. 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 indicating the presence of appreciable quantity of water in *G. xanthochymus* fruit. The extractive values give an idea about the chemical constitution of the drug and also help in estimation of specific constituents soluble in particular solvents [19]. The extractive values of leaves revealed that majority of the chemical constituents were alcohol soluble while in fruit water soluble. Ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with drug. Preliminary phytochemical analysis indicated presence of mainly glycoside, flavonoid, and tannins. HPTLC fingerprint profile along with their R_f values were recorded, which would serve as a reference standard for the scientist engaged in research on the medicinal properties of plant.

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

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