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Research Article

PHARMACOGNOSTICAL, SEM AND XRF PROFILE OF THE LEAVES OF *Artocarpus heterophyllus* Lam. (Moraceae) – A CONTRIBUTION TO COMBAT THE NTD

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ABSTRACT

Objective: To study in detail the micromorphology including Scanning Electron Microscopy and phyto, physicochemical analysis along with determination of trace elements by X-Ray Fluorescence spectrometer of the leaves of *Artocarpus heterophyllus* family-Moraceae which possesses various bioactive components and many traditional and pharmacologically validated uses in the treatment of many diseases including NTD.
Methods: Macroscopy, microscopy including SEM, physicochemical analysis, preliminary phytochemical screening, XRF and other WHO recommended parameters for standardizations were performed.
Results: Leaves are Obovate elliptic – elliptic, alternate 5-25cm, Width 4-12 cm broad. Dark green with entire margin, cuspidate apex and symmetrical base with ridge and furrowed petiole. Microscopic evaluation revealed the presence of anomocytic stomata in lower epidermis and apostomatic upper epidermis, unicellular trichomes which are arised from thick walled circular, lignified ring of ten radially elongated epidermal rosette cells, xylem vessels, phloem and fibres. SEM of midrib showed many folded appearance. No diagnostic feature and new kind of microcostituents not previously recognized and apparently simple structure which may be extremly complex was observed. Identification of inorganic minerals of the leaves of *A.heterophyllus* by XRF showed the presence of minerals Calcium (39.4%), Potassium (29.6%), Magnesium (2.06%), Manganese (0.13%), Chlorine (2%) and Iron (0.99%).
Vein islet numbers, vein termination numbers, stomatal number, stomatal index and other physico chemical tests like ash values, loss on drying, extractive values were determined. Preliminary phytochemical screening showed the presence of sterols, tannins, proteins and aminoacids, flavonoids, terpenoids, mucilage, saponin, carbohydrates and absence of alkaloids, glycosides, fixed and volatile oil.
Conclusion: The microscopic using histological identification, microscopic constants and other phyto, physico chemical examinations of the leaves of *Artocarpus heterophyllus* Lam. can be used as a rapid, inexpensive botanical identification technique and is useful in standardization, hence would be of immense value in authentication of the leaf as it proved to have wide panel of pharmacological and ethno medical use including prevention and treatment of NTD.

Keywords: *Artocarpus heterophyllus*, Moraceae, Microscopical evaluation, Scanning Electron Microscopy (SEM), X-Ray Fluorescence spectrometer (XRF).

INTRODUCTION

The prevalence of infectious disease in the tropics is of great public health problem, particularly as many diseases that are unique to the tropics and thus the funding for the development of effective drugs are insufficient. WHO estimates that nearly one billion people suffers from one or more of the identified NTDs, such as cholera, Buruli ulcer, cysticercosis, hydatidosis, dracunculiasis (guinea-worm disease), soil-transmitted helminthiasis, leishmaniasis, etc. without including the enormous toll of worldwide diseases such as HIV/AIDS and hepatitis C [1]. The economic, cultural, and social toll of these diseases is tremendous. Tropical humid climate facilitates the occurrences of many skin infections and other diseases [2].

The immune system present in plants is surprisingly similar to our own immune system and plants exhibit similar techniques of pattern recognisation in their encounters with bacterial, viral, fungal and parasitic infections [3, 4].

The *A.heterophyllus* is a species of tree of the mulberry family (Moraceae) is known by other names Jackfruit (Eng). [5]. Leaf is used for asthma, ring worm infestation, gallstones, abscesses, wound healing, antishyphillic, anthelmintic, lactogogue, ear ache, antiulcer, anticariogenic, adsorbent, antibacterial, anti-inflammatory, anemia, dermatitis, cough, diarrhea, fever, sedative and digestive[6-8]. The leaves used as hypoglycaemic [9, 10], α-amylase [11], Antioxidant and antibacterial [12], anticariogenic [13] and wound healing [8].

It was reported that fresh leaves contains: Four isoprenylated flavonoids artoheterophyllin A, B, C and D, together with 16 known compounds in the ethanol extract of the twigs of *A.heterophyllus* by HPLC method [14] and n-Octadec-enoyl derivatives [15]. In short, there is good level of traditional and experimental evidences to support various claims and advantages of this widely available plant. An investigation to explore its pharmacognostic examination is inevitable. Hence, in this work we report an attempt on microscopic evaluation, physicochemical determination and phytochemical screening for the standardization and quality assurance purposes of this cultivar.

MATERIALS AND METHODS

Chemicals

Formalin, acetic acid, ethyl alcohol, chloral hydrate, toludine blue, phloroglucinol, glycerin, hydrochloric acid and all other chemicals used in this study were of analytical grade.

Plant collection and authentication

The leaves of the healthy plant *Artocarpus heterophyllus* Lam. selected for our study was collected from Suchindram, Nagercoil District, Tamil Nadu, India during the month of August 2012 and was authenticated by Dr. Stephen, Department of Botany, American College, Madurai and Dr. P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai,Tamil Nadu, India.

Macroscopic analysis

Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc was noted [16].

Microscopic analysis

Transverse section midrib region of fresh leaf pieces were cut and fixed in FAA and then dehydrated by employing graded series of ethyl alcohol and tertiary butyl alcohol [17]. Sections were taken using microtome. Permanent mount was prepared using saffranin fast green double staining technique [18]. In order to supplement the descriptive part the photomicrographs in different magnifications of all necessary cells and tissues were taken with NIKON Coolpix 8400 digital camera and Labphot2 microscopic unit.

Powder microscopy

Coarse powder of the leaf was used to study the microscopical characters of the leaf powder [19, 20].

Physicochemical analysis

Total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying, extractive values and leaf constants such as vein islet numbers, vein terminal number, stomatal number and stomatal index, palisade ratio were determined [21].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure [22].

Scanning electron microscopic study

Scanning electron microscopy is a complementary technique and importance in pharmacognostic evaluation [23].

SEM sample preparation

Sample for SEM analysis were mounted on the specimen stub using carbon adhesive sheet. Small sample were mounted with 1sq. cm glass slide And kept in carbon adhesive sheet.. Samples were coated with gold to a thickness of 100 AO using Hitachi vacuum evaporator. Coated sample were analysed in a Hitachi Scanning electron Microscope 3000 H model.

Elemental analysis by XRF Spectrometer

We have quantitatively determined the trace elements present in the *A.heterophyllus* leaves by X-Ray flouresence spectrometer (XRF) which has the advantage generally being non-destructive, multi elemental, fast & cost effective [24, 25].

Preparation of solid sample

Mix equal volume of powder and binder pressed up to 30 ton made into pellet. The binder must be free from contaminant element and low absorption. It must stable under vacuum and irradiation conditions.

RESULTS

Macroscopy

Artocarpus heterophyllus is a large evergreen tree, 10-15m in height. Leaves broad (5-25 cm× 3.5-12 cm), alternate, dorsiventral, obovate elliptic – elliptic, decurrent, globular, entire and dark green in colour, cuspidate apex and symmetrical base with ridge and furrowed petiole. Fruit is succulent, flavourful and aromatic, syncarp with green to yellow brown exterior rind, stem straight, cylindrical, and covered with smooth or slightly rough bark, green or black in color exuding milky latex. Inflorescence solitary axillary, cauliflorous and ramiflorous on short leafy shoots. Seeds are oblong-ellipsoid [6] (Fig 1).

Microscopy of the leaf

Transverse section (T.S) of the leaves through the midrib showed the following tissue systems.

Shape

Leaves are dorsiventral with prominent midrib, long elliptic or ovate, acute or acuminate. In transactional view it is Plano convex shape with slightly elevated adaxial side and comparatively thick, semicircular, broad abaxial side. (Fig 2)

Epidermis

Upper epidermal cells were thick polygonal, wavy anticlinal walls, apostomatic and lower epidermal cells were thin, small squarish. The stomata were anamocytic (Fig 5).

Mesophyll

It contains short cylindrical, compact two layered palisade cells with 500µm thick. Lower part is spongy parenchyma. Lateral veins are with central group of xylem and phloem with upper and lower cylinders of tannin bearing bundle sheath extensions (Fig 4). Complex multi stranded collateral vascular bundle present in the midrib region. Xylem elements are short, wide angular mixed with lignified fibres with wide lumen. Outer part of the bundle contains continues sheath of phloem with exterior sclerenchyma continues or discontinuous thick sheath. Three larger in central, much smaller in the adaxial zone and slightly larger Vascular bundle in between were observed (Fig 3). Ground tissue was 5 to 8 small compact thick walled parenchymatous cell and inner zone is thin walled, compact, angular. Outer part with dense accumulation of tannin.

Calcium oxalate druses were observed both in lamina and phloem and near the fibre zone of midrib (Fig 6). Unicellular covering trichome is present. The epidermal from which it arises is thick walled, circular, lignified and as a ring of 10 radially elongated rosette cells (Fig 7). Lateral veins and narrow vein islets are thick prominent and forms dense reticulations. The islets are polygonal in outline with thick and straight boundaries and almost all contains mostly short, thick and some forked vein termination.

Petiole

Transverse section of petiole is deeply ridged, furrowed with wide deep adaxial groove, 2mm thick. Epidermis vertically elongated, rectangular thick walled cells. Ground tissue wider zone of small compact thick walled and inner zone of parenchyma cells with dense deposition of tannin. Vascular strands 14 wedges shaped discrete vascular bundles with narrow interfascicular parenchymatous gaps with 1 or 2 medullary bundles in regular rings. Xylem occurs as fairly long, compact, parallel lines of wide, circular thick walled elements. Phloem Occurs as semi-circular cap on the outer part of the xylem strand. Medullary bundle contains a circular cluster of xylem elements partially surrounded by phloem elements (Fig 8). Ground tissue Parenchymatous in nature. Calcium oxalate druses are abundant especially around the vascular cylinder and less towards periphery (Fig 9). Short, thick, triangular buried deeply in the epi and subepidermal regions. Crystal masses were seen in these short conical trichomes

Powder microscopy

The analysis of the dried powder of the leaf showed wavy walled epidermal cells of surface view with thick and straight anticlinal walls, anamocytic stomata with 4-5 subsidiary cells, unicellular covering trichome, spiral and annular xylem vessels, pericyclic fibres, calcium oxalate crystal druses (Fig 11).

Physicochemical analysis

Physicochemical parameters were found as follows: total ash 12.09%w/w, acid insoluble ash 1.98, water soluble ash 6.04%w/w, sulphated ash 4.5%w/w, ethanol soluble extractive value 5.23%w/w, water soluble extractive value 5.63%w/w, petroleum ether soluble extractive 1.8%, loss on drying 1.01%w/w and foreign organic matter was nil. Leaf constants were as follows vein islet number 13, vein termination number 5, stomatal number (lower epidermis) 50.88, stomatal index (lower epidermis) 17.6, and palisade number 3.125.

Preliminary phytochemical screening

Preliminary phytochemical screening showed the presence of flavonoids, terpenoids, sterols, mucilage, tannin, saponins, carbohydrates, reducing sugars, proteins and amino acids and

absence of alkaloids, cyanogenetic glycosides, anthroquinone glycosides, cardiac glycosides, fixed and volatile oil.

SEM of leaf

Scanning Electron Microscopy of midrib showed many folded appearance. No diagnostic feature and new kind of micro-constituents not previously recognized and apparently simple structure which may be extremely complex was observed (Fig 10).

XRF analysis of leaf

X-Ray Fluorescence Spectrometer (XRF) showed the presence of minerals Calcium (39.4%), Potassium (29.6%), Magnesium (2.06%), Manganese (0.13%), chlorine (2%), Iron (0.99%).

DISCUSSION

NTD are endemic in 149 countries with differing populations, economics, political and legal arrangement, health regulation, tradition, cultures, climates, infrastructure and geographies. Overall it is estimated that millions of people require preventive chemotherapy, a public health intervention for the treatment of NTD. Now a day's pharmaceutical companies and academic partners have joined this endeavor there by more activities directed at control are in progress and many health benefits can be achieved [1]. So the utilization or exploring the available sources of medicinal plants which are enriched with bioactive compounds and have been tested traditionally to reduce illness, social exclusion and mortality may be innovative flexible and cost effective way. Hence we have undertaken this study to serve as a tool for developing standards for identification, quality and purity of *A.heterophyllus* leaves.

Adulteration and misidentification of medicinal plants can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The past decade has witnessed the introduction and implementation of new Good Manufacturing Practices (GMP) in quality control of raw materials, intermediates and finished products of botanical origin .The initial step in quality control of medicinal plants is ensuring the authenticity of the desired species for the intended use. It can be conducted via a variety of techniques, namely macro and microscopic identification and chemical analysis especially description of microscopic botanical aspects to determine definitively the proper species of plant material while it is still in its non extracted form. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. These characters are especially important for identification of powdered drugs, because in these cases most of the morphological diagnostic features are lost [26]. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials [27]. The macroscopic and organoleptic characters of the leaf can serve as diagnostic parameters. Presence of Anamocytic stomata in the lower epidermis, unicellular covering trichomes were observed. The epidermal from which the trichomes arises is thick walled, circular, lignified and as a ring of 10 radially elongated rosette cells. Calcium oxalate druses were also observed. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. The ash values are particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter). Acid insoluble ash provides information about non-physiological ash produced due do adherence of inorganic dirt, dust to the crude drug. Increased acid insoluble ash indicates adulteration due do dirt, sand (or) soil [28]. The extractive values are primarily useful for the determination of exhausted or adulterated drug and helpful in the detection of adulteration [29]. Preliminary phytochemical screening will reveal the useful information about the chemical nature of the drug. Phytochemical evaluation and molecular characterization of plants is an important task in medicinal botany and drug discovery [30]. Preliminary phytochemical screening showed the presence of sterols, flavonoids, terpenoids, saponins, mucilage, reducing sugars, carbohydrates, protein and aminoacids and absence of alkaloids, fixed & volatile oil and glycosides. It is also used often as diagnostic feature to avoid misleading by over simplified descriptions and one may find new kinds of microstructures not previously recognised and apparently simple structures may be extremely complex. Remarkably, poor conventional descriptions enabling taxonomic process of reducing a complex pattern to a few simple characters. SEM plays a vital role when a specimen needs to be satisfactorily defined in terms of characters. For most biological materials, maximum information is obtained by employing light and electron microscopy jointly and an attempt was made by applying SEM [31]. Scanning Electron Microscopy of midrib showed many folded appearance. No diagnostic feature and new kind of micro-constituents not previously recognized and apparently simple structure which may be extremely complex was observed. Trace elements are considered the "inorganic switches" in various medicinal systems. This concept has gained ground in Ayurveda and the traditional Indian medicinal systems [32]. Mineral contents of various medicinal plants correlated with their therapeutic action by numerous studies [33]. X-Ray Fluorescence Spectrometer (XRF) showed the presence of minerals Calcium (39.4%), Potassium (29.6%), Magnesium (2.06%), Manganese (0.13%), chlorine (2%), Iron (0.99%).

CONCLUSION

The present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant especially to control some of the neglected tropical diseases. Microscopical evaluation, XRF and physicochemical standards and preliminary phytochemical reports can be useful to substantiate and authenticate drug.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

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Figure 1: Habit of *A.heterophyllus* Lam

Figure 2: Dorsal and ventral view of the leaves of *A.heterophyllus*

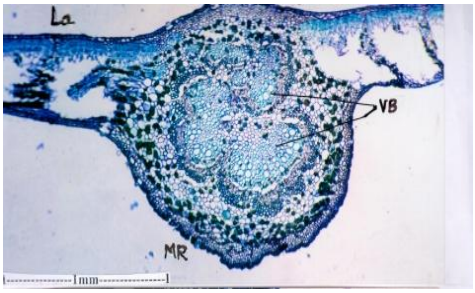

Figure 3: T.S of the leaf of *A.heterophyllus* through midrib (4×)

La- Lamina, VB- Vascular Bundles, MR- Midrib

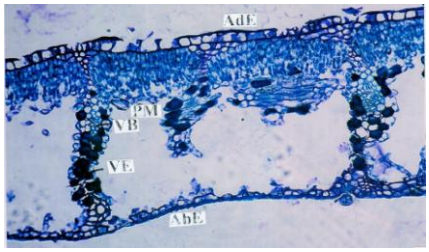

Figure 4: T.S of Lamina (10×)

Ade- Adaxial Epidermis, PM- Palisade Mesophyll, VB- Vascular Bundle, VE- Vertical Extension, AbE-Abaxial Epidermis

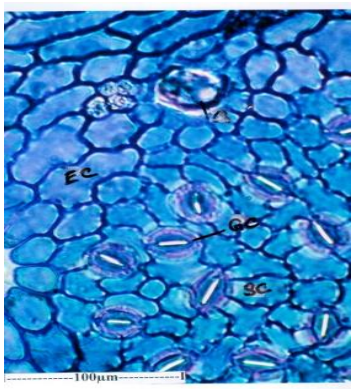

Figure 5: Lower epidermis surface view (40×)

EC- Epidermal cell, GC- Guard cell, SC- Subsidiary cell, CR- Crystal

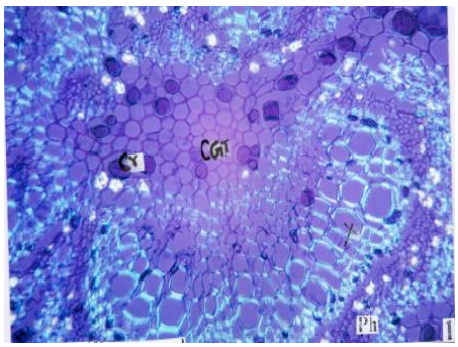

Figure 6: Crystals in the Medullary ray (16×)

CR- Crystals, CGT- Cortical Ground Tissue, Ph- Phloem, X- Xylem

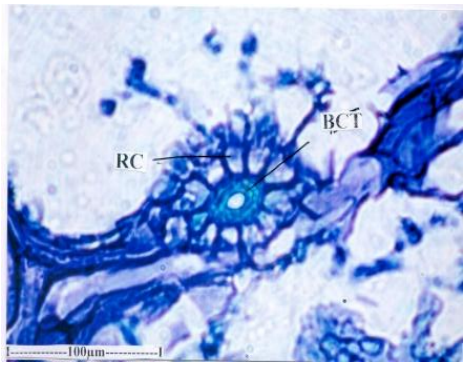

Figure 7: Epidermis showing BCT (40×)

BCT- Basal Cell Trichome with cystolith, RC- Rosette cells

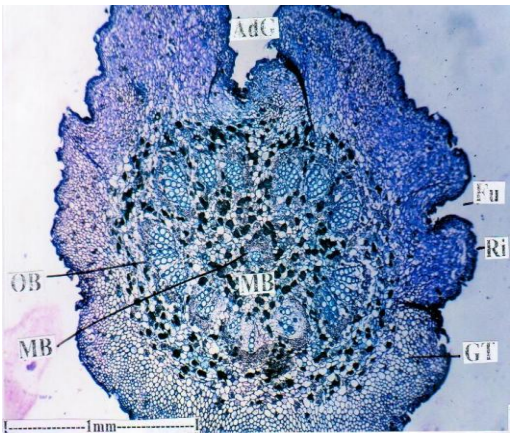

Figure 8: T.S of Petiole (4×)

AdG- Adaxial Groove, OB- Outer Bundles, MB- Median Bundles, GT- Ground Tissue, Ri- Ridge, Fu- Furrow

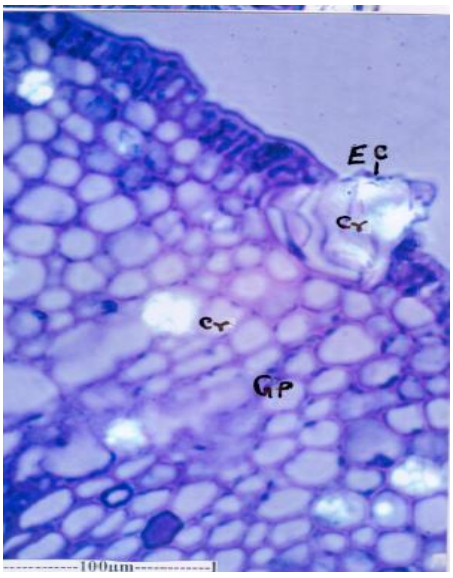

Figure 9: Crystals in outer ground tissue of Petiole (16×)

EC- Epidermal cell with crystal, GP- Ground Parenchyma, CR- Crystal

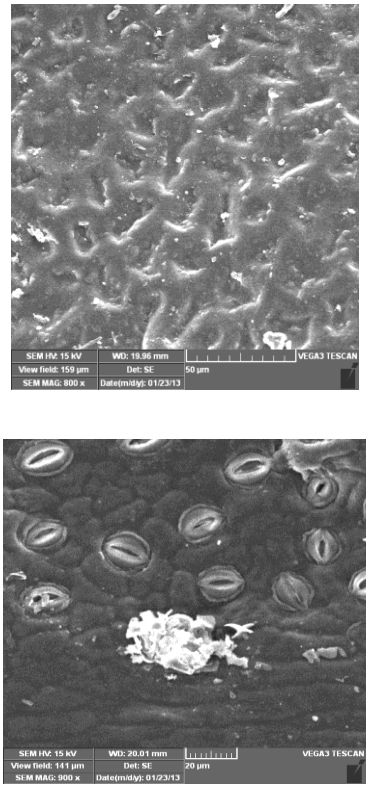

Figure 10: SEM- *A.heterophyllus* leaf

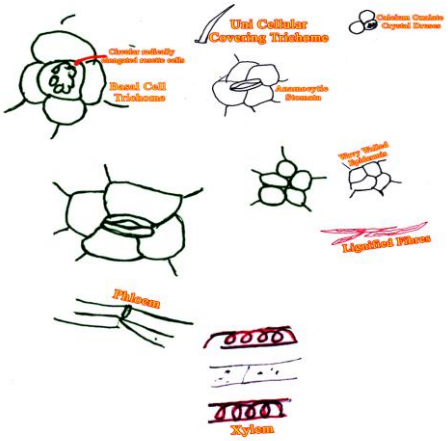

Figure 11: Powder microscopy of *A.heterophyllus* leaves

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