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Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES ON THE LEAVES OF *Psidium guajava Linn*- HAFSI VARIETY

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ABSTRACT

Objective: To explore the micro morphology and physio chemical parameters of the leaves of *Psidium guajava* Linn. (Myrtaceae) – Hafsi variety.

Methods**:** Macroscopy, microscopy, physicochemical analysis, preliminary phytochemical screening and other WHO recommended parameters for standardizations were performed.

Results**:** Leaves (5-15cm × 4-6 cm) are dorsiventral, oblong – elliptic, dull grey to yellow green with entire margin, obtuse to bluntly acuminate apex and rounded to subcuneate base with short petiole. Microscopic evaluation revealed the presence of paracytic stomata, multilayered layers of adaxial epidermis squarish cells with frequent calcium oxalate druses, druses in palisade and spongy parenchyma, secretory cavity, and parenchymatous bundle sheath cells in lateral vein and phloem found as small nests at the ends of xylem.

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, volatile oil, terpenoids, saponin, carbohydrates and absence of alkaloids, mucilage, glycosides, fixed oil.

Conclusion**:** Microscopic analysis was informative and provides useful information in the botanical identification, standardization for purity & quality and immense value in authentication of the leaf.

Keywords: Psidium guajava, Myrtaceae, Microscopical evaluation, Physicochemical analysis.

INTRODUCTION

Psidium guajava .Linn commonly called as poor man apple. The leaves of *P. guajava* really do not have any match as a cheap natural and easily available plant. It is traditionally known to be useful for the treatment of wide panel of diseases like ulcers, wounds, astringent, antiemetic, cholera, epilepsy etc [1]. Leaf is traditionally used for antispasmodic, anodyne, febrifuge[2], scurvy [3], malaria [4], antiseptic[5], antibacterial [6-8], antifungal [9] dysentery, diarrhoea[10,11], anti-inflammatory [12,13], gout[14], hypoglycaemic[15], headache, fever, gonorrhoea, dysmenorrheal[16], haemostat[17], antihypertensive[18], analgesic[19], hepatoprotective[20] and anticoagulant[21].

It was reported that fresh leaves contains: Guajavarin, isoquercetin, hyperin, quercetrin, quercetin 3-o gentiobioside [22]. Leaves also contains two triterpenoids , guavanoic acid and guava coumaric acid along with six known compounds 2 alpha hydroxy ursolic acid, jacoumaric acid, isoneriucoumaric acid, asiatic acid, ilelatifol D and β- sitosterol – 3-o – beta D glucopyranoside [23]. 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 *Psidium guajava* Linn. (Hafsi) selected for our study was collected from Horticulture Department, Madurai, Tamil Nadu, India 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 [24].

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 [25] .Sections were taken using microtome. Permanent mount was prepared using saffranin fast green double staining technique [26]. 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 [27, 28].

Physicochemical analysis

Total ash, acid insoluble ash, water soluble 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 [29-31].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure [32, 33].

RESULTS

Macroscopy

Psidium guajava is a large dicotyledonous- shrub or small evergreen tree, generally 3-10m high with many branches and crooked stems (Fig 1). Leaves (5-15cm × 4-6 cm) are opposite, simple, stipules absent, oblong – elliptic, dull grey to yellow green with entire margin, obtuse to bluntly acuminate apex and rounded to subcuneate base with short petiole (Fig 2). Flowers.

Figure 1: Habit of *P.guajava* L

Figure 2: Dorsal and ventral view of the leaves of *P.guajava*- HAFSI VARIETY

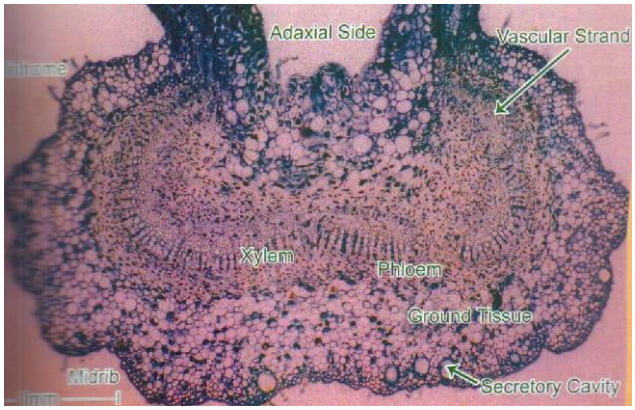

Figure 3: T.S through MIDRIB of *P.guajava* L. leaves (HAFSI)

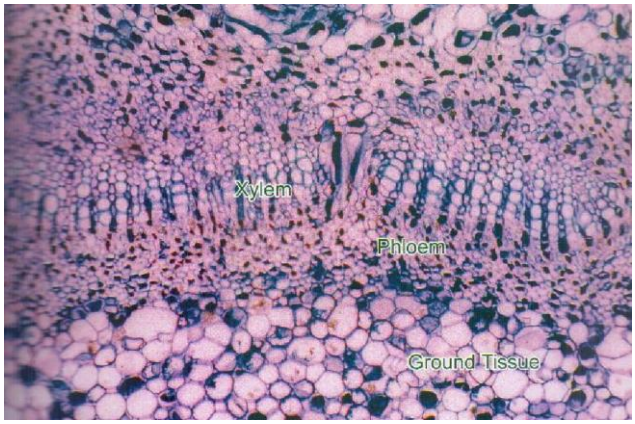

Figure 4: T.S through MIDRIB of *P.guajava* L. leaves – A portion enlarged- (HAFSI)

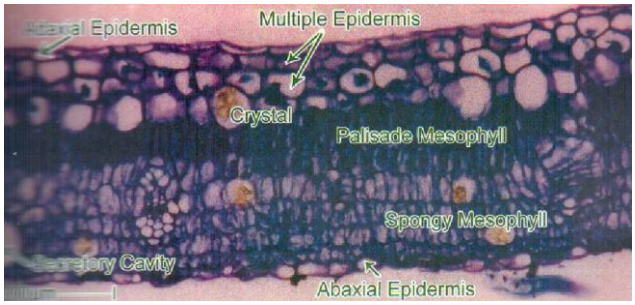

Figure 5: T.S of LAMINA (HAFSI)

are white, borne singly or in small clusters, 2-3 cm wide, with 4 or 5 white petals which are quickly shed, and a prominent tuft of perhaps 250 white Stamens. Fruit is small, 3 to 6 cm long, pear-shaped, reddish-yellow when ripe.

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,. 1.4 mm thick, broadly concave adaxial side and wavy shallow ridges with furrow and lamina being vertical in orientation (Fig 3).

Vascular bundle**:** Wide, thin deeply bowl shaped. Xylem thin walled angular in outline occur in short parallel lines (1.9 mm in horizontal plane and 150µm thick. Phloem element occurs at the end of the xylem lines as small nests (Fig 4).

Lateral vein**:** Elliptical collateral vascular bundle with parenchymatous bundle sheath.

Mesophyll**:** Palisade zone is one or two layered, short, cylindrical compact cells and five layers of short vertically compact cells

showing stratified arrangement of spongy parenchyma. Dilated cells contain calcium oxalate druses both in palisade and spongy parenchyma cells.

Ground tissue**:** Parenchymatous, thin walled less compact, less tanniferous. Secretory cavities are more frequent in the abaxial part.

Epidermis: 230µm thick, smooth and even. The adaxial epidermis is thin with narrow tubular cells. The subdermal layers of cells are dilated, squarish or rectangular without tannins, four layered. Calcium oxalate druses in the dilated cells are frequently seen in the adaxial epidermis (Fig 5).

Powder microscopy: The analysis of the dried powder of the leaf showed paracytic stomata, calcium oxalate druses, secretory cavities, parenchymal cells and fragment of palisade mesophyll with druses, xylem and phloem cells and fibres.

Physicochemical analysis

Physicochemical parameters were found as follows: total ash 11.06%w/w, acid insoluble ash 1.50, water soluble ash 2.83%w/w, ethanol soluble extractive value 18.18%w/w, water soluble extractive value 23.24%w/w, petroleum ether soluble extractive 2.69%, benzene soluble extractive 4.4%w/w, ethyl acetate soluble extractive 5.66%w/w, chloroform soluble extractive 5.18%w/w, loss on drying 9.9%w/w and foreign organic matter was nil. Leaf constants were as follows vein islet number 3.3, vein termination number 4.5, stomatal number (lower epidermis) 44.6, stomatal number (upper epidermis) 39.6, stomatal index (lower epidermis) 19.1, stomatal index (upper epidermis) 21.4.

Preliminary phytochemical screening

Preliminary phytochemical screening showed the presence of flavonoids, terpenoids, sterols, tannin, volatile oil , saponins, proteins and amino acids, carbohydrates, reducing sugars, and absence of alkaloids, cyanogenetic glycosides, anthroquinone glycosides, cardiac glycosides, mucilage and fixed oil.

DISCUSSION

Sensory evaluation plays a key role in determining the suitability or denunciation of a crude drug. Organoleptic testing of a crude drug is mainly for qualitative evaluation based on the observation of morphological and sensory profile. In this report, various morphological, microscopical, physicochemical standards have been developed. Hence we have undertaken this study to serve as a tool for developing standards for identification, quality and purity of

P.guajava leaves.

Adulteration and misidentification of crude drugs can cause serious health problems to consumers and legal problems for the pharmaceutical industries. 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 [28]. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials [34]. The macroscopic and organoleptic characters of the leaf can serve as diagnostic parameters [35]. Microscopic evaluation showed. Xylem-thin walled angular in outline occur in short parallel lines (1.9 mm in horizontal plane and 150µm thick. Phloem element occurs at the end of the xylem lines as small nests. Lateral vein contains elliptical collateral vascular bundle with parenchymatous bundlesheath. Palisade zone is one or two layered, short, cylindrical compact cells and five layers of short vertically compact cells showing stratified arrangement of spongy parenchyma. Dilated cells contain calcium oxalate druses both in palisade and spongy parenchyma cells. The adaxial epidermis is thin with narrow tubular cells. The subdermal layers of cells are dilated, squarish or rectangular without tannins, four layered. Calcium oxalate druses in the dilated cells are frequently seen in the adaxial epidermis. Secretory cavities are more frequent in the abaxial part. 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) [36]. 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. The extractive values are primarily useful for the determination of exhausted or adulterated drug and helpful in the detection of adulteration [37]. Phytochemical evaluation and molecular characterization of plants is an important task in medicinal botany and drug discovery [38]. Preliminary phytochemical screening showed the presence of sterols, flavonoids, terpenoids, saponins, volatile oil, protein and aminoacids, reducing sugars, carbohydrates, and absence of alkaloids, fixed oil, mucilage and glycosides. Dried powder of the leaf showed paracytic stomata, three layers of wide rectangular cells, calcium oxalate druses, secretory cavity, conical and flagellate trichome, parenchymal cells and fragment of palisade mesophyll.

CONCLUSION

The study of Pharmacognostical features of *Psidium guajava* Linn. (Hafsi) had shown the standards which will be useful for the detection of its identity and authenticity. The other study viz. physical evaluation, preliminary phytochemical test add to its quality control and quality assurance for proper identification.

Conflict of interest statement:

We declare that we have no conflict of interest.

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