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**Research Article**

# PHYTOCHEMICAL INVESTIGATION AND PHARMACOGNOSTICAL STANDARDISATION OF THE LEAVES OF *Psidium guajava Linn*- RED FLESHED VARIETY

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**ABSTRACT**

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

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

Results**:** Leaves (5-15cm × 4-5 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, two layers of wide rectangular sub epidermal cells with frequent druses, palisade mesophyll, parenchymal cells with tannin and numerous secrtory cavities. 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 and 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, Red fleshed variety, Microscopical evaluation, Physiochemical 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], anti bacterial [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. (Red fleshed) selected for our study was collected from Horticulture Department, Madurai, Tamilnadu, 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 × 4-5cm) 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*- (RED FLESHED)**

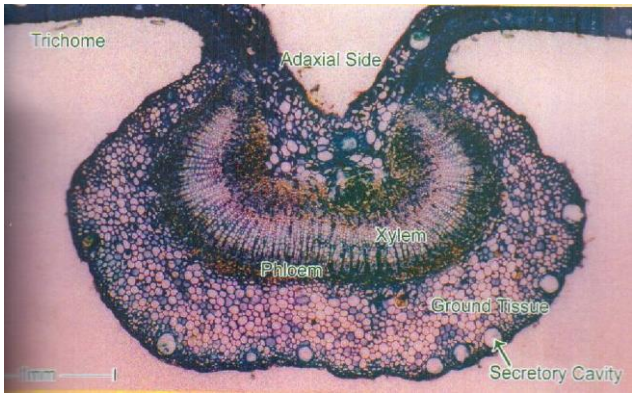


**Figure 3: T.S through MIDRIB of *P.guajava* L. leaves (RED FLESHED)**

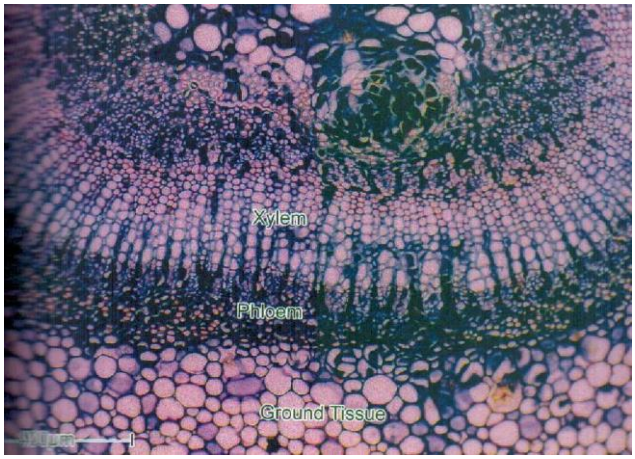


**Figure 4: T.S through MIDRIB OF *P.guajava* L. leaves – A portion enlarged- (RED FLESHED)**

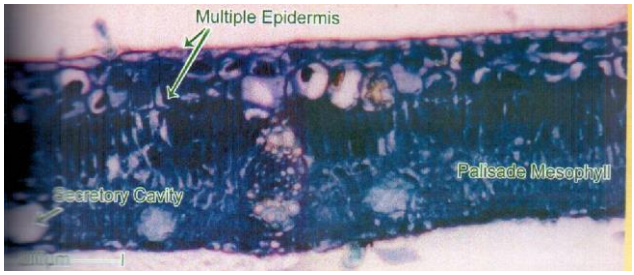


**Figure 5: T.S of LAMINA (RED FLESHED)**

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, conical and concave adaxial side with spreading lamina and horizontally extented, semicircular abaxial side with more or less even margin (Fig 3).

Vascular bundle**:** Vascular strand is deep, wide and urn shaped, 1mm wide, 200µm thick. Xylem elements are thin walled arranged in dense parallel rows**.** Metaxylem 2µm in wide. Phloem parenchyma cells have dense content of tannin (Fig 4).

Lateral vein**:** The vascular strand of lateral vein is vertically elongated and collateral with sclernchymatous small bundle caps.

Mesophyll**:** Narrow and cylindrical and compact single row of palisade cells (60µm in height). Four to five layered small lobed and loosely arranged spongy parenchyma cells. Mesophyll tissues are having dark tannin accumulation**.**

Ground tissue**:** Wide and numerous secretory cavities along the periphery of the parenchymatous ground tissue are seen (100µm) with reduced content of tannin in the ground parenchyma.

Epidermis: 200µm thick, smooth and even. The adaxial epidermis is narrow with thin walled cylindrical cells. Two rows of wide and rectangular subepidermal layer of cells are present with freguent druses. (Fig 5).

**Powder microscopy** The analysis of the dried powder of the leaf showed paracytic stomata, parenchymal cells with tannin, xylem and phloem, fragment of palisade mesophyll, secretory cavities.

**Physicochemical analysis**

Physicochemical parameters were found as follows: total ash 11.10%w/w, acid insoluble ash 1.5, water soluble ash 2.81%w/w, ethanol soluble extractive value 19.44%w/w, water soluble extractive value 25.06%w/w, petroleum ether soluble extractive 2.68%, benzene soluble extractive 4.32%w/w, ethyl acetate soluble extractive 5.7%w/w, chloroform soluble extractive 5.28%w/w, loss on drying 9.9%w/w and foreign organic matter was nil. Leaf constants were as follows vein islet number 2.5, vein termination number 4, stomatal number (lower epidermis) 44.7, stomatal number (upper epidermis) 31.4, stomatal index (lower epidermis) 19.4, stomatal index (upper epidermis) 20.4.

**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 conical and concave adaxial side with spreading lamina and horizontally extented, semicircular abaxial side. Vascular strand is deep, wide and urn shaped. Xylem elements are thin walled arranged in dense parallel rows. Phloem parenchyma cells have dense content of tannin. Narrow and cylindrical and compact single row of palisade cells (60µm in height). Four to five layered small lobed and loosely arranged spongy parenchyma cells. Mesophyll tissues are having dark tannin accumulation.

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, secretory cavity, conical and flagellate trichome, parenchymal cells and fragment of palisade mesophyll.

## CONCLUSION

The study of Pharmacognostical features of *Psidium guajava* Linn. (Red fleshed) 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.

**Acknowledgement**

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