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Preliminary Physico-phytochemical Study and Pharmacognostical Standardization of *Psidium guajava* Leaves

Krishanu Samanta, Romi Yadav, Rishikant Tripathi, Ashok Kumar, and Emdad Hossain*

Pharmacy College, Itaura, Chandeshwar, Azamgarh 276128, Uttar Pradesh, India

ABSTRACT

Psidium guajava L. (Family- Myrtaceae) possess great medicinal importance. It is used for treatment of various diseases like diarrhea, gastroenteritis, dysentery, diabetes, hypertension, caries, wounds, pain and fever. It also possesses anti-microbial, anti-malarial, antitussive, hepatoprotective effects etc. It is very important to standardize the plant part phrmacognostically for its utilization in different formulation. The present study deals with the pharmacognostical characterization along with preliminary phytochemical screening for understanding the active components in the plant. In preliminary phytochemical screening, presence of alkaloids, steroids, cardiac glycosides, flavonoids, triterpenoids were observed in methanol extract of the leaves. A typical sickle shaped lignified xylem layers were found in transverse section along with numerous calcium oxalate crystals, unicellular covering trichomes, glandular trichomes, cork cells, straight walled polygonal upper epidermal cells, found in powder microscopy.

Keywords: *Psidium guajava*, Myrtaceae, steroids, covering trichomes

Email: hossainemdad@yahoo.com

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INTRODUCTION

Since the primitive age, different medicinal plants were extensively used for treatment of different diseases. Those were utilized in different systems of medicines all over the world which include Ayurveda, Chinese medicine, Homeopathy, Siddha, Unani and many others. Though amazing development could be possible through advanced research in Allopath system of medicine but even today nearly eighty percent of individuals from developing countries are using traditional medicine obtained from medicinal plants because of lack of affording capacity and others [1].

Under the family Myrtaceae, *Psidium guajava* L. brought its importance for its different traditional uses throughout India. It is a small evergreen tree. It is a native to Mexico and well cultivated all over India especially in West Bengal [2-3]. It is used for treatment of various diseases like diarrhea, gastroenteritis, dysentery, diabetes, hypertension, caries, wounds, pain and fever. It also possesses anti-microbial, anticonvulsant, anti-inflammatory, anti-malarial, antitussive, hepatoprotective effects etc. [3-4].

Other traditional uses include decoction of leaves to cure mental disorder; thick decoction of root as paste, applied on the painful area due to arthritis. The plant extract has ability to reduce headache, stop vomiting. It can be used as heart tonic and cures constipation, physical and mental deformities. The tincture of guava along with honey is curing dry cough, common cough due to cold. It cures indigestion, acidity, swelling of the stomach caused by indigestion. It is beneficial in diarrhea and dysentery in children and burning sensation. The seeds can be used with rose water and sugar candy in enlarged liver [5].

It is very important to standardize the plant part phrmacognostically for its utilization in different formulation. The present study dealt with the pharmacognostical characterization along with preliminary phytochemical screening for understanding the active components in the plant which may be helpful to develop the individual monograph.

MATERIAL AND METHODS

Plant material

Fresh leaves of *Psidium guajava* were collected from fields of Chiraiyakot, District Mau, Uttar Pradesh, India in February 2012 and authenticated by Prof. N.K. Dubey, Taxonomist, Department of Botany Banaras Hindu University, Varanasi, Uttar Pradesh, India. A voucher specimen has been preserved in Natural Product laboratory, Department of Pharmaceutical Chemistry, Pharmacy College, Azamgarh, India for future reference (Voucher specimen no. NKD 2012-08). The leaves parts were dried under shade and powdered (40 mesh size) and stored in airtight containers. The macroscopic characters were studies as per given procedure in WHO guidelines on quality control methods for medicinal plants materials [6]. Fluorescence analysis of powdered leaves was carried out according to the method of described by Kokoski et al. [7] and Pratt & Chase [8].

Macroscopical studies

The leaves of the plant were studied for their macroscopic characters such as size, shape, margin, apex, surface, colour, odour, taste, nature and texture.

Microscopical studies

For microscopical studies the fresh leaf was fixed with formalin-acetic acid-alcohol mixture for about eighteen hours. Thin section was made by sharp razor and stained by safranine or phloroglucinol-hydrochloric acid and observed under compound microscope for pharmacognostic characterization. Photomicrographs of different magnification were taken with Samsung S750 digital microscopic unit. A details powder microscopic study along with different reagents was done with the powdered leaves [9].

Physicochemical studies

The ash values (total ash, acid insoluble ash, water soluble ash) were determined according to the official methods of Indian Pharmacopoeia [10]. The loss of drying, foaming index, swelling index and extractive values in different solvents (Petroleum ether 60-80ºC, benzene, chloroform, methanol and water) were performed according to the official methods prescribed in Indian Herbal Pharmacopeia [11] and the WHO guidelines [6].

Extraction method and preliminary phytochemical screening

For the phytochemical screening, the powdered leaves were extracted with methanol through cold maceration technique where 500 g of powdered leaves was kept with 1.5 liter of methanol in a closed flat bottomed flask for seven days; the extract was filtered and marc was re-extracted for two more time in similar fashion. All extracts were mixed together and were concentrated in a rotary vacuum evaporator below 40ºC and subsequently dried in high vacuum to get solid crude methanol extract. Phytochemical screening of the methanol extract of *Psidium guajava* leaves was performed for the detection of various phytoconstituents such as carbohydrates, protein, amino acid, steroids, alkaloids, cardiac glycosides, anthraquinone glycosides, cyanogenic glycosides, coumarin glycosides, tannins, flavonoids, gums, triterpenoids and saponins as per standard procedure [12].

RESULTS

Macroscopical study

In the macroscopical study of the leaves of *Psidium guajava*, it was found simple, entire at margin, pinnate type reticulate venation, opposite phyllotaxy, petiolate, oval-oblong in shape, acute at apex, to be light green in color, rough, characteristics smell and acrid in taste. It was 7 to 15 cm in length and 3.5 to 6 cm in width.

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Microscopical character

In transverse section of the midrib, adaxial and abaxial epidermis was found. 5-7 layers of lignified xylem were found in a sequence to form sickle shape where proto-xylems are in upper side and meta-xylem was in abaxial side. Xylem cells were sandwiched between layers of phloem which were non-lignified. Collenchymas were present mostly in abaxial side above the epithelial cells (Figure 1).

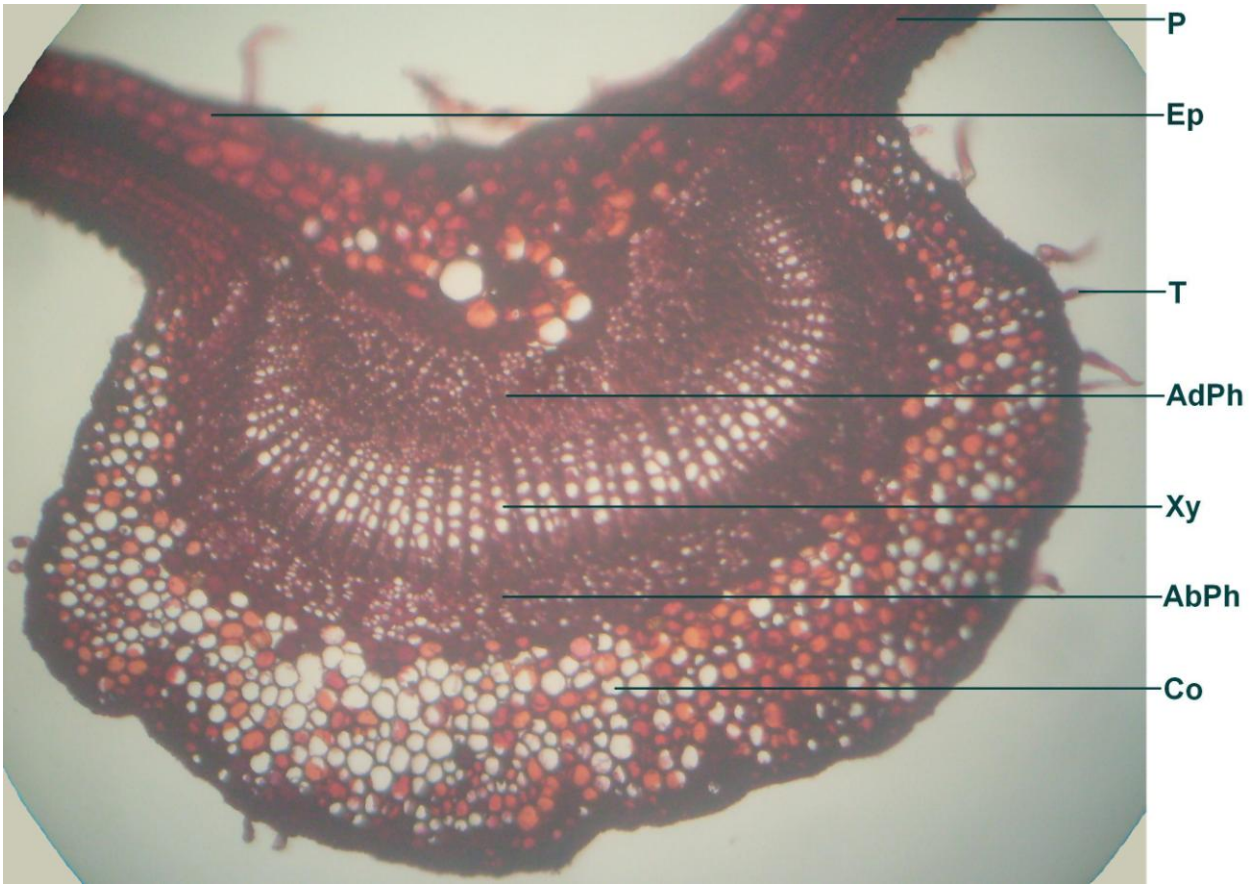

Figure 1: Transverse section of leaf of *Psidium guajava*
P- Parenchyma; Ep-Epidermis; T- Trichome; AdPh- Adaxial phloem; AbPh- Abaxial phloem; Xy- Xylem; Co- Collenchyma

In microscopical studies, the trichomes on the leaf epidermis were found unicellular and vermiform over adaxial and abaxial part. Straight walled polygonal epidermal cells were found on adaxial side of the lamina. Numerous anomocytic stomata occurred uniformly throughout the abaxial surface along with wavy walled epidermal cells (Figure 2).

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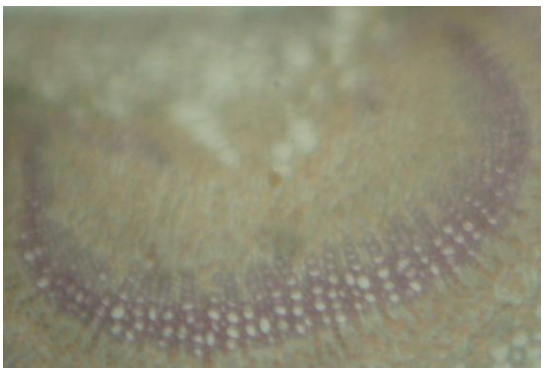
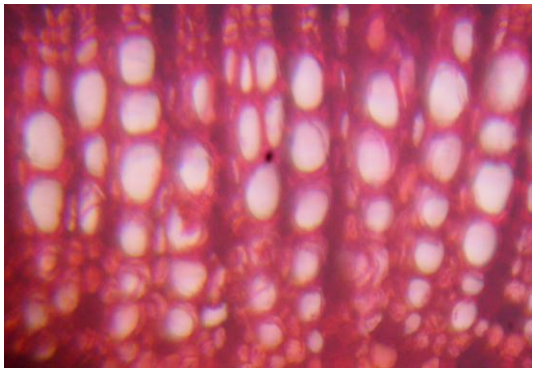
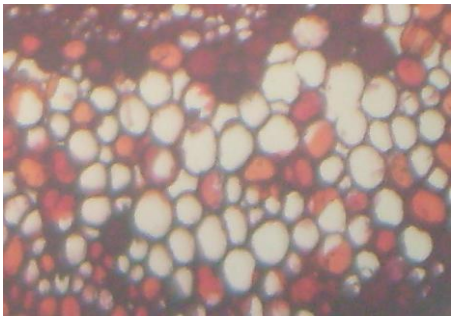


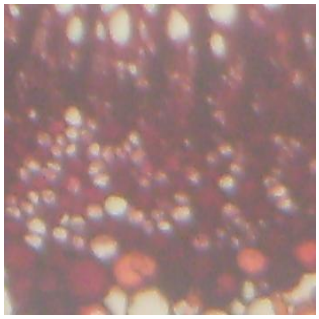
a. Sickle shaped xylems



b. Xylem fibers

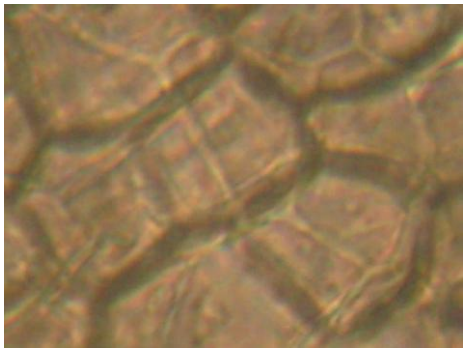


c. Collenchyma trichome

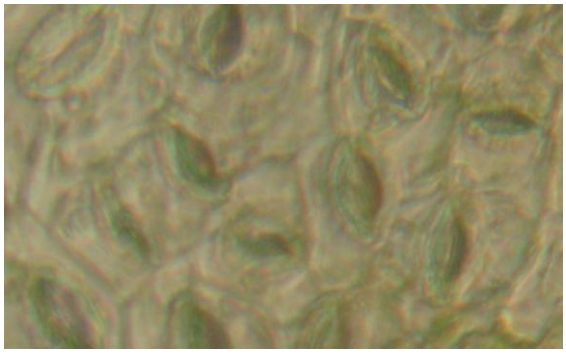


d. Phloem cells

e. Trichomes



f. Upper epidermis



g. Stomata with lower epidermis

Figure 2: Photomicrographs of different microscopic character of *Psidium guajava* leaf

In powder microscopy, numerous calcium oxalate crystals, unicellular trichomes, glandular trichomes, cork cells, straight walled polygonal upper epidermal cells, stone cells, stomata along with wavy walled lower epidermal cells and xylems were found (Figure 3).

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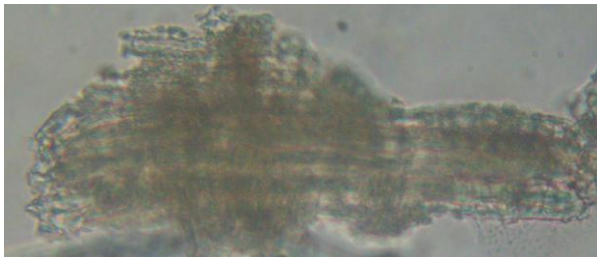
a. Calcium oxalate crystals

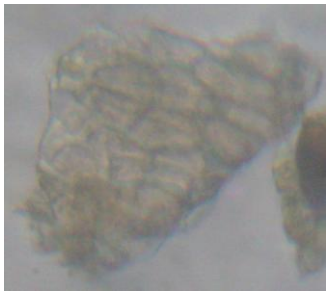
b. Unicellular trichome

c. Glandular trichome



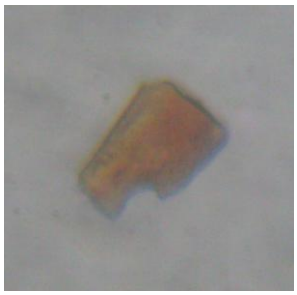
d. Xylem vessels

e. Stomata with wavy epidermal cells



f. Polygonal epidermal cells

g. Cork cell



h. Stone cell

Figure 3: Photomicrographs of different microscopic character found in powdered *Psidium guajava* leaves

Physicochemical studies

The values of the physical constant like ash values, loss on drying, extractive value were determined. Extractive value and color of powdered leaves with different chemical reagents under ultraviolet light were investigated. Ash values, loss on drying, foaming index, swelling index were mentioned in table 1. Extractive values and effect of ultraviolet light on powdered leaves were depicted in table 2 and table 3 respectively.

Table 1: Physical parameters for powdered leaves of *Psidium guajava*

S.No.	Parameters	Results
1.	Total ash (mg/g)	11.95
2.	Acid insoluble ash (mg/g)	9.08
3.	Water soluble ash (mg/g)	2.03
4.	Loss on drying (mg/g)	5.10
5.	Foaming index	>1000
6.	Swelling index	1.0

Table 2: Extractive values for various solvents for powdered leaves of *Psidium guajava*

S.No.	Solvent	Extractive value (% w/w)
1.	Water	6.40
2.	Methanol	21.24
3.	Chloroform	5.06
4.	Petroleum ether (40-60˚C)	2.56
5.	Benzene	3.71

Table 3: Effect of different chemical agents on powdered *Psidium guajava* leaves

Treatment	Color at Ultra violet light	
	Short UV	Long UV
Blank	Grey	Black
Powder + Ruthenium red solution	Mud green	Dark brown
Powder + 1N sodium hydroxide	Dark mud green	Dark brown
Powder + Dilute sulphuric acid	Mud green	Grey
Powder + Conc. sulphuric acid	Dark green	Dark brown
Powder + 10% sodium hydroxide	Mud green	Dark brown
Powder + 10% potassium hydroxide	Light green	Dark brown
Powder + 5% Ferric chloride	Light green	Dark brown
Powder + Distill water	Light brown	Light green
Powder + Toluene	Dark green	Dark brown
Powder + Iodine solution	Dark green	Dark brown
Powder + Acetic acid	Mud green	Light brown
Powder + Potassium ferry cyanide	Light brown	Dark brown

Phytochemical Screening

Preliminary phytochemical screening showed the presence of alkaloids, steroids, cardiac glycosides, flavonoids, triterpenoids.

DISCUSSION

In theoretical survey it was found that the plant possesses several traditional and pharmacological uses. Pharmacognostical standardization was essential for proper utilization of the plant for pharmaceutical uses. Presence of alkaloids, steroids, cardiac glycosides, flavonoids, triterpenoids showed the leaves are rich source of secondary metabolites responsible different pharmacological activities. Sickle shaped xylems layers were unique characteristics of the plant leaves. Unicellular covering trichomes along with glandular trichomes and straight walled polygonal epidermal cells were specific features in the powder microscopy.

CONCLUSION

Preliminary physico-phytochemical study of the leaves of *Psidium guajava* including macroscopic, other physical values and parameters will help to identify the species of plant;

phytochemical screening will help the presence of secondary metabolites, which are responsible for the pharmacological importance of the plant.

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