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Pharmacognostic Evaluation of *Psidium guajava* Linn. Leaves (Myrtaceae)

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ABSTRACT

The leaves of *Psidium guajava* (Myrtaceae) (guava) are known to have many medicinal properties. In tropical and sub-tropical countries these leaves are used to treat many disorders such as diarrhoea, cough, gastrointestinal disorders, vomiting, wounds, dysentery, ulcers, toothache and as an antiseptic. In the present study pharmacognostical investigations including morphology, microscopy (both general and quantitative), physico-chemical, preliminary phytochemical and fluorescence studies have been done to establish the specific identity of drugs. Phytochemicals observed with simple qualitative tests were alkaloids, tannins, flavonoids, phytosterols, and glycosides. They could have particular medicinal effects individually or in combined forms on various ailments. The plant has been extensively studied in terms of pharmacological activity of its major components. The findings may provide useful information with regards to its identification and standardization in future.

Keywords: fluorescence study, pharmacognosy, physico-chemical studies, phytochemical, quantitative leaf microscopy

INTRODUCTION

The medicinal plant *Psidium guajava* Linn. is commonly known as guava. The genus *Psidium* has more than 100 species throughout the globe. Two varieties, *pyriferum* and *pomiferum*, are found in India. It has several vernacular names: perale or seebe (kannada), amrud, jamphal, yellow guava, apple guava, bayabas, kalimbahin, tayabas, and guayabas in Sanskrit. The French call it goyave or goyavier; the Dutch guyaba, goeajaaba; the Suninamese, guvae or goejaba; the Portuguese and Brazilians, goiaba or goaibeira and the Hawaiians call it guava or kuawa (Morton 1987). Guava fruits are edible with a rich source of vitamin C (Wilson 1980). Seeds yield fatty oil, bark and leaves are used for tanning while bark decoction is used to treat diarrhoea (Prakash 2008).

In folk medicine, root, bark and leaf extracts are used to treat gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat and inflamed gums (Gutiérrez *et al*. 2008). Guavas are free from fat and cholesterol (except seeds). They are also an excellent source of fiber, potassium and vitamin A (Kamath *et al*. 2008; Parle and Broka 2009). Leaves are especially used to treat diabetes mellitus, painful menstruation, hypertension, as an antiseptic (Morton 1987), and treatment of coughs and colds (Khare 2007). The leaves of guava are used as an astringent (Ticzon 1997). Locally, a decoction of leaves is applied with much benefit to the *prolapsus ani* of children (Nadkarni and Nadkarni 1999). Indians also employ it to treat sore throats, vomiting, stomach upsets and vertigo. The leaves are used as a health tea. Leaves contain copious amounts of phenolics, which inhibit peroxidation in living bodies and therefore can be expected to prevent various chronic diseases (Kimura *et al*. 1985). *P. guajava* possesses useful medicinal properties: anti-diarrhoeal, anti-oxidant, hepatoprotective, anti-allergy, anti-microbial (Iwu 1993), anti-genotoxic, cardioprotective, anti-cough, anti-spasmodic and anti-hyperglycemic properties (Gutiérrez *et al*. 2008). Thus, guava fruit possesses a wide range of useful medicinal properties, which can be clinically exploited (Milid and Ekta 2009). The stem, bark and root bark of *P. guajava* are astringent. Unripe fruit is indigestible, causes vomiting and feverishness. The leaves are rich in tannin, and have antiseptic properties (Hernández 1980). The anti-inflammatory and analgesic activities of *P. guajava* were investigated in rats using the carrageenan-induced hind paw edema model (Muruganandan *et al*. 2000). The leaves are also used for several other ailments, including diabetes (van Wyk *et al*. 1997). The young leaves and shoots are also used for inflammation of the kidney and kidney problems (Ticzon 1997). Some of the ethnomedicinal uses include the crushing of leaves and the application of liquids from them onto wounds, cuts, ulcers, boils, skin and soft tissue infectious sites and rheumatic places (Bala 2006) and the chewing of the leaves to relieve toothache, oral ulcers, inflamed gums, throat and chest pains, treatment of leucorrhea, diarrhea, dysentery, convulsions and epilepsy, as well as the use of decoctions and infusions as a douche for vaginal discharges and to tighten and tone vaginal walls after childbirth (Burkil 1994).

The plant has been identified as having many medicinally important phyto-constituents. The fruit contains saponin combined with oleanolic acid. Morin-3-*O*-α-L-lyxopyranoside, morin-3-*O*-α-L-arabopyranoside and flavonoids, guaijavarin and quercetin were identified from leaves (Arima 2002). Aroma-active volatiles such as (*Z*)-3-hexenal, 3-sulfanyl-L-hexanol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3-sulfanylhexyl acetate, hexanal, ethyl butanoate, cinnamyl acetate and methional were also identified (Steinhaus *et al*. 2009). Two triterpenoids, betulinic acid and lupeol, were isolated from the leaf extract of *P. guajava* and their potential antimicrobial and phytotoxic activities were evaluated (Ghosh *et al*. 2010). Two triterpenoids, 20β-acetoxy-2α,3β-dihydroxyurs-12-en-28-oic acid (guavanoic acid), and 2α,3β-dihydroxy-24-*p*-*z*-coumaroyloxyurs-12-en-28-oic acid (guavacoumaric acid), along with six known com-

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pounds (2α-hydroxyursolic acid, jacoumaric acid, isoneriucoumaric acid, asiatic acid, ilelatifol D and β-sitosterol-3-*O*-β-D-glucopyranoside) have been isolated from the leaves (Begum *et al*. 2001).

Pharmacognostic studies provide useful information about leaf macroscopy, microscopy and behavior of leaf powder following treatment with different chemical reagents, fluorescence characters under ultra violet light after treatment with various chemicals. No concrete scientific study has been reported to prove the folklore claim in the utility of *P. guajava* leaves in the treatment of various diseases and hence the objective of the present study was to correlate the ethnobotanical evidence with a scientific study. These studies also suggested that the observed pharmacognostic and physiochemical parameters are of great value in quality control and formulation development.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Psidium guajava* Linn. (Myrtaceae) were collected in and around Savanur, Haveri and from Shankarghatta, Shivamogga, Karnataka in May 2010. The taxonomic identification of the plant was confirmed by Dr. Prashant Kumar Jha, Department of Botany, ALN Rao Memorial Medical College, Koppa (voucher specimen number SPS126). Fresh leaves were collected and dried under shade for 15 days. They were powdered using a mechanical grinder and were further used for other studies.

Leaf macroscopy

The plant was morphologically examined for the shape of leaves, apex, base, margin, colour, surfaces, vention, presence or absence of petiole, lamina, texture, odour and taste (Trease and Evans 2002).

Leaf microscopy

The outer epidermal membranous layer were cleared in chloral hydrate, mounted with glycerin and observed under compound microscope (Trinocular microscope, RCM-20XLT, Besto, India). The presence or absence of the following was observed: paracytic type of stomata on abaxial (dorsal surface), secretory cells among ground tissue of midrib portion, xylem, phloem, fibers (stained with phloroglucinol + HCl, iodine and saffaranine green) and epidermal hairs (type of trichome and distribution). The transverse sections of the fresh leaves through the lamina and midrib were observed after clearing and staining, respectively. Anatomical sections surface preparation of the fresh leaves and the powdered samples for the microscopy and chemomicroscopy were carried out according to the methods outlined by African Pharmacopoeia (1986).

Powder studies

The leaf powder was treated with different chemical reagents such as 1% HCl, 1% NaCl and distilled water, and colour reactions were noted down to carry out fluorescent analysis. Quantitative investigations were done to determine the palisade ratio, stomata number, stomata index, vein – islet number and veinlet termination number microscopically (Trease and Evans 2002). Physicochemical parameters such as moisture content, total ash, acid-insoluble ash, water-soluble ash, methanol and water-soluble extractive values were evaluated (British Pharmacopoeia 1980). Phytochemical studies of the leaves were carried out for the presence of different constituent's viz. alkaloids, flavonoid, phenolics, saponins, phytosterols, carbohydrates and protein (Gondim *et al*. 2009).

RESULTS AND DISCUSSION

Taxonomical identification

P. guajava is a low evergreen small tree or shrub up to 1.83-7.62 m high, with wide spreading branches and square, downy twigs. This tree is a native of tropical America, which is both a tropical and sub-tropical plant. The branches are crooked with opposite leaves. The flowers are white; 1-3 flowered on the leaf axils. Petals are 4-5 petals, free. Stamens are numerous. Ovary is 2-many celled. The fruit is globose or pear-shaped berry turning to reddish-yellow after ripening (Gamble 1921).

Macroscopic characteristics of leaf

The leaf is simple on short petiole. They are 8-12 × 3-5 cm in size and ovate in shape. Upper surface is green and glabrous while lower surface is pale green and softly pubescent with prominent principal nerves. The apex is acuminate. The odour and taste are specific (**Fig. 1**).

Microscopic characteristics of the leaf

The surface preparation of both surfaces of leaves reveals the presence of stomata only on lower surfaces (**Fig. 2**). They are paracytic type and are traversed with unicellular to multicellular uniseriate trichomes and starch grains in between. The transverse section of the leaves is dorsi-ventral in nature. The broader portion of midrib is covered with vascular bundle in centre surrounded by fibres. Trichomes are present on both surfaces in midrib region. The detailed study shows a layer of both upper and lower epidermis consisting of oval to rectangular shaped cells (**Fig. 3**). The deposition of cuticle is observed on upper epidermis. The layer of upper epidermis is followed by a layer of tooth-shaped cells in lamina portion while 2-5 layered collenchymas cells in midrib region. The mesophyll cells of lamina portion are composed of usually single layered palisade cells (rarely 2-layered) in continuation with spongy parenchyma cells. They are interrupted with vessels and are filled with chlorophyll contents. In midrib region, hypodermis is followed by a wide zone of ground tissue composed of parenchyma cells and is filled with scarce distribution of starch grains at places. The prismatic crystals of calcium oxalate are also seen in ground tissue portion. Schizolysigenous types of secretory cells are observed in ground tissue portion. The vascular bundle was found surrounded by lignified fibres (**Fig. 4**).

Fluorescence and quantitative studies

The fluorescence characteristics, being commonly applied parameters in the analysis of crude drugs were employed. The colour of powder after treating with 1% NaOH, 1% HCl and distilled water and as such under day light and long UV light were analysed. The florescence behavior was noted as shown in **Table 1**. The quantitative microscopic studies revealed the values like palisade ratio, stomata number, stomata index, vein-islet number and veinlet termination number as displayed in **Table 2**. The colour change in florescence studies was distinctive and reproducible revealing the properties of treated chemicals to the phyto-constituents.

Physico-chemical and phytochemical studies

Physico-chemical studies of leaves expose the presence of total inorganic salts present with drugs in terms of total ash and their insolubilities with 6N HCl and solubility with water. The quantity of metabolites expected to be dissolved with water and methanol are given in terms of extractives values while the moisture content present with powder is in reference to loss on drying (**Table 3**). The preliminary qualitative studies for the presence or absence of various metabolites are given in **Table 4**. The total ash value for a crude drug is not always reliable, since there is a possibility of presence of non-physiological substances such as early matters. So, the parameters such as acid insoluble, water soluble and sulphated ash values were performed. Our results correlate with the fact that the alcoholic extractive

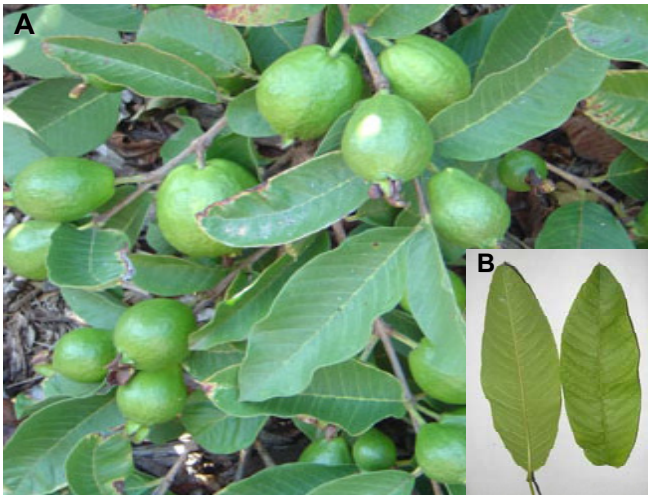

Fig. 1 Morphology of *Psidium guajava* plant (**A**) along with adaxial and abaxial surfaces of the leaf (**B**).

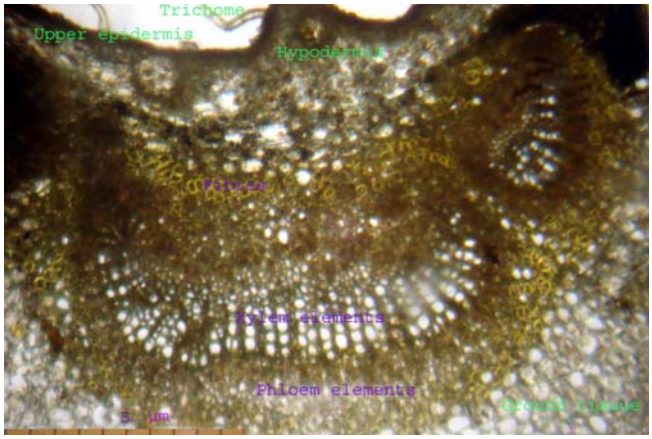

Fig. 2 Microscopy of *Psidium guajava* leaf showing outline of TS stained with iodine.

Table 1 Fluorescence behaviour of leaves of *Psidium guajava.*

Treament	Daylight	Long UV light
Powder (P) as such	Dark green	Fluorescent dark green
P+1% HCl*	Orange	Fluorescent greenish
P+1% NaOH*	Red	Fluorescent red
P+ Distilled H_2O	Orange	Fluorescent light orange

* 85%, s.d. Fine Chem. Ltd., Mumbai.

Table 2 Quantitative leaf microscopy of *Psidium guajava*.

Parameter	Range	Mean*
Palisade ratio	12-15	13.65 ± 0.45
Stomata number Upper surface	0	0
Stomata number Lower surface	68-83	65.34 ± 6.71
Stomata index Upper surface	0	0
Stomata index Lower surface	12.47-14.46	15.08 ± 0.23
Vein islet number	13-16	14.23 ± 0.43
Veinlet termination number	8-13	12.82 ± 0.19

* Mean value of 10 counts

Table 3 Physicochemical analysis of leaves of *Psidium guajava.*

Parameters	Values (%) obtained on dry weight basis (w/w)
Loss on drying	10.00
Total ash	4.75
Acid-insoluble ash	0.25
Water-soluble ash	2.75
Methanol-soluble extractives	20.00
Water-soluble extractives	14.00

value being higher reveals the absence, or present in smaller quantity of water soluble constituents such as sugars, glycosides and tannins (Prabhu *et al*. 2009).

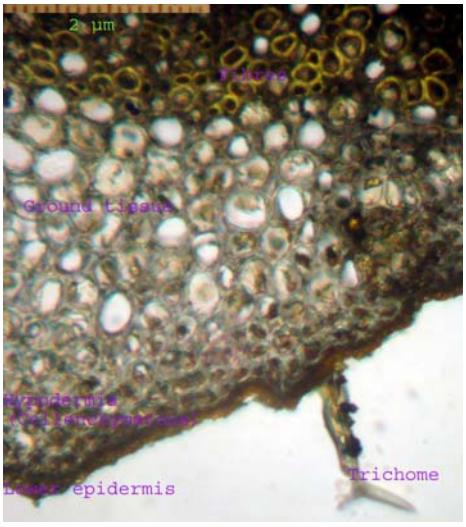

Fig. 3 Microscopy of *Psidium guajava* leaf showing lower epidermis with fibres and trichome.

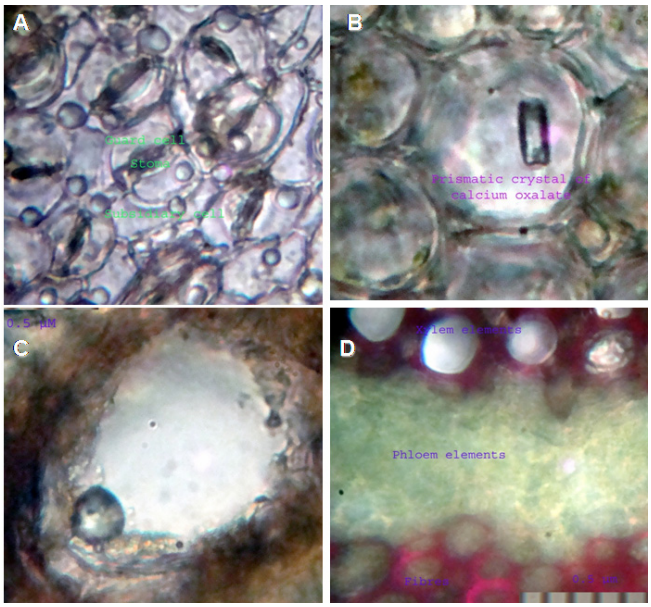

Fig. 4 Microscopy of *Psidium guajava* leaf showing- Paracytic type of stomata on abaxial surface (**A**); Prismatic crystal of calcium oxalate in ground tissue (**B**); Secretory cells among ground tissue of midrib portion (**C**) and Xylem, phloem elements along with fibres (**D**).

Table 4 Phytochemical examination of leaves of *Psidium guajava*.

Qualitative tests	Result
Carbohydrates	–
Protein	–
Anthraquinone glycoside	+
Alkaloid	–
Flavonoid	+ + +
Phenolics	+
Saponins	+ +
Phytosterols	+
Tannins	–

\+ = present; – = absent

CONCLUDING REMARKS

An extensive literature survey revealed that guava, acclaimed as ‘poor man’s apple of the tropics’, has a long history of traditional use for a wide range of diseases. The

fruit as well as its juice is freely consumed for its great taste and nutritional benefits. Much of the traditional uses have been validated by scientific research. The plant has been extensively studied in terms of pharmacological activity of its major components. In this regard, further studies need to be carried out to explore *P. guajava* leaves for its potential in preventing and treating diseases. The present study may be useful to supplement information with regard to its identification and standardization, and in carrying out further research and revalidation of its use in the Ayurvedic System of medicine.

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