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Comparative pharmacognostical evaluation of nine different varieties of the leaves of *Psidium guajava* Linn.

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Abstract

Objective: To explore the micro morphology and Physio chemical parameters of nine different varieties of the leaves of *Psidium guajava* Linn. (Myrtaceae). **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. Morphology of the leaves showed no difference in all the varieties. A noteworthy feature Myrtaceae family is the presence of secretory cavities. Microscopic evaluation revealed that secretory cavities were absent in Bangalore, Chittidhar and it is present in the lower side in case of Anakapalli, Hafsi, Lucknow-46, Nagpur seedless and Red fleshed, but it was 80µm wide and present in the upper portion in Smooth green. Narrow secretory cavities observed at the periphery of the midrib in Lucknow-49. Calcium oxalate druses in dilated cells are present in Hafsi and Lucknow-46 alone. All varieties contain invariably multiple epidermis. Tannin is abundant in the tissue, but it is very less in Lucknow-49 and dark tannin accumulation seen in Red fleshed. In vascular bundle xylem less lignified, uniseriate thick walled in Anakapalli, thin walled dark elliptical sclerenchyma in Bangalore variety. Powder microscopy showed the presence of paracytic stomata, fragment of epidermis, conical and flagellate trichomes, fragment of palisade mesophyll, secretory cavity (absent in Bangalore, Chittidhar varieties), calcium oxalate crystals (present in Hafsi and Lucknow -46 varieties) and parenchymal cells. Vein islet & Termination number, stomatal number & 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 amino acids, 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 different varieties of the leaves.

Keywords: *Psidium guajava*, Myrtaceae, Lucknow-46, Lucknow-49, Microscopical evaluation, Physicochemical analysis..

Introduction

Psidium guajava. Linn commonly called as a 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 a wide panel of diseases like ulcers, wounds, astringent, antiemetic, cholera, epilepsy etc.¹ Leaf is traditionally used for antispasmodic, anodyne, febrifuge², scurvy³, malaria⁴,

antiseptic⁵, antibacterial⁶⁻⁸, antifungal⁹ dysentery, diarrhoea^{10,11}, anti-inflammatory^{12,13}, gout¹⁴, hypoglycaemic¹⁵, headache, fever, gonorrhoea, dysmenorrhoea¹⁶, haemostat¹⁷, antihypertensive¹⁸, analgesic¹⁹, hepatoprotective²⁰ and anticoagulant²¹.

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, isoneriuoumaric 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. Pharmacognostical and phytochemical studies of nine different varieties of *P. guajava* leaves was previously reported.²⁴⁻³² In the present study attempts have been made to compare gross macroscopical and microscopical characters, physicochemical properties of nine different varieties of the leaves of *P. guajava* as important pharmacognostic parameters in herbal drug standardization process.

Materials and Methods

Chemicals

Formalin, acetic acid, ethyl alcohol, chloral hydrate, toluidine 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. (Nine varieties) selected for our study was collected from Horticulture Department, Madurai, Tamil Nadu, India and Dr. Stephen, Department of Botany, American college, Madurai for plant authentication and Dr. P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai, Tamil Nadu, India for microscopic work.

Macroscopic analysis

Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc was noted.³³

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.³⁴ Sections were taken using microtome. Permanent mount was prepared using saffranin fast green double staining technique.³⁵ 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.^{36, 37}

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 were determined.^{38, 39}

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure.^{40, 41}

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 (5-13cm × 4-5 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. Morphology of the leaves showed no difference in all the varieties. (Fig 2). Flowers 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.



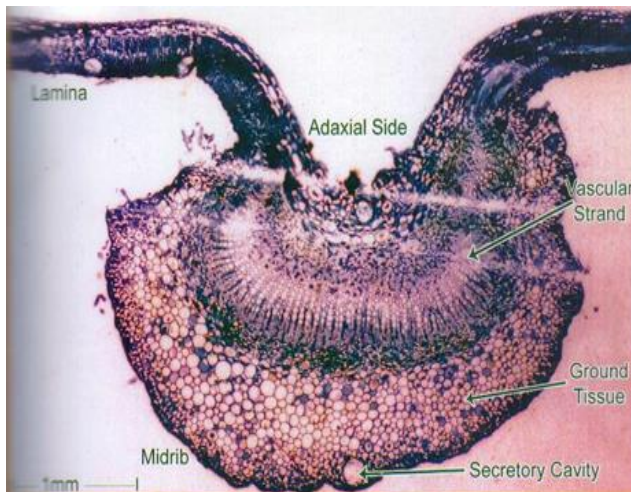
Figure 1: Habit of *P. guajava* L



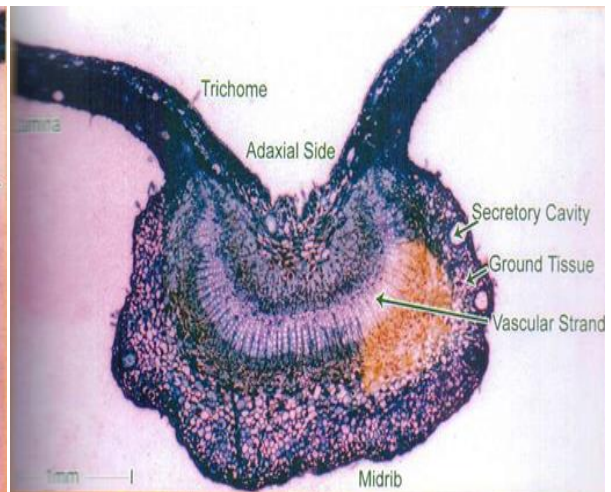
Figure 2: Dorsal and ventral view of nine different varieties of the *P. guajava*

Microscopy of the leaf

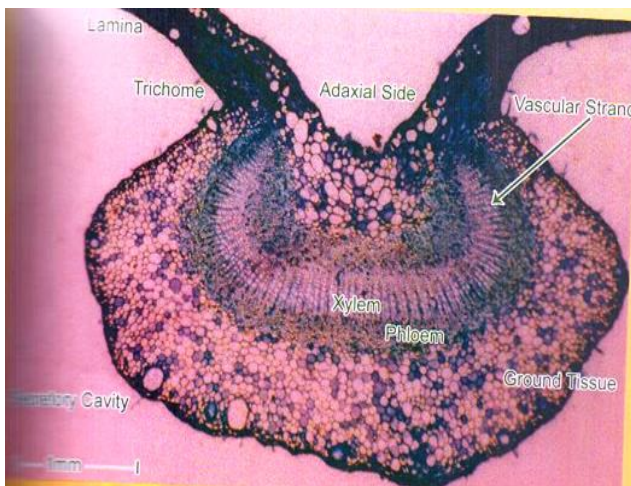
Transverse section (T.S) of the leaves of nine varieties through the midrib showed the special feature (Table 1) & (Fig 3).



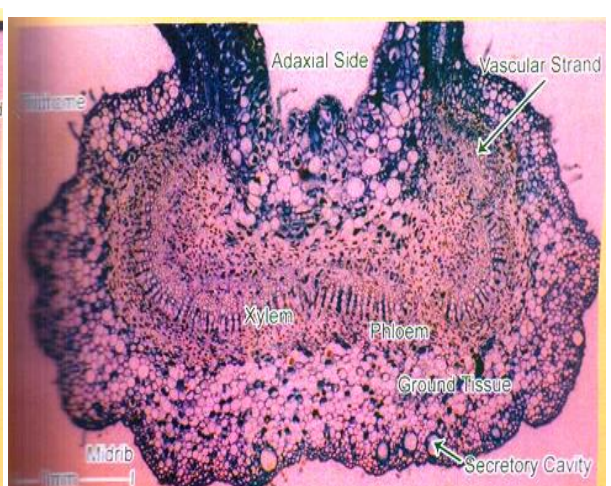
Anakapalli



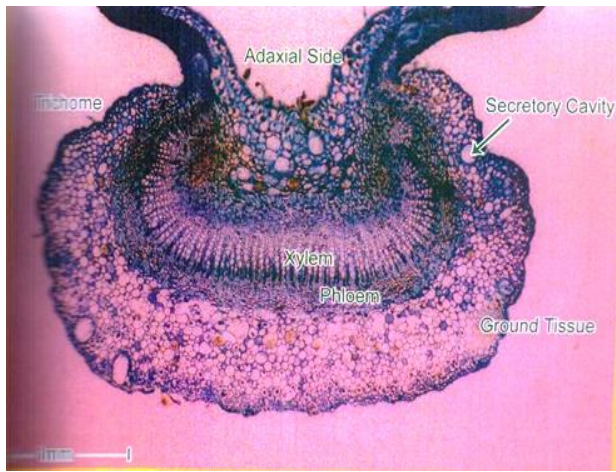
Bangalore



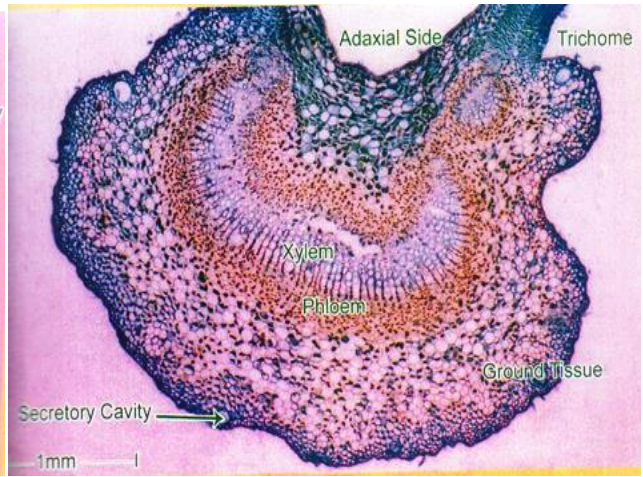
Chittidhar



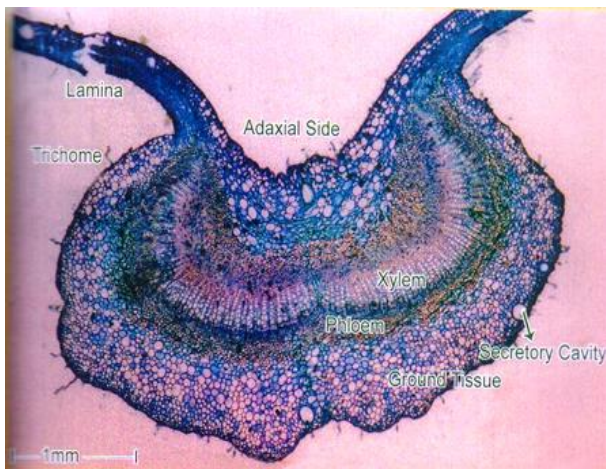
Hafsi



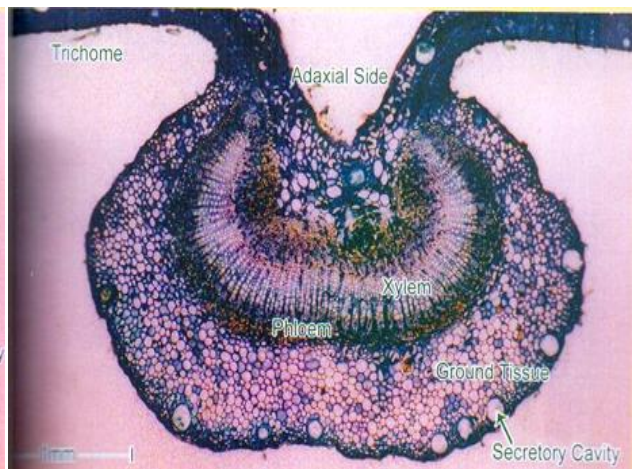
Lucknow-46



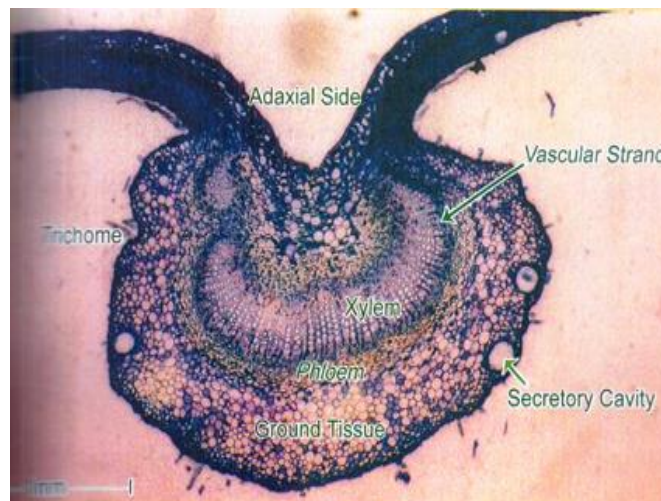
Lucknow-49



Nagpur seedless



Red fleshed



Smooth green

Figure 3: T.S. of midrib of nine different varieties of the *P. quajava* leaves

Table 1: Microscopy of 9 different varieties of *P. guajava* leaves showing special features

| Parameters | Anakapalli | Bangalore | Chittidhar | Hafsi | Lucknow-46 | Lucknow-49 | Nagpur seedless | Red fleshed | Smooth green |
|------------------|---|---|------------|---|--|---|--|---|-----------------------------|
| Vascular bundle | Xylem less lignified, uniseriate, thick walled fibres | Xylem dense parallel lines, thin walled containing dark elliptical sclerenchyma cells in between the lines. Phloem- seen in small cluster at the end of each xylem row (30µm wide). | | Phloem elements occur at the ends of xylem lines as small nests. | Xylem fairly wide, thin walled angular in outline occur in compact radial lines. | | | Xylem – dense parallel rows. | |
| Epidermis | | | | | Tannin content is not evident. Calcium oxalate druses are common with in dilated ground cells. | Narrow secretory cavities seen in the periphery | Lacking of tannin | | |
| Mesophyll | | | | Dilated cells contain calcium oxalate druses both palisade and spongy parenchyma. | Calcium oxalate druses in the dilated cells. | Tannin content is less. | | Single row palisade cells. Dark tannin accumulation | |
| Lateral vein | | Vascular strand of lateral vein are prominent collateral with thick adaxial and abaxial sclerenchyma sheath. | | Elliptical, parenchymatous bundle sheath. | Vascular strand of lateral vein vertically stretched collateral with sclerenchyma bundle caps. | | Bundles projects predominantly below the surface having thick mass of xylem, small groups of phloem, and thick pillar of bundle sheath | | |
| Secretory cavity | in lower side | Absent | Absent | In lower side | In lower side | Periphery of the midrib. | In lower side | In lower side | 80µm wide in upper portion. |

Table 2: Stomatal number of 9 different varieties of *P. guajava* leaves

| Range | Anakapalli | Bangalore | Chittidhar | Hafsi | Lucknow-46 | Lucknow-49 | Nagpur seedless | Red fleshed | Smooth green |
|-------|------------|-----------|------------|-------|------------|------------|-----------------|-------------|--------------|
| | UE | LE | UE | LE | UE | LE | UE | LE | UE |
| Min | 29 | 41 | 32 | 40 | 36 | 42 | 38 | 42 | 35 |
| Avg | 30.7 | 43 | 34.3 | 42.4 | 37.8 | 44 | 39.6 | 44.6 | 36.2 |
| Max | 43 | 45 | 36 | 44 | 40 | 46 | 42 | 48 | 38 |

UE-Upper Epidermis LE-Lower Epidermis

Table 3: Stomatal index of 9 different varieties of *P. guajava* leaves

| Range | Anakapalli | | Bangalore | | Chittidhar | | Hafsi | | Lucknow-46 | | Lucknow-49 | | Nagpur seedless | | Red fleshed | | Smooth green | |
|-------|------------|------|-----------|------|------------|------|-------|------|------------|------|------------|------|-----------------|------|-------------|------|--------------|------|
| | UE | LE | UE | LE | UE | LE | UE | LE | UE | LE | UE | LE | UE | LE | UE | LE | UE | LE |
| Min | 20 | 19.1 | 20.9 | 19.2 | 21.2 | 19.4 | 21.0 | 19.0 | 19.9 | 19.1 | 19.8 | 19.2 | 20.8 | 18.7 | 20.1 | 19.2 | 20.1 | 19.0 |
| Avg | 20.2 | 19.3 | 21.0 | 19.4 | 21.6 | 19.8 | 21.4 | 19.1 | 20.1 | 19.3 | 20.0 | 19.5 | 21.0 | 19.0 | 20.4 | 19.4 | 20.3 | 19.1 |
| Max | 20.5 | 19.6 | 21.2 | 19.6 | 21.8 | 20.0 | 21.8 | 19.2 | 20.3 | 19.5 | 20.2 | 19.6 | 21.2 | 19.2 | 20.6 | 19.6 | 20.6 | 19.2 |

UE-Upper Epidermis LE-Lower Epidermis

Table 4: Vein islet and vein termination number of 9 different varieties of *P. guajava* leaves

| Range | Anakapalli | | Bangalore | | Chittidhar | | Hafsi | | Lucknow-46 | | Lucknow-49 | | Nagpur seedless | | Red fleshed | | Smooth green | |
|-------|------------|-----|-----------|-----|------------|-----|-------|-----|------------|-----|------------|-----|-----------------|-----|-------------|----|--------------|-----|
| | VI | VT | VI | VT | VI | VT | VI | VT | VI | VT | VI | VT | VI | VT | VI | VT | VI | VT |
| Max | 2 | 4 | 2 | 4 | 3 | 4 | 3 | 4 | 3 | 4 | 2 | 4 | 2 | 3 | 2 | 3 | 3 | 4 |
| Avg | 3 | 4.3 | 3 | 4.5 | 3.5 | 4.8 | 3.3 | 4.5 | 3.5 | 4.3 | 2.5 | 4.3 | 2.3 | 3.5 | 2.5 | 4 | 3.3 | 4.3 |
| Min | 4 | 5 | 4 | 5 | 4 | 5 | 4 | 5 | 4 | 5 | 3 | 5 | 4 | 4 | 3 | 5 | 4 | 5 |

VI- Vein Islet VT- Vein Termination

Table 5: Extractive values of 9 different varieties of *P. guajava* leaves

| Solvent | Extractive value % (Individual solvent) | | | | | | | | |
|-----------------|---|-----------|------------|-------|------------|------------|-----------------|-------------|--------------|
| | Anakapalli | Bangalore | Chittidhar | Hafsi | Lucknow-46 | Lucknow-49 | Nagpur seedless | Red fleshed | Smooth green |
| Petroleum ether | 2.63 | 2.64 | 2.62 | 2.69 | 2.61 | 2.66 | 2.70 | 2.68 | 2.65 |
| Benzene | 4.26 | 4.20 | 4.18 | 4.40 | 4.42 | 4.31 | 4.40 | 4.32 | 4.40 |
| Ethyl acetate | 5.55 | 5.68 | 5.66 | 5.66 | 5.61 | 5.74 | 5.66 | 5.70 | 5.66 |
| Chloform | 5.24 | 5.30 | 5.28 | 5.18 | 5.20 | 5.36 | 5.24 | 5.28 | 5.30 |
| Ethanol | 18.66 | 18.90 | 19.12 | 18.18 | 17.98 | 19.99 | 18.76 | 19.44 | 18.42 |
| Water | 20.10 | 21.20 | 20.62 | 23.24 | 20.46 | 25.15 | 22.26 | 25.06 | 20.16 |

Table 6: Ash values of 9 different varieties of *P. guajava* leaves

| Range | Ash values % | | | | | | | | | | | | | | |
|-------|--------------|------|------|-----------|------|------|------------|------|------|-------|------|------|------------|------|------|
| | Anakapalli | | | Bangalore | | | Chittidhar | | | Hafsi | | | Lucknow-46 | | |
| | T | AI | WS | T | AI | WS | T | AI | WS | T | AI | WS | T | AI | WS |
| Min | 10.78 | 1.46 | 2.52 | 10.76 | 1.47 | 2.53 | 10.77 | 1.46 | 2.48 | 10.74 | 1.46 | 2.52 | 10.70 | 1.48 | 2.50 |
| Avg | 11.10 | 1.51 | 2.8 | 11.09 | 1.49 | 2.80 | 11.08 | 1.49 | 2.79 | 11.06 | 1.50 | 2.83 | 11.05 | 1.51 | 2.79 |
| Max | 11.49 | 1.56 | 3.02 | 11.50 | 1.52 | 3.04 | 11.52 | 1.52 | 3.01 | 11.50 | 1.54 | 3.08 | 11.46 | 1.55 | 3.01 |

| Range | Ash values % | | | | | | | | | | | |
|-------|--------------|------|------|-----------------|------|------|-------------|------|------|--------------|------|------|
| | Lucknow-49 | | | Nagpur seedless | | | Red fleshed | | | Smooth green | | |
| | T | AI | WS | T | AI | WS | T | AI | WS | T | AI | WS |
| Min | 10.80 | 1.49 | 2.54 | 10.70 | 1.45 | 2.48 | 10.72 | 1.46 | 2.50 | 10.79 | 1.47 | 2.51 |
| Avg | 11.12 | 1.51 | 2.83 | 11.05 | 1.50 | 2.77 | 11.10 | 1.50 | 2.81 | 11.11 | 1.51 | 2.81 |
| Max | 11.58 | 1.54 | 3.06 | 11.44 | 1.56 | 3.02 | 11.49 | 1.54 | 3.08 | 11.60 | 1.54 | 3.02 |

T- Total ash **AI-** Acid insoluble ash **WS-** Water Soluble ash

Table 7: Loss on drying of 9 different varieties of *P. guajava* leaves

| Range | Loss on Drying (%) w/w | | | | | | | | | |
|-------|------------------------|-----------|------------|-------|------------|------------|-----------------|-------------|--------------|--|
| | Anakapalli | Bangalore | Chittidhar | Hafsi | Lucknow-46 | Lucknow-49 | Nagpur seedless | Red fleshed | Smooth green | |
| Min | 9.3 | 9.6 | 9.2 | 9.2 | 9.1 | 9.5 | 9.3 | 9.2 | 9.3 | |
| Avg | 9.8 | 9.9 | 9.8 | 9.7 | 9.7 | 9.8 | 9.8 | 9.9 | 10.0 | |
| Max | 10.5 | 10.2 | 10.1 | 10.3 | 10.0 | 10.3 | 10.4 | 10.3 | 10.4 | |

Powder microscopy

The analysis of the dried powder of the leaf showed paracytic stomata, three layers of wide rectangular cells, conical and flagellate trichome, calcium oxalate druses (present only in hafsi and Lucknow-46 varieties), secretory cavity (absent in Bangalore and chittidhar varieties), parenchymal cells and fragment of palisade mesophyll.

Physiochemical 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 and tabulated (Table : 2 to 7). Foreign organic matter of all varieties was nil.

Preliminary phytochemical screening

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.

Discussion

Herbal medicine is gradually getting popular among people in both developing and developed countries because of its less /no adverse side effects. Quality evaluation of herbal materials for safety and efficacy by using multi-component systems and acceptable analytical methodologies is fundamental as it strengthens their quality, safety and efficacy.^{42, 43}

With respect to quality control, correct identification of the species or different variety of the same species concerned from commonly available adulterants or substitutes, in fresh, dried or powdered state is of prime importance.⁴⁴ Hence we have undertaken this study to serve as a tool for developing standards for identification, quality and purity of nine different varieties of *P. guajava* leaves.

The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials^[45]. Morphology of the leaves showed no difference in all the varieties. A noteworthy feature of this family Myrtaceae is

the presence of secretory cavities. Microscopic evaluation revealed that secretory cavities were absent in Bangalore, Chittidhar and it is present in the lower side in case of Anakapalli, Hafsi, Lucknow-46, Nagpur seedless and Red fleshed, but it was 80µm wide and present in upper portion in the case of Smooth green. Narrow secretory cavities observed in the periphery of the midrib in Lucknow-49. Calcium oxalate druses in dilated cells are present in Hafsi and Lucknow-46 alone. All varieties contain invariably multiple epidermis. Tannin is abundant in the tissue, but it is very less in Lucknow-49 and dark tannin accumulation seen in Red fleshed. In vascular bundle xylem less lignified, uniseriate thick walled in Anakapalli, thin walled dark elliptical sclerenchyma in Bangalore variety. Powder microscopy showed presence of paracytic stomata, fragment of epidermis, conical and flagellate trichomes, fragment of palisade mesophyll, secretory cavity (absent in Bangalore, Chittidhar varieties), calcium oxalate crystals (present in Hafsi and Lucknow -46 varieties) and parenchymal cells. The ash values (total, acid insoluble and water soluble ash), loss on drying and extractive values were evaluated and showed marginal difference in all nine varieties. Leaf constants such as vein islet numbers, vein terminal number, stomatal number and stomatal index were evaluated and showed little different in all varieties. Phytochemical evaluation and molecular characterization of plants is an important task in medicinal botany and drug discovery.⁴⁶ 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. The analysis of the dried powder of the leaf showed paracytic stomata, three layers of wide rectangular cells, conical and flagellate trichome, calcium oxalate druses (present only in hafsi and Lucknow-46 varieties), secretory cavity (absent in Bangalore and Chittidhar varieties), parenchymal cells and fragment of palisade mesophyll.

Conclusion

The study of Pharmacognostical features of *Psidium guajava* Linn. (Nine varieties) 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 adds to its quality control and quality assurance for proper identification.

Acknowledgement

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Conflict of interest statement

We declare that we have no conflict of interest.

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