

DRUG RESEARCH

Pharmacognostic & Physico-Chemical Evaluation Of *Psidium Guajava* L.

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Abstract

Psidium guajava L. belonging to the family Myrtaceae is known as Guava. It is a common fruit tree, cultivated and naturalized throughout India. The leaves are used as an application in wounds, ulcers and as an astringent to bowels in Unani. Besides, it is also used as digestive, febrifuge and antispasmodic, in cerebral affections, nephritis, rheumatism, epilepsy and chorea. Leaves contain catechol and pyrogallol tannins besides oil and vitamins.

Due to growing urge for introduction of well known indigenous drugs into Homoeopathy, the leaves of *P. guajava* are also being adopted. In the present, pharmacognostic studies of leaf comprising macro- and microscopic characters, organoleptic and powder microscopic features, besides physico-chemical constants viz. moisture content, ash and extractive values for raw drugs and parameters such as weight per ml., total solids, alcohol content and chromatographic profiles of TLC, HPTLC, along with UV absorbance for mother tincture are detailed.

Introduction

Psidium guajava L. belonging to the family Myrtaceae is commonly known as *guava* in English, *amrud* in Hindi, *koyya* in Tamil and *tella jama* in Telugu. Guava is also referred to as the apple of the tropics. It is a native of tropical America and cultivated and naturalized throughout India¹. It is an arborescent shrub or a small tree, upto 8 m high. The guava leaves are used as an application in wounds, ulcers, and as an astringent for bowels (Unani). The young leaves are used as a tonic in the diseases of the digestive functions. The decoction of leaves has been used in Cholera and also as a febrifuge and antispasmodic. Infusion of leaves is used in Cerebral affections, Nephritis and Cachexia. The pounded leaves are locally applied in Rheumatism and their extract in Epilepsy and Chorea. Though traditionally the medicinal use of leaves of *P. guajava* finds mention only in Unani system, their folklore utilities have been elaborate⁵. Leaves are reported to contain catechol and pyrogallol tannins. A new flavonol glycoside quercetin 4' glucuronide was reported⁴. Oil of leaves is aromatic and also used as a flavouring agent. Besides, they also contain carotene, vitamins B1, B2 and B6 and vitamin C. Antibacterial activity is reported by Rabe and Van Staden⁸.

Presently, there is a growing urge to introduce indigenous drugs into Homoeopathic system.

In view of this leaves of *P. guajava* is one of the drug also being tried. Review of literature reveals that there is no reported work on pharmacognostic and physico-chemical aspects of the leaves of *P. guajava*. Hence a detailed study on these aspects is presented.

Material and Methods

Leaves of *Psidium guajava* L. was collected from Botanic garden, Department of Botany, Osmania University, Hyderabad. Fresh leaves were fixed in Carnoy's fixative. Subsequently epidermal peels of leaf and petiole were prepared following the method of Leelavathi and Ramayya⁶. Microtome sections of 10-15 μ m thick of leaf and petiole were prepared following the traditional techniques³. Sections were stained with crystal violet and basic fuchsin. Powder studies were made following Youngken⁹ and Johansen³.

The air dried leaves were powdered to obtain coarse powder 10/44 (sieve size). It was subjected to determination of moisture content (L.O.D.), Ash values and extractive values. The above parameters were determined in accordance with the procedures given in Homoeopathic Pharmacopoeia of India². The mother tincture was prepared by percolation technique². Alcohol content for the preparation of mother tincture was fixed as per maximum extractive value (M.E.V.).

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The alcoholic extract (mother tincture) was subjected for a) Physico-chemical constants, b) Chromatography (TLC/HPTLC), c) U.V. absorbance.

All chemicals and solvents used were of analytical grade. Silica gel G (E Merck) was used for thin layer chromatography (T.L.C.). U.V. spectra was recorded using spectrophotometer, Shimadzu make (courtesy CFTRI, Hyderabad)

Physico-chemical Constants

Physico-Chemical parameters viz., organoleptic characters, weight per ml. total solids, alcohol content and pH value, were determined as per the guidelines laid down in the Homoeopathic Pharmacopoeia of India².

Chromatography (T.L.C.)

For TLC 25 ml. alcoholic extract was evaporated on water bath to remove alcohol. The remaining aqueous part was extracted with 25 ml. chloroform (three times). All the three fractions were combined and concentrated to 2ml. 15 μ m was applied on activated silica gel G coated TLC plate. It was developed using chloroform and benzene with few drops of acetic acid (1:1v/v) on mobile phase and 10% FeCl₃ solution was sprayed for visualization.

UV Absorbance

For UV absorbance, the mother tincture was diluted with 99 parts 70% alcohol, scanned in the range of 200-300 nm. The peaks of maximum absorption are given in Table 6.

HPTLC Studies

Precoated high performance TLC plates (silica gel 60 F 254 as stationary phase) were used. For sample application, CAMAG Linomat IV sample applicator was employed. The speed of application was maintained at 4mm/sec. The solvent system used for development was chloroform, acetic acid, methanol and water (6.2 : 2.1 : 2.0 : 0.8 v/v). Methanolic sulphuric acid and anisaldehyde H₂SO₄ were used for derivatisation. The Rf values of the various components before and after derivatisation are provided in Table 5.

OBSERVATIONS

Pharmacognostic Studies

Macroscopy : Leaves 10-15 cm long, oblong or el-

liptic oblong, entire glabrous above, pubescent beneath, pellucid punctuate; lateral nerves 10-20 pairs, prominent beneath, strongly curved near the edge and joined by intro marginal veins, petioles 2.5-7.5 mm long.

Microscopy: Adaxially epidermal cells polygonal isodiametric or anisodiametric with sides slightly thick, straight to curved; surface striated; contents scanty or dense in few. Epidermal cells 5,45,000 per sq. cm. Stomata are absent on adaxial side. Trichomes 1) Unicellular conical hair, longer, distributed all over surface. 2) Unicellular flagellate conical hair, long, flagellate conical, all over, more on vens.

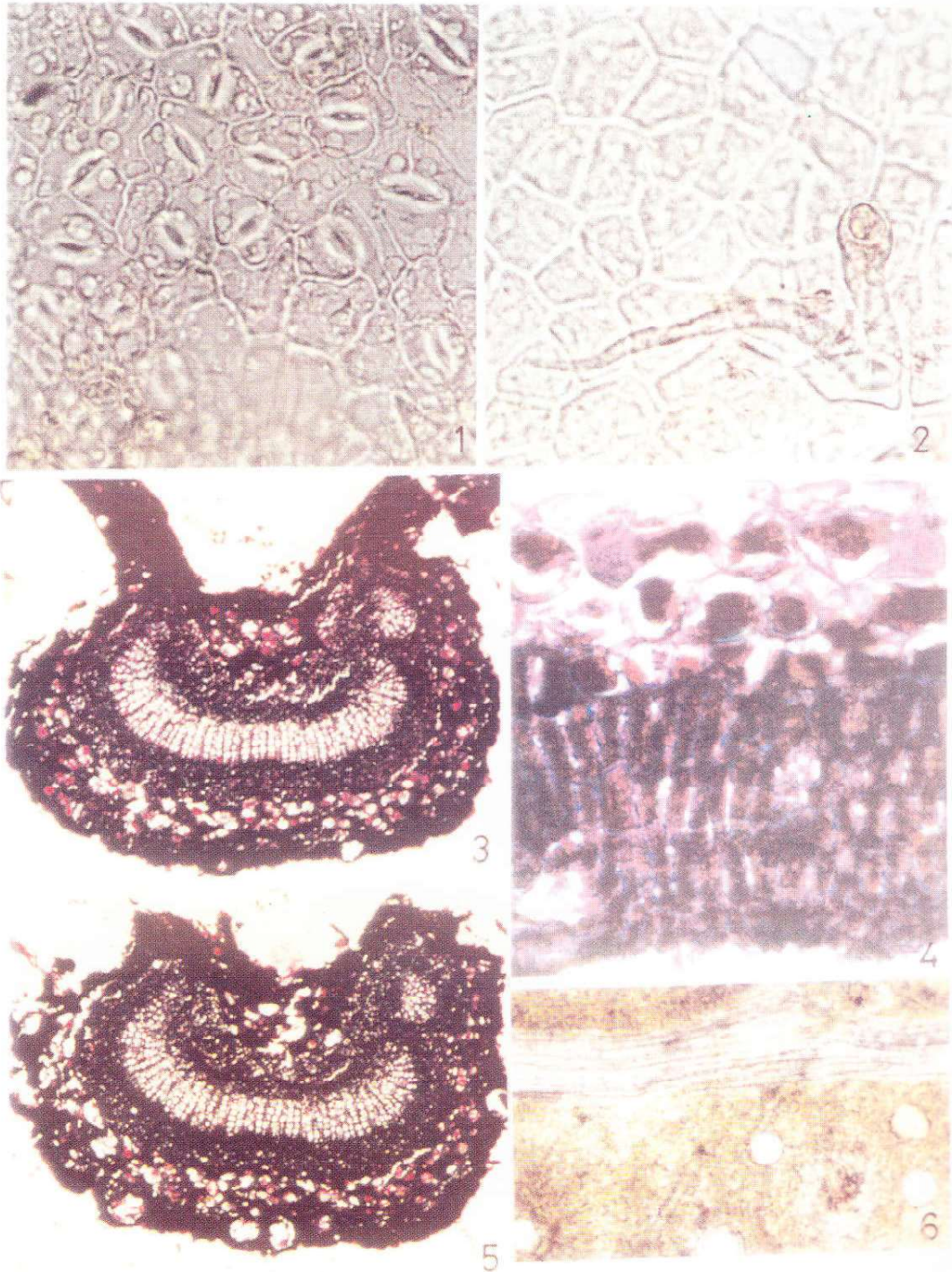
Abaxially cells similar as on adaxial, sides thin with curved or wavy anticlinal walls; contents scanty but with tannins in few; interspersed by secretory cavities. Epidermal cells 10,20,000 per sq. cm. Stomata mostly paracytic, few anisocytic and anomocytic; paracytic subsidiaries longer, distinct. Stomatal pore large, contents dense; Stomatal frequency: 2,90,000 per sq. cm. Stomatal Index: 22.13; Stomatal size; 19-24 μ m (21) long and 12-16 μ m (15) wide. Trichomes are similar to those described on adaxial side.

Transection (TS):

In T.S. midvein is grooved on adaxial side and prominently ribbed towards abaxial. Secondary and tertiary veins ribbed abaxially. Midvein kidney-shaped, laterally upto 1.5 mm and vertically 1mm thick; Lamina isobilateral, 151-173 μ m thick. Covered by conical and flagellate hair. Secretory cavities conspicuously present on abaxial.

Epidermis is 1-layered, adaxially large, barrel shaped or tabular to polygonal, covered by a thick cuticle and often dispersed with conical hairs. Abaxially cells smaller, interrupted by stomata, which are sunken. Mesophyll is characterized adaxially with a hypoderm, consisting of 3-4 layered tabular to barrel shaped cells often containing tannins, sphaeraphides and resins and interrupted by secretory cavities. Palisade is abaxial, 4-5 layered, cells cylindrical, 11-27 μ m (19) long and 5.5-8 μ m (7) wide; filled with chloroplasts, tannins and sphaeraphides and interrupted by secretory cavities.

Ground tissue at midvein of collenchyma, parenchyma and sclerenchyma tissues. Collenchyma: abaxially 1-layered and as a group of cells on adaxial side, 5-19 μ m (13) in diameter; angular or lamellar, contents dense. Parenchyma:



PSIDIUM GUAJAVA

- | | |
|--|-------|
| 1. Leaf epidermis, Abaxial surface | x 485 |
| 2. Leaf epidermis, Abaxial surface | x 418 |
| 3. Leaf midvein in Transection | x 69 |
| 4. Leaf lamina in transection | x 45 |
| 5. Petiole in transection | x 56 |
| 6. Leaf surface showing secretory cavities | x 104 |

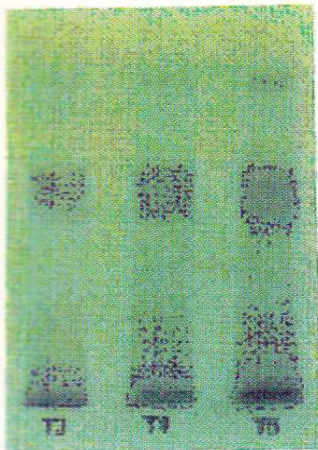


Fig.1

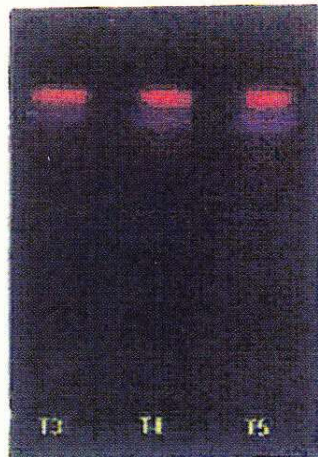


Fig.2



Fig.3

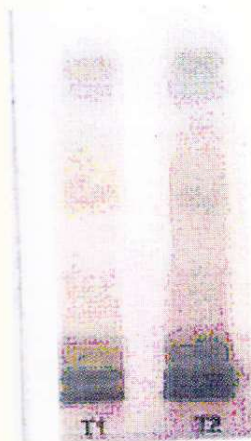


Fig.4

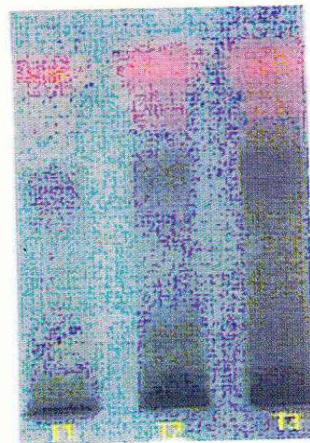


Fig.5



Fig.6

HPTLC CHROMATOGRAMS OF MOTHER TINCTURE OF PSIDIUM GUAJAVA

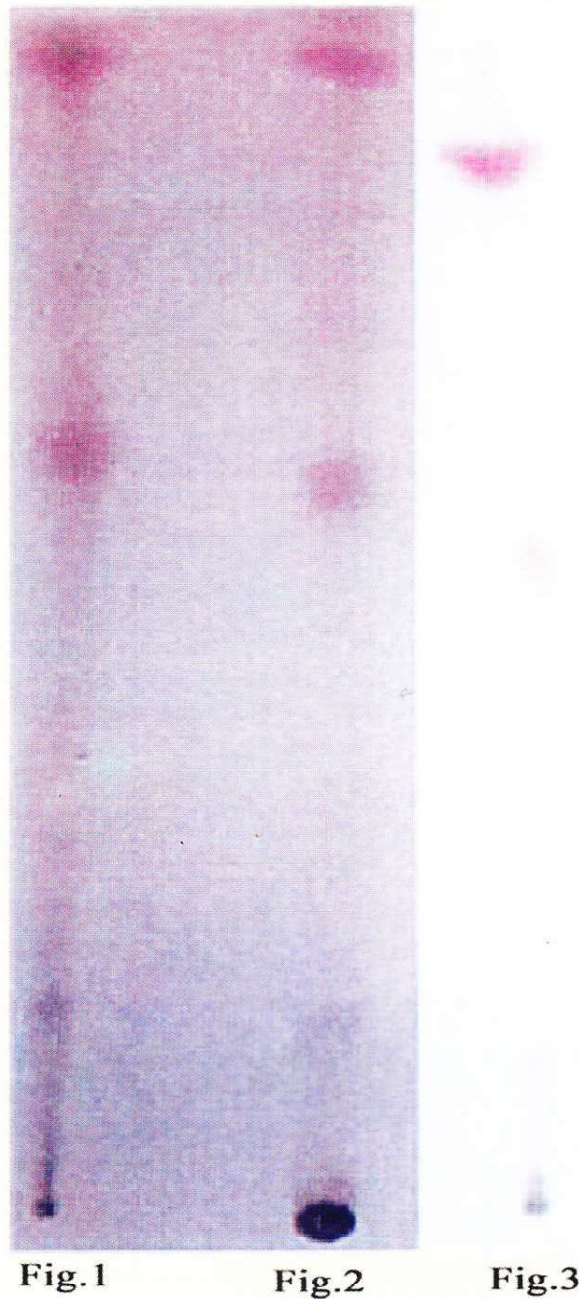
- Fig. 1 Images of chromatograms at 254 nm. before derivatization.
- Fig. 2 Images of chromatograms at 366 nm. before derivatization.
- Fig. 3 Images of chromatograms at 366 nm. after Spraying with Methanolic H_2SO_4 .
- Fig. 4 Visible images of chromatograms after derivatization with Methanolic H_2SO_4 .
- Fig. 5 Images of chromatograms at 366 nm. after Spraying with anisaldehyde H_2SO_4 .
- Fig. 6 Visible images of chromatograms after derivatization with anisaldehyde H_2SO_4 .

Mobile phase - Chloroform : Acetic acid : Methanol : Water
(6.3 : 2.1 : 2 : 0.8)

Stationary phase - TLC plates Silica gel 60F 254 E. Merck.

Sample concentration - T1 - 5 UL, T2 - 10 UL, T3 - 15 UL,
T4 - 20 UL, T5 - 25 UL,

T. L. C. PROFILES OF *PSIDIUM GUAJAVA*



TLC PROFILE OF *PSIDIUM GUAJAVA*

Fig. 1 *Psidium guajava* mother tincture (Chloroform extract)

Fig. 2 70% Alcoholic extract of *Psidium*.

Fig. 3 Refined figure of Chloroform extract.

S.S. Chloroform : Benzene, few drops of Acetic acid. (1:1 v/v).

Detecting agent : 10% Ferric chloride solution.

8-10 layered on abaxial and 6-8 layered on adaxial; interspersed with secretory canals, sphaeraphidal and tanniferous cells; 13-68 μm (11) in diameter, walls lignified, contents scanty to slightly dense with tannins and crystals of calcium oxalate in few.

Vascular tissue of mid vein consists of a central wedge or sigma shaped bundle, 918-950 μm laterally long and 183-205 μm thick; endarch, bicollateral, closed, pericyclic with two small adaxial bundles. Xylem elements in radial rows, secondary walls helical, scalariform and reticulate. Phloem on either sides with phloem parenchyma, sieve cells and fibers.

Petiole

Epidermal cells polygonal iso- to anisodiametric, sides thin, straight to curved, surface finely striated; contents slightly dense, in some with tannins and crystals of calcium oxalate; interspersed with secretory cavities; Stomata absent. Surface covered with few conical and flagellate hairs.

Transection

In T.S. 3-4 mm in diameter, elongated laterally with an adaxial groove; covered by conical or flagellate hairs.

Epidermis is unilayered, polygonal to barrel shaped; walls slightly thick, contents tanniferous. Collenchyma is 1-layered on the abaxial and 1-2 layered on the adaxial, cells polygonal to spherical, angular or lamellar; in few with tannins and chloroplasts. Parenchyma is 12-16 layered

adaxially and 18-22 celled on the abaxial; polygonal to spherical, 11-63 μm (32) in diameter; contents slightly dense, in few with tannins and sphaeraphides, often interspersed with secretory cavities. Sclerenchyma is 8-12 layered, enclosing the vascular bundle; cells polygonal, walls lignified, thick, 8-19 μm (13) in diameter, contents slightly dense, with tannins in few. Fibers in longitudinal section long, non-libiriform and septate.

Vascular tissue consists of a single large sigma shaped bundle at the center; endarch, bicollateral, conjoint and pericyclic; 2065-2151 μm long and 481-537 μm thick vertically. Xylary cells arranged in radial rows, few clustered, polygonal to spherical, 5.5-22 μm in diameter; walls thick, lignified, associated with xylem parenchyma; secondary walls mostly helical, few scalariform; phloem scanty, on either sides of xylem and enclosed by sclerenchymatous pericycle.

Powder Studies

Microscopy: Trichomes conical and flagellate conical, either whole or fragments many. Pieces either whole or fragmented sphaeraphides. Fragments of epidermis with wavy sides with stomata. Pieces of leaf dark and greenish with tannins. Pieces of sclerenchymatous fibers with xylary tissue. Pieces of leaves with secretory cavities.

Organoleptic Characters

Colour	:	Light dull green
Touch	:	Smooth or finely coarse
Odour	:	No characteristic
Taste	:	No characteristic

PHYSICO-CHEMICAL STUDIES

TABLE - 1

Standardisation of Raw Drug

S.No.	Parameters	Quantitative values
1.	Moisure content (L.O.D. at 105 C)	12.7% W/W
2.	Ash value (total)	11.827% W/W
3.	Acid insoluble ash	1.59% W/W
4.	Water soluble ash	3.135% W/W
5.	Water soluble extractive	15.0% W/W
6.	Alcohol soluble extractive	23.5% W/W

TABLE - 2

Determination of M.E.V. using different strengths of alcohol

Sl.No.	Strength(s) of alcohol (% V/V)	Extractive value	Remarks
1.	30%	23.79%	70% alcohol Used for preparation of Q on the basis of No. of spots and stability.
2.	40%	27.25%	
3.	50%	20.15%	
4.	60%	19.50%	
5.	70%	11.80%	
6.	75%	22.45%	
7.	80%	16.50%	
8.	90%	12.00%	
9.	Absolute	23.50%	

TABLE - 3

Formulation and preparation of mother tincture

Coarse powder of leaves of <i>P. guajava</i>	:	100g
Purified water	:	300 ml.
Strong alcohol	:	735 ml.
To make one thousand ml. of the mother tinctures		

TABLE - 4

Standardisation of mother tincture

S.No.	Parameters	Observations
1.	Organoleptic characters	Clear, non-viscous Tab brown Fruity and aromatic
	(a) Appearance	
	(b) Colour	
	(c) Odour	Absent
2.	Sediments	
3.	Weight per ml	0.887 g.
4.	Total Solids	2.34%
5.	pH at room temperature	4-4.5
6.	Alcohol content	68%

TABLE - 5

Chromatographic result of : *Psidium guajava* Mother tincture (Q)

Extract	:	Chloroform extract of Q
Adsorbent	:	Silica gel G
Layer thickness of plate	:	0.4 mm in wet conditions

Solvent system	Detecting agent	No. of spots	Rf values	Colour of spots	
Chloroform : Benzene, a few sol. Drops of CH ₃ COOH (1:1 V/V)	10% FeCl ₃ Sol.	13	0.5	Pink	
			0.9		
			0.24		
			0.28		
			0.39		
			0.45		
			0.51		
			0.59		Grey
			0.69		
			0.73		Green
			0.82		
			0.87		Pink
			0.92		

TABLE - 6

UV Absorbance of alcoholic extract of leaves of *P. guajava*

Mother tincture	No. of peaks	UV absorbance
Psidium guajava Q	5	259.5 nm 254.3 nm 239.4 nm 221.5 nm 212.5 nm

Discussion

Psidium guajava is also known as the apple of the tropics and grows under a wide variety of climatic conditions. Guava is one of the richest natural sources of vitamin C besides pectin and other nutrients. Leaves are reported to contain catechol and pyrogallol types of tannins¹. The leaves are popularly used in tanning leathers.

a) Pharmacognostic features :

The leaves are light green, finely pubescent and chartaceous. Epidermal cells in surface show striations on adaxial side. Stomata occur only on lower surface. Paracytic type being dominant besides anisocytic and anomocytic. Unicellular conical and uniseriate flagellate conical hairs occur on either surfaces. Stomatal index is 22.13.

Transectionally midvein is kidney shaped being prominently ribbed towards abaxial and grooved adaxially. Lamina wing is 151-173 μm in thickness. Secretory cavities appear conspicuously on the abaxial surface. An adaxial hypoderm of 3-4 layers is conspicuous and confirms earlier observations of Metcalfe and Chalk⁷. Palisade is 4 layered and is confined to abaxial side. Secretory cavities interrupt the mesophyll. Cells of mesophyll often contain tannins and sphaeraphides besides chloroplasts.

Ground parenchyma at midvein is interspersed with secretory cavities besides sphaeraphidal and tanniniferous idioblasts. A sclerenchymatous cap of 8-10 celled thick encloses the midvein bundle.

Central vascular bundle is sigma shaped, 918-950 μm laterally long and 183-205 μm vertically thick; endarch, and bicollateral. Secondary walls of xylary elements possess helical and scalariform thickenings.

Petiole

In surface epidermal cells are polygonal with straight to curved sides. Epidermal cells often possess tannins and prismatic crystals of calcium oxalate. The epidermal surface also is dispersed with some conical and flagellate hairs.

In transverse section 3-4 mm in diameter, laterally elongated with an adaxial groove. Epidermis is 1-layered and hypodermal collenchyma is 1-2 layered. Ground parenchyma is predominant, interspersed with cells containing tannins and sphaeraphides. 8-12 layered sclerenchyma encloses the central vascular bundle.

Vascular tissue is made of a single large sigma shaped bundle which is bicollateral and pericyclic. Phloem is scanty and present on either sides of xylem.

b) Physico-chemical

Alcohol extract and mother tincture have shown positive tests for tannins. The data pertaining to physico-chemical studies of raw drugs is presented in table 1. Formulation of mother tincture is given in table 2 & 3. Physico-chemical constants of mother tincture are summarized in Table 4. TLC study on Chloroform extract of the mother tincture reveals 3 prominent spots on spraying with 10% Ferric chloride solution and heating at 110 C (Table 5). It is evident from the raw drug studies that the value of acid insoluble ash falls within the acceptable range.

HPTLC profile shows absorbance in the short and long wave length of UV light before derivitisation while after derivitisation i.e. subsequent to spraying with methanolic sulfuric acid and anisaldehyde sulfuric acid separately, reveals eight bands with Rf values 0.15, 0.19, 0.28, 0.51, 0.57, 0.66, 0.79 and 0.94 correspondingly in both.

The diluted mother tincture (1 ml Q diluted with 99 ml of alcohol) reveals large number of peaks in the UV region. It is clear that 70% alcohol used for preparation of mother tincture is the best alcohol in accordance with Homoeopathy philosophy. However, five major peaks amongst them are reported.

Acknowledgements

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References

1. Anonymous, 1969, Wealth of India, Raw material, Vol. 8, Publication and Information Directorate, CSIR, New Delhi.
2. Anonymous, 1971, Homoeopathic Pharmacopoeia of India, Vol 1, Ministry of Health and Family Welfare, Govt. of India, New Delhi.
3. Johnson, D.A. 1940, Plant Microtechnique, Mc Graw Hill Book Col., New York.
4. Kaudil, F.E. et al., 1997. Flavonoids from *Psidium guajava*, Asian Journal of Chemistry, 9(4), 871-872.
5. Kirtikar, K.R. AND b.d. Basu, 1980, Indian Medicinal Plants, Vol. 2, Bishen Singh Mahendra Pal Singh, Dehradun.
6. Leelavathi, A. and N. Ramayya, 1975, Rapid, isolation of leaf epidermis by double treatment method, Geobios, Vol. 2, 117-119.
7. Metcalf, C.R. and L. Chalk, 1950, Anatomy of the Dicotyledeons, Vol. 1, Oxford University Press, London.
8. Rabe, T. and J. Van Staden, 1997, Antibacterial activity of South African plants used for medicinal purposes, J. Ethnopharmacology, 56(1), 81-87.
9. Youngken, H.W., 1951, Pharmaceutical Botany, Rep. Ed. International Book Distributors, Dehradun.