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Research Article

A PHARMACOGNOSTICAL APPROACH FOR STUDY OF *PSIDIUM GUAJAVA* LINN

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ABSTRACT

This study deals with the detailed pharmacognostical evaluation of the most frequently encountered species *Psidium guajava* Linn (Myrtaceae). Morphology of the young and mature leaves have been studied with keeping the aim to aid pharmacognostic and taxonomic species identification using Leica DME Microscope at the 10x & 10x magnifications, WHO recommended physiochemical, morphological and histological parameters presented in this paper may be proposed as parameters to establishes the authenticity of *Psidium guajava* Linn and can possibly help to differentiate the drug from its other species/varieties.

Keywords: *Psidium guajava;* Stomatal number; Stomatal index; Vein islet and Vein termination number.

INTRODUCTION

Herbal medicine has been used in India for thousands of years worldwide during last few decades as evidenced by rapidly growing global and national market of herbal drugs.[1] For centuries, nature has been an enormous source of agents of medical importance. *Psidium guajava*, a tropical fruit guava of the family Myrtaceae, is widely recognized as a plant of many herbal medicines.[2] Guava, is an evergreen shrub or small tree 2 to 8 m in height and up to 40 cm in diameter at breast height. Plants may have a single stem, especially if crowded in secondary forest, but individuals receiving ample light usually develop secondary stems arising from the main stem near the ground. The branches and stems are usually crooked and have a smoothes, cream to reddish-brown bark between thin, irregular scales that peel off. Guava bark is 5 to 8 mm thick.[3] The sapwood is light brown and the heartwood is reddish brown, hard, heavy (specific gravity of 0.8), and strong.[4] The roots are slender. The young twigs are four-angled, slightly winged, and green, turning brown with age. The leathery and light green or yellow green opposite leaves have petioles 4 to 7 mm long, and elliptic or oblong blades, 8 to 14 cm long that are short or round pointed at both ends. The foliage is aromatic when crushed. Fruits (berries) are globose or pear-shaped, with a prominent, persistent calyx.[5] The first step towards ensuring quality of starting material is authentication followed by creating numerical values of standards for comparison. Pharmacognostical parameters for easy identification like leaf constants, microscopy & physico chemical analyses are few of the basic protocol for standardization of herbals. Hence, in the present work the pharmacognostical standardization has been performed for the mature and young leaf of the plant.

MATERIALS AND METHODS

Collection and Authentication

The plant and their parts were collected from Mesra, Ranchi, Jharkhand, India in the month of August and September and was authentified by Dr. S. Jha, Professor, Department of Pharmaceutical sciences, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India. Plant was dried in the shade for 15 days and packed tightly in Poly Vinyl Chloride jar and stored in dark place.

For the microscopic studies, transverse sections were prepared and stained.[6, 7] The leaves were boiled separately with saturated chloral hydrate solution for surface studies and quantitative microscopical observation of leaf.

Quantitative Microscopy

A piece of leaf was cut in the middle portion and boiled in chloral hydrate solution or treated with chlorinated soda. Upper and lower epidermis were peeled out and mounted on glycerin on a glass slide. Then by using stage micrometer and camera Lucida, one square mm was drawn in a black sheet. Then by the help of microscope 5 x & 10 x magnification and camera Lucida the number of stomata and epidermal cells were counted within the square. Leaf fragments were observed for the presence and quantification of epidermal cells, stomata (type and distribution), palisade cells, vein islet number and vein termination number. Stomatal index was calculated as the percentage of number of stomata present per number of epidermal cells and each stoma was counted as one cell.[8, 9]

Physico Chemical Properties

About 2.5 g of the powdered drug of both mature and young leaf was accurately weighed in a tarred silica crucible. The powder was incinerated gradually by increasing the heat until free from carbon and cooled before weighing. The ignition was repeated to get constant weight. The percentage of total ash was calculated with reference to air dried leaf powder. However, the total ash was boiled with 2 N HCL and water to calculate, acid insoluble ash and water soluble ash respectively. The moisture content was also determined.[10, 11]

Extractive value

The coarsely powdered air dried drug was macerated with water and alcohol. Then the extractive value in water and alcohol was calculated and percentage of extractive value was calculated with reference to the air dried leaf powder.[9, 11]

Anatomical Study

The transverse section of leaf passing through midrib and lamina was dipped in 30% alcohol for 2-3 minutes then in 50% and 70% alcohol for the same time. Again the section was transferred in 90% alcohol to 70%, 50% and 30% alcohol for the same time. After that the section was transferred in a solution of safranin for 30 sec and was then washed thrice with plain water to prepared slide.[12, 13] However, powder microscopy was also performed using chloral hydrate, water and a mixture of 1:1 Phloroglucinol + conc. HCL. The well known identifying characters of leaf were determined under Leica DME Microscope at the 10x & 10x magnifications. [14]

RESULTS AND DISCUSSION

A comparative study to understand the differences in pharmacognostical parameters of young and mature leaves forms the basis of study. The average stomatal index on upper epidermis is more than on lower epidermis for mature leaf and found opposite in case of young leaf (Table 1). It is obvious from the Table 2 that mature leaves contain more vein-islet and vein termination number than young one. Infact, young leaves contain more moisture, moreover, water and alcohol soluble compounds are present nearly

in same amount in both young and mature leaves (Table 3). TS of the lamina show polygonal tubular cells with straight anticlinal cell wall in its upper and lower epidermis. While, unicellular epidermal trichome is found in the base, however, one layered palisade cells is prevalent below upper epidermis. In addition, TS of midribs show gutter shaped xylem and phloem and beneath the phloem pericycle present, which, contain collenchymatous cells. The covering, unicellular trichomes are present at upper surface and under surface; moreover, remaining tissue of the midribs is filled with parenchyma (Fig 1). The powder microscopy also reveals the presence of abundant unicellular trichome, paracytic stomata, xylem vessel, calcium oxalate crystals and few crystal sheath (Fig 2).

Table 1: Determination of Stomatal number and Stomatal index of young and mature leaves of *Psidium guajava* Linn.

Part Used	Young Leaf*			Mature Leaf#		
	Stomatal number/mm²	Experimental cell/mm²	Stomatal index	Stomatal number/mm²	Experimental cell/mm²	Stomatal index
Lower	25	46	35.2	22	43	34.9
Epidermis	20	50	28.5	19	35	35.1
	24	48	33.3	24	48	33.3
	23	53	30.2	23	50	31.5
	18	45	28.5	17	41	29.3
	19	42	31.1	20	43	31.7
Upper Epidermis	16	32	33.3	15	38	28.3
	12	26	31.5	12	35	25.5
	20	37	35.0	14	39	26.4
	15	33	31.2	16	42	27.5
	17	38	30.9	13	40	24.5
	18	35	33.9	14	37	27.4

*Lower epidermis – Average Stomatal number – 21.1, Average Stomatal index – 31.1; Upper epidermis – Average Stomata number – 16.3, Average Stomatal index- 32.8.
Lower epidermis – Average Stomatal number - 20.8, Average Stomatal index – 32.6; Upper epidermis – Average Stomatal number - 14.0, Average Stomatal index- 26.6.

Table 2: Determination of Vein islet number and Vein termination number of both young and mature leaves of *Psidium guajava* Linn.

Sl. No.	Young Leaf*		Mature Leaf#	
	No. of vein islet per mm²	No. of vein termination per mm²	No. of vein islet per mm²	No. of vein termination per mm²
1	12	30	14	35
2	15	29	13	39
3	14	32	15	37
4	15	30	14	36
5	13	33	16	37
6	12	31	12	38
7	13	29	14	34
8	15	33	16	38
9	14	32	15	37
10	15	34	13	36

*Average vein islet number - 13.8; Average vein termination number: 31.3.
Average vein islet no.14.3; Average vein termination number: 36.7.

Table 3: Physio-chemical parameter of young and mature leaf of *Psidium guajava* Linn.

Parameters (%)	Young leaf	Mature leaf
Ash value	6.2	7.0
Water soluble ash value	16.0	11.36
Acid insoluble ash value	57.9	16.0
Water soluble extractive value	5.4	5.2
Alcohol soluble extractive value	4.9	4.7
Moisture Content	14.0	10.75

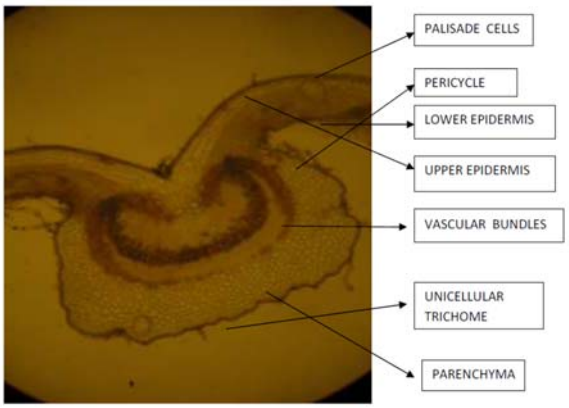

Fig. 1: T. S. of *Psidium guajava* Linn. Leaf (10X)

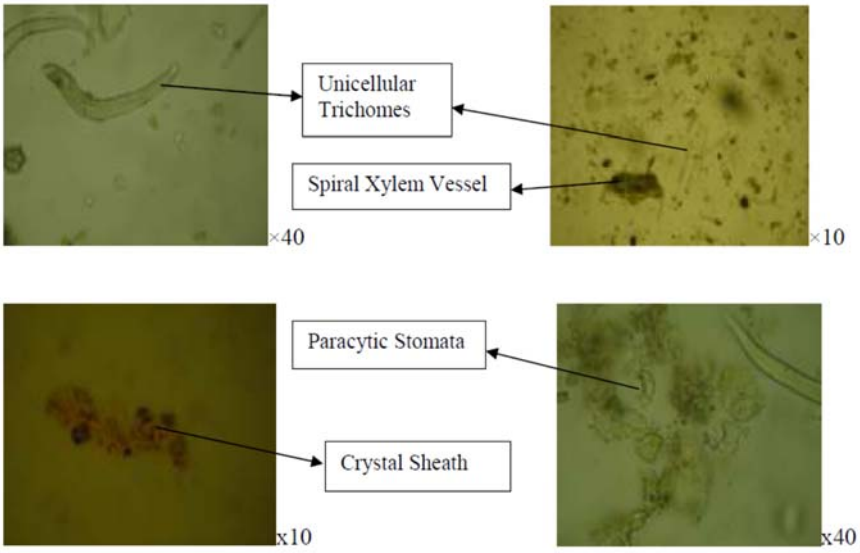

Fig. 2: Powder characterstics of *Psidium guajava* leaf powder

CONCLUSION

The pharmacognostic parameters, which are being reported, could be useful in the identification and standardization of a crude drug. The data produced in the present investigation is also helpful in the preparation of the crude drug's monograph and inclusion in various pharmacopoeias. However, the comparative data will help in understanding the changes that occur, when the leaves mature.

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