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Research Article

EVALUATION OF PHARMACOGNOSTICAL AND PHYTOCHEMICAL PROPERTIES OF THE LEAVES OF *Psidium guajava Linn*- SMOOTH GREEN VARIETY

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ABSTRACT

Objective: To explore the micro morphology and physio chemical parameters of the leaves of *Psidium guajava* Linn. (Myrtaceae) – Smooth green variety.

Methods**:** Macroscopy, microscopy, physicochemical analysis, preliminary phytochemical screening and other WHO recommended parameters for standardizations were performed.

Results**:** Leaves (5-12cm × 4-5 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. Microscopic evaluation revealed the presence of paracytic stomata, slightly dilated square shaped epidermal cells, secretory cavities, palisade mesophyll and parenchymal cells. Vein islet numbers, vein termination numbers, stomatal number, stomatal 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 aminoacids, 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 leaf.

Keywords: Psidium guajava, Myrtaceae, Microscopical evaluation, Physiochemical analysis.

INTRODUCTION

Psidium guajava .Linn commonly called as 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 wide panel of diseases like ulcers, wounds, astringent, antiemetic, cholera, epilepsy etc [1]. Leaf is traditionally used for antispasmodic, anodyne, febrifuge[2], scurvy [3], malaria [4], antiseptic[5], antibacterial[6-8], antifungal [9] dysentery, diarrhoea[10,11], anti-inflammatory [12,13], gout[14], hypoglycaemic[15], headache, fever, gonorrhoea, dysmenorrheal[16], haemostat[17], antihypertensive[18], analgesic[19], hepatoprotective[20] and anticoagulant[21].

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, isoneriucoumaric 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.

MATERIALS AND METHODS

Chemicals

Formalin, acetic acid, ethyl alcohol, chloral hydrate, toludine 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. (Smooth green) selected for our study was collected from Horticulture Department, Madurai, Tamilnadu, India and was authenticated by **Dr.Stephen,** Department of Botany, American college, Madurai and **Dr. P. Jayaraman**, Director of Plant Anatomy Research Institute, Tambaram, Chennai, Tamilnadu, India.

Macroscopic analysis

Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc was noted [24].

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 [25] .Sections were taken using microtome. Permanent mount was prepared using saffranin fast green double staining technique [26]. 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 [27, 28].

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, palisade ratio were determined [29- 31].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure [32, 33].

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 (5-12cm × 4-5cm) 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 (Fig 2). Flowers

Figure 1: Habit of *P.guajava* L

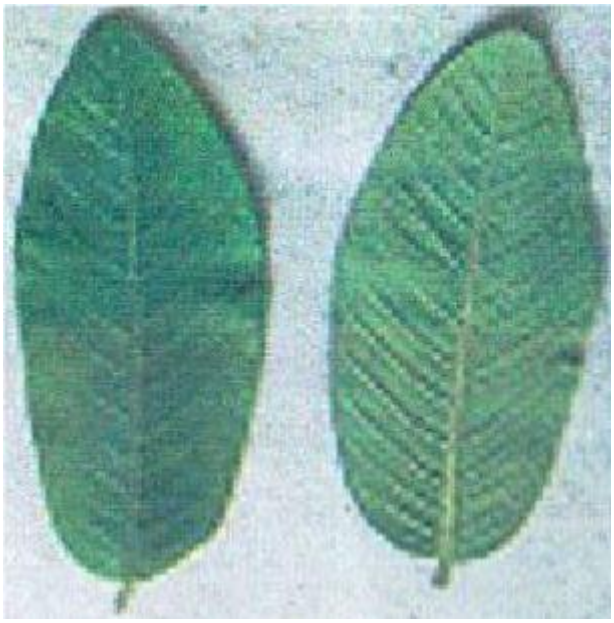

Figure 2: Dorsal and ventral view of the leaves of *P.guajava*- (SMOOTH GREEN)

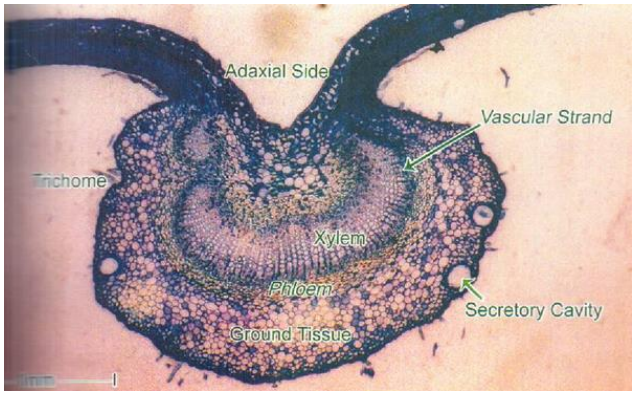

Figure 3: T.S through MIDRIB of *P.guajava* L. leaves (SMOOTH GREEN)

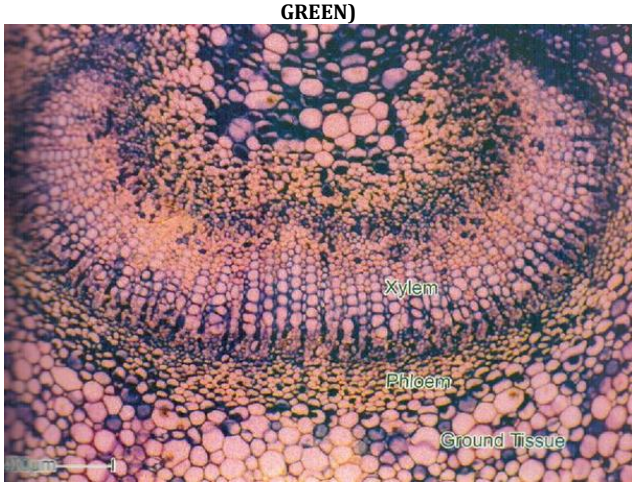

Figure 4: T.S through MIDRIB of *P.guajava* L. leaves – A portion enlarged- (SMOOTH GREEN)

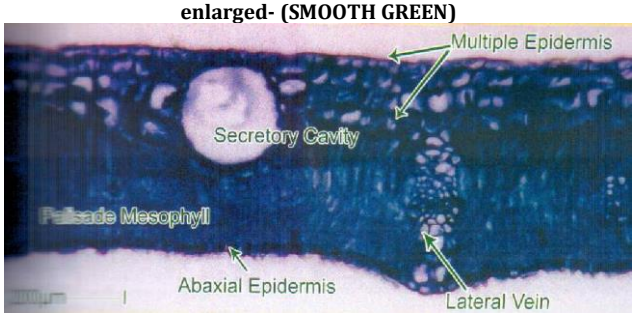

Figure 5: T.S of LAMINA (SMOOTH GREEN)

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.

Microscopy of the leaf

Transverse section (T.S) of the leaves through the midrib showed the following tissue systems.

Shape: Leaves are dorsiventral with prominent midrib, 1.1mm thick, shallow wide, concave adaxial side with spreading lamina and semicircular abaxial side with more or less even outline (Fig 3).

Vascular bundle**:** Vascular strand is broad and arc shaped, 1mm wide, 200µm thick. Xylem elements are thin walled and 20µm wide. Phloem zone is narrow and occurs opposite to metaxylem element (Fig 4).

Lateral vein**:** The vascular strand of lateral vein is vertically elongated and collateral with parenchymatous small bundle caps.

Mesophyll**:** Short compact two layers of palisade cells (50µm in height). Short three layer of palisade like spongy parenchyma.

Ground tissue**:** Parenchymatous, thin walled circular and compact, wide circular secretory cavities are frequently seen in the periphery.

Epidermis: Smooth and even in the raised lower side of the lateral vein. The adaxial epidermis is narrow with thinwalled elongated cells. Three layers of subepidermal cell which are slightly dilated and square shaped (Fig 5).

Powder microscopy: The analysis of the dried powder of the leaf showed paracytic stomata, secretory cavities, xylem and phloem cells, fibres, slightly dilated square shaped epidermal cells, parenchymal cells and fragment of palisade mesophyll (Fig 6).

Physicochemical analysis

Physicochemical parameters were found as follows: total ash 11.11%w/w, acid insoluble ash 1.51, water soluble ash 2.81%w/w, ethanol soluble extractive value 18.42%w/w, water soluble extractive value 20.16%w/w, petroleum ether soluble extractive 2.65%, benzene soluble extractive 4.4%w/w, ethyl acetate soluble extractive 5.66%w/w, chloroform soluble extractive 5.3%w/w, loss on drying 10%w/w and foreign organic matter was nil. Leaf constants were as follows vein islet number 3.3, vein termination number 4.3, stomatal number (lower epidermis) 49.7, stomatal number (upper epidermis) 35.1, stomatal index (lower epidermis) 19.1, stomatal index (upper epidermis) 20.3.

Preliminary phytochemical screening

Preliminary phytochemical screening showed the presence of flavonoids, terpenoids, sterols, tannin, volatile oil , saponins, proteins and amino acids, carbohydrates, reducing sugars, and absence of alkaloids, cyanogenetic glycosides, anthroquinone glycosides, cardiac glycosides, mucilage and fixed oil.

DISCUSSION

Sensory evaluation plays a key role in determining the suitability or denunciation of a crude drug. Organoleptic testing of a crude drug is mainly for qualitative evaluation based on the observation of morphological and sensory profile. In this report, various morphological, microscopical, physicochemical standards have been developed. Hence we have undertaken this study to serve as a tool for developing standards for identification, quality and purity of

P.guajava leaves.

Adulteration and misidentification of crude drugs can cause serious health problems to consumers and legal problems for the pharmaceutical industries. It can be conducted via a variety of techniques, namely macro and microscopic identification and chemical analysis especially description of microscopic botanical aspects to determine definitively the proper species of plant material while it is still in its non extracted form. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. These characters are especially important for identification of powdered drugs, because in these cases most of the morphological diagnostic features are lost [28]. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials [34]. The macroscopic and organoleptic characters of the leaf can serve as diagnostic parameters [35]. Microscopic evaluation showed thick, shallow wide, concave adaxial side with spreading lamina and semicircular abaxial side. Vascular strand is broad and arc shaped. Phloem zone is narrow and occurs opposite to metaxylem element. The vascular strand of lateral vein is vertically elongated and collateral with parenchymatous small bundle caps. Ground tissue - Parenchymatous, thin walled circular and compact, wide circular secretory cavities are frequently seen in the periphery. The ash values are particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter) [36]. Acid insoluble ash provides information about non-physiological ash produced due do adherence of inorganic dirt, dust to the crude drug. Increased acid insoluble ash indicates adulteration due do dirt, sand (or) soil. The extractive values are primarily useful for the determination of exhausted or adulterated drug and helpful in the detection of adulteration [37]. Phytochemical evaluation and molecular characterization of plants is an important task in medicinal botany and drug discovery [38]. 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. Dried powder of the leaf showed paracytic stomata, three layers of wide rectangular cells, secretory cavity, conical and flagellate trichome, parenchymal cells and fragment of palisade mesophyll.

CONCLUSION

The study of Pharmacognostical features of *Psidium guajava* Linn. (Smooth green) 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 add to its quality control and quality assurance for proper identification.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

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REFERENCES

1. Anonymous. *Wealth of India* Raw materials. Vol II. National Institute of Science Communication and Information Resources (NISCAIR), CSIR: New Delhi; 2005,293.
2. Warrier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants– a Compendium of 500 Species. Vol. IV. Arya Vaidya Sala, Orient Longman Limited: Kottakal; 1995, 371.
3. Nadkarni KM, Nadkarni AK. Indian Materia Medica. Vol. I. 3rd ed, Popular Prakasham: Mumbai; 1976, 1019.
4. Nundkumar N, Ojewole JA. Studies on the antiplasmodial properties of some South African medicinal plants used as antimalarial remedies in Zulu folk medicine. Methods Find Exp Clin Pharmacol 2002; 24(7):397-401.
5. Hernandez, Dolores F. Plants of the Philippines. 2nd ed. M&L Licudine Enterprises: Phillipines; 1980.
6. Gnan SO, Demello MT. Inhibition of Staphylococcus aureus by aqueous Goiaba extracts. J Ethno pharmacol 1999; 68:103-8.
7. Oliver-Bever, Bep. Medicinal Plants in tropical West Africa. Cambridge University Press: Cambridge; 1986.
8. Jaiarj P, Khoohaswan P, Wongkrajang Y, Peungvicha P, Suriyawong P, Saraya MLS. Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. J Ethno pharmacol 1999; 67(2): 203-12.
9. Dutta BK, Rahman I, Das TK. *In vitro* study on antifungal property of common fruit plants. Biomedicine 2000; 20(3): 187-89.
10. Lin J. Puckree T, Mvelase TP. Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. J Ethno pharmacol 2002; 79(1): 53-6.
11. Ticzon, Romeo. Ticzon Herbal Medicine Encyclopaedia. Ticzon Publishing: Philippines; 1997.
12. Muruganandan S, Srinivasan K, Tandan SK, Jawahar Lal, Chandra S, Raviprakash V. Anti-inflammatory and analgesic activities of some medicinal plants. Journal of Medicinal and Aromatic Plant Sciences 2001; 22/23: 56-8.

13. Kavimani S, Karpagam RI. Jaykar B. Anti-inflammatory activity of volatile oil of *Psidium guajava*. Indian Journal of Pharmaceutical Sciences 1997; 59(3): 142-44.
14. Conway, Peter. Tree Medicine – a comprehensive guide to the healing power of over 170 trees. Judy Piatkus (Publishers) Ltd: 2001.
15. Wyk, Ben-Erik van, Oudtshoorn, Bosch van, Gericke N. Medicinal Plants of South Africa. 1st ed. Briza Publications: Pretoria, South Africa; 1997.
16. Jain SK. Dictionary of Indian folk medicine and Ethnobotany. Deep Publications: New Delhi; 1991.
17. Qian H, Nihorimbere V. Antioxidant power of phytochemicals from *Psidium guajava* leaf. J Zheji Univ Sci 2004; 5(6): 676-83.
18. Ojewole JA. Hypoglycemic and hypotensive effects of *Psidium guajava* Linn leaf aqueous extract. Methods Find Exp Clin Pharmacol 2005; 27(10): 689-95.
19. Ojewole JA. Anti inflammatory and analgesic effects of *Psidium guajava* Linn leaf aqueous extract in rats and mice. Methods Find Exp Clin Pharmacol 2006; 28(7): 441-46.
20. Roy CK, Kamath JV, Asad M. Hepatoprotective activity of *Psidium guajava* Linn leaf extract. Indian J Exp Biol 2006; 44(4): 305-11.
21. Hsieh CL, Lin YC, Yen GC, Chen HY. Preventive effects of guava (*Psidium guajava*) leaves and its active compound against α-dicarbonyl compounds induced blood coagulation. Food chemistry 2007; 103(2): 528-35.
22. Lozoya X, Meckes M, Abouzaid M, Tortoriello J, Nozzolilloc, Arnason JT. Quercetin glycosides in *Psidium guajava* L leaves and determination of spasmolytic principle. Arch Med Res 1994; 25(1): 11-15.
23. Begum S, Hassan SI, Siddiqui BS, Shaheen F, Ghayur MN, Gilani AH. Triterpenoids from the leaves of *Psidium guajava*. Phytochemistry 2002; 61(4): 399-403.
24. Kokate CK, Gokhale SB, Purohit AP. Pharmacognosy. 32nd ed. Nirali Prakashan: New Delhi; 2005.111-13.
25. Asokan J. Botanical microtechnique principles and practice.1st edn. Plant anatomy research centre: Chennai; 2007.
26. Johansen DA. Plant Microtechnique. MC Graw hill: Newyork; 1940, 523.
27. Evan WC. Trease and Evans Pharmacognosy. 15th edn. Elsevier: London Saunders; 2002. 544.
28. WHO. Quality control methods for medicinal plant materials. World Health Organisation: Geneva; 1998.
29. Anonymous. The Ayurvedic Pharmacopoeia of India. Part –I, Vol 11, 1st ed. Ministry of Health and family welfare, Department of Indian system of medicine and Homeopathy: New Delhi; 1999, 142-145
30. Anonymous. The Indian Pharmacopoeia. Vol 11. Ministry of Health and family welfare: New Delhi; 1996, A- 53, 54, 89.
31. Wallis TE. Text book of Pharmacognosy. 5th ed. CBS Publishers and Distributors: New Delhi; 1997. 111-17, 561-63.
32. Mukherjee PK. Quality control of Herbal drugs- An approach to evaluation of botanicals 1st edn. Business Horizon: New Delhi; 2002.
33. Kokate CK, Purohit AP, Gokhale SB. Practical Pharmacognosy. 4th ed. Vallabh prakashan: New Delhi; 1997. 117-19, 123-25.
34. Patel S, Zaveri M. Pharmacognostic study of the root of *Justicia gendarussa* Burm. J Trad Med 2011; 6(2): 61-72.
35. Singh S, Machawal L, Chauhan MG. Pharmacognostic study of male leaves of *Trichosanthes dioica* Roxb. with special emphasis on microscopic technique. *J Pharmacognosy Phytother* 2010; 2(5): 71-75.
36. Nayak BS, Patel KN. Pharmacognostic studies of the *Jatropha curcas* leaves. Int J Pharm Tech Res 2010; 2(1): 140-143.
37. Thomas S, Patil DA, Patil AG, Chandra N. Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* L. fruit. J Herb Toxicol 2008; 2(2): 51-54.
38. Yashvanth S. Rani SS. Rao AS. Madhavendra SS. Anatomical exploration of *Leucas aspera* (Willd) links a medicinal herb and its pharmacognostic relevance. *J Pharma Res* 2011; 4(12):4777-79.