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Pharmacognostical and Phyto-physicochemical profile of the leaves of *Piper betle* L. var Pachaikodi (Piperaceae) - Valuable assessment of its quality

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ABSTRACT

Objective: To study in detail the micromorphology and physicochemical analysis of the leaves of *Piper betle* L. var Pachaikodi family Piperaceae. **Methods:** Macroscopy, microscopy, physicochemical analysis, preliminary phytochemical screening and other WHO recommended parameters for standardizations were performed. **Results:** Leaves are cordate, alternate 12-18cm long and 9-11 cm broad. Aromatic, dark green with entire margin, acuminate apex and unequal base with stout petiole (5-6.5cm).Microscopic evaluation revealed the presence of cyclocytic stomata in lower epidermis and apostomatic upper epidermis, hypodermis, secretory cells, pearl glandular trichomes, xylem vessels, phloem, fibres. 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 steroids, tannins, proteins and aminoacids, flavonoids, terpenoids, mucilage, volatile oil, saponin , carbohydrates and absence of alkaloids, fixed oil. **Conclusions:** The microscopic using histological identification , microscopic constants and other physico chemical examinations of the leaves of *Piper betle* L. var pachaikodi can be used as a rapid, inexpensive botanical identification technique and is useful in standardization, hence would be of immense value in authentication of the leaf.

1. Introduction

Plant materials are used throughout the world as home remedies, over the counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the world drug market. It is therefore important to establish their quality. In this study we selected a widely available plant betel leaf, *Piper betle* L. The deep green heart shaped leaves of betel vine are popularly known as *Pann*, in India and vettrilai in Tamilnadu. It belongs to the family Piperaceae, black pepper family. It was reported that fresh leaves contains: moisture 85.4, protein 3.1, fat 0.8, carbohydrate 6.1, fibre 2.3, calcium 230mg, phosphorous 40mg, iron 7mg, ionisable iron 3.5mg, iodine 3.4 μg, carotene (as vitamin A) 9600 IU, Thiamine 70 μg, riboflavin 30 μg, nicotininc acid 0.7mg and vitamin C 5mg/100g. They have a high content of potassium nitrate (0.26-0.42%). The sugars identified in betel leaves include glucose, fructose, maltose and sucrose. The average content of free reducing sugars in different types of betel leaves varies from 0.38-1.46%. It also contains the enzyme like diastase and catalase. Steam distillation of leaves gives essential oil (0.7-2.6%), the amount and nature of which is the important factor determining the aromatic value of the leaf. The oil consists of phenols and terpenes. It was also reported that the leaves contain vitamins and significant amounts of all the essential amino acids except lysine, histidine and arginine which occur in traces [1].There are 100 varieties of betel vine in the world of which about 40 are found in India. The most probable origin of betel vine is Malaysia. The plant is much more popular in India than in any other country since antiquity [2]. The leaves used as antiseptic [3], to stop excessive bleeding during mensturation[4], stimulant[5], to

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relieve throat pain[6], carminative, expectorant[7]. The leaf juice is used for fever, cough, fatigue, asthma, to disinfect wounds externally [8]. The scanning electron microscopy analysis confirmed the inhibitory effect of the leaf extract towards Streptococcus mutans[9], wound healing activity in rabbits as external application[10], hypocholesteromic activity[11], cardiovascular,anti-inflammatory, immunomodulatory,antiulcer, hepatoprotective[12], neuroprotective effect[13].

In short, there is good level of traditional and experimental evidences to support various claims and advantages of this widely available plant. As mentioned earlier several reports have been published on the effects of the plant extract and chemical constituents on different biological activities in vitro and in vivo. An investigation to explore its pharmacognostic examination is inevitable. In Tamilnadu, (South India), *Piper betle* L.var pachaikodi is widely cultivated. 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.

2. Materials and methods

2.1 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.

2.2: Plant collection and authentication:

The leaves of the healthy plant *Piper betle* L. var Pachaikodi selected for our study was collected from Solavandan, Madurai District, Tamilnadu, India and identified by Dr. R. Arulmozhian, Professor-Horticulture, AC &RI, Tamilnadu Agricultural University, Thiruchirapalli-620 009 and Prof. Dr.P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai, Tamilnadu, India. A voucher specimen of the plant has been deposited at the herbarium of Tamil University, Thanjavur, Tamilnadu, India (TUH-276).

2.3: Macroscopic analysis:

Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour , taste etc was noted[14].

2.4: 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 [15] .Sections were taken using microtome. Permanent mount was prepared using saffranin fast green double staining technique [16]. 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.

2.5: Powder microscopy: Coarse powder of the leaf was used to study the microscopical characters of the leaf powder [17, 18].

2.6: Physicochemical analysis:

Total ash, acid insoluble ash, water soluble ash, sulphated 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 [17,18].

2.7: Preliminary phytochemical screening:

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure [19].

3.Results

3.1 Macroscopy:

Piper betle is a dioecious creeper with semi woody stem, climbing by short adventitious roots belonging to the family Piperaceae.(Fig 1). The shape of the leaves is slightly cordate, alternately arranged, 5-7 veins are arising from the base rising to the tip. It is aromatic, 12-18cm long, 9-11cm wide, dark green, glabrous with entire margin. Apex is acuminate with often unequal base. Petiole is stout, 5.5-6.5cm long. Inflorescence is spike. Male flowers are dense with two stamens and female long, pendulous having single ovary. Fruits are rarely produced, fleshy often sunk in the fleshy spike, forming nodule like structure.

Figure 1 HABTT OF P. betle L.var Pachaikedi

3.2 Microscopy of the leaf:

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

Shape: The adaxial(Upper) side is flat and abaxial(lower) side is flask shaped with 2 to 3 layers of collenchyma cells in the lower end. (Fig 2)

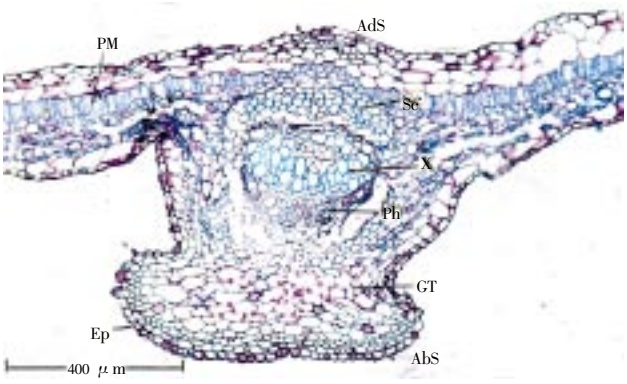


Figure 2. T.S of P. betle L. var Pachaikodi LEAF THROUGH THE MIDRIB.

Epidermis: Upper epidermal cells were wide rectangular 15 μm thick, apostomatic and lower epidermal cells were narrow, spindle shaped 10 μm thick, stomatiferous. A layer of wide thin walled hypodermis present on both sides. The stomata were cycolcytic. (Fig 3, 4, 5).

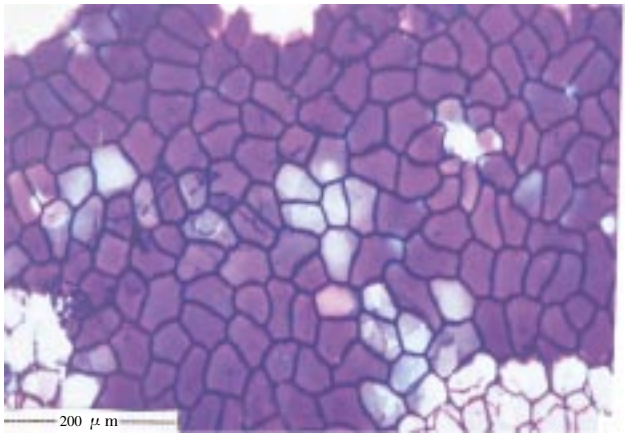


Figure 3. APOSTOMATIC UPPER EPIDERMIS SURFACE VIEW.

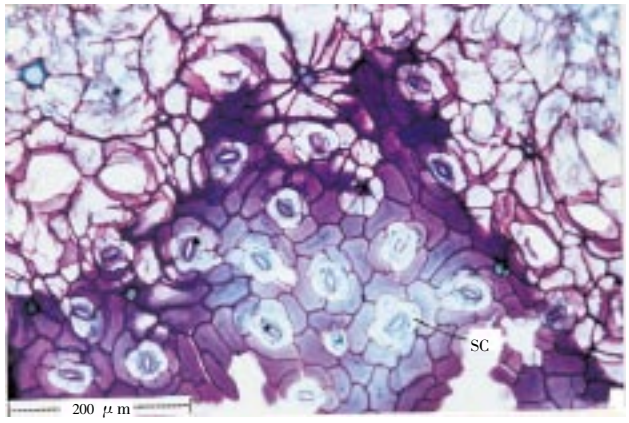


Figure 4. LOWER EPIDERMIS SHOWING STOMATA

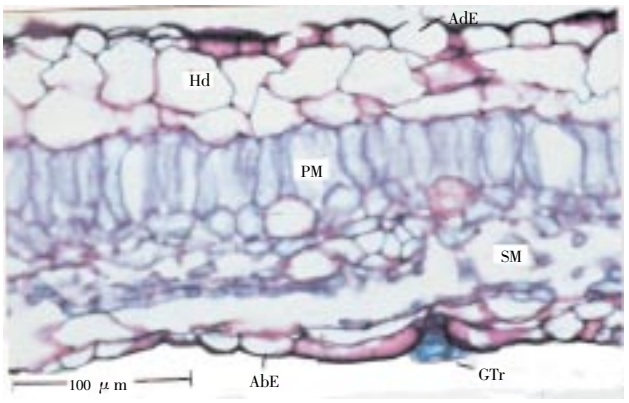
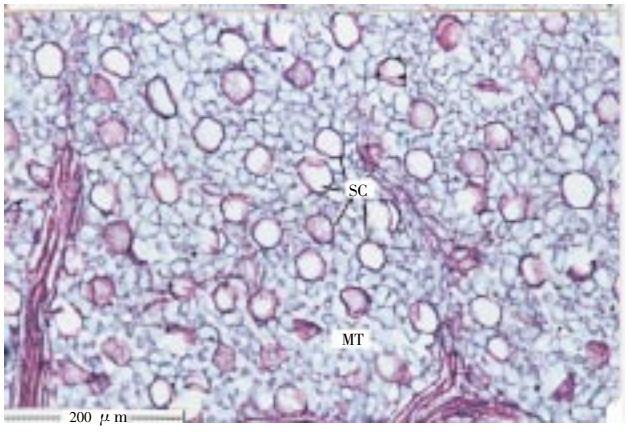
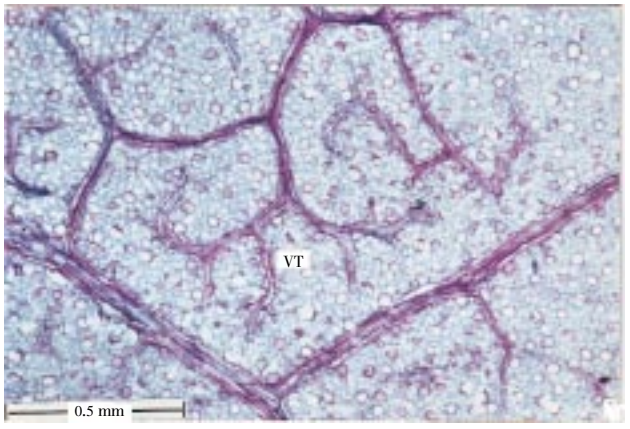


Figure 5. T.S OF LAMINA SHOWING GLANDULAR TRICHOME

Mesophyll: It contains wide cylindrical, compact single layered palisade cells of 35 μm height and 4 or 5 layers of spherical or lobed loosely arranged spongy mesophyll. Fairly wide, circular and abundant secretory cells were present. (Fig 6) Spherical, single celled, sessile secretory glandular trichomes sparsely seen in a sunken pit (Fig 5). The vascular bundle was single, large, bowl shaped. Thick walled xylem elements were present. Phloem occurs as thick arc beneath the xylem. Top portion has a group of sclerenchyma cells (Fig 1). The lateral veins were thick and straight. The vein islets were not distinct. The terminals were mostly forked once or twice and some of them form dendroid vein endings. (Fig 7)



Figuer 6. PARADERMAL SECTION SHOWING SECRETORY CELLS IN THE MESOPHYLL



Figuer 7. VENATION PATTERN

Petiole: Transverse section of petiole is semicircular in shape with a shallow depression in the adaxial side and 2.6mm in diameter. Epidermis is papillate beneath of which a broad zone of 7-8 layered collenchymas cells. Ground tissue composed of compact parenchymatous cells. 5 or 10 separate collateral circular or ovate and vascular bundle is present as a ring. One or more wide circular lysigenous secretory mucus canals are seen in the centre of the petiole (Fig 8).

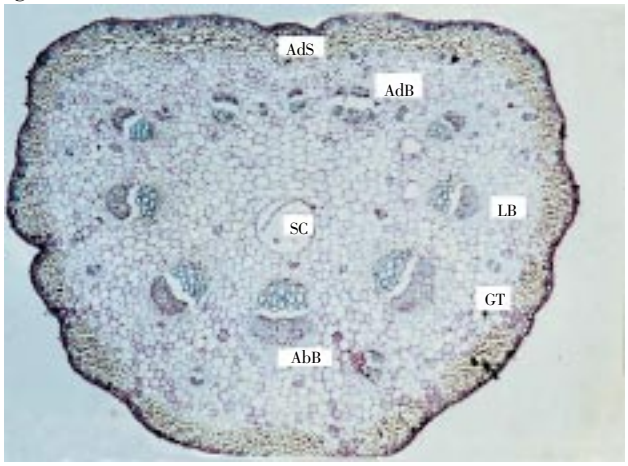


Figure 8. T. SOF PETIOLE

3.3 Powder microscopy

The analysis of the dried powder of the leaf showed polyhedral epidermal cells of surface view with thick and straight anticlinal walls, cyclocytic stomata with 4-5 subsidiary cells, spherical, single celled sessile secretory glandular trichomes, epidermal cells with underlying palisade cells, spiral and annular xylem vessels, fibres, mucus cells, phloem with companion cells.(Fig 9).

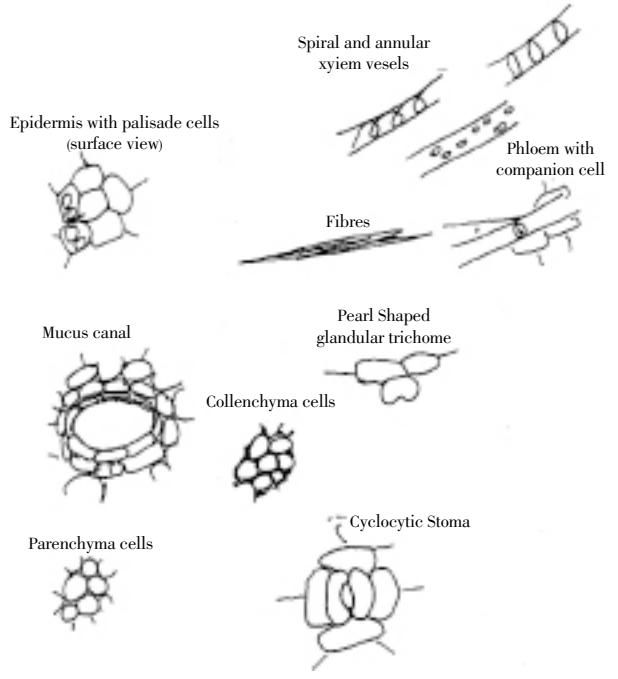


Figure 9. POWDER MICROSCOPY OF THE LEAF OF P. betle var Pachaikodi (Hand drawing)

3.4 Physicochemical analysis:

Physicochemical parameters were found as follows: total ash 11.73%w/w, acid insoluble ash 0.46% w/w, water soluble ash 1.34%w/w, sulphated ash 4.5%w/w, ethanlol soluble extractive value 11.73%w/w, water soluble extractive value 19.2%w/w, loss on drying 9.5%w/w and foreign organic matter was nil. Leaf constants were as follows vein islet number 3, vein termination number 5, stomatal number (lower epidermis) 21.66, stomatal index (lower epidermis 13, and palisade number 4.5.

3.5 Preliminary phytochemical screening:

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

4. Discussion

Adulteration and misidentification of medicinal plants can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The past decade has witnessed the introduction and implementation of new Good Manufacturing Practices (GMP) in quality control of raw materials, intermediates and finished products of botanical origin .The initial step in quality control of medicinal plants is ensuring the authenticity of the desired species for the intended use. 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 [20]. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials. In our present work we selected the plant P.betle L. var. pachaikodi (Piperaceae). The macroscopic and organoleptic characters of the leaf can serve as diagnostic parameters. Presence of cyclocytic stomata in the lower epidermis, numerous more or less uniform secretory cells(40 μm), two layers of hypodermis under the epidermis, few pearl glands, thick lateral vein terminals mostly forked once or twice and some of them form dendroid endings, thick walled fibres are diagnostic value. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. The ash values is particularly

important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter). The extractive values are primarily useful for the determination of exhausted or adulterated drug. Preliminary phytochemical screening will reveal the useful information about the chemical nature of the drug. Preliminary phytochemical screening showed the presence of volatile oil, steroids, flavonoids, terpenoids, saponins, mucilage, reducing sugars, carbohydrates, protein and aminoacids and absence of alkaloids, fixed oils and glycosides.

In conclusion, the present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. Microscopical evaluation and physicochemical standards and preliminary phytochemical reports can be useful to substantiate and authenticate drug [21].

Conflict of interest statement:

We declare that we have no conflict of interest.

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