

## PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF *PIPER BETLE* LINN LEAF

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### ABSTRACT

**Objective:** The present work has been done to establish various pharmacognostical, physicochemical and phytochemical parameters of the leaf of *Piper betle* Linn. (Bangla variety) belonging to family Piperaceae. This could serve as a measure of authentication and quality control of the crude drug.

**Methods:** Organoleptic, anatomical, microscopic, physicochemical analysis and preliminary phytochemical investigation of leaves were performed. Petroleum ether (40-60 °C), chloroform, ethanolic and aqueous extracts of *P. betle* dried leaves powder were prepared by using continuous hot percolation technique for phytochemical screening.

**Results:** Presence of cyclocytic stomata and abundant secretory cells in mesophyll cells are main diagnostic characters of the leaf. Various physicochemical parameters like ash values and extractive values were determined. Phytochemical investigation of an ethanolic and aqueous extract of leaves showed the presence of carbohydrates, proteins, phytosterols, saponins, flavonoids, alkaloids, volatile oil, tannins and phenols. However, petroleum ether and chloroform extract of leaves showed positive tests only for phytosterols and alkaloids.

**Conclusion:** Study of various macroscopic, histological characters and physicochemical constants can serve as a rapid, effective, inexpensive method for identification and standardization of *P. betle* Linn leaves. The qualitative phytochemical investigation revealed ethanolic and aqueous extract of leaf contained a large number of plant secondary metabolites, which are of great therapeutic value. Therefore, aqueous and ethanolic extracts of *P. betle* Linn. leaves of Bangla variety can be used for isolating useful secondary plant metabolites for future drug discovery purpose.

**Keywords:** Epidermis, Mesophyll cells, Microscopy, Piperaceae, *Piper betle*, Standardization

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### INTRODUCTION

Herbal resources are natural treasures for traditional medicine, folk medicines, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates and chemical precursors for synthetic drugs [1]. The two main objectives of using plants as a therapeutic agent are either to isolate a bioactive phytoconstituent for direct use as drug e.g. digoxin, morphine, vinblastine, reserpine, etc. or to synthesize some novel bioactive compound by using phytoconstituent as a precursor e.g. metformin, nabilone, verapamil, etc [2]. In developed countries like Germany and France, several prescriptions contain herbs and herbal extracts. The traditional system of medicines like Ayurveda, Homeopathy, Unani and Sidha contains 90 to 95 % plant based prescriptions. Approximately 80 % medicinal products are of plant origin, and their sale exceeds US \$ 65 billion. Recent years, people showed a revival interest in ethnomedicinal plants as a source of medicine because of their better cultural acceptability, better compatibility with the human body and lesser side effects [3-5]. Plants belonging to the genus *Piper* have high commercial, economical and medicinal value. They have a reputed position in the Indian Ayurvedic system of medicine and in folklore medicine of Latin America and West Indies [6] *P. betle* L. is a glabrous, dioecious and shade loving perennial root climber belonging to family Piperaceae [7]. *P. betle* leaf is indigenous to most areas of South and Southeast Asia. It is generally found in hot and moist climatic condition. In India, it is cultivated mainly in Bihar, Bengal, Orissa, Tamilnadu and Karnataka. It is also found in Sri Lanka. The deep green heart-shaped leaves of *P. betle* are popularly known as paan in India and have various vernacular names in different Indian languages as shown in table [8-11].

In Ayurvedic preparations, *P. betle* leaf extract is used along with different medicines due to its better effects besides its independent use as medicine. In Susrta Samhita and Bhabaprakash tambool leaves have been described as aromatic, sharp, hot, acrid and beneficial for voice, laxative, appetizer, besides this, they pacify vata and aggravate pitta [12]. National Botanical Research Institute

at Lucknow in India has been classified five main cultivars of *P. betle* Linn. i.e. Bangla, Desawari, Kapori, Meetha and Sanchi [13].



Fig. 1: Habitat of *Piper betle* Linn

The leaves contained  $\beta$  and  $\gamma$ -sitosterol, hentriacontane, pentatriacontane, n-triacontanol, stearic acid and chavicol. The essential oil from leaves contained carvacrol, eugenol, chavicol, allyl catechol, cineole, estragol, caryophyllene, cardinene, p-cymene and eugenol methyl ether [14]. The phenolic compounds eugenol and hydroxychavicol isolated from leaf extract of plant reported to possess dose-dependent antimutagenic effect. Essential oil extracted from *P. betle* leaves showed anthelmintic activity [15]. Different extracts of leaves mainly aqueous, ethanolic and methanolic extract possess anticancer, antiulcer, antidiabetic, antifungal, antimalarial, antioxidant, analgesic, anti-inflammatory, antimicrobial activities and wound healing activity [16-22]. A lot of work has been carried out on Meetha, Sanchi and Desawari variety of paan. However, the maximum concentration of phenolic biomarker compounds *v. i. z.* eugenol, hydroxychavicol, chavibetol etc. are present in Bangla variety of plant. Due to presence of a variety of important

phytoconstituents in *P. betle* Linn. (bangla variety) leaves, it has been extensively used, as a source of material for various traditional as well as modern drug formulations. Other varieties of *P. betle* Linn. like kapoori, sanchi, desawari, desi paan, has been well explored for their standardization, physicochemical evaluation, and phytochemical screening [23, 24].

However, macroscopic, microscopic evaluation, physicochemical, phytochemical studies of bangla variety of plant have not been well explored. Therefore, in present research work, authentication, pharmacognostical, physicochemical and phytochemicals investigation of leaves of *P. betle* Linn. (bangla variety) has been carried out for its standardization.

**Table 1: Vernacular names of *Piper betle* Linn**

Traditional system of medicine/different languages	Synonym of <i>Piper betle</i> Linn
Ayurvedic	Taambula, Naagvallari, Naagini, Taambulvalli, Saptashiraa, Bhujangalataa.
Unani	Paan, Tambool
Sidha/Tamil	Vettilai Nagavalli, Kammaaruvetritai
Hindi, Bengal, Gujraji, Urdu	Paan
Konkani	Phodi paan
Malayalam	Vettila
Marathi	Vidyache pan
Telugu	Tamalapaka

## MATERIALS AND METHODS

All chemicals used were SD. Fine Chem. Ltd., Mumbai; AR grade.

### Collection of *Piper betle* Linn. leaf

*Piper betle* leaves were collected from the local market, jind (Haryana) and authenticated. Leaves were dried under shade for 10 d. The dried leaves were powdered and stored in desiccators for further analysis.

### Authentication of *Piper betle* Linn. leaf

*P. betle* Linn. leaf was authenticated by Dr. Sunita Garg, Chief Scientist at Raw Material Herbarium and Museum, Delhi (RHMD) CSIR-NISCAIR, Delhi. A voucher specimen number is NISCAIR/RHMD/Consult/2014/2466/45-2.

### Pharmacognostical study

The pharmacognostical study has been done by performing morphological and microscopic analysis of leaves as per WHO guidelines. [25-27].

### Morphological features

Morphological features of the leaf such as the presence of foreign organic matter, color, odor, size, shape and taste were studied.

### Microscopic analysis

Transverse section of midrib, petiole and lamina of fresh leaf were cut by using potato pith method. The sections were cleared by boiling with chloral hydrate solution and stained with a mixture of phloroglucinol and hydrochloric acid (1:1), and studied under a compound microscope (10 X and 45 X).

### Powder microscopy

Powder microscopy has been performed by using coarse powder of leaf. This study was used for identification of various diagnostic characters of leaf powder.

### Physicochemical parameters

Physicochemical constants such as total ash value, acid insoluble ash, water-soluble ash, sulfated ash and loss on drying were determined as per WHO guidelines. Alcohol soluble extractive value has been determined by making an extract of dried powder leaves of *P. betle* L. by cold maceration technique using alcohol as a solvent while for water-soluble extractive value euqinhcet noitarecam toh , nevlos sa retaw mroforolhc gnisu desu saw [26].

### Preliminary phytochemicals analysis

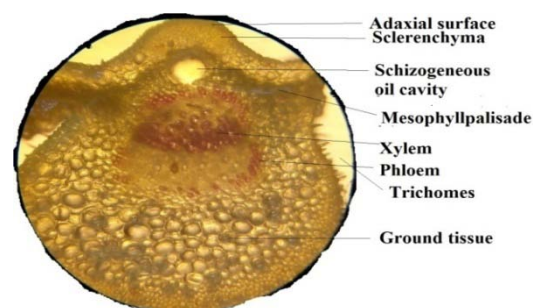
For phytochemical analysis, four types of extract *v. i. z.* petroleum ether (40-60 °C), chloroform, ethanol and aqueous extract were prepared by using successive solvent extraction method of dried powder of leaves of *P. betle* L. These extracts were screened for the

presence of various phytochemical constituents in the leaves of *P. betle* L. [27]. In addition to phytochemical screening, percentage yield of each extract were also calculated.

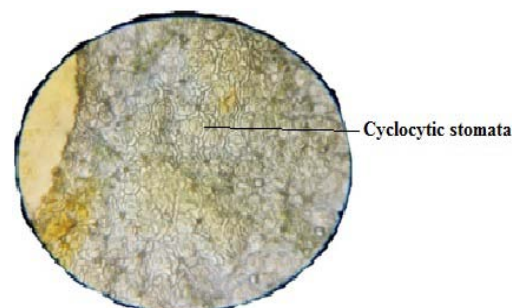
## RESULTS

### Macroscopic analysis

*P. betle* Linn. is a perennial creeper with woody stem belonging to family Piperaceae. Leaf is dorsiventral, mesomorphic with a prominent midrib on both sides. Description of morphological characters is compiled in table 2.



**Fig. 2: TS of midrib**



**Fig. 3: Surface view of upper lamina**

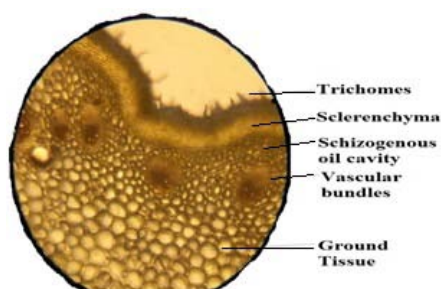
### TS of petiole of *P. betle* leaf

Transverse section of the petiole (fig. 4) was semicircular with a shallow depression on the adaxial side and somewhat round at abaxial side. Schizigenous oil cavities )secretory canal (were present in the center of the petiole. Pappilate epidermis beneath of which a wide zone of 6-8 layered collenchymas cells was present. Parenchymatous cells were present in the ground tissue. Vascular

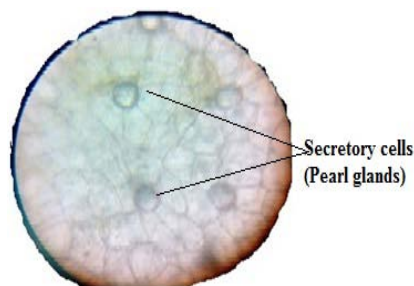
bundles were present as a ring showing 6-10 in number. Para dermal section of leaf showed the presence of pearl like secretory cells (fig 5).

**Table 2: Morphological characters of *Piper bettle* Linn leaf**

S. No.	Characters	<i>Piper bettle</i> Linn
1.	Dimensions	Length of leaf: 8-16 cm Width of leaf: 6-12 cm
2.	Color and condition	Green to dark green and fresh leaves
3.	Lamina	Simple and reticulate
	Composition and venation	Entire and acuminate
	Margin and apex	Broadly cordate leaves with rounded base
	Base	Thick lamina with smooth and glabrous surface
	Surface and texture	Long petiole 1.5 to 4.5 cm long
	Petiole	Stipulate leaves
	Leaf base	Alternately arranged leaves
	Phyllotaxis	Aromatic
4.	Taste	Characteristic and pleasant
5.	Odor	



**Fig. 4: TS of petiole**



**Fig. 5: Paradermal section**

#### Powder microscopy

Leaf powder was grayish green in color having slippery touch. Main diagnostic characters of leaf were secretory cells, mucus canal, calcium oxalate crystals, spiral and annular xylem vessels (fig. 6). The stomata were cyclocytic with three to five subsidiary cells around the guard cells in the epidermal cells.

#### Physicochemical analysis

Results of various physicochemical investigations are compiled in table 3.

#### Phytochemical analysis

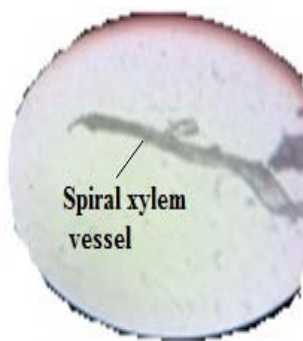
Results of phytochemical analysis and percentage yield of each extract are compiled in table 4 & 5.

**Table 3: Various physicochemical parameters**

Physicochemical parameters	% w/w
Total ash value	20.73
Acid insoluble ash	1.07
Sulphated ash	5.8
Water soluble ash	2.06
Loss on drying	6.2
Alcohol soluble extractive	18.5
Water soluble extractive	26.6
foreign organic matter	0.87



**a) Annular xylem vessel**



**b) Spiral xylem vessel**



**c) Glandular trichome**

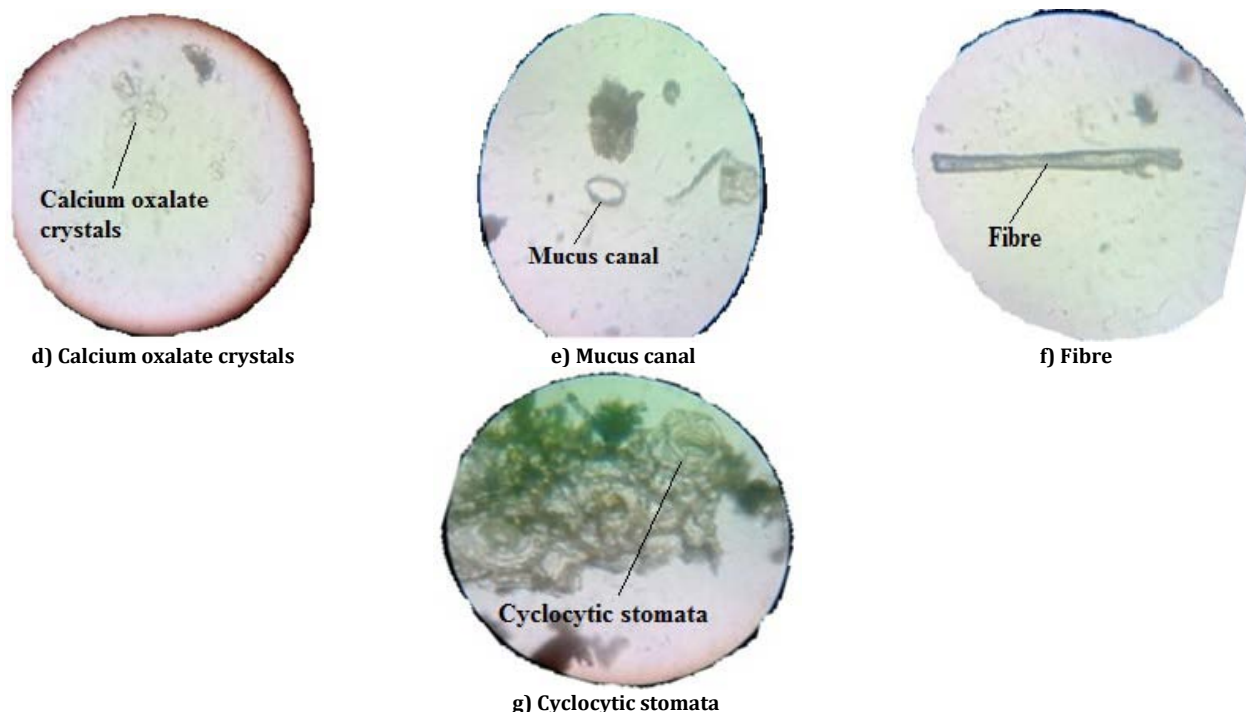
Fig. 6(a-g): Various diagnostic characteristics of powdered leaf of *P. betle* Linn

Table 4: Phytochemicals present in different extract

Phytochemicals	Petroleum ether (40-60 °C) extract	Chloroform extract	Ethanol extract	Aqueous extract
Carbohydrates	-	-	++	++
Proteins	-	-	++	++
Fats and oils	-	-	-	-
Phytosterols	++	+	++	-
Glycosides	-	-	-	-
Saponins	-	-	-	+
Flavonoids	-	-	++	-
Alkaloids	-	+	+	+
Tannis and phenol	-	-	++	++
Volatile oils	-	-	-	++

+ indicates presence, - indicates absence

Table 5: Percentage yield of various extracts of *Piper betle* Linn

S. No.	Extract	Nature of extract	Color	% Yield
1	Petroleum ether	Semisolid	Dark green	17.8
2	Chloroform	Semisolid	Dark green	18.9
3	Ethanol	Semisolid	Dark green	21.45
4	Aqueous	Solid	Dark brown	26.56

## DISCUSSION

Study of various organoleptic and microscopic characteristics is simplest, cheapest and frequent method of identification of the plant. The presence of cyclocytic stomata, mucus canal and annular and spiral xylem vessels and a paradermal section of leaf showed the presence of pearl like abundant secretory cells are important demarcating characters of *P. betle* L. (bangla variety) which makes it distinguished from other varieties of *P. betle* L. Plant. In kapoori variety, main microscopic characteristics of the leaf are multiple epidermises with thick cuticle hypodermal cells followed by a small arc of sclerenchyma and silicified cells in the epidermis of upper lamina [24]. The main anatomical and microscopical characteristics of *Piper betle* L. var. Sirugamani are single ovate collateral vascular bundles, cylindrical finger like glandular trichomes, vertically oblong palisade cells and spongy mesophyll of 3 to 4 layers with secretory

cells [28]. Sessile secretory glandular trichomes, 5 to 10 separate collateral circular vascular bundle in petiole transverse section and single circular mucus canal are main demarcating characters of *Piper betle* L. var. Pachaikodi [29]. Similarly, Khan *et al.* studied different cultivars of *Piper betle* L. (desi, kali, haldia, sanchi, meetha, and birkoli variety) of the eastern region of India cultivated in bihar, odisa and west bengal states [30]. Physicochemical constant evaluation such as ash value and extractive values serves as an important parameter to detect any kind of adulteration and presence of foreign inorganic matter such as metallic and earthy matter. The highest water-soluble extractive value indicates the presence of more water-soluble phytoconstituents in leaves. These parameters can be used for identification and standardization of the crude drug. Preliminary phytochemical screening of plant will provide information regarding chemical nature of plant such as presence and absence of various phytoconstituent. *P. betle* Linn. leaf is excellent bio-source of

flavonoids and phenolic compounds. Aqueous and ethanolic extract of the plant shows the presence of maximum secondary plant metabolites and therefore, can be used for isolation of pure compounds for novel drug discovery purpose.

#### CONCLUSION

Present research attempts to provide a pharmacognostical, physicochemical and phytochemical account of *P. betle* Linn. leaf (bangla variety) that unambiguously useful for identification, authentication, and standardization of drug in crude form. *P. betle* Linn. leaf (bangla variety) may further be exposed for its pharmacological potential on the basis of its phytochemical analysis of constituents.

#### CONFLICT OF INTERESTS

Declared none

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