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Pharmacognostical and physicochemical studies of *Piper Betle* Linn leaf (Assam variety)

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Abstract
Piper betle leaf is widely used in Assam and its adjoining areas since ancient days as a stimulizer and also as worship item. Its common name is betel in English, paan in India and Bangladesh. Different research works related to Ayurvedic consequence, geographical distribution and cultivation, morphoanatomy, phytochemistry, biological activities, along with tradomedicinal uses which signify the tremendous potential of "*Piper betle*." to come out as Green medicine. In the pharmacognostic study of *Piper betle* Linn leaf some major studies like macroscopy, microscopy, phsicochemical screening was carried out. The anatomy of the leaf was studied by taking different sections which showed cyclocytic stomata, etc. Physicochemical parameters of the powder were also carried out. These studies are important in the way of acceptability of herbal drugs in present scenario of lacking regulatory laws to control quality of herbal drugs. The objective of present study is to revels the potential effect of this plant leaf in the development of therapeutically active herbal drugs.

Keywords: piper betle, microscopic, macroscopic, physicochemical, standardization, TLC etc.

Introduction
In Ayurveda betel leaf extract is frequently used as an adjuvant & mixed with different medicines possibly for better effects beside its independent use as medicine [1]. In Susrta Samhita (Ch 28-46, 279-280) tambool leaves have been described as aromatic, sharp, hot, acrid and beneficial for voice, laxative, appetizer, beside this they pacify vata and aggravate pitta. Similar characteristics have been described in Bhabaprakash (Sloka180- 183).
In addition to these, the aphrodisiac effect of betel chewing has been indicated in ancient texts. Pan also believed to provide strength to heart and regulate blood. Its utility as anticoagulant, anti-inflammatory and anti-microbial is emphasized at several references [2]. In ayurveda it acts as vata and kapha suppressant. It also helps in expelling out the mucus from the respiratory tract because of its hot potency. According to Unani system the leaf has a sharp taste and good smell improves taste and appetite, tonic to brain, heart and liver, lessens the thirst, clear the throat and purify the blood. In 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].
However macroscopic microscopic evaluation, pharmacognostics, physicochemical studies of Assam variety of plant have not been well explored. Study of various microscopic, macroscopic, histological characters and physicochemical constant can serve as a rapid, effective, inexpensive, method for identification and standardization of *Piper betle* leaves [4]. Therefore in present research work authentication, pharmacognostical, physicochemical investigation of leaves of *Piper betle* (Assam variety) has been carried out for its standardization.

Materials and methods
Plant collection and extraction
Fresh leaves of *piper betle* (Family: Piperaceae) were Collected from Govt. Ayurvedic College campus, Guwahati. The plant was identified by Botanical Survey of India, Shillong, Meghalaya. The fresh and matured leaves were used for pharmacognostics study. The leaves were separated, washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottles [5].

Pharmacognostic studies
Macroscopic characteristics
Macroscopic studies of the plant was carried-out as per the methods described in Evans. The plant was macroscopically examined for shape, size, surface characteristics, texture, color, consistency, odour, taste, etc [6].

Microscopic characteristics
Microscopic studies were done by preparing a thin hand section of midrib and lamina region of leaf of *piper betle*. The section was stained by safranine. The powder of the dried leaf was used for observation of microscopic characters [6].

Thin layer chromatography
Thin layer chromatography was carried out with the methanolic extract and maximum spots been separated on precoated silicagel G TLC plate with trial and error methods [7].

Physicochemical properties
Physicochemical parameters were determined as per guidelines of WHO. Total cash value, loss on drying, water soluble ash, acid insoluble ash, solubility of the extract in different solvents, melting point, boiling point, pH, heavy metal analysis, petroleum ether soluble extractive, alcohol soluble extractive and water soluble extractive values were determined [8].

Result and discussion

Macroscopic characteristics

The beetel leaf is yellowish green to dark green in colour with glossy upper surface with a characteristic odour and pleasant taste. The betel leaves are aeromatic with varied taste, ranging from sweet to pungent.

As per the Figure 1, the betel leaf is a heart shaped with different size. The size of the leaf varies with different cultivator from 7-15cm in length and 5-14cm in width. Betel leaves are simple alternate stipulate petiolate with 0.77 to 3.7cm, ovate oblong broadly ovate cordate or obliquely elliptic entire glabrous coriaceous10 to 17 cm long and 5 to 10 cm broad acuminate oblique and rounded base

Fig 1: *piper betle* leaf.

Microscopic characteristics

The leaves are hypostomatic, cyclocytic stomatal complexes are common which the characteristic feature of the Piperaceae is. Transverse section of leaf through midrib shows four layered upper and two layered lower epidermis. The cuticle is thick on the upper epidermis and thin on the lower epidermis. The cells of the outer epidermal layers on both sides of the leaf are small, that possess tannins and oils. The sub epidermal cells on the abaxial side are enlarged and they store water. Crystal and oil reserves are found in the sub epidermal cells on both sides. The palisade layer are well distinguished they are double layered shortwide compact cells and mesophyll cells are 3-4 layered and small lobed. Thick walled irregular secretory cells. The trichomes are glandular which have unicellular apical cell and a short pedicel. The pedicle has thicker wall, surrounded by 5 or 6 epidermal cell arranged in a rosette disc like manner.The vascular bundles located at the centre of midrib portion single ovate collateral cells and a thick phloem was observed. Microscopic study of *piper beetle* leaf.

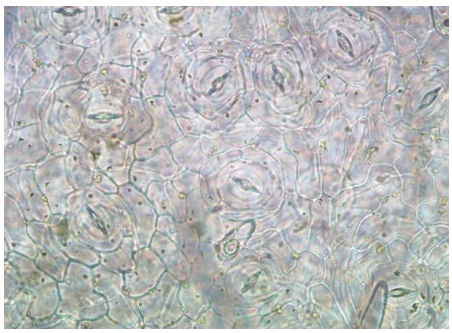

Fig 2: Stomata (at 40X zoom)

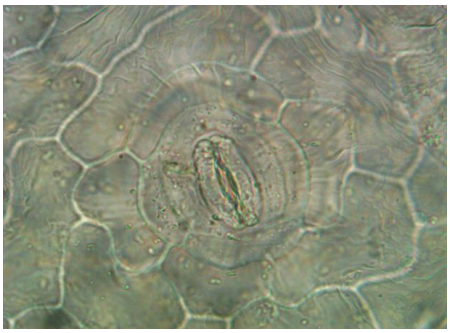

Fig 3: Stomata (at 100X zoom)

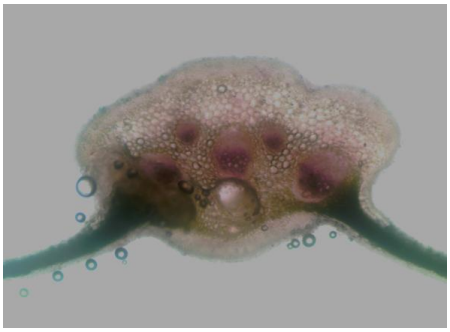

Fig 4: T.S. of the leaf

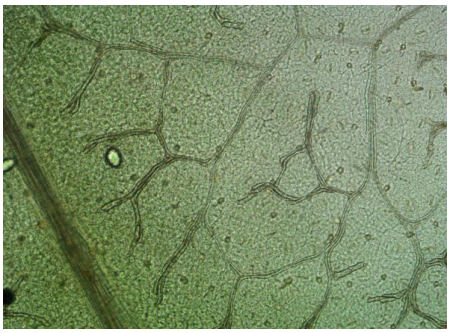

Fig 5: Vein termination

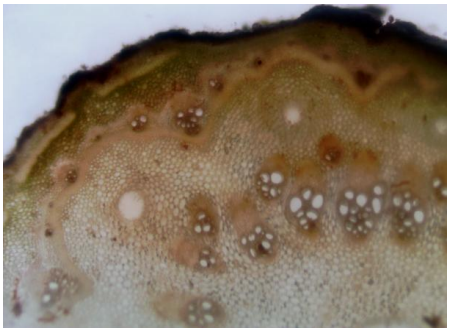

Fig 6: T.S. of petiole of *piper beetle* leaf

Physicochemical properties

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs [8]. Studies of physicochemical constant can serve as a valuable source of information and are usually used in judging the purity and quality of the drug. The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The ash

value was determined by three different forms viz., total ash, acid-insoluble methanol followed by ethanol and water. The variation in extractable matter in various solvents is suggestive of the fact the formation of the bioactive principle of the medicinal plant is influenced by number of intrinsic and extrinsic factors. High alcohol soluble and water soluble extractive values reveal the presence of polar substance like phenols, tannins and glycosides. All these parameters are useful for the compilation of a suitable monograph for its proper identification (Table 1).

Table 1: Physicochemical characterization of leaves of ***piper beetle***

Parameters	Sample	Value
Loss on drying	Crude powder	17.5%
Total ash	Crude powder	14.5%
Acid insoluble ash	Crude powder	2.3%
Water soluble ash	Crude powder	2.6%
Alcohol soluble extractive	Crude powder	17.5%
Water soluble extractive	Crude powder	25%

Figure: Chromatographic Profile of Crude Extract of *piper betle leaf*

Fig 7: TLC of methanolic extract at 254 nm

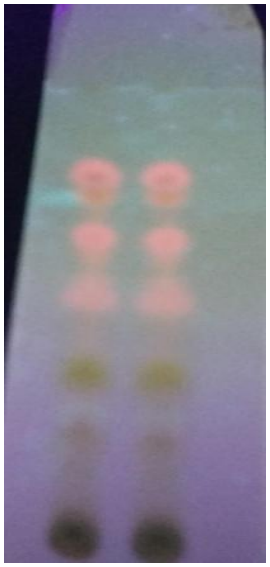

Fig 8: TLC of methanolic extract at 365 nm

The details of solvent system and the Rf values are mentioned in the Table-2.

Table 2

Extract	Solvent System	Visibility	No. of Spots	Rf Values
Methanol	Dichloromethane	UV/Iodine	4	0.62 0.48 0.31 0.25
Methanol	Dichloromethane	Fluorescent	5	0.78 0.71 0.59 0.49 0.32

Discussion

The standardization of a crude drug is an integral part for establishing its correct identity. Microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials [9]. No detailed standardized work has been reported in literature for this plant of this region. Apart from its spiritual uses the leaves of this plant were used by local people since ancient time as in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part for establishing its correct identity. As there is a vast variation in potency of herbs depending upon its Geographical locations, there is utmost need for the plant to get it standardized. Microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. The pharmacognostic standards for leaves of *piper betle* found in this North Eastern region are carried out for the first time in this study. Leaves powder subjected for microscopic, pharmacognostical and physicochemical analysis provides relevant information which may be helpful in authentication of the crude drug and check adulteration for quality control of raw material. The current observation will be helpful in differentiating the leaves of this species from closely related species of same genus and family.

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