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Comparative Pharmacognostic Study of Leaves of Two Varieties of *Piper betle* L. namely Calcutta Bangla and Assam Kapoori

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ABSTRACT

A large number of varieties of *P. betle* (family- Piperaceae) are used as a source of essential oil, medicine and in day to day life for chewing and other purposes. These varieties represent landraces and hence pose problem in taxonomic identification and classification. Pharmacognostic studies like organoleptic, macroscopic, microscopic characters and anatomical features provide supporting data for this purpose. Comparative study of two varieties of *P. betle* namely Calcutta Bangla and Assam Kapoori are presented here. These studies indicated stomata and trichome characters can be used to distinguish these two varieties even in crude powder form.

Key words: *P. betle*, Stomata, Trichomes, Macro morphology, Microscopic study, Anatomy, Powder study, Vein islet and Vein termination number, Oil glands, Tracheids and Vessels.

1. Introduction

The betel leaf commonly known as 'Paan' or 'Nagvalli' (family-Piperaceae) is an evergreen and perennial creeper^[1]. Significance of leaves has been explained in relationship to every sphere of human life including social, culture, religious and is very much relevant even in modern days^[2]. From ancient time betel are chewed along with areca nut, slaked lime, cardamom and clove in many Asian countries^[3]. Review of literature indicate variety of medicinal properties of betel leaf which include antioxidant, antifungal, antiulcerogenic, antiplatelet, antidiabetic, immunomodulatory, antileishmanial, antiamebic, anti-inflammatory, antifilarial and antimicrobial^[4], antifertility^[5], antihyperglycemic^[6], antidermatophytic^[7], antinaceptive^[8] and radioprotective property^[9].

In spite of their great importance in day to day human life, a scientific documentation of all varieties with respect to pharmacognostic investigation is not complete. Although major review by Nagori (2011)^[4] and some reports by Patra (2011)^[10] and Seetha Lakshmi (2010)^[11] are available, the present study supports to broaden the data base of betel leaf.

About 85 different varieties of betel are grown in different parts of India. Broadly they are grouped into Deshawari, Meetha, Sanchi, Bangla and Kapoori etc^[12]. Out of these varieties Calcutta Bangla (CB) and Assam Kapoori (AK) are selected for the present study. Both these varieties are highly aromatic with strong flavor and pungent taste. But they differ with each other in many respects. The present investigation gives a comparative account of organoleptic studies of lamina and petiole.

2. Materials and methods

2.1 Macroscopic, Microscopic, and Anatomical studies

Fresh leaves of Calcutta Bangla variety were obtained from local market where as Assam Kapoori leaves were collected and identified by from 'Panvel Sanshodhan Kendra' Ramtek. For organoleptic and anatomical studies leaves were fixed in FAA (5:5:90). Further thoroughly cleaned, shed dried and powdered leaves were subjected to physical and chemical analysis. Microscopic studies were carried on dechlorophyllised (10% KOH), leaf segments according to the method given by Trease and Evans (2002)^[13]; Khendelwal (2008)^[14] and Salikutti Thomas (2006)^[15].

3. Results and Discussion

3.1 Macro morphology

Data of different aspects of macro morphological characters is compiled in Table-1. Although the two varieties belong to same species, they differ with each other in many respects; CB leaves are large in size, dark green with shiny adaxial surface whereas AK leaves are smaller and dark olive green in color.

1. **Shape:** CB leaves are cordate with petiole attached to shallow cleft while AK leaves are more or less round in outline with distinct symmetrical lobes at base giving it reniform structure (Photo slide 1- A, B).
2. **Venation:** Both the varieties show reticulate, multicostate convergent type of venation with five major veins and pronounced ramification of veinlets.
3. **Apex:** In AK leaf apex is obtuse while in CB the lamina

terminates into small, sharp, acute tip hence described as cuspidate apex.

4. **Leaf base:** In both cases leaf bases have symmetrical lobes but in CB lobing is not prominent while in AK leaf shows deep notch giving reniform shape.
5. **Surface:** In both varieties adaxial surface is glabrous whereas lower surface are pubescent. However in AK, adaxial surface has prominent wrinkled appearance hence described as rugose.
6. **Texture:** In both cases leaves are thick but AK is more coriaceous than CB.

Table 1: Comparative macroscopic study of CB and AK leaf

Sr. no.	Plant part and characters	Calcutta Bangla	Assam Kapoori
1.	Duration	Evergreen	Evergreen
2.	Petiole Shape Color	Quill shaped & grooved. Light green.	Quill shaped & grooved. Light green and pink.
3.	Lamina color shape composition incision venation margin apex base surface • adaxial • abaxial texture	Dark green Cordate Simple Absent Reticulate multicostate convergent. Entire Cuspidate Cordate Glabrous, shiny Pubescent Less coriaceous	Dark olive green. Reniform Simple Absent Reticulate multicostate convergent Entire Obtuse Reniform Glabrous & rugose Pubescent More coriaceous

3.2 Microscopic studies

3.2.1 Stomata: In both the varieties anomocytic type of stomata are observed which are characterized by presence of 4 to 5 number of subsidiary cells.

Stomatal index signifies proportion of number of epidermal cell converted into stomata. These values can be used to distinguish between the leaves of closely related CB (8.8) and AK (7.3). (Table 2)

3.2.2 Trichome: In both CB and AK varieties thin walled, tubular, blunt trichome are observed only on abaxial surface, but these varieties differ with each other in following respects.

1. Trichomes in CB are comparatively larger in size than AK.
2. In midrib region the trichome in CB are unicellular while in AK they are multicellular and pointed. (Table 2).

3.2.3 Vein islet number (VI) and Vein termination number (VT): Venation in *P. betle* varieties is reticulate convergent type. The primary 5 major vein undergo secondary, tertiary, quaternary ramification which form small vein islet. Vein islet number differ in different species but it constant character in given species. Although CB and AK leaves exhibited similar vein islet number. Vein termination number in CB is more (8.5) as compared to AK, indicating excessive ramification of vein islet in CB. (Table 2)

Table 2: Microscopic studies Comparative study of stomata, trichome and leaf constant

P. betle		CB	AK
Stomata	Type	Anomocytic	Anomocytic
	Size	25.3×18μ	28×19.5μ
	SI	8.8	7.3
Trichome	Type	Lamina- unicellular glandular	Lamina- unicellular glandular
		Midrib- unicellular glandular.	Midrib- biseriate multicellular nonglandular and glandular
		Petiole- unicellular glandular.	Petiole- uniseriate multicellular nonglandular and glandular
	Size	38×15.4 μ	33.6×14 μ
Vein characters	VI	2.25	2.25
	VT	8.5	5.9

3.3 Anatomy of Leaf and Petiole

3.3.1 Leaf

The leaves of CB and AK are typically dorsiventral bound by cuticularised thin walled upper and lower epidermis. However on abaxial surface, the outermost thin walled cell layer is followed by 2-3 layers of parenchyma cells, hence referred as multicellular epidermis- characteristic feature of *Piper betle*. The multilayered epidermis is followed by heterogeneous mesophyll layer. Both the varieties show the single layer of palisade cells which are elongated and perpendicular to the surface of leaf blade and characterized by abundance of chloroplast. Spongy mesophyll consists of 3-4 layers of chlorenchymatous irregular cells arranged compactly.

T.S. through midrib show upper & lower epidermis and followed by prominent collenchymas, however the midrib in CB is smooth and round in shape at lower side. While, in AK the rib is squarish in shape having many angles at lower side which is pointed on adaxial side.

In both the varieties epidermal layer is traversed by unicellular glandular trichome on adaxial regions. Glandular trichomes are arranged on 4 to 6 rosette disc like configuration of epidermal cells. Stomata are present on abaxial side only. Oil glands are interspersed in lower and upper parenchymatous epidermis. The lower surface of midrib in AK shows presence of tector/multicellular nonglandular trichomes whereas they are glandular and unicellular in CB. The trichomes are absent on the upper epidermis.

3.3.1.1 Vascular Bundle (VB) of Midrib:

In both the varieties V. B. is large in size with xylem toward adaxial surface and phloem toward abaxial side. It is collateral and surrounded by parenchymatous cells, in which oil glands are present on lower side. Patches of multilayered sclerenchyma can be seen over the vascular bundle on adaxial side.

3.3.2 Petiole

In Calcutta Bangla T. S. of petiole shows a distinct groove on adaxial side, margin is wavy having shallow ridges corresponding to multicostate vein. Epidermis is covered by glandular uniseriate trichomes. Epidermis is single layered with cuticle. It is followed by large patches of 4-5 cell layers thick sclerenchyma interrupted by parenchyma in between.

Vascular bundles are arranged in 2 rings. Inner ring consists of large vascular bundle while outer ring contain smaller vascular bundle each alternating with large vascular bundle. Secretary cell and schizogenous oil cavities are randomly dispersed in parenchymatous ground tissue.

Individual vascular bundle is conjoint, collateral with xylem facing inner side while phloem is situated on the outer side.

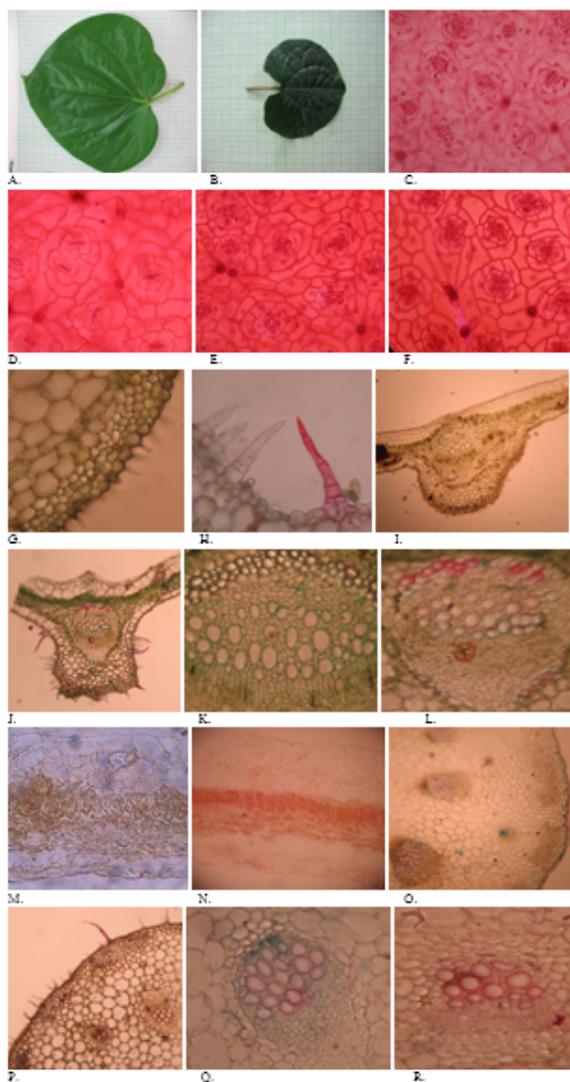
In Assam Kapoori, the petiole is omega shaped with 2 major grooves on upper & highly undulating margin on lower side. Petiole is heavily covered by multicellular, uniseriate, pointed trichomes, epidermis is single layered followed by discontinuous ring of sclerenchyma. V.B. are of 2-types, large and small and are arranged in C-shaped arc.

3.4 Powder Study

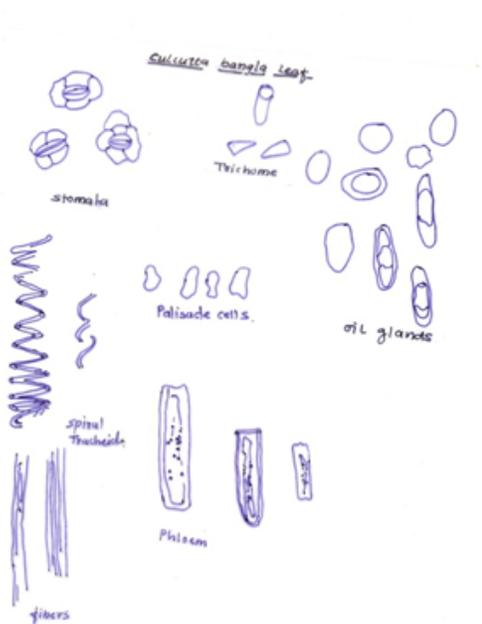
Characteristic microscopic feature of powdered material of leaf and petiole of CB and AK included presence of

- Epidermal cells- thin walled with irregular shapes.
- Palisade cells- thin walled rectangular.
- Oil glands- large in numbers, big or small and isolated.
- Stomata- anomocytic
- Trichomes- uni or multicellular, pointed or blunt.

In macerated powder material, tracheids, vessels and tracheidal fibers were observed. Predominantly the tracheids and vessel members exhibited spiral lignin ornamentation. (Photo Slide-2)



PHOTOSLIDE 1:
A. Culcutta leaf
B. Assam kapoori leaf
C. Stomata of CB
D. Trichome of CB
E. Stomata of AK
F. Trichome of AK
G. Trichome of CB on leaflet epidermis
H. Trichome of AK on leaflet epidermis
I. T.S of CB leaf through mid rib
J. T.S of AK leaf through mid rib
K. VB of CB leaf midrib
L. VB of AK leaf midrib
M. T.S of CB leaf lamina
N. T.S of AK leaf lamina
O. T.S of CB petiole
P. T.S. of AK petiole
Q. VB of CB petiole
R. VB of AK petiole



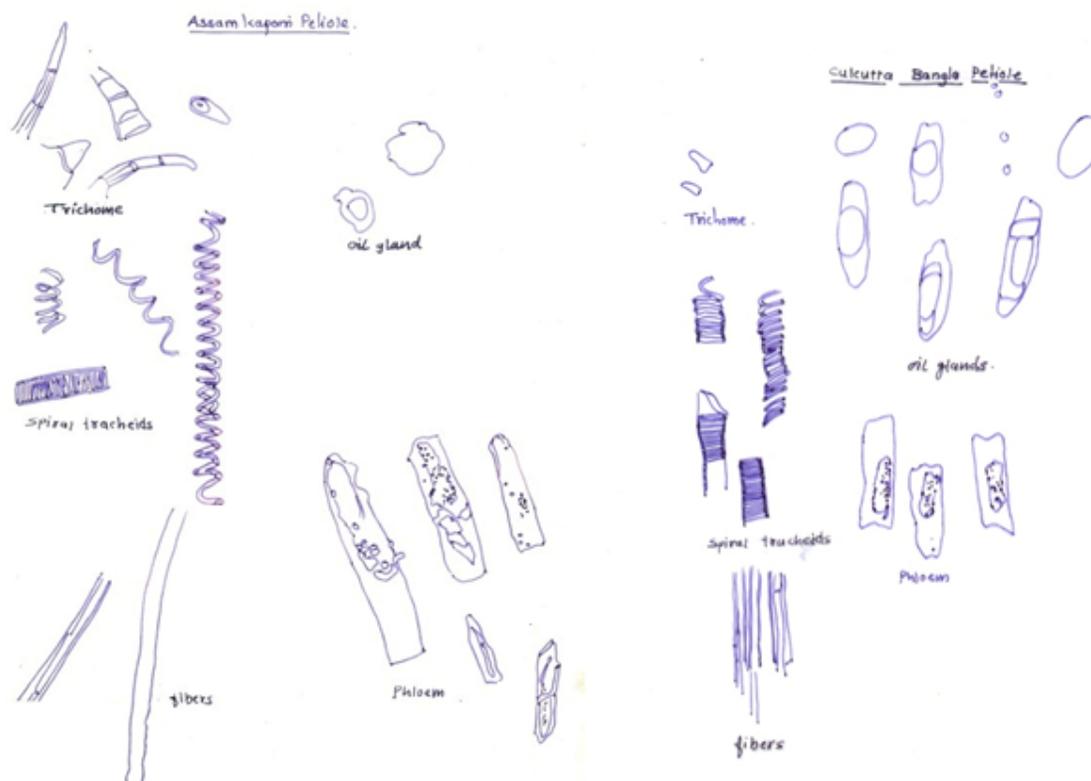


Photo Slide 2: Microscopic feature of Powder material of leaf and petiole of CB and AK.

4. Conclusion

As different varieties of *P. betle* have great commercial importance, pharmacognostic characterization and validation play an important role in authentication of this biological source to be used in essential oil and medicine industry.

In the present study differences shown by Calcutta Bangla and Assam Kapoori occur in leaf morphology like shape, apex, base, surface and texture of leaf. Additionally trichome, size and distribution of stomata and vein termination numbers are found to be distinguishable identifying characters of leaf as well as powdered material. Previous reports by Seetha Lakshmi and Naidu (2010) [11] indicated presence of cycloctytic type of stomata and glandular trichome in Kapoori tuni and Jaleswar variety of *P. betle* leaf. Hence it can be concluded that trichome and stomata can be used to successfully differentiate the large number of *P. betle* varieties.

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