

Title	Morphological and Anatomical studies on <i>Piper betle</i> L. cultivated in three different localities in Myanmar
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Morphological and Anatomical studies on *Piper betle* L. cultivated in three different localities in Myanmar

Win Win Khaing*

Abstract

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Key words: *Piper betle* L., Morphology, Anatomy, three different localities

Introduction

Piper betle L. is native to Malay Archipelago (Chibber 1912). It was commonly grown throughout the Eastern Tropics but not known as a wild plant (Dassanayake 1985). The nomenclature of betel vine is the general craze with growers to name the varieties after their village or town. Numerous cultivars exist with leaves differing in size, shape and colour. Some cultivars have red veins and petioles. The aroma of betel leaf is due to the presence of essential oils, consisting of phenols and terpenes (Lakshmi & Naidu 2010).

It is extensively cultivated in India, Bangladish, Pakistan, Malaysia, Indonesia, Vietnan, Laos, Kampu-cha, Thailand, Myanmar and Singapore, etc. for its leaves which are used as masticatory (Ravindran 2000 and Kumar *et al.* 2010). As a masticatory, it is credited with many properties: it is aromatic, digestive, stimulant and carminative (Anonymous 1969).

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Piper betle L. is a perennial creeper belonging to the family Piperaceae, cultivated in Myanmar for its leaves, used mainly for chewing and medicinal uses. It is a perennial dioecious, semiwoody climber. Stems were strongly swollen at the nodes, and papillose when young. Leaves were alternate, simple and yellowish green to bright green in colour.

Metcalf and Chalk (1950) mentioned that stem and petiole of *Piper betle* L. show spherical secretory cells with suberized walls, containing mucilage. Several species of *Piper*: e.g. *P. betle* L. and *P. nigrum* L. are distinguished by mucilage canals in petiole and axis. Hairs are mostly uniseriate. Small, spherical, shortly stalked glands, and sometimes deciduous peral glands, also occur. Stomata, nearly always confined to the lower surface, are often surrounded by a rosette of numerous epidermal cells, whilst others are cruciferous.

Morphological and various medicinal uses, and planting techniques of betel vine were studied by other researchers. However, the anatomical study of *Piper betle* L. is scanty. It is for this reasons, it is needed to study morphological and anatomical characters of *Piper betle* L.

The aims and objectives of this research are to compare the morphological and anatomical characters of *Piper betle* L. from three different localities, and to provide the differences and similarities of their morphological and anatomical structure of different localities in Myanmar.

Materials and Methods

The species studied in this research were collected from Pantanaw Township, Ayeyawady Region, Twan tay Township, Yangon Region and Ye U Township, Sagaing Region during November 2012 to November 2013. After the collection, some of the specimens were studied in fresh stage and others were pressed and dried at room temperature as well as preserved in 50% ethyl alcohol for further study.

After killing and fixation, the small portions of stems, leaves and roots were washed in the running water for overnight and dehydrated through a series of tertiary butyl alcohol solutions. Then they were infiltrated in a series of paraffin wax of which melting points were 49°C, 54.5°C or 55 °C and 60°C respectively. The specimens were then embedded in the 60°C paraffin wax and cut into 15-25 µm thick sections by using a rotary microtome. These thin sections were double-stained in

the combination of safranin and fast green solutions. After staining, the sections were permanently mounted under a cover slip with a Canada balsam in xylol and kept dried for a few weeks on slide tray. The dehydration, infiltration, embedding, staining and mounting were made according to Johansen's (1940) method.

The parts of specimens were macerated by warming in a mixture of 50% hydrogen peroxide solution and 50% glacial acetic acid solution according to Jaffery method (1917). The vessels elements and fibers were studied and measured.

Results

1. Taxonomical studies

1.1 *Piper betle* L. Sp. Pl. 28. 1753. (Figure 1 A - F)

Family : Piperaceae

Scientific name: *Piper betle* L.

Common name: Betel vine

Myanmar name: Kun

Perennial herbs, 2.0-6.0 m height; root climbers; older stems ridged, the younger stems smooth, tumid at the nodes, adventitious roots present, slightly red streaks present in Pantanaw and Twan tay. Leaves simple, alternate, petiolate, stipulate, reticulate venation; blades ovate oblong, 6.0-19.0 cm by 4.0-13.0 cm from Pantanaw, 6.0-14.0 cm by 4.5-11.0 cm from Twan tay and 11.0-21.5 cm by 6.8-15.0 cm from Ye U, cordate at the base, entire along the margin, acuminate at the apex from Pantanaw and acute at the apex from Twan tay and Ye U, glossy upper surface and coriaceous, green from Pantanaw and Twan tay, and yellowish green from Ye U, 7 veined from Pantanaw and Twan tay, 7- to 9 veined from Ye U; petiole 2.5-7.0 cm by 0.2-0.5 cm from Pantanaw, 3.5-7.0 cm by 0.3-0.5 cm from Twan tay and 2.2-8.8 cm by 0.2-0.5 cm from Ye U, slightly canaliculate above, slightly red streaks present from Pantanaw and Twan tay; stipules bud scale, lanceolate, creamy, 1.5 - 2.5 cm long from Pantanaw, 2.5 - 3.0 cm long from Twan tay and 2.0 - 2.8 cm long from Ye U (Table 1).

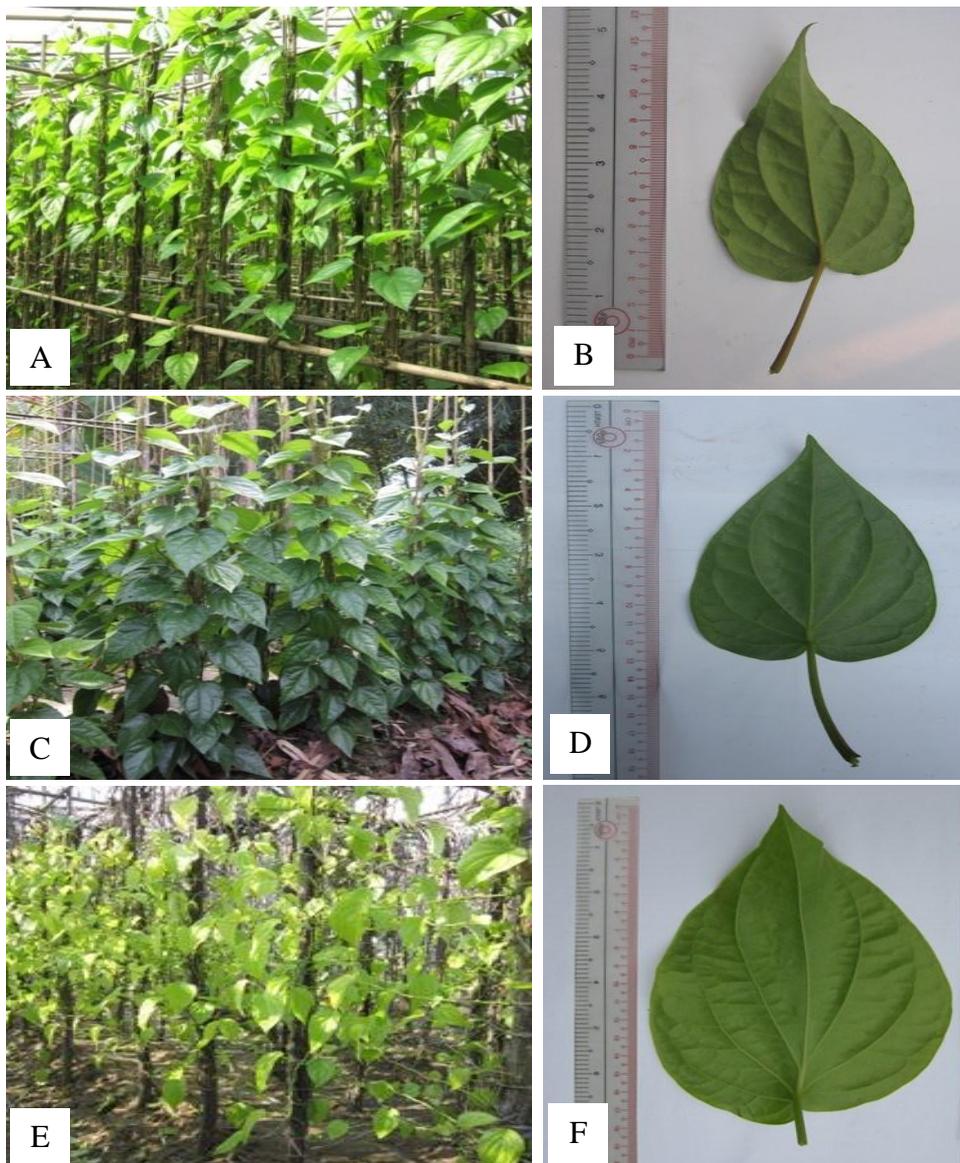


Figure 1 Morphological Characters of *Piper betle* L.

A. Habit and B. Dorsal surface of leaf (Kun sein from Pantanaw)

C. Habit and D. Dorsal surface of leaf (Kun sein from Twan tay)

E. Habit and F. Dorsal surface of leaf (Kun nuwar from Ye U)

2. Anatomical Studies

2.1 Internal structure of the leaves of *Piper betle* L. from three different localities (Figure 2, 3, and 4)

Petiole

In transverse section, petioles (Figure 2A, 3A, and 4A) were heart-shaped in outline, 3.0 - 3.5 mm in length and 2.0 - 2.5 mm in width from three different localities, distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System: In transverse section, the epidermal cells 1-layered, cells barrel or rectangular in shape, outer wall convex, anticlinal walls straight; cuticle thin.

Ground Tissue System: Differentiated into collenchymatous and parenchymatous tissues, collenchymatous tissue composed of 7- to 9-layered from Pantanaw, 6- to 8- layered from Twan tay and 6- to 9- layered from Ye U. Parenchymatous tissue composed of many layered in thick, cells were polygonal in shape. A large mucilage canal was embedded in the center of parenchymatous tissues and 3 - 6 mucilage canals were arranged in a ring. Each canal situated opposite to the vascular bundles.

Vascular Tissue System: Vascular bundles arranged in a ring, embedded in ground tissue, circular or oval shaped in transverse section, colletral type, variable numbers of large bundles alternating with small bundles (Table 2). Each bundle surrounded by continuous ring of 2- to 3- layered of parenchymatous bundle sheaths. Phloem tissues composed of sieve tube elements and companion cells; xylem tissues composed of scalariform and spirally thickened vessels, tracheids, fibers and xylem parenchyma cells. Vessels were measured 85.0 - 750.0 μm in length and 15.0 - 40.0 μm in width from Pantanaw, 150.0 - 500.0 μm in length and 15.0 - 20.0 μm in width from Twan tay and 75.0 - 1250.0 μm in length and 5.0 - 25.0 μm in width from Ye U. Tracheids were 310.0 - 650.0 μm in length, 5.0 - 15.0 μm in width from Pantanaw, 85.0 - 200.0 μm in length, 10.0 - 20.0 μm in width from Twan tay and 25.0 - 1250.0 μm in length, 5.0 - 10.0 μm in width from Ye U. Fibers were measured 245.0 - 760.0 μm in length and 10.0 - 15.0 μm in width from Pantanaw, 275.0 - 1250.0 μm in length and 10.0 - 25.0 μm in width from Twan tay and 200.0 - 300.0 μm in length and 10.0 - 15.0 μm in width from Ye U (Table 5).

Lamina

In the transverse section, the laminae (Figure 2B, 3B, and 4B) were dorsiventral with reticulate venation, 140.0 - 200.0 μm thick from Pantanaw and Twan tay and 155.0 - 220.0 μm thick from Ye U, distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System: In transverse section, adaxial epidermis composed of 1 outer epidermal layer and 2 inner hypodermal layers, and abaxial epidermis composed of 1 outer epidermal layer and 1 inner hypodermal layer; both epidermal cells rectangular or barrel in shape, outer wall convex; adaxial hypodermal layers, cells were rectangular in shape, thin and straight or slightly curved anticlinal walls. Lower hypodermal cells were mostly similar to those of the adaxial hypodermal cells. Secretory cells present in hypodermal layer. Cuticle thin on both surfaces.

Ground Tissue System: Mesophyll differentiated into palisade parenchyma at upper side and spongy parenchyma at the lower side; palisade cells one-layered, the cells elongated in shape, compactly arranged; the spongy parenchyma cells 4 -to 5- layered.

Vascular Tissue System: Vascular bundles of lateral veins were embedded in the mesophyll tissues. They were collateral type and different in size according to their position; bundle sheath distinct and composed of parenchymatous cells, rounded or oval in shape. Phloem composed of sieve tubes, companion cells and phloem parenchyma. Xylem composed of scalariform and spirally thickened vessels, tracheids, fibers and xylem parenchyma. Vessels were measured 50.0 - 700.0 μm in length and 5.0 - 65.0 μm in width from Pantanaw, 150.0 - 450.0 μm in length and 15.0 - 50.0 μm in width from Twan tay and 130.0 - 500.0 μm in length and 10.0 - 15.0 μm in width from Ye U. Tracheids were 35.0 - 175.0 μm in length, 5.0 - 10.0 μm in width from Pantanaw, 60.0 - 400.0 μm in length, 5.0 - 25.0 μm in width from Twan tay and 90.0 - 500.0 μm in length, 5.0 - 25.0 μm in width from Ye U. Fibers were measured 200.0 - 1200.0 μm in length, 10.0 - 15.0 μm in width from Pantanaw, 250.0 - 1000.0 μm in length, 5.0 - 25.0 μm in width from Twan tay and 225.0 - 460.0 μm in length, 5.0 - 15.0 μm in width from Ye U (Table 6).

Midrib

In transverse section, midribs (Figure 2C, 3C, and 4C) were subcircular, distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System: In transverse section, both adaxial and abaxial epidermal cells 1- layered, cells oval to barrel in shape, outer wall convex, anticlinal walls straight; cuticle thin, smooth on both surfaces.

Ground Tissue System: Lying internal to the epidermis made up of two types of cells, collenchyma and parenchyma; adaxial and abaxial side collenchymatous cells different layered from three different localities, cells oval, rounded, irregular or polygonal in shape; adaxial and abaxial side parenchymatous cells different layered from three different localities (Table 2), cells oval, polygonal in shape; intercellular space absent. A single mucilage canal was embedded adaxial side of parenchymatous cells. Secretory cells were found more abundantly in Twan tay than in Pantanaw and Ye U.

Vascular Tissue System: A large vascular bundle is embedded in ground tissue, circular or oval shape, 200.0 - 250.0 μm long and 225.0 - 240.0 μm wide in Pantanaw, 288.0 - 336.0 μm long and 240.0 - 312.0 μm wide in Twan tay and 200.0 - 250.0 μm long and 190.0 - 235.0 μm wide in Ye U, colletral type, surrounded by continuous or discontinuous ring of pericyclic parenchymatous cells, 2- to 3 - layered, polygonal in shape. Phloem tissues were composed of sieve tube elements and companion cells; xylem tissues composed of scalariform and spirally thickened vessels, tracheids, fibers and xylem parenchyma. Vessels were measured 340.0 - 800.0 μm in length and 5.0 - 30.0 μm in width from Pantanaw, 110.0 - 600.0 μm in length and 10.0 - 20.0 μm in width from Twan tay and 50.0 - 500.0 μm in length and 5.0 - 15.0 μm in width from Ye U. Tracheids were 25.0 - 200.0 μm in length, 5.0 - 10.0 μm in width from Pantanaw, 20.0 - 300.0 μm in length, 5.0 - 10.0 μm in width from Twan tay and 50.0 - 175.0 μm in length, 5.0 - 10.0 μm in width from Ye U. Fibers were measured 250.0 - 850.0 μm in length, 5.0 - 15.0 μm in width from Pantanaw, 100.0 - 1050.0 μm in length, 7.5 - 10.0 μm in width from Twan tay and 150.0 - 575.0 μm in length, 5.0 - 7.5 μm in width from Ye U (Table 7).

2.2 Internal structure of the stem of *Piper betle* L. from three different localities (Figure 5 A - F)

In transverse section, stems (Figure 5 A, C, and E) were oval - shaped in outline, distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System: In transverse section, epidermal cells 1-layered, oval to barrel in shape, outer wall convex, anticlinal walls straight; cuticles thick, smooth.

Ground Tissue System: Differentiated into collenchymatous and parenchymatous tissues, the outer cortex collenchymatous tissue composed of 5- to 8- layered from all localities, cells polygonal in shape, forming a continuous ring beneath the hypodermis, if interrupted them the patches are separated by the extension of inner cortex. The inner cortex parenchymatous tissue composed of 6- to 10- layered from all localities, cells were polygonal in shape. Endodermis and pericycle were indistinct. Pith several cells wide, different layered from three different localities, parenchymatous, cells were circular or polygonal in shape. A large mucilage canal was embedded in center of pith parenchymatous tissues. In peripheral region, a ring of 9- to 10-mucilage canals were present (Table 3).

Vascular Tissue System: Vascular bundles embedded in ground tissue, oval shape in transverse section, occurred in a ring of two rows, cortical and medullary bundles, the inner medullary bundles are larger than the outer cortical bundles; cortical bundles small and 24- to 25- bundles from Pantanaw and Twan tay, 27- to 28- bundles from Ye U, one third of them are larger than the others, all embedded in a wavy ring of 5- to 9- cells thick sclerenchymatous tissues, cells were polygonal with heavily thickened walls; medullary bundles were 14 - 15 from Pantanaw and Twan tay, and 17 - 18 from Ye U. Both of vascular bundles were collateral with phloem above and xylem below, separated by cambium; cambium composed of 4 - 5 layers of tangentially elongated rectangular cells; bundle sheath present and composed of parenchymatous cells, round or oval in shape (Table 3). Phloem tissues composed of sieve tube elements and companion cells; xylem tissues composed of scalariform and spirally thickened vessels, tracheids, fibers and xylem parenchyma cells. Vessels were measured 50.0 - 350.0 μm in length and 20.0 - 50.0 μm in width from Pantanaw, 160.0 - 650.0 μm in length and 10.0 - 40.0 μm in width from Twan tay and 185.0 -

400.0 μm in length and 50.0 - 90.0 μm in width from Ye U. Tracheids were 200.0 - 300.0 μm in length, 5.0 - 10.0 μm in width from Pantanaw, 110.0 - 350.0 μm in length, 10.0 - 15.0 μm in width from Twan tay and 160.0 - 350.0 μm in length, 5.0 - 30.0 μm in width from Ye U. Fibers were measured 300.0 - 925.0 μm in length and 10.0 - 20.0 μm in width from Pantanaw, 265.0 - 1000.0 μm in length and 10.0 - 15.0 μm in width from Twan tay and 260.0 - 1075.0 μm in length and 10.0 - 30.0 μm in width from Ye U (Table 8).

2.3 Internal structure of the root of *Piper betle* L. from three different localities (Figure 6 A - F)

In transverse section, the root (Figure 6 A, C, and E) were circular in outline. Distinguishable into dermal, ground and vascular tissue system.

Dermal Tissue System: The root epiblema uniseriate, Parenchymatous.

Ground Tissue System: Composed of cortex, endodermis and pericycle. Cortex homogenous parenchymatous cells, cells were polygonal or oval in shape, many circular or rhomboidal crystals occurred throughout the cortical parenchyma cells. Endodermis 1- layered continuous parenchymatous barrel-shaped cells; pericycle uniseriate, discontinuous, cells barrel-shaped. Pith parenchymatous cells present in Pantanaw. The other two localities (Twan tay and Ye U), pith were inconspicuous, central part of root occupied by xylem parenchyma. Mucilage canal absent.

Vascular Tissue System: In transverse section, vascular cylinder tetrarch to pentarch, 4 - 5 xylem plates from Pantanaw, tetrarch, 4 xylem plates, from Twan tay and triarch to tetrarch, 3 - 4 xylem plates from Ye U, bifurcated once or twice and radiating from the center, the plates being separated by rays, phloem distributed above the xylem beneath the pericycle; phloem composed of sieve tubes and companion cells; xylem towards the centre, xylem composed of vessel elements, tracheids, xylem fibres and xylem parenchyma. Vessels were measured 95.5 - 260.0 μm in length and 15.0 - 125.0 μm in width from Pantanaw, 75.0 - 375.0 μm in length and 30.0 - 95.0 μm in width from Twan tay and 65.0 - 200.0 μm in length and 25.0 - 130.0 μm in width from Ye U. Tracheids were 50.0 - 225.0 μm in length, 15.0 - 25.0 μm in width from Pantanaw, 50.0 - 300.0 μm in length, 10.0 - 20.0 μm in width from Twan tay and 60.0 - 300.0 μm in length, 10.0 - 15.0 μm in width from Ye U (Table 9).

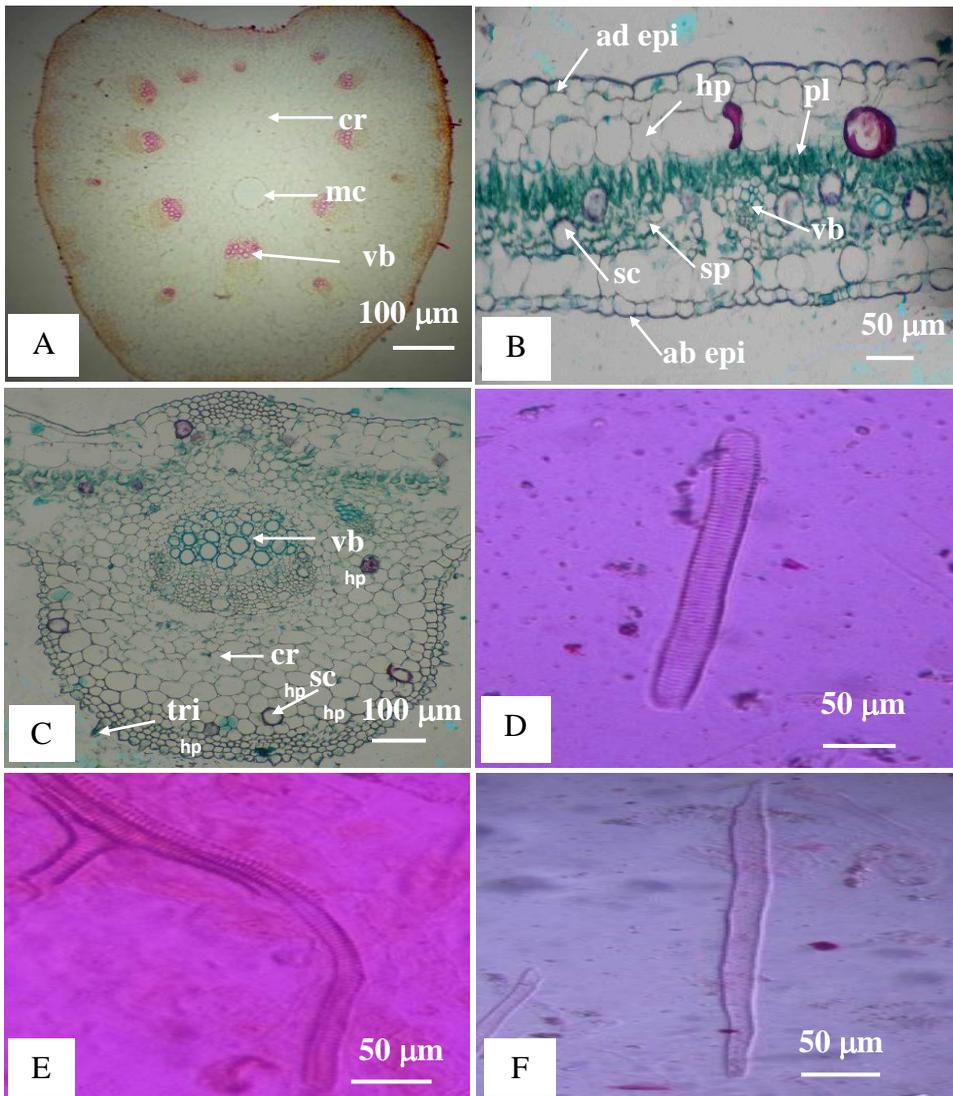


Figure 2 Internal structure and macerated elements of leaves of *Piper betle* L. from Pantanaw

- A. T.S of petiole showing a ring of vascular bundle
 B. T.S of lamina showing epidermal layer, palisade and spongy mesophyll cells
 C. T.S of midrib with a large vascular bundle D. vessel E & F. tracheal elements
 (ab epi = abaxial epidermal cell, ad epi= adaxial epidermal cell, cr = cortex,
 hp = hypodermal cell, mc = mucilage canal, pl = palisade parenchyma cell,
 sc = secretory cell, sp = spongy parenchyma cell, tri = trichome,
 vb = vascular bundle)

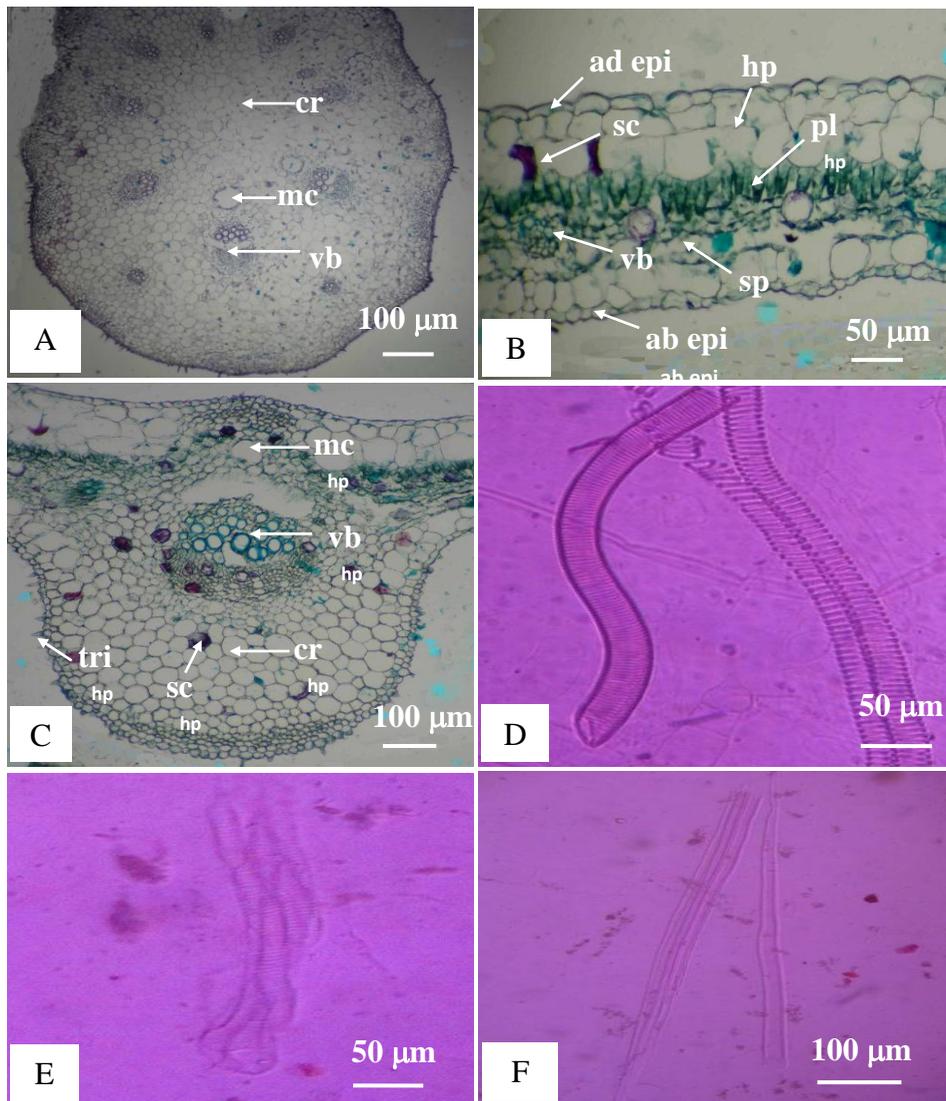


Figure 3 Internal structure and macerated elements of leaves of *Piper betle* L. from Twan tay

- A. T.S of petiole showing a ring of vascular bundle
 B. T.S of lamina showing epidermal layer, palisade and spongy mesophyll cells
 C. T.S of midrib with a large vascular bundle D. vessel E & F. tracheal elements
 (ab epi = abaxial epidermal cell, ad epi= adaxial epidermal cell, cr = cortex,
 hp = hypodermal cell, mc = mucilage canal, pl = palisade parenchyma cell,
 sc = secretory cell, sp = spongy parenchyma cell, tri = trichome,
 vb = vascular bundle)

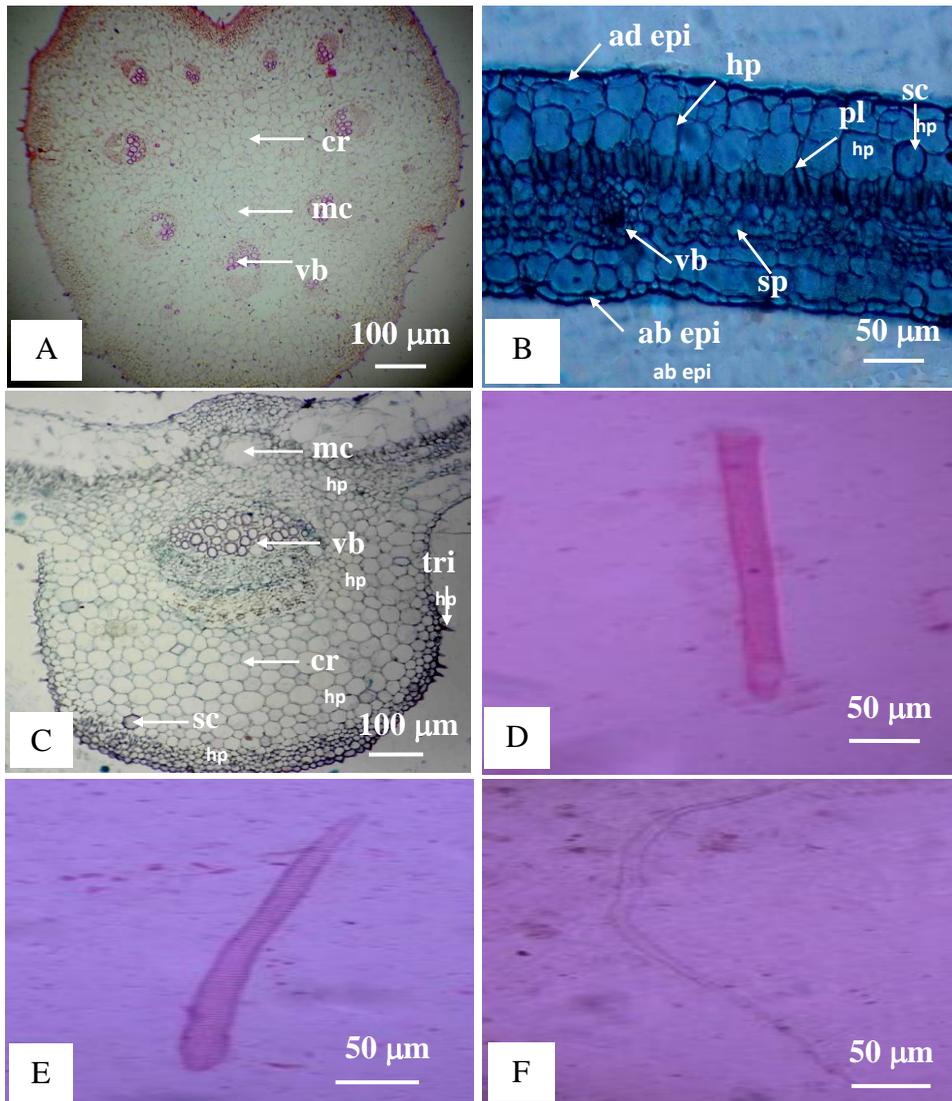


Figure 4 Internal structure and macerated elements of leaves of *Piper betle* L. from Ye U

- A. T.S of petiole showing a ring of vascular bundle
 B. T.S of lamina showing epidermal layer, palisade and spongy mesophyll cells
 C. T.S of midrib with a large vascular bundle
 D. vessel
 E & F. Tracheal elements
 (ab epi = abaxial epidermal cell, ad epi= adaxial epidermal cell, cr = cortex, hp = hypodermis cell, mc = mucilage canal, pl = palisade parenchyma cell, sc = secretory cell, sp = spongy parenchyma cell, tri = trichome, vb = vascular bundle)

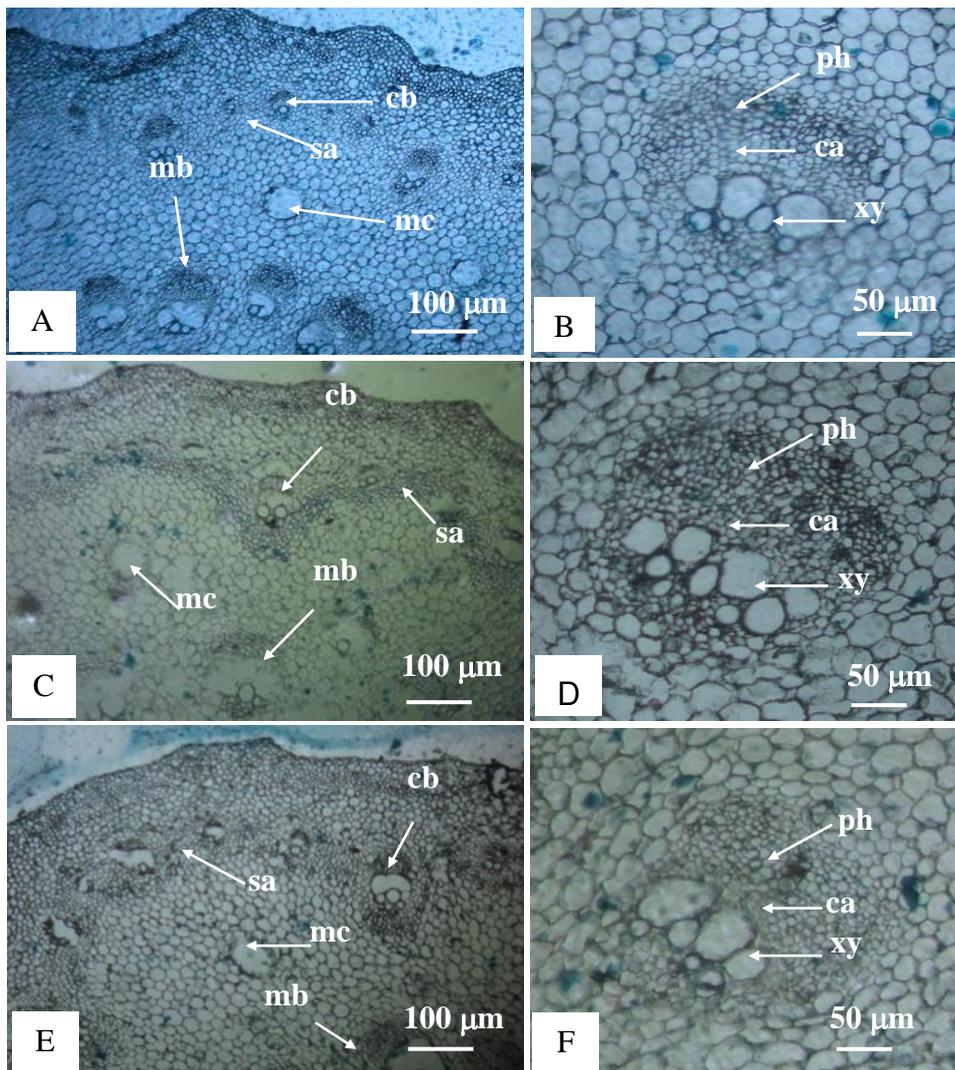


Figure 5 Internal structure of the stem of *Piper betle* L. from Pantanaw, Twan tay, Ye U

A. T.S of a portion of stem from Pantanaw

B. Close up view of vascular bundle from Pantanaw

C. T.S of a portion of stem from Twan tay

D. Close up view of vascular bundle from Twan tay

E. T.S of a portion of stem from Ye U

F. Close up view of vascular bundle from Ye U

(ca = cambium, cb = cortical bundle, mb = medullary bundle,

mc = mucilaginous cannal, ph = phloem, sa = sclerenchyma, xy = xylem)

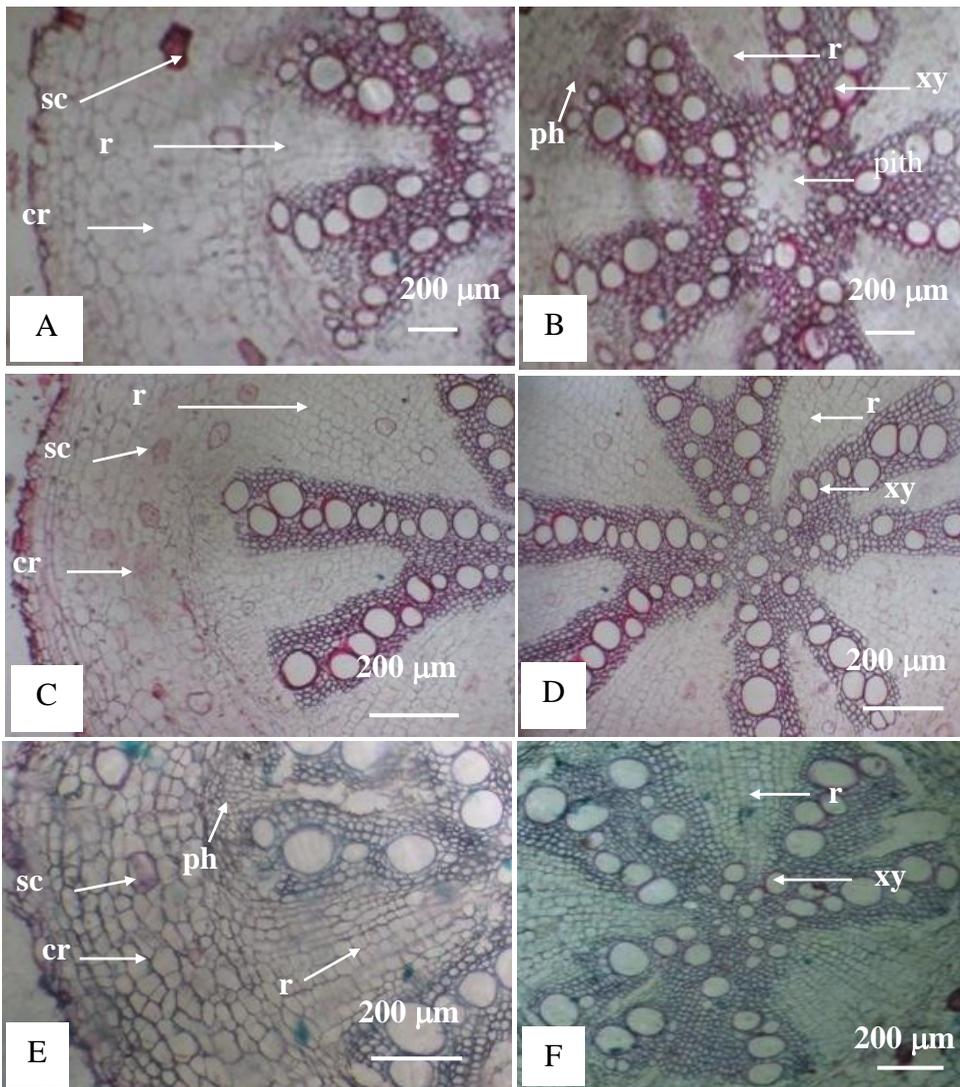


Figure 6 Internal structure of the root of *Piper betle* L. from Pantanaw, Twan tay and Ye U

- A. T.S of a portion of root from Pantanaw
 - B. Close up view of vascular bundle from Pantanaw
 - C. T.S of a portion of root from Twan tay
 - D. Close up view of vascular bundle from Twan tay
 - E. T.S of a portion of root from Ye U
 - F. Close up view of vascular bundle from Ye U
- (cr = cortex, ph = phloem, r = ray, sc = secretory cell, xy = xylem)

Table 1 Morphological Characters of the Leaves of *Piper betle* L. from three different localities

Locality & Local Name	Size of Leaves (cm)	No of vein	Colour and Texture of Leaves	Size of Petioles (cm)
Pantanaw, Kun sein	6.0-19.0 by 4.0-13.0	7	Green, glossy upper surface and coriaceous	2.5-7.0 by 0.2-0.5
Twan tay, Kun sein	6.0-14.0 by 4.5-11.0	7	Green, glossy upper surface and coriaceous	3.5-7.0 by 0.3-0.5
Ye U, Kun nuwar	11.0-21.5 by 6.8-15.0	7 to 9	Yellowish green, glossy upper surface and coriaceous	2.2-8.8 by 0.2-0.5

Table 2 Anatomical Characters of the Leaves of *Piper betle* L. from three different localities

Localities & Local Name	Petiole				Lamina thickness (µm)
	size (mm)	Collenchyma	Vascular bundle	Accessory bundle	
Pantanaw, Kun sein	3.0- 3.5 x 2.0- 2.5	7- to 9- layered	7-9 large bundles	4-10 small bundles	140.0-200.0
Twan tay, Kun sein	3.0- 3.5 x 2.0- 2.5	6- to 8- layered	6-7 large bundles	6-8 small bundles	140.0- 200.0
Ye U, Kun nuwar	3.0- 3.5 x 2.0- 2.5	6- to 9- layered	5-7 large bundles	5-7 small bundles	155.0-220.0

Table 2 Continued.

Localities & Local Name	Midrib Size		Collenchyma		Parenchyma	
	Length (μm)	Width (μm)	Below the adaxial epidermis	Above the abaxial epidermis	Between the adaxial epidermis and vascular strands	Between the abaxial epidermis and vascular strands
Pantanaw, Kun sein	850.0-1075.0	725.0-875.0	6- to 9- layered	4- to 5- layered	3- to 6- layered	7- to 11- layered
Twan tay, Kun sein	875.0-1000.0	750.0-975.0	4- to 6- layered	3- to 4- layered	3- to 6- layered	7- to 10- layered
Ye U, Kun nuwar	1087.5-1125.0	900.0-925.0	4- to 8- layered	2- to 5- layered	5- to 8- layered	7- to 10- layered

Table 3 Anatomical Characters of the Stems of *Piper betle* L. from three different localities

Localities & Local Name	Shape and Size (mm)	Pith	Vascular bundle		
			Type	Cortical bundle	Medullary bundle
Pantanaw, Kun sein	Oval in outline 3.0-4.0 by 2.0-3.0	16- to 34- layered	Collateral, two concentric rings	small and 24- to 25	14-15
Twan tay, Kun sein	Oval in outline 3.0-4.0 by 2.0-3.0	15- to 34- layered	Collateral, two concentric rings	small and 24- to 25	14-15
Ye U, Kun nuwar	Oval in outline 4.0-4.5 by 3.0-3.5	19- to 38- layered	Collateral, two concentric rings	small and 27- to 28	17-18

Table 4 Anatomical Characters of the Roots of *Piper betle* L. from three different localities

Localities & Local Name	Epiblema	Parenchymatous cells	Endodermis	Pericycle	Pith	Vascular system
Pantanaw, Kun sein	Uniseriate	10- to 15- layered	One-layered	Uniseriate	10- to 12- layered	Tetrarch to pentarch, 500.0-825.0 μm in length
Twan tay, Kun sein	Uniseriate	7- to 17- layered	One-layered	Uniseriate	Inconspicuous	Tetrarch, 525.0-950.0 μm in length
Ye U, Kun nuwar	Uniseriate	10- to 14- layered	One-layered	Uniseriate	Inconspicuous	Triarch to tetrarch, 500.0-510.0 μm in length

Table 5 Macerated elements of the Petiole of *Piper betle* L. from three different localities

Locality & Local Name	Vessel elements		Tracheids		Fibers	
	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)
Pantanaw, Kun sein	85.0 - 750.0	15.0 - 40.0	310.0 - 650.0	5.0 - 15.0	245.0 - 760.0	10.0 - 15.0
Twan tay, Kun sein	150.0 - 500.0	15.0 - 20.0	85.0 - 200.0	10.0 - 20.0	275.0 - 1250.0	10.0 - 25.0
Ye U, Kun nuwar	75.0 - 1250.0	5.0 - 25.0	25.0 - 1250.0	5.0 - 10.0	200.0 - 300.0	10.0 - 15.0

Table 6 Macerated elements of the Lamina of *Piper betle* L. from three different localities

Locality & Local Name	Vessel elements		Tracheids		Fibers	
	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)
Pantanaw, Kun sein	50.0 - 700.0	5.0 - 65.0	35.0 - 175.0	5.0 - 10.0	200.0 - 1200.0	10.0 - 15.0
Twan tay, Kun sein	150.0 - 450.0	15.0 - 50.0	60.0 - 400.0	5.0 - 25.0	250.0 - 1000.0	5.0 - 25.0
Ye U, Kun nuwar	130.0 - 500.0	10.0 - 15.0	90.0 - 500.0	5.0 - 25.0	225.0 - 460.0	5.0 - 15.0

Table 7 Macerated elements of the Midrib of *Piper betle* L. from three different localities

Locality & Local Name	Vessel elements		Tracheids		Fibers	
	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)
Pantanaw, Kun sein	340.0 - 800.0	5.0 - 30.0	25.0 - 200.0	5.0 - 10.0	250.0 - 850.0	5.0 - 15.0
Twan tay, Kun sein	110.0 - 600.0	10.0 - 20.0	20.0 - 300.0	5.0 - 10.0	100.0 - 1050.0	7.5 - 10.0
Ye U, Kun nuwar	50.0 - 500.0	5.0 - 15.0	50.0 - 175.0	5.0 - 10.0	150.0 - 575.0	5.0 - 7.5

Table 8 Macerated elements of the stem of *Piper betle* L. from three different localities

Locality & Local Name	Vessel elements		Tracheids		Fibers	
	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)
Pantanaw, Kun sein	50.0 - 350.0	20.0 - 50.0	200.0 - 300.0	5.0 - 10.0	300.0 - 925.0	10.0 - 20.0
Twan tay, Kun sein	160.0 - 650.0	10.0 - 40.0	110.0 - 350.0	10.0 - 15.0	265.0 - 1000.0	10.0 - 15.0
Ye U, Kun nuwar	185.0 - 400.0	50.0 - 90.0	160.0 - 350.0	5.0 - 30.0	260.0 - 1075.0	10.0 - 30.0

Table 9 Macerated elements of the root of *Piper betle* L. from three different localities

Locality & Local Name	Vessel elements		Tracheids	
	Length (μm)	Width (μm)	Length (μm)	Width (μm)
Pantanaw, Kun sein	95.5 - 260.0	15.0 - 125.0	50.0 - 225.0	15.0 - 25.0
Twan tay, Kun sein	75.0 - 375.0	30.0 - 95.0	50.0 - 300.0	10.0 - 20.0
Ye U, Kun nuwar	65.0 - 200.0	25.0 - 130.0	60.0 - 300.0	10.0 - 15.0

Discussion and Conclusion

Morphological and anatomical characters of the leaves, stems and roots of three different localities viz., Pantanaw (Kun sein), Twan tay (Kun sein) and Ye U (Kun nuwar) were studied.

In this study, the *Piper betle* L. from all localities were perennial herbs, root climbers, tumid at the nodes, adventitious roots present. The specimens from three different localities were cultivated plants harvesting for green leaves. The colors of leaves were found to be green from Pantanaw and Twan tay, yellowish green from Ye U. The textures of leaves were found to be glossy upper surface and coriaceous. The shapes of leaves were found to be ovate oblong. The sizes of leaves were found to be different in all localities (Table 1). The shortest length of leaves was Kun sein from Twan tay and the longest length of leaves was Kun nuwar

from Ye U. The slightly red streaks were present in the petioles and stems of Kun sein from Pantanaw and Twan tay.

In the present study, the leaves were found to be simple, alternate, petiolate, stipulate, coriaceous which were agreed with previous researchers (Chibber 1912; Yongqian *et al.* 1999). The baldes were 7 veins from Pantanaw and Twan tay, and 7- to 9- veined from Ye U.

In this research, the transverse sections of laminae, midribs, petioles, stems and roots showed the three tissue systems viz. dermal tissue system, ground tissue system and vascular tissues system.

The transverse sections of petioles of *Piper betle* L. were found to be heart-shaped. Vascular tissues were separated, variable number of large bundles alternating with small bundles, collateral type, circular or oval in shape (Table 2). These were agreed with Metcalfe and Chalk (1950), Lakshmi and Naidu (2010), Selvadurai *et al.* (2011) and Raman *et al.* (2012).

In transverse sections, the thickness of laminae were the same in Kun sein from Pantanaw and Twan tay, and the laminae of Kun nuwar from Ye U were the thickest (Table 2). The stomata were found to be anisocytic type. The ground tissue of lamina was differentiated into upper palisade and lower spongy parenchyma. Palisades were one-layered and composed of elongated cells, and cells were compactly arranged. The spongy layers were 4- to 5- layered, cells were rounded to oval in shape, secretory cells were present in the mesophyll from three different localities and these characters were in agreement with Chibber (1912), Lakshmi and Naidu (2010), Selvadurai *et al.* (2011) and Raman *et al.* (2012).

In this research, the transverse sections of midribs of *Piper betle* L. from three localities were subcircular. The ground tissue of midribs was differentiated into two types of tissues, collenchymatous and parenchymatous, collenchymatous cells below the adaxial epidermis and above the abaxial epidermis were different layered, the main mass of midrib was thin-walled parenchymatous cells (Tabel 2). Secretory cells were found more abundantly in Twan tay than Pantanaw and Ye U. A large vascular bundle was present, collateral type and circular or oval in shape, a single mucilage canal was found in the adaxial side of three

localities; these characters were in agreement with Lakshmi and Naidu (2010), and Pradhan *et al.* (2013).

According to the present study, the transverse sections of stems of *Piper betle* L. were oval - shaped in outline. The ground tissues differentiated into collenchymatous and parenchymatous, the outer cortex collenchymatous tissue forming a continuous ring beneath the hypodermis, if interrupted them the patches are separated by the extension of inner cortex. The inner cortex parenchymatous tissue composed of 6- to 10-layered from three different localities. Pith parenchymatous tissue composed of different layered from three different localities. A large mucilage canal was embedded in center of pith parenchymatous tissues. A ring of 9- to 10- mucilage canal was also observed in the peripheral region. Endodermis and pericycle were indistinct of all three localities. Vascular bundles of stems were two concentric rings. They were collateral type and variable number (Table 3). These characters were in agreement with Metcalfe and Chalk (1950) and Raman *et al.* (2012).

In transverse section of roots of *Piper betle* L. were circular in outline. Many circular or rhomboidal secretory cells were observed in the cortical layers.

The roots of *Piper betle* L. is hexarch, 6 xylem plates, bifurcated once or twice (Chibber 1912, Metcalfe and Chalk 1950). The vascular bundles were observed in tetrarch to pentarch in Pantanaw, tetrarch in Twan tay and triarch to tetrarch in Ye U (Table 4). Among three localities, the largest vascular strand is Kun sein from Twan tay and shortest vascular strand is Kun nuwar from Ye U (Table 4).

In the present study, pith parenchyma were only found in *Piper betle* L. from Pantanaw. In *Piper betle* L. from Twan tay and Ye U, pith were inconspicuous and central portions of roots were xylem parenchyma (Table 4). In the present study, mucilage canal was absent in the pith, these characters were similar to previous researcher, Metcalfe and Chalk, (1950).

The morphological characters and anatomical characters of *Piper betle* L. from three different localities were described. According to the result of present investigation, these can provide the information of similarities and differences of each locality. In addition, these will partially fulfill in the field of modern taxonomy.

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