MORPHOLOGICAL AND HISTOLOGICAL CHARACTERIZATION AND PRELIMINARY PHYTOCHEMICAL EXAMINATION ON RHIZOMES OF *CURCUMA PETIOLATA* ROXB.

Htay Htay Lwin[1], Khin San Nu[2], Ohnmar[3], Aye Aye Aung[4], San San Maw[5]

Abstract

The plant *Curcuma petiolata* Roxb., Myanmar name "Marlar" belongs to the family Zingiberaceae. The plants are widely distributed in Myanmar. It was collected from Dawei University Campus, Tanintharyi Region from July to October (2018). In this study, identification of collected plant is carried out by using available literatures. In morphological study, the plant is perennial rhizomatous herb. Leaves are oblong lanceolate. Inflorescences are lateral spike. Flowers are yellow, sessile, zygomorphic, trimerous, epigynous. Calyx of 3 sepals, fused. Corolla of 3 petals, fused. One fertile stamen, 2 lateral staminodes, other staminodes, fused to form a labellum. Ovary tricarpellary, syncarpous, axile placentation, disc present. In histological study, the anticlinal walls in the epidermal cell of both surfaces are straight. Tetracytic stomata present on both surfaces but more abundant on lower surface. Oil cells contain in mesophyll layers of lamina and parenchymatous layer of midrib, leaf sheath and rhizome. Vascular bundles of lamina, midrib, leaf sheath, root and rhizome are collateral and closed type. Prismatic calcium oxalate crystals present in mesophyll tissues of lamina and ground tissues of midrib, leaf sheath, root and rhizome. These has been investigated and presented as diagnostic characters for identification and medicinal purposes. Phytochemical tests of *Curcuma petiolata* Roxb. rhizomes showed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, proteins, reducing sugars, saponins, starch, steroids, tannins and terpenoids. Due to the presence of these chemical constituents, these documents were highlighted to know the effective biological activity of *Curcuma petiolata* Roxb. rhizomes.

Keyword: *Curcuma petiolata* Roxb., morphological and histological characters, phytochemical properties.

Introduction

The plant *Curcuma petiolata* Roxb. belongs to the family Zingiberaceae. This family consists of 50 genera and 1300 species (Heywood, *et al*., 2007 and Trease and Evans, 2009). The member of this family distributed in South and South East Asia, some species in America and subtropical and warm-temperate Asia (Te-lin and Larsen, 2000).

In Myanmar, this family consists of about 18 genera and 125 species (Hundley and Chit ko ko, 1961). According to the Kress, *et al*., (2003), genus *Curcuma* contains 24 species of Myanmar are listed in Zingiberaceae. The genus *Curcuma* is one of the

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largest genera in the Zingiberaceae, most *Curcuma* grows well in loose and sandy soil in shaded areas (Sirirugsa, *et al*., 2007). The rhizomes of these species are used in traditional medicines (Perry, 1980). *Curcuma* species possess antioxidant activity and the pharmacological effects. Bioactive components such as curcuminoids are responsible for anti-oxidative and anti-inflammatory properties, wound healing, hypoglycemia and antimicrobial activities (Beghel, *et al*., 2013).

Curcuma petiolata Roxb. is one of *Curcuma* species which widely cultivated as an ornamental plant and has long been used as a folk botanical in Asia (Perry, 1980). The *Curcuma petiolata* Roxb. rhizomes extract contain high amount of curcumins with potent DPPH radical scavenging, ferrous reducing power and inhibition of lipid peroxidation activities (Thakam, *et al.*, 2012). **Curcumin has a wide range of biological functions, especially the anticancer activity including bladder cancer (Gao, *et al*., 2012), pancreatic cancer (Plengsuriyakarn, *et al*., 2012), prostate cancer (Zhou, *et al*., 2014**).

The aim and objectives are to study the morphological and histological characters and to examine the phytochemical constituents of *Curcuma petiolata* Roxb.

Materials and Methods

Morphological study of *Curcuma petiolata* Roxb.

The specimens of *Curcuma petiolata* Roxb. were collected from Dawei University Campus, Tanintharyi Region from July to October (2018). The morphological study of plant was undertaken with the help of available literatures (Hooker, 1894; Kirtikar and Basu, 1975 and Dassanayake, 1983).

Histological study of *Curcuma petiolata* Roxb.

In histological studies, free hand section of lamina, midribs, petioles, leaf sheath, roots and rhizomes from the fresh specimens were prepared by using chloral hydrate solution for clearing reagents, safranin solution for testing lignin and iodine solution B.P for testing starch. These characters were determined according to the literature of Tomlinson (1969), Pandey (1998) and Trease and Evens (2009).

Phytochemical investigation of *Curcuma petiolata* Roxb. rhizomes

In this investigation, preliminary phytochemical tests were carried out at Department of Botany, Hpa-an University according to the methods of Marini Bettolo, *et al*., (1981), Central Council for Research in Unani Medicine (1987) and Sasikala and Sundaraganapathy (2017).

Results

Morphological characters of *Curcuma petiolata* Roxb.

Perennial rhizomatous herbs, tubers present. Leaves emerge from the rhizome, oblong-lanceolate. Both surfaces are glabrous. Inflorescences are lateral spike, oblong. Flowers are yellow, floral bracts oveate-lanceolate, pale green, coma bracts lanceolate, red, sessile, bracteolate, bisexual, irregular, zygomorphic, trimerous, cyclic, epigynous. Calyx of 3 sepals, fused, calyx tube funnel-shaped, the lobes minute, white, superior. Corolla of 3 petals, fused, corolla tube, infundibuliform, the narrow tube long and tubular, corolla lobes oblong, pale yellow, posterior lobe hood-shaped, minute extension, superior. One fertile stamen, 2 lateral staminodes, other

staminodes, fused to form a labellum, the filament flattened, contracted at apex, each anther thecous with a basal spur, 2 lateral staminodes free, elliptic-oblong, yellow, the labellum obovate or orbicular, the tip bilobed, strap-shaped medium part, yellow with bright yellow at the centre, superior. Ovary inferior tricarpellary, syncarpous, trilocular, axile placentation, many ovule in each ovule, pubescent, style long and filiform, extending between two anther lobes and protruding out as a stigma. Disc present.

Morphological characters of *Curcuma petiolata* Roxb.

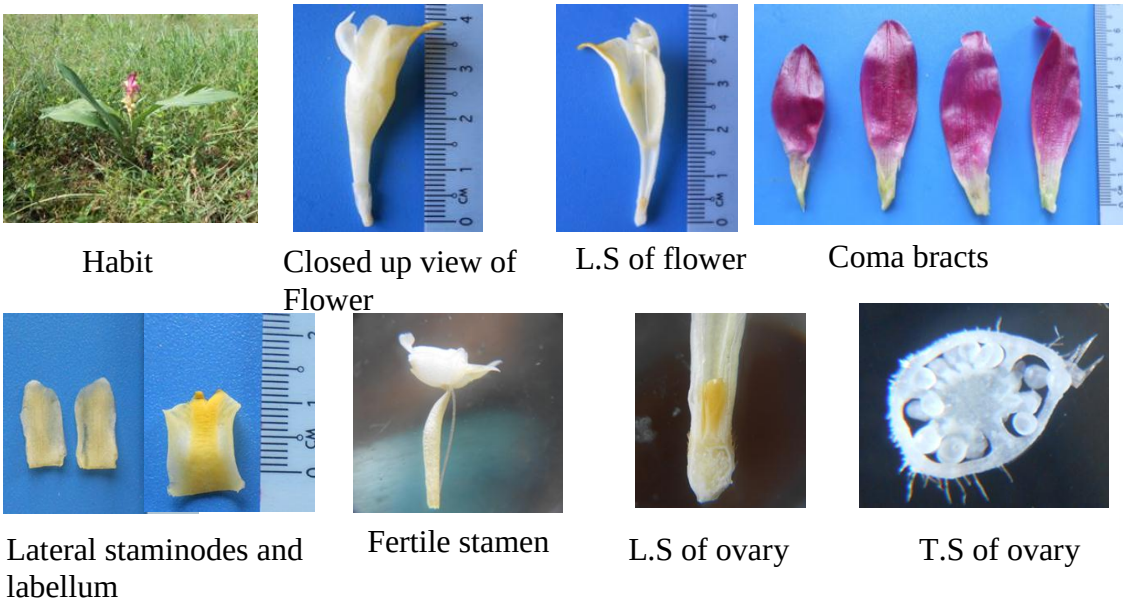


Figure. 1 Morphological characters of *Curcuma petiolata* Roxb.

Histological characters of *Curcuma petiolata* Roxb.

Lamina

In surface view, the cuticle is thin and smooth. The anticlinal wall in the epidermal cells of both surfaces is straight. The cells are polygonal in shape, thin walled, parenchymatous. Tetracytic stomata are present on both surfaces but more abundant on lower surface. Prismatic calcium oxalate crystals and silica are present.

In transverse section of lamina, cuticle layers are thin and smooth on both surfaces. Epidermal cells barrel shaped. Only one layer of hypodermis is present on upper side and two-three layers on lower side. These cells are polygonal in shaped in adaxial side and irregular in abaxial side. Palisade parenchymatous layers found beneath the upper hypodermis are two-three layers. The spongy mesophyll cells are four-five layers, the cells loosely arranged, irregular in shape, thin-walled parenchymatous cells and intercellular spaces. Vascular bundles are embedded in the mesophyll cells. They are collateral and closed type. Prismatic calcium oxalate crystals and silica are present in mesophyll cells.

Midrib

In transverse section, the midrib is V-shaped in outline. Both upper and lower epidermal cells are barrel shaped, thin-walled, parenchymatous cells. Below the epidermis, both upper and lower collenchymatous cells are 2 – 3 layers, polygonal in shape. 4 – 10 layers of parenchymatous cells are found above main vascular bundles. These cells are polygonal in shape and thin-walled. Vascular bundles are arranged in three rows, developing unequally at different levels. The main vascular bundles are between alternating with air canals and embedded in chlorenchyma. The abaxial conducting system consists of an arc of vascular bundles of different sizes that are circular in outline. The adaxial conducting system consists of 1 – 4 vascular bundles (subsidiary vascular bundle) that are similar in appearance to the main vascular bundles but are smaller in size. The main vascular bundles are furnished with a massive fibrous or sclerenchymatous sheath above the xylem and below the phloem. Abaxial bundles are enveloped within almost a complete fibrous sheath. Air canals contain a loose network of lobed cells. Prismatic calcium oxalate crystals are scattered in the ground tissues.

Petiole

In transverse section, the petiole is crescent-shaped in outline. The epidermal cells of adaxial surfaces are barrel shaped and those of abaxial surfaces are rounded in shaped and thin-walled. In adaxial region, the collenchymatous cells are 1 – 4 layers and 4 – 5 layers in abaxial region, polygonal shaped. 5 – 15 layers of parenchymatous cells are found above main vascular bundles. These cells are polygonal in shape and thin-walled. Vascular bundles are arranged in three rows that are similar in appearance to midrib. Prismatic calcium oxalate crystals are scattered in the ground tissues.

Leaf sheath

In transverse section, both adaxial and abaxial epidermal cells are barrel in shape, thin-walled, parenchymatous cells. The abaxial epidermal cells are smaller than adaxial cells. Above the abaxial epidermal layer, 1 – 3 layers of collenchymatous cells are polygonal in shape. Parenchymatous cells are 4 – 5 layers in abaxial region and 7 – 10 layered in adaxial region. Both are polygonal in shape. Vascular bundles are arranged in three rows similar in appearance to the main vascular bundles but are smaller in size. The main vascular bundles are furnished with a massive fibrous or sclerenchymatous sheath above the xylem and below the phloem. Abaxial bundles are enveloped within almost a complete fibrous sheath. Air canals contain a loose network of lobed cells. Oil cells are embedded in parenchymatous layers. Prismatic calcium oxalate crystals are scattered in the ground tissues.

Root

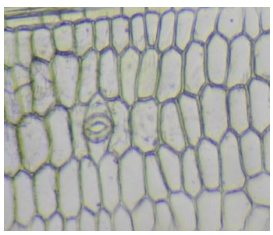
In transverse section, the root is more or less circular in outline. The roots hairs are present. The epiblema layer is only one layer, barrel shaped and thin-walled, parenchymatous cells. Below the epiblema layer, 5 – 6 layers of outer cortex which are polygonal in shape, thin-walled, parenchymatous cells. In the middle cortical layers composed of 6 – 7 layers of aerenchymatous cells, polygonal shaped, thin-walled, intercellular spaces present. Inner cortical layers made up of 1 – 2 layers, polygonal shaped, thin-walled, parenchymatous cells. The endodermis is one layer,

barrel-shaped, thin-walled parenchymatous cells. Pericycle layer lie below endodermal layers are 1 – 2 layers, barrel shaped. Vascular bundle is polyarch. Prismatic calcium oxalate crystals are scattered in the ground tissues.

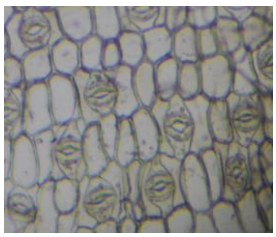
Rhizome

In transverse section, periderm consists of 7 – 10 layers, thin-walled, parenchymatous cells, rectangular to irregular in shape. Periderm composed of phellem or cork, phellogen or cork cambium and phelloderm or secondary cortex. Cortex 35 – 45 layers, thin-walled, parenchymatous cells, polygonal in shape. Endodermal cells lie the inner region of cortex layer, 2 – 5 layers, thin-walled parenchymatous cells. Vascular bundles are collateral and all scattered throughout the ground tissue. Vascular bundles are furnished with a massive fibrous or sclerenchymatous sheath above the xylem and below the phloem. Oil cells are embedded in parenchymatous layers. Prismatic calcium oxalate crystals are scattered in the ground tissues and starch grains are present.

Histological characters of *Curcuma petiolata* Roxb.



Upper surface view of lamina (X400)

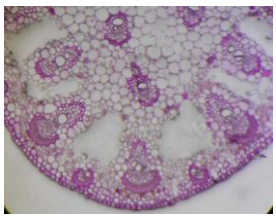


Lower surface view of lamina (X400)

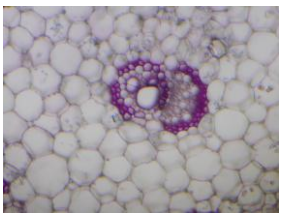
T.S of lamina (X100)

T.S of midrib in outline (X40)

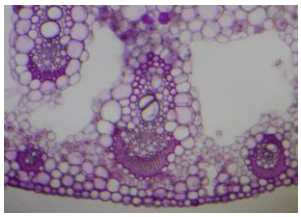
T.S of midrib showing cortex layer with subsidiary vascular bundle (X200)



T.S of midrib showing vascular bundles alternating with air canals (X100)

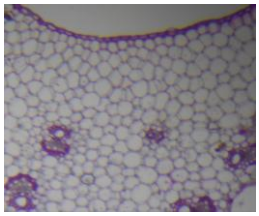


T.S of midrib showing subsidiary vascular bundle (X400)

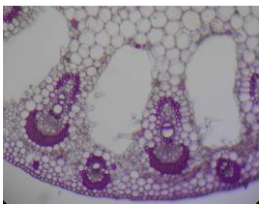


T.S of midrib showing main vascular bundle (X200)

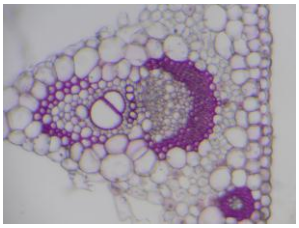
T.S of petiole in outline (X40)



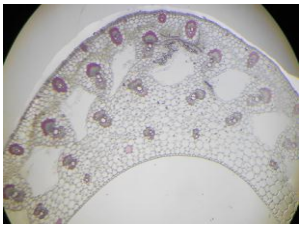
T.S of petiole showing cortex layer with



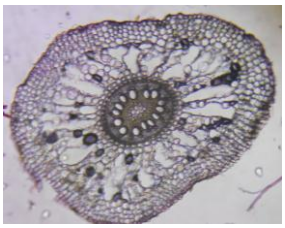
T.S of petiole showing air canals (X200)



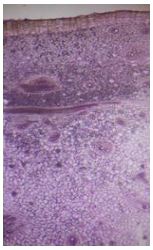
T.S of petiole showing main vascular bundle (X400)



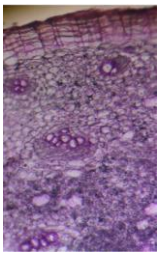
T.S of Leaf sheath showing vascular bundles (X100)



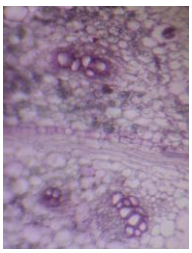
T.S of root outline (X40)



T.S of rhizome in outline (X40)



T.S of rhizome showing periderm layers (X200)



T.S of rhizome showing endodermis and vascular bundles (X200)

Figure.2 Histological characters of *Curcuma petiolata* Roxb.

Phytochemical investigation of *Curcuma petiolata* Roxb. rhizomes

Preliminary phytochemical tests indicated the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, proteins, reducing sugars, saponins, starch, steroids, tannins and terpenoids of *Curcuma petiolata* Roxb. rhizomes.

Table 1. Phytochemical test of *Curcuma petiolata* Roxb. rhizomes

No	Test	Extract	Test reagents	Observation	Results
1.	Alkaloid	EtOH	1.Dragendorff's reagent 2. Mayer's reagent 3.Wagner's reagent 4. Hager's reagent	Orange brown ppt White ppt Reddish brown ppt Yellow ppt	+ + + +
2.	α-amino acids	H_2O	Ninhydrin reagent	Pink spot	+
3.	Carbohydrate	H_2O	Benedict's solution	Brick red ppt	+
4.	Flavonoid	EtOH	HCl / Mg	Pink color	+
5.	Glycoside	EtOH	H_2O + NaOH	Yellow color	+
6.	Phenolic compound	EtOH	H_2O + 10% $FeCl_3$	Green color	+
7.	Protein	H_2O	Millon's reagent	White ppt turns red on heating	+

8.	Reducing sugar	H_2O	Fehling's solution A and B	Brick red ppt	+
9.	Saponin	H_2O	H_2O	Frothing	+
10.	Starch	H_2O	Iodine solution	Blue black	+
11.	Steroid	EtOH	$CHCl_3$ + conc:H_2SO_4	Green color	+
12.	Tannin	H_2O	5% $FeCl_3$ + H_2SO_4	Yellow brown ppt	+
13.	Terpenoid	EtOH	$CHCl_3$ + conc:H_2SO_4	Pink color	+

(+) = Present

Discussion

In this research, investigation of morphological and histological characters and preliminary phytochemical examination of *curcuma petiolata* Roxb. were carried out.

Curcuma petiolata Roxb.was perennial rhizomatous herb. Leaves emerge from the rhizome. Inflorescences are lateral spike. Flowers are yellow, floral green, coma bracts red, epigynous. Calyx of 3 sepals, fused, Corolla of 3 petals, fused. One fertile stamen, 2 lateral staminodes, other staminodes, fused to form a labellum. Ovary inferior, axile placentation, style long and filiform, extending between two anther lobes and protruding out as a stigma. These characters agreed with those of (Hooker, 1894; Kirtikar and Basu, 1975 and Dassanayake, 1996).

In histological study, anticlinal walls of both upper and lower surfaces of lamina are straight. Tetracytic stomata are present on both surfaces but lower surface is more abundant than upper ones. Vascular bundle of lamina is collateral and closed type. Vascular bundles of midrib and leaf sheath are arranged in three rows. The main vascular bundles are between alternating with air canals. The abaxial conducting system consists of an arc of vascular bundles of different sizes that are circular in outline. The adaxial conducting system consists of vascular bundles that are similar in appearance to the main vascular bundles but are smaller in size. In lamina, oil cells are embedded in mesophyll and parenchymatous layers in midrib and leaf sheath. Prismatic calcium oxalate crystals are scattered in mesophyll in lamina and ground tissue in midrib and leaf sheath. These characters are agreed with those reported by Esau (1953), Tomlinson (1969), Pandey and Chadha (1998) and Ravindran, *et al*., (2007).

In root, cortex layer lies below epiblema layer. Only one layer of endodermis and 1 – 2 layers of pericycle are present. Vascular bundle is polyarch in root. Prismatic calcium oxalate crystals are present within the ground tissues. These characters are agreed with those of Tomlinson (1969).

In rhizome, periderm layers present. Vascular bundles are scattered throughout the ground tissue. Oil cells are embedded in parenchymatous layers. Prismatic calcium oxalate crystals are scattered in the ground tissues and starch grains are present. These characters are agreed with those of Tomlinson (1969).

Khin Tar yar Myint, *et al*., (2018) stated that preliminary phytochemical test of *Curcuma petiolata* Roxb. rhizomes showed the presence of alkaloids, α–amino

acid, carbohydrate, flavonoids, phenolic compound, reducing sugar, steroid and terpenoid.

In this research, the powdered sample of *Curcuma petiolata* Roxb. rhizomes contained alkaloids, α- amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, proteins, reducing sugars, saponins, starch, steroids, tannins and terpenoids.

Conclusion

Myanmar name Marlar was perennial rhizomatous herb. It belongs to family Zingiberaceae. Leaves are distichous. Inflorescences are lateral spikes. Flower is yellow. Androecium of two whorls, stamens and staminodes. Ovary inferior and disc present.

In histological study tetracytic stomata are present on both surfaces. Vascular bundle is collateral and closed type. Oil cells are embedded in mesophyll of lamina and parenchymatous layers in midrib and leaf sheath and rhizomes. Prismatic calcium oxalate crystals are scattered in mesophyll in lamina and ground tissue in midrib and leaf sheath, roots and Rhizomes. Starch grains are present in rhizomes. The above mentioned characters can be used for identification in medicinal plant.

According to the Dash (2016), alkaloids has addictive or pain killing or poisonous effect and sometimes help in important cure. Saponins may help to prevent colon cancer. Flavonoids possess antiallergic, anti-inflammatory, antiviral and antioxidant activities. Steroids are used to suppress various allergic, inflammatory and autoimmune disorders.

Due to the presence of active constituents such as alkaloids, α- amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, proteins, reducing sugars, saponins, starch, steroids, tannins and terpenoids, these documents were highlighted to know effective medicinal bioactivities of *Curcuma petiolata* Roxb. rhizomes.

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