

MORPHOLOGICAL AND HISTOLOGICAL CHARACTERS OF *BOESENBERGIA ROTUNDA* (L.) MANSF.

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Abstract

Boesenbergia rotunda (L.) Mansf. is locally known as Seik-Phoo belongs to the family Zingiberaceae. These specimens were collected from Shwe-Thin-Phyu Village, Ye-Zin, Mandalay Region, in 2018. These plants have been used as a remedy in traditional medicine and also as vegetables and spices in the preparation of appetizing meal by local people in these collected areas. This study was conducted to identify the morphological and histological characters of *Boesenbergia rotunda* (L.) Mansf. In morphological studies, it is an aromatic perennial herb which has bright yellow rhizomes and finger-like tuberous roots; leaves alternate, biseriate; inflorescences spike, terminal on a leafy shoot; flowers pinkish purple, bisexual, irregular, epigynous. In histological studies, tetracytic stomata are present on both surfaces of lamina, midribs, petioles and leaf sheaths. The three types of vascular bundles such as main arc, abaxial arc and adaxial arc are also found in midribs, petioles and leaf sheaths. Prismatic calcium oxalate crystals and oil cells are found in lamina, midribs, petioles and leaf sheaths. Starch grains and oil cells are abundantly found in tuberous roots and rhizomes. In diagnostic characters, oil cells, fibers, fiber-tracheids and fragments of vessels (pitted, spiral thickening, reticulate thickening and scalariform thickening) are found in both powdered of aerial shoots and subterranean organs. Moreover, fragments of epidermal cells with stomata and calcium oxalate crystals (prism) are also found in aerial shoot while starch grains in subterranean organs.

Keywords: *Boesenbergia rotunda*, Prismatic Calcium oxalate crystals, tetracytic

Introduction

Boesenbergia rotunda is a ginger species belonging to the family Zingiberaceae that grows in Southeast Asia, India, Srilanka, and Southern China. (Baker, 1890). In Myanmar, it grows wild in Bago Region, Mandalay Region, Yangon Region, Sagaing Region and Chin State. It is a very common plant growing naturally in damp, shaded parts of the low-land or hill slopes.

Boesenbergia rotunda (L.) Mansf. is commonly known as Seik-Phoo in Myanmar and fingerroot in English (Hundley and Chit Ko Ko, 1987 and Kress et al., 2003). It is known as fingerroot because the plant consists of a small globular shaped central rhizome with fleshy long and thick tubers sprout all in the same direction like fingers. (Yaya Rukauadi, 2015).

Boesenbergia rotunda is small herbaceous plant with slender rhizomes and few leaves. The fresh rhizomes have a characteristic aroma and a slightly pungent taste. It is commonly used in Southeast Asia as a food ingredient, a folk medicine for the treatment of several diseases such as aphthous ulcer, dry mouth, stomach discomfort, leucorrhoea and dysentery. (Burkill, 1935)

The species taken into study are widely spread in moist and shady places of some areas in Myanmar. Aerial shoots and subterranean organs of Seik-Phoo have been used as a condiment in food such as curry and soup due to its aromatic flavour, which promotes appetite and also used as traditional medicines for dysentery and stomach discomfort by local people in this collected area. Therefore, *Boesenbergia rotunda* (L.) Mansf. was chosen and studied due to the poor data available in this plants.

The aim and objectives of this chapter is to identify the vegetative and reproductive parts and to characterize the histological characters of leaves, rhizomes and roots of the plant *Boesenbergia rotunda* (L.) Mansf.

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Material and Methods

1. Collection, Classification and Identification

Collection

The specimens were mainly collected from Shwe-Thin-Phyu Village, Ye-Zin, Mandalay Region, during the month of September, 2018.

Classification and Identification

Morphological characters of vegetative and reproductive parts of the specimens were identified by using literatures of key to the families of Myanmar Flowering plants, 1994; Flora of Java, Backer, 1968; Flora of Ceylon, Dassannayake, 1983 and Flora of China, Wu-Te-lin, 2000. Herbarium of *Boesenbergia rotunda* (L.) Mansf. was deposited in the Herbarium of Botany Department, Bago University.

2. Histological examination of different plant parts and powders.

Microscopical examination

Different plant parts such as aerial parts and underground parts were washed, dried, powdered and kept in an airtight bottle for microscopical examination. The fresh different plant parts were examined by the free hand sections. Powders were examined to get standardization for medicine. The free hand sections and powdered samples were analyzed by using the following reagents.

1. Chloral hydrate solution B.P. as clearing reagent.
2. Dilute glycerine B.P. for mounting reagent.
3. Phloroglucinol HCL B.P. for lignin test.
4. Iodine solution B.P. for testing starch.
5. Sudan III solution B.P. for testing oils and
6. Conc: H₂SO₄ for testing calcium oxalate crystals.

Results

Morphological Characters of *Boesenbergia rotunda* (L.) Mansf.

Scientific Name - *Boesenbergia rotunda* (L.) Mansf.

Family Name - Zingiberaceae

Myanmar Name - Seik-Phoo

English Name - Finger Root

Flowering Period - July to September

Aromatic Perennial herbs; leafy shoot 3.5-8.5 m in height. Rhizomes globose with several finger-like tuberous roots. Leaves alternate, simple, biseriate, aromatic; leaf sheath reddish green; ligules triangular; petioles long, channeled. Inflorescences spike, terminal on a leafy shoot. Flowers pinkish purple, bisexual, zygomorphic, 3-merous, epigynous; calyx 3, synsepalous, tubular, bifid, pinkish white; corolla 3, synpetalous, tubes long and white, lobes pink, oblong, incurved, the posterior lobe larger than the lateral twos; staminodes are broadly obovate, molted purple; the labellum oblong-obovate, apex crenate, undulate, plicate, the upper half pink and the lower half

pale pink with red-violet dots within it. Fertile stamen one, epipetalous. Pistils 3, tricarpeal, syncarpous, tri-locular, axile placentation, ovary ovoid, inferior; the styles filiform, passing through the channel along the fertile stamen, the stigma funnel-shaped. Fruits are not seen (Fig.1-4).

1.4.2 Histological characters of Leaves, Roots and Rhizomes of *Boesenbergia rotunda* (L.) Mansf.

Lamina

In surface view, upper epidermal cells are transverse extended and hexagonal, more or less isodiametric. Anticlinal walls are straight. Lower epidermal cells are polygonal-shaped, anticlinal walls are straight. Prismatic calcium oxalate crystals and oil cells are found in lower surface. Tetracytic stomata are present in both surfaces but more numerous in the lower surface (Figure 5-6).

In transverse section, the cuticles are present on both surfaces. The upper epidermal cells are rectangular-shaped. The lower epidermal cells are rectangular to polygonal-shaped. The epidermal cells of both surfaces are thin-walled, compactly arranged.

The hypodermal cells are one-layered, colourless and found on both sides. The mesophyll composed of palisade parenchyma and spongy mesophyll cells. Below the upper hypodermis is one-layered thick, short conical palisade cells which are compactly arranged. The spongy mesophyll cells are two layers of irregular-shaped, thin-walled parenchymatous cells. Prismatic calcium oxalate crystals are present in the spongy mesophyll cells.

The vascular bundles are collateral and closed type. Xylem lies towards the upper epidermis and composed of vessels, tracheids, fibers, fiber-tracheids and xylem parenchyma. Phloem lies towards the lower epidermis and consists of sieve tube, companion cells and phloem parenchyma cells. Each bundle is surrounded by sclerenchymatous sheath, which is distinct from the neighbouring cells. (Figure. 7).

Midribs

In surface view, the epidermis of both surfaces are made up of thin-walled, parenchymatous cells. The upper epidermal cells are rectangular to polygonal-shaped. The lower epidermal cells are rectangular and elongated. Prismatic calcium oxalate crystals, oil cells and tetracytic stomata are present on both surfaces but more numerous in the lower surface. (Figure 8 - 9).

In transverse sections, the adaxial surfaces of midrib are concave and abaxial surfaces are convex in basal region, middle region and apical region. Epidermal cells are single-layered, barrel-shaped, parenchymatous cells, compactly arranged. Under the epidermis, collenchymatous cells are 1-2 layers in thickness on both sides. They are polygonal-shaped. Parenchymatous cells are thin-walled, irregular, polygonal-shaped, compactly arranged. The large air canals are abundantly present. Prismatic calcium oxalate crystals are present in the parenchymatous cells that surround the air canal.

The vascular bundles of midrib are oval-shaped, collateral and closed type. The vascular tissue of middle and basal regions consists of main vascular bundles, abaxial bundles and adaxial bundles. The apical region consists of only one main vascular bundle. Xylem composed of vessels, tracheids, fibers, fiber tracheids and xylem parenchyma. Phloem consists of sieve tube, companion cells and phloem parenchyma cells. (Figure 10)

Leaf sheaths

In surface view, epidermal cells are rectangular to polygonal shaped, thin-walled, parenchymatous cells. The upper epidermal cells are rectangular and elongated. Prismatic calcium oxalate crystals, oil cells and tetracytic stomata are present on both surfaces but more numerous on the lower surface. (Figure 11-12)

In transverse section, the leaf sheaths are overlapping. The cuticle is thin, the epidermal cells are barrel-shaped, parenchymatous, compactly arranged. Below the epidermis are the 1-2 layers collenchymatous cells on both sides. The parenchymatous cells are rounded to polygonal-shaped. The vascular bundles of leaf sheaths are oval-shaped, collateral and closed type. The vascular tissue consists of main vascular bundles, abaxial bundles and adaxial bundles. The adaxial bundles are divided into center arc and upper arc. Xylem composed of vessels, tracheids, fiber, fiber-tracheids and xylem parenchyma. Phloem consists of sieve tube, companion cells and phloem parenchyma cells. (Figure 13)

Petioles

In surface view, the epidermal cells of both surfaces are made up of parenchymatous cells. The upper epidermal cells are rectangular to polygonal-shaped. The lower epidermal cells are rectangular and elongated. Prismatic calcium oxalate crystals, oil cells and tetracytic stomata are present on both surfaces but more numerous on the lower surface. (Figure 14 - 15)

In transverse section, the petioles are more or less U-shaped, the upper surface distinctly grooved, the lower surface rounded. The cuticle is thin. The epidermal cells are barrel-shaped, parenchymatous, compactly arranged. Below the epidermis are the collenchymatous cells 1-2 layers on both sides. The parenchymatous cells are rounded to polygonal-shaped. The parenchymatous cells surround the large air canal that contains prismatic calcium oxalate crystals.

The vascular bundles of petioles are oval-shaped, collateral and closed type. The vascular tissue consists of main vascular bundles, abaxial bundles and adaxial bundles. The adaxial bundles are divided into center arc and upper arc. Xylem composed of vessels, tracheids, fiber, fiber-tracheids and xylem parenchyma. Phloem consists of sieve tube, companion cells and phloem parenchyma cells. (Figure 16)

Adventitious roots

In surface view, epidermal cells are rectangular and elongated, thin walled, parenchymatous cells. (Figure 17)

In transverse section, the adventitious roots are circular in outline. The epiblema is single layered, barrel-shaped, parenchymatous cells with numerous unicellular root hairs. The exodermis lies below the epiblema layer. The exodermis consists of 3-4 layers of parenchymatous cells, rectangular to polygonal-shaped. The cortex consists of parenchymatous cells, about 10-12 layers, oval to rounded in shape. Endodermis and pericycle are single layered, barrel-shaped, thin walled, parenchymatous cells. Pith lies in the center and composed of parenchymatous cells. Vascular bundles are radial types, polyarch. The xylem is exarch and composed of vessels, tracheids, fibers, fiber-tracheids and xylem parenchyma. Phloem composed of sieve tubes, companion cells and phloem parenchyma. (Figure 18-19)

Tuberous roots

In surface view, epidermal cells are rectangular-shaped, thin walled, parenchymatous cells. It contains numerous yellowish oil cells. (Figure 20)

In transverse section, the epiblema is single-layered, barrel-shaped, thin walled parenchymatous cells. The exodermis consists of 4-6 layers of parenchymatous cells, rectangular-shaped. The cortex contains 18-20 layers of parenchymatous cells, oval to rounded in shape. Endodermis and pericycle consist of single-layered, barrel-shaped parenchymatous cells. Pith lies in the center and composed of parenchymatous cells which are oval to rounded in shape. The parenchymatous cells contain numerous starch grains and yellowish oil cells. Starch grains are oval-shaped and eccentric.

Vascular bundles are radial type, and polyarch. The xylem is exarch composed of vessels, tracheids, fibers, fiber- tracheids and xylem parenchyma. Phloem composed of sieve tube, companion cells and phloem parenchyma. (Figure 21-22)

Rhizomes

In surface view, epidermal cells are polygonal-shaped. The anticlinal walls are straight and smooth. The yellowish oil cells are scattered throughout the section. (Figure 23)

In transverse section, the rhizomes are circular in outline. The epidermal cells are single layered, barrel-shaped. Periderm consists of outer cork and inner cork. The outer cork about 16 layers of parenchymatous cells, rectangular-shaped. The inner cork about 13 layers of parenchymatous cells, rectangular-shaped, tangentially flattened. The cortex is composed of 30-35 layers of thin-walled parenchymatous cells, oval to rounded in shape. Both endodermis and pericycle consists of single layered, barrel-shaped, parenchymatous cells. The stellar region is composed of parenchymatous cells which are rounded to oval shaped. The ground parenchymatous cells contain numerous starch grains and yellowish oil cells. (Figure 24)

Vascular bundles are scattered throughout the cortical and stellar regions. Vascular bundles are collateral and closed type. Xylem composed of vessels, tracheids, fiber tracheids, fibers and xylem parenchyma. Phloem consists of sieve tube, companion cells and phloem parenchyma. (Figures 25 - 26)

Diagnostic characters of powdered aerial shoot and subterranean organs of *Boesenbergia rotunda* (L.) Mansf.

Both powdered aerial shoot and subterranean organs contain oil cells, fibers, fiber-tracheids, vessels (pitted, spiral thickening, reticulate thickening and scalariform thickening). Moreover, fragments of epidermal cells with stomata and calcium oxalate crystals (prism) are also found in aerial shoot while starch grains in subterranean organs. (Figures 27 - 37)



Figure 1 Habit



Figure. 2 Close up view of flower



Figure 3 Flower



Figure 4 Rhizome and Tuberous roots

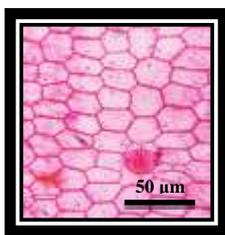


Figure 5 Surface view of upper epidermal cells with tetracytic stoma

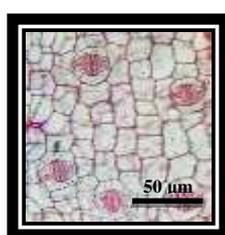


Figure 6 Surface view of lower epidermal cells with tetracytic stomata

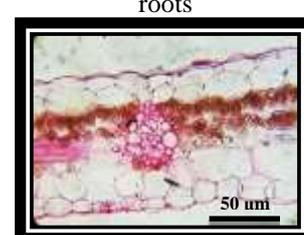


Figure 7 Transverse section of Lamina

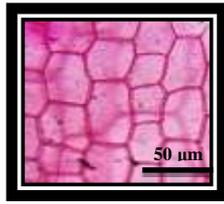


Figure 8. Upper surface of midrib

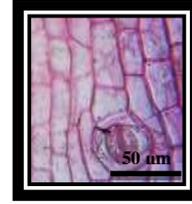
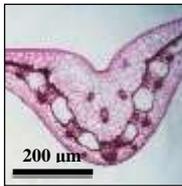
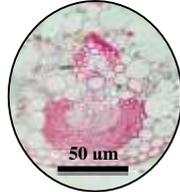


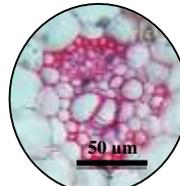
Figure 9 Lower surface of midrib showing tetracytic stoma



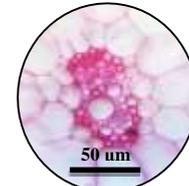
T.S of midrib



main bundle



adaxial bundle



abaxial bundle

Figure 10 T.S of midrib showing main vaascular bundles, adaxial bundles and abaxial bundles

Leaf sheath (Surface view)

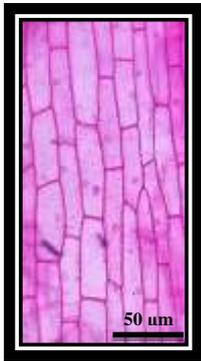
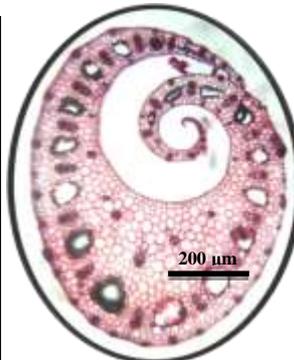


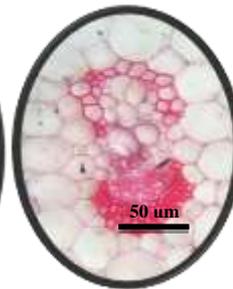
Figure 11 Surface view of upper epidermal cells



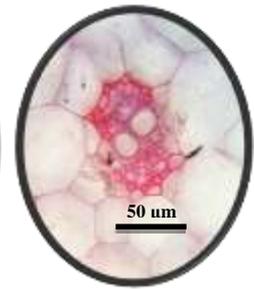
Figure 12 Surface view of lower epidermal cells with tetracytic stoma



T.S of leaf sheath



main bundle



adaxial bundle

Figure 13 T.S of leaf sheath showing main vaascular bundles and adaxial bundles

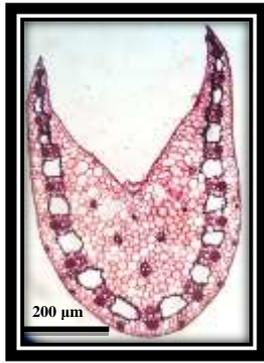
Petiole (Surface view)



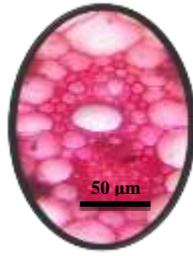
Figure 14 Surface view of upper epidermal cells



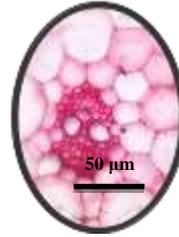
Figure 15. Surface view of lower epidermal cells with tetracytic stoma



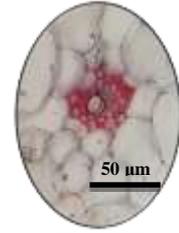
T.S of petiole



main bundle



adaxial bundle (centre arc)



adaxial bundle (upper arc)

Figure 16 T.S of petiole showing main vaascular bundles and adaxial bundles

Adventitious roots (Surface view)



Figure 17 Surface view of epidermal cells

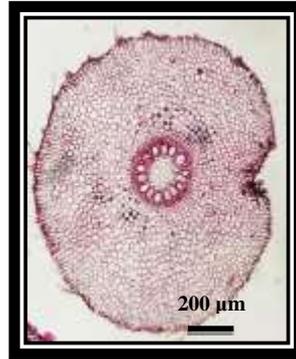


Figure 18 T.S of adventitious root

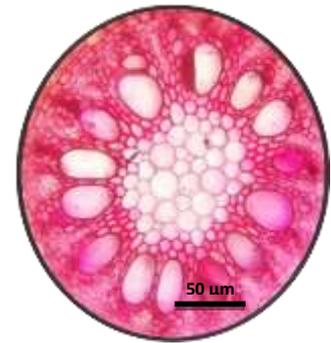


Figure 19 T.S of adventitious root showing vascular cylinder

Tuberous roots (Surface view)



Figure 20 Surface view of tuberous roots

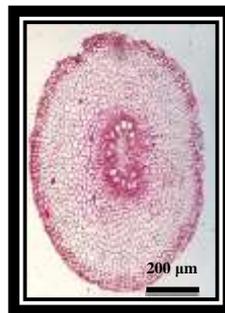


Figure 21 T.S of tuberous roots



Figure 22 T.S of tuberous roots showing vascular

Rhizome (Surface view)

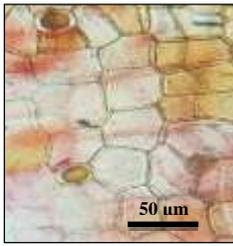


Figure 23 Surface view of lower epidermal cells with oil cells

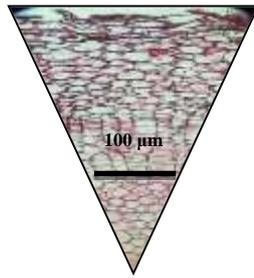


Figure 24 T.S of rhizome showing cork and cortex layers



Figure 25 Close up view of vascular bundle in cortical region



Figure 26.Close up view of vascular bundle in stellar region

Diagnostic Characters of Powdered Aerial Shoots of *Boesenbergia rotunda* (L.) Mansf.



Figure 27 Tracheid



Figure 28 Fibres



Figure 29 Fibre-tracheids



Figure 30. Spiral



Figure 31. Fragments epidermal cells



Figure 32 Pitted vessels

Diagnostic Characters of Powdered Subterranean Organs of *Boesenbergia rotunda* (L.) Mansf.

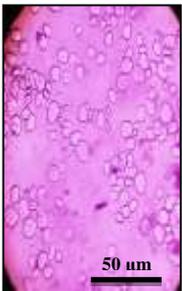


Figure 33 Starch grains



Figure 34 Fibre-tracheids, fibres and fragment of tracheids



Figure 35. Pitted vessels



Figure 36 spiral thickening



Figure 37 Reticulate thickening

Discussion

In this research, the morphological studies on both vegetative and reproductive parts as well as the microscopical examination of the fresh leaves, rhizomes and roots and powdered aerial shoots and subterranean organs were undertaken.

In the morphological study, the plants are aromatic perennial herbs, reddish sheath at the base. This features agreed with the habit of *Boesenbergia rotunda* (L.) Mansf. given by Backer, 1968; Dassanayake, 1983; Guzman & Siemonsma, 1999; Wu-Te-lin, 2000).

Rhizomes globose with long finger-like tuberous roots. Rhizomes and tuberous roots are yellow-brown outside and bright yellow inside, aromatic. This finding was in accordance with that of Guzman & Siemonsma, 1999; Wu-Telin, 2000.

Leaves alternate, biseriate, aromatic; leaf sheaths reddish green; ligules triangular; petioles long, channeled; lamina elliptical oblong to broadly lanceolate. This features were in agreement with that given by Backer, 1968; Dassanayake, 1983; Guzman & Siemonsma, 1999; Wu-Telin, 2000.

Inflorescence spike, terminal on leafy shoot, peduncle very short. These characters were in agreement with these given by Dassanayake, 1963; Guzman & Siemonsma, 1999; Wu-Telin, 2000.

Flowers situated in the axil of a bract and a bracteole; bracts and bracteoles oblong-lanceolate; calyx tubular, apex bifid; corolla with white tube, incurved, pink lobes at apex; labellum oblong-obovate, panduriform, apex crenate, undulate plicate, upper half pink, lower half pale pink with red-violet dots within it; staminodes broadly obovate, anther dithecous, pale yellow; gynoecium tri-locular, style filiform. These characters were agreement with those described by Guzman & Siemonsma, 1999.

In surface view of lamina, anticlinal walls of upper and lower epidermal cells are straight. Stomata are of tetracytic type. These features were in accordance with Khatijah et al., 2001.

In transverse section of lamina, the cuticle is thin, epidermal cells are polygonal in shape. These features were agreed with Tomlinson, 1969. The adaxial epidermis is papillate. Hypodermis present on both surfaces, which contain calcium oxalate crystals and oil cells. The mesophyll is differentiated into palisade and spongy layers. The palisade cells are conical-shaped on both sides. The spongy mesophyll cells are rounded or elliptic and may be loosely arranged. These microscopical features of leaves were similar with the findings of Olanrewaju, 1970.

The smallest vascular bundles attached to abaxial surface while the largest attached to both surfaces. Bundle sheath present above and below larger veins but present only below smaller veins. These characters of leaves were agreed with Tomlinson, 1969 and Khatijah et al., 2001.

In transverse section of midrib, adaxial surfaces are slightly curved and grooved. The abaxial surfaces are arced to V-shaped in the middle and basal portion. The vascular tissue consists of vascular bundles arranged in several arcs. Air lacunae are present between the bundles. These characters were in accordance with khatijah et al., 2001.

According to in agreements of Olanrewaju 1970, it was found that the axial epidermis of the leaf sheath and petiole is differentiated into costal and intercostals areas. Both the costal and intercostals cells are rectangular but the intercostals cells are more wider than costal cells. In the transverse section of the leaf sheaths and petioles, the cuticle is thin and the epidermal cells are rectangular. The ground tissue is composed mainly of large parenchymatous cells. The vascular bundles have been grouped into main arc I, abaxial arc II, adaxial arc III and IV. A single row of air canals, which vary in size from the center to the margins of leaf sheath and petiole. In transverse section of root, the epiblema is made up of parenchymatous cells. The cortex is composed of

parenchymatous cells. The central pith consists of rounded and thin walled parenchymatous cells. The vascular bundles are radially arranged and polyarch. The xylem is exarch. In the transverse section of rhizome, the periderm is composed of thin-walled cells. The cells of inner layers are arranged in parallel. The cortex is composed of thin-walled parenchymatous cells. The vascular bundles are scattered throughout the cortical and stellar regions. Prismatic calcium oxalate crystals and oil cells are present in lamina, petioles and leaf sheaths. Starch grains are most abundant in rhizomes and tuberous roots. These features were in accordance with Tomlinson, 1969 and Olanrewaju, 1970. Starch grains are simple, spherical or ellipsoidal; hilum eccentric. These features were similar with the findings of Tomlinson, 1969.

In diagnostic characters, oil cells, fibers, fiber-tracheids, vessels (pitted, spiral thickening, reticulate thickening and scalariform thickening) are found in both powdered of aerial shoots and subterranean organs. Moreover, fragments of epidermal cells with stomata and calcium oxalate crystals (prism) are also found in aerial shoot while starch grains in subterranean organs. These characters were agreement with that given by Tomlinson, 1969.

Vegetative period was from June to July. Flowering and fruiting period was July to September. But Surapon et al., 2017 stated that the period of flowering and fruiting is June to September. These differences may depend on the ecological variation of areas.

Conclusion

The morphological and histological studies are ones of the very important parameters in the pharmacognostic study. These parameters are needed for standardization and authentication of medicinal plants with the help of which adulteration and substitution can prevented. Therefore, the morphological and histological characters have been studied for the identification of *Boesenbergia rotunda* (L.) Mansf. in this study.

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