

Study on Anatomy and Preliminary Phytochemical Investigation of leaves of *Dendrobium findalayanum* Par & Rchb.f.

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Abstract

Dendrobium findalayanum Par & Rchb.f. belongs to the family Orchidaceae and it is a pharmaceutically valuable, ornamental epiphytic orchid. It is distributed in Tanintharyi Region and Shan State. It is known as Cho-chin-thit-khwa in Myanmar. It was collected from Maw Taung area, Tanintharyi Township, Tanintharyi Region. The morphology and anatomy of the plant have been studied and identified at the Department of Botany, Myeik University. The anatomy of medicinally important organs leaves were prepared and examined with the help of microscope. The collected plant samples were washed and dried at room temperature. After drying, the samples were grounded to get powder and stored in an air-tight container for chemical and pharmacological studies. In the phytochemical test, alkaloids, α -amino acids, glycosides, flavonoids, phenolic compounds, steroids, terpenoids, reducing sugar saponins, starch and carbohydrates were present. Tannins and cyanogenic glycoside were absent.

Introduction

Orchidaceae is one of the largest families of the flowering plants in the world. They comprise somewhere between 18,000 and 25,000 different species. Orchids are distributed all over the world and grown in nearly every habitat, ranging from tropical rainforest to semi-desert, temperate, grassland and arctic tundra. However, by far the largest concentration is found in the tropics, particularly in mountainous regions. Myanmar is still endowed with diverse forest resources. Although a recent revision of all flowering of Myanmar lists 11,800 species in 273 families (Kress, *et al.*, 2003) and current estimates range from 13,000 to 15,000 species. According to Kress, *et al.*, 2003, there are 841 orchid species and Kurzweil & Saw Lwin, 2014 stated that 800 orchid species and 150 genera are currently known in Myanmar. The genus *Dendrobium* comprises about 900 species (Bechtel, *et al.*, 1992; Mabberley, 1993) that exhibits a wide range of morphology and anatomy. *Dendrobium* is distributed throughout tropical Asia, some Pacific Islands, and Australasia (Dressler, 1993). *Dendrobium findalayanum* is also called Findlay's *Dendrobium*. This species was described by Parish & Rchb.f. in 1874. *Dendrobium findalayanum* is native to Southeast Asia. In Myanmar, these plants are found in the regions near Tenasserim, Taunggyi and Inle Lake. Moreover this habitat extends through the mountains of northern Thailand to Laos. The plants usually appear in mixed forests at the heights of 1000-1700 m. These secondary metabolites contribute significantly towards the biological activities of medicinal orchids such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial activities (Negi, *et al.* 2011). Flavonoids are also present in this plant as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity (Rio, *et al.*, 1997 and Salah, *et al.*, 1995). It also helps in managing diabetes induced oxidative stress. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties (Rabi, *et al.*, 2009 and Wagner, *et al.*, 2003). In addition, terpenoids can be used as protective substance in storing agriculture products as they are known to have insecticidal properties as well (Sultana and Ata, 2008). Saponins possess the unique property of precipitating and coagulating red blood cells

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(Okwu, 2004 and Sodipo,*et al.*, 2000) and steroids are responsible for cholesterol-reducing properties. Steroids are also helpful in regulating the immune response (Shah,*et al.*, 2009). The aims and objectives of the research are to examine the histological characters of leaves of *Dendrobiumfindalayanum* and to investigate the preliminary phytochemical constituents of the leaves of *Dendrobiumfindalayanum*.

Materials and Methods

Dendrobiumfindalayanum was collected from Maw Taung area, Tanintharyi Township, Tanintharyi Region during the flowering period from December 2017 to February 2018. This species was illustrated, taxonomic details and identified with the help of relevant literatures by Backer (1963), Holtum (1964) and Grant (1966). The leaves of *Dendrobiumfindalayanum* were used to study the anatomical characters. For the anatomical studies, the material was selected from the plants grown in Home Garden. Hand Cut sections were taken to study leaves of the Plant. The sections were stained with aqueous safranin to visualize the important tissues. The sections and the peels were observed under Motic Image microscope and the taking photographs. The powdered samples were examined by standardizing methods according to Willis (1967) and Trease and Evans (2002). These results were shown in Table 1. The collected leaves and pseudobulbs of *Dendrobiumfindalayanum* were repeatedly washed with tap water and finally with pure water. The sliced samples were dried under shades for three weeks. The leaves were dried at room temperature and made into powder using blender. Then, dried samples were stored in air tight containers for the phytochemical investigation. Phytochemical analysis was concerned with the presence or absence of alkaloids, α -amino acid, carbohydrates, reducing sugar, glycoside, phenolic compound, starch, saponins, tannins, steroids, terpenoids and flavonoids were investigated by the methods of British Pharmacopoeia (1965) and Trease and Evans (2002). Preliminary phytochemical examination was carried out at the Department of Botany, Myeik University. These results were shown in Table 2.

RESULTS

1. Outstanding characters of *Dendrobiumfindalayanum*

Sympodial epiphyte, ever green stem long, the knobs club-shaped, large upwards and tapering, pear-shaped; leaf oblong lanceolate, acute margin entire and the tip acute both surface glabrous; inflorescence raceme, lateral upper portion of the stems, 2 to 3 flower per node, peduncle very short cylindrical, green, lavender, resupinate, slightly fragrant, pedicel cylindrical, pale purple; flower bracteates acute, dull white, glabrous, sepals 3, lavender, oblong lanceolate acute, fused of the base, forming mentum lavender, obtuse; petals 3, 2 lateral petals oblong ovate, slightly lavender with faintly purple tip; labellum with deeply yellow in center and purple at pointed end, finely pubescent on the upper surface, column short with stripe; anther green yellow, terminal, and 2 celled, pollinia 4 in pairs; stigmatic surface concaves, ovary inferior. These results were shown in Figure 1.

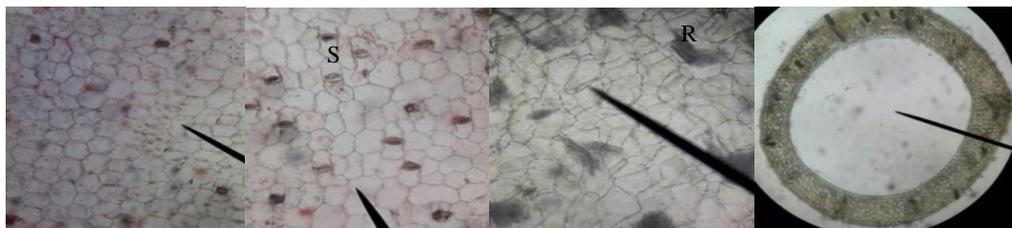


Figure 1. Habit

Figure 2. Inflorescence

Microscopical characters of leaves of *Dendrobium findalayanum*

In surface view, the epidermal cells of both the surfaces are parenchymatous and thin walled. The cell walls of the upper surfaces and lower surfaces are polygonal to barrel in shape. Stomata are present on the lower surfaces and absent on the upper surface. They are paracytic type. The guard cells are reniform in shaped and contain abundant of chloroplast. In Cross section of lamina, the cuticle layer of the upper surface is thick. The upper cuticle layer is thicker than the lower one. The upper epidermal cells are polygonal to barrel shape. The lower epidermal cells are similar in shape and size. Mesophyll layer is homogeneous, not differentiated into palisade and spongy parenchyma. It consists of polygonal to oval shaped parenchymatous cells. The mesophyll cell consists of 8-10 layered in thickness of cells, which are oval to circular in shape and loosely arranged. Assimilatory cells are filled with chloroplast pigment. Simple water storage cells with mucilage are abundant in plants cell and air cavity is also present. Vascular Bundles is collateral vascular bundles are present in a single series. Large midrib vascular bundle is located in centre and, small and large vascular bundles are on the either side of it. All vascular bundles are associated with sclerenchymatous cell. The phloem cap is made up of sclerenchyma, consists of 3 layers, whereas xylem cap only 2. Each bundle is surrounded by a compact layer of thick-walled sclerenchymatous sheath, distinct from the neighboring cells. Phloem consists of sieve tubes, companion cells, fibres of varying length and parenchyma. Xylem possesses tracheids with helical thickenings and xylem fibres. The results were shown in Figure.2. In cross section of midrib, the epidermal cells of both surfaces are parenchymatous and elongate along the length of the midrib. The lower epidermal cells are comparatively smaller than the upper epidermis. The midrib is slightly curved inwards and cuticle thick. The epidermal cells of both the surfaces are polygonal to rectangular in shape. Mesophyll, the parenchyma cells are 4-5 layered in thickness above the vascular bundle and 5-7 layers in thickness below the vascular bundle. They are thin-walled and rounded to oval in shape. The vascular bundle is more or less rounded in outline. Each vascular bundle is surrounded by a compact layer of sclerenchymatous cells, known as the bundle sheath in layers. The cells are thick-walled, and lignified. Vascular Bundles is collateral and close type of vascular bundles are present. Each bundle is surrounded by a compact layer of thick-walled sclerenchymatous sheath, distinct from the neighboring cells. The xylem cells are hexagonal, thick-walled, and lignified, composed of vessels, tracheids, fibers, and xylem parenchyma. The phloem cells were thin-walled and composed of sieve-tube, companion cells and phloem parenchyma cells. These results are shown in Figure.2. In cross section of leaf sheath, the cuticle layers are thin and smooth. Raphids crystals are abundant in leaf sheath. The mesophyll cells are made up of parenchymatous cells. The parenchymatous tissues consist of 5-7 layered in thickness. The parenchyma cells are round to oval in shape. Intercellular spaces are numerous among the tissue. The vascular bundle is collateral and close type. Each vascular bundle is surrounded by a compact layer of the sclerenchymatous cells known as the bundle sheath. The cells are thick-walled and lignified. These results were shown in Figure2.



Upper and lower surface of leaves

Surface view and outline of leaf sheath

S= Stoma, R=Raphids,

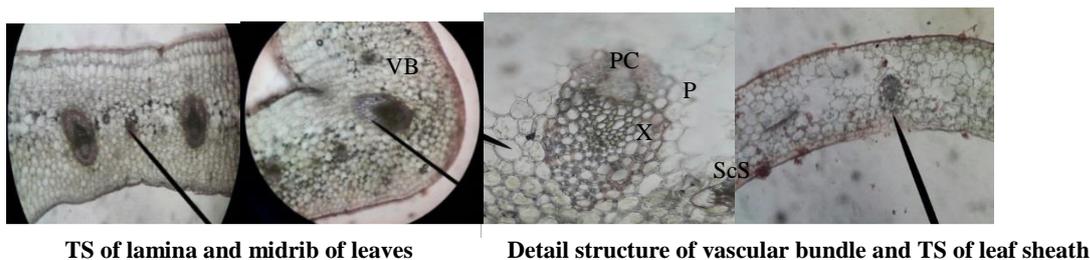


Figure 3. Microscopical characters of leaves of *Dendrobium findalayanum*
 VB= Vascular Bundle, PC= Phloem Cap, P= Phloem, ScS= sclerenchymatous sheath

Preliminary phytochemical investigation of leaves of *Dendrobium findalayanum*

The preliminary phytochemical examination of the leaves and pseudobulbs of *Dendrobium findalayanum* indicated the presence of carbohydrates, glycosides, α -amino acids, flavonoids, steroids and terpenoids. These results were shown in Table 2 and Figure 5 and 6

Table 2. Phytochemical constituents of leaves of *Dendrobium findalayanum*

No	Chemical constituents	Solvents	Reagents	Observation	Results
1.	Alkaloid	1 % HCl	Mayer's reagent	Turbid	+
			Wagner's reagent	Brown ppt.	+
			Dragendorff's reagent	Reddish brown ppt.	+
			Hager's reagent	Turbid	+
2.	Carbohydrates	H ₂ O	10% α -Naphthol & H ₂ SO ₄ Conc.	Purple ring	+
3.	Glycoside	H ₂ O	10% Lead acetate solution	White ppt.	+
4.	Phenol	H ₂ O	5% FeCl ₃ Solution	Greenish brown color	+
5.	α -amino acid	H ₂ O	Ninhydrin reagent	Light purple color	+
6.	Saponin	H ₂ O	H ₂ O	Persistent foam	+
7.	Tannin	H ₂ O	1% Gelatin & 10% NaCl Solution	No ppt.	-
8.	Flavonoid	70% Ethanol	Mg ribbon & HCl Conc.	Pink color	+
9.	Steroid	Petroleum ether	Acetic anhydrite & H ₂ SO ₄ Conc.	Bluish green	+
10.	Terpenoid	Petroleum ether	Acetic anhydrite & H ₂ SO ₄ Conc.	Pink color	+
11.	Reducing sugar	H ₂ O	Fehling's Solution	No color	-
12.	Starch	H ₂ O	Iodine Solution	No color	-
13.	Cyanogenic glycoside	Powder	H ₂ O, H ₂ SO ₄ Conc. and Sodium picrate paper	No color change	-

(+) = presence (-) = absence

Discussion and Conclusion

The anatomical characters and phytochemical of leaves of *Dendrobium findalayanum* are presented in this research work. In microscopical studies, the epidermal cells of both surfaces of leaves are polygonal in shape. Paracytic type of stomata was present on lower surface and absent on the upper one. Mesophyll cell is homogeneous, not differentiated into palisade and spongy parenchyma. It consists of barrel and circular to oval shaped cells with intercellular space. Assimilatory cells are rich with chloroplast. This finding agreed with those mentioned by Metcalf and Chalk (1950), Pandey (1993) and Ramesh (2012). Vascular bundles are arranged

in single series with layer, midrib vascular bundle at the center. This finding agreed with those mention by Metcalf and Chalk, (1950), Pandey (1993), Ramesh (2012) and Carlsward (1997). The phloem cap present. Xylem comprises tracheids and fibres. Phloem consists of sieve tube elements, companion cells, fibers and parenchymatous cell. This finding agreed with those mention by Easu (1965), Pandey (1993) and Ramesh (2012). The preliminary phytochemical examination of the leaves and pseudobulbs of *Dendrobium findlayanum* showed that presence of alkaloids, carbohydrate, glycoside, phenolic compounds, α -amino acid, saponin, flavonoid, steroid and terpenoids except the tannin, reducing sugar, starch and cyanogenic glycoside. These results have obtained from my research study. In conclusion, the study focused on the morphological, anatomical and phytochemical of the *Dendrobium findlayanum*. The morphological, anatomical and phytochemical findings could also be supported for further research. Today, medicinal orchids become rare in their habitats because of excessive collections and over-exploitation from their natural habitats. Therefore, it is essential to conserve and propagate these plants.

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