

## RESEARCH ARTICLE

# Phyto - chemical and morpho-anatomical studies with ecological implications in *Coelogyne flaccida* Lindl. (Orchid) of India

■ K. Ravichandra Reddy, G. Ramesh and S. M. Khasim

### SUMMARY

The ecological adaptability of *Coelogyne flaccida* Lindl. is based on morphological, anatomical and preliminary phytochemical compounds present in the leaf, stem/pseudo bulb and root. The anatomical features of leaf showing absorbing trichomes, large mid-rib vascular bundle, abundant small vascular bundles on the adaxial side, and also the adaxial cells below the epidermis showing chloroplast pigment. The pseudo bulb showing the cortical cells with pitted thickening and the root with fibrous mat on the exodermis. The preliminary phytochemicals, recorded in the leaf are flavonoids, steroids, terpenoids and tannins. From the observations it is showing, the Darjeeling readings are more when compared to Kerala collection due to its elevation and climatic factors.

**Key Words :** Anatomy, Phytochemical, *Cflaccida*, Root, Stem, Leaf

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The Orchidaceae is one of the largest families of flowering plants. It comprises about 779 genera and 22,500 species (Mabberley, 2008). In India,

with 1141 species spreading over 166 genera (Kumar and Manilal, 1994), it represents the one of the largest flowering plant family and contributes about 10 % of Indian flora. For the present investigation *coelogyne flaccida* Lindl. collected from Darjeeling and Kerala forest. *coelogyne* is a genus of over 200 species and in India 20 are recorded (Kumar and Manilal, 1994). Micro morphology and phytochemical. Parameters of leaf, stem/pseudo bulb and root are thoroughly studied from the ecological point of view (Sanford, 1974; Kaushik, 1983 and Mohana Rao and Khasim, 1986). The importance of plant-derived drugs is multifaceted, encompassing their historical efficacy, role in drug discovery, cultural

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significance, accessibility and potential to address a wide spectrum of health conditions while promoting holistic well-being and sustainability (Marrelli, 2021). Phytochemical studies are a crucial link between traditional herbal remedies deeply rooted in diverse cultures and Modern Science- Therefore based on the above evidences the present investigation deals with *coelogyne flaccid* anatomy and Phytochemical implications.

## MATERIAL AND METHODS

### Study area :

The plant material *Coelogyne flaccid* Lindl. Is collected from Kalimpong (Darjeeling), at an elevation of 2250 m and from Palavara (Kerala) at an elevation of 980 m (Table 1). The collected material is dried and the herbariomis deposited in the Botany Department, Acharya Nagarjuna University with voucher specimen ANUBOTH 1400 and 1401, for further reference.

### Anatomy :

The fresh material is fixed in FAA (0.05;0.05.9 of formalin, acetic acid and 70% ethanol), later, these were preserved in 70% ethanol before processing. Leaf, stem, pseudo bulb and root are dehydrated in alcohol and xylene series, infiltrated and embedded in 15-20 mm Paraffin wax melting point (60-62°C) and section with rotary microtome thickness of double staining was done by safranin and fast green combination and sections were mounted in DPX mount (Khasim, 2002; Vijayaraghavan and Shukla, 1990). optical Photomicroscope (Motic 2.0, 6 megapixels) was used to take anatomy photographs.

### Phytochemical :

Fresh leaves were collected from matured plant and subsequently subjected to a careful process that include rinsing, cutting the leaf into small pieces and shade dried for a duration of 30-40 days. The plant material was finely powdered using an electric blender, the powder is carefully stored in air-tight glass container, approximately 50 gm. of powder is placed in 500 ml of n-hexane, acetone, ethyl acetate and methanol. The extractions were conducted through the cold extraction method with gentle stirring at room temperature, spanning 48 hrs. the extract was filtered using Whatman No. 41 filter paper, after which they were transferred to glass jars and preserved at a temperature of 4°C.

The quantitative phyto-chemical screening of crude

extract were utilized to perform qualitative analysis of both primary (Carbohydrates and proteins) and secondary metabolites include (Alkaloids, flavonoids, phenols, saponins, tannins and terpenoids) by standardized protocol (Harborne, 1973 and Trease *et al.*, 1996).

### Test for proteins (Biuret test) :

A few ml of plant extract was taken and 1 ml of 40% NaOH solution and 2 ml of 1% CuSO<sub>4</sub> were added. The appearance of violet colour indicates the presence of proteins.

### Test for alkaloids (Dragendorff's test) :

About 5ml of 1% HCl was added to 0.5gm of extract. Later the mixture was boiled and filtered to 1 ml of filtrate, 2-3 drops of Dragendorff's reagent were added. The formation of reddish-brown precipitate denotes the presence of alkaloids.

### Test for flavonoids (1% AlCl<sub>3</sub> Test) :

A few drops of 1% AlCl<sub>3</sub> were added to the plant extracts.

The appearance of yellow colour indicates the presence of flavonoids.

### Test for phenols (10% FeCl<sub>3</sub> test) :

A few drops of 10% aqueous FeCl<sub>3</sub> solution were added to the plant extract. The appearance of blue or green colour indicates the presence of phenols.

### Test for saponins (Frothing test) :

About 5ml of distilled water was added to 1ml of plant extract and mixed well. The formation of persistent froth indicates the presence of saponins.

### Test for steroids (Liebermann-Burchard test) :

About 1 ml of plant extract was dissolved in 1 ml of chloroform and acetic anhydride. A few drops of concentrated HCl were added to the above extract. The appearance of a brownish-green ring at the interface indicates the presence of steroids.

### Test for terpenoids (Salkowskistest) :

A few drops of plant extract were allowed to dissolve in 2 ml of chloroform. Concentrated H<sub>2</sub>SO<sub>4</sub> was added to form a lower layer. The appearance of reddish-brown colour at the interface denotes the presence of terpenoids.

**Test for tannins (Iodine Test) :**

A few drops of iodine solution were added to 2 ml of plant extract. The appearance of faint bluish colour indicates the presence of tannins.

**RESULTS AND DISCUSSION**

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

**Anatomy leaf :**

In surface view the epidermal cells are Polygonal in shape and stomata are paracytic and confined to abaxial surface. In outline, the leaf is almost flat, slightly angular in the mid-rib region. Cuticle is well developed on both the surfaces, the adaxial epidermal cells are comparatively larger than the abaxial epidermal cells (Fig. 1A). The thickness of the cuticle is 0.08  $\mu\text{m}$  (Table 2). The length of the absorbing trichome is 0.059

$\mu\text{m}$  (Fig. 1A). The chloroplast is clearly seen in the cells of the mesophyll tissue cells (Fig. 1B) some of the mesophyll cells are showing cellulosic thickening the substomatal chamber is larger in the sample collected from Darjeeling ( $T_2$ ). The vascular bundles are arranged in single series, larger vascular bundle in the centre and other small and large laminar vascular bundles on either side of it. The mid-rib vascular bundle is 0.0584  $\mu\text{m}$  in size ( $T_2$ ). All the vascular bundles are associated with sclerenchyma tissue. Phloem cap made up of sclerenchymatous cells 4-cells and xylem cap 2-cells ( $T_2$ ). The phloem consists of sieve tube, Companion cells, fibres and parenchyma. According to Dahlgren and Rasmussen 1983 vessel type and their occurrence.

**Pseudo bulb :**

In cross section, pseudo bulb is circular in outline the epidermal cells are irregular in shape and having leaf sheath at the outer surface, The cortex is well developed,

**Table 1 : Details of collection of orchid plant material**

Sr. No.	Species	Place, Altitude and date of collection	Host tree	Voucher No.
	Family : ORCHIDACEAE			
	Subfamily : EPIDENDROIDEAE			
	Tribe : ARETHUSEAE.	Kalimpong (Darjeeling), 2250 m; Nov.2023	<i>Alnusnepalensis</i>	ANUBOTH1400
	Subtribe : COELOGYNINAE <i>COELOGYNAE</i>	Palavara (Kerala), 980m; Oct.2023	<i>Azadirachta indica</i>	ANUBOTH1401
1.	<i>Coelogyne flaccida</i>			

**Table 2 : Leaf: Anatomical features in *Coelogyne flaccida* (in  $\mu\text{m}$ )**

Taxa.	Absorbing trichome	Cuticle thickness	Stomatal width (two guard cells including pore)	Stomatal length (only guard cell)	Midrib vb. size	Laminar vb. size	Water storage cell	Sub stomatal chamber size	No. of ph. cap layers	No. of xy. cap layers
<i>Cflaccida</i> (a)	0.059	0.08	0.016	0.087	0.058	0.062	0.041	0.022	5	3
<i>Cflaccida</i> (b)	0.051	0.06	0.013	0.079	0.050	0.058	0.040	0.021	2	3

**Table 3 : Stem/Pseudobulb: Anatomical features in *Coelogyne flaccida* (in  $\mu\text{m}$ )**

Taxa	Cuticle thickness	Water storage cells	Size of vb.	No. of ph. cap layers	No. of xy. cap layers	Length of tracheid/ vessel member	Length of xy. fibre	Length of ph. fibre
<i>Cflaccida</i> (a)	0.005	0.029	0.049	4	2	0.028	0.031	0.043
<i>Cflaccida</i> (b)	0.005	0.026	0.045	3	2	0.027	0.031	0.042

**Table 4 : Root : Anatomical features in *Coelogyne flaccida* (in  $\mu\text{m}$ )**

Taxa	No. of velamen layers	Fibrous mats/ tilosomes	Exodermis cell lignifications	Passage cell size	Endodermis cell lignification	Vascular cylinder diameter	No. of protoxylem poles
<i>Cflaccida</i> (a)	1	+	0.022	0.004	0.006	0.062	7
<i>Cflaccida</i> (b)	1	-	0.019	0.004	0.005	0.060	5
a-Darjeeling collection		b-Kerala collection					

**Table 5 : Morphological and anatomical features of ecological interest in *Coelogyne flaccida***

Taxa	Habitat	External features	Ade cells size; Stomata, distribution; ssc	Absorbing trichomes	Water storage cells and other tracheoidal elements; fb, vlt	No. of velamen layers	Fibrous mats/tilosomes
<i>Cflaccida</i> (a)	E	Thick leaves, fleshy pseudo bulbs	Adecells comparatively larger; with 2 or 4 subsidiary cells, prominent stomatal ledges,h;well-developedssc	+	Simple water storage cells with abundant mucilage; fb present; vltabundant	Single layered	+
<i>Cflaccida</i> (b)	E	Thickleaves, fleshy pseudo bulb	ade cells comparatively larger;with3subsidiary cells,h;ssc present	+	Simple water storage ells with mucilages;fbabsent	Single-layered	+

a–Darjeeling collection b –Kerala Collection

**Table 6: Phyto-chemical profile of different solvent extracts of *Coelogyne flaccid* leaf**

Phyto-chemical Constituents	Test/reagents	Nhexane		Acetone		Ethylacetate		Methanol	
		<i>Cflaccida</i> (a)	<i>Cflaccida</i> (b)	<i>Cflaccida</i> (a)	<i>Cflaccida</i> (b)	<i>Cflaccida</i> (a)	<i>Cflaccida</i> (b)	<i>Cflaccida</i> (a)	<i>Cflaccida</i> (b)
Carbohydrates	Benedict's test	-	-	-	-	-	-	+	+
Proteins	Biuret test	-	-	-	-	+	-	+	+
Alkaloids	Dragendorff's test	-	-	+	-	-	-	+	+
Flavonoids	1%AlCl <sub>3</sub> test	-	-	-	-	+	+	+	+
Phenols	10%FeCl <sub>3</sub> test	-	-	+	-	-	-	+	+
Saponins	Frothing test	+	-	+	-	-	-	+	+
Steroids	Liebermann–Burchand test	-	-	-	-	+	-	+	+
Tannins	Iodine test	-	-	-	-	+	+	+	+
Terpenoids	Salkowski test	-	-	-	-	-	-	+	+

a–Darjeeling collection

b –Kerala collection

the entire ground tissue consists of parenchymatous cells (Ramesh, 2014), the majority of the ground tissue possess in outer walls and are mucilagenous. According to (Oliveira and Sajo, 1999) and (Shushan, 1959) the outer layer of the cortex comprising smaller vascular bundles and the inner cortex with larger vascular bundles. The vascular bundles are irregular and are scattered (Fig. 1C). The number of 6 a players towards phloemis 4 and xylemis 2(75), xylem comprises tracheids with helical thickenings, cells with pitted thickening is clearly seen in the sample of Darjeeling collection (Fig. 1D), showing the ecological parameters ( $T_5$ ).

### Root :

In cross section, root is circular in outline. The velamen is single-layered, but ruptured in the mature roots, the inner tangential walls of velamen cell is highly thickened and form fibrous mats (Fig. 1E). Exodermis is formed of long thick-walled and short thin walled cells which are not alternating to each other. Few cells are unligified, called as passage cell. The endodermis is well

developed with lignified cells and interrupted at the Protoxylem poles. In vascular cylinder, pericycle is single layered with thin-walled cells Phloem strands with xylem strands (Fig. 1F) the diameter of the vascular cylinder is  $0.062 \mu\text{m}$  ( $T_5$ ), pith cells are polygonal in shape merging with vascular sclerenchyma (Benzing, 1986). Phyto-chemical analysis preliminary phyto-chemical screening of *C. flaccid* in four different solvent extracts revealed presence of various phyto-chemicals, encompassing primary and secondary metabolites ( $T_6$ ). The methanol leaf extract exhibited the highest number of compounds, followed by the ethyl acetate and acetone extracts, specifically, the methanol leaf extract of *C. flaccid* exhibited the presence of phyto-chemical such as proteins, carbohydrates, alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids. The ethyl acetate leaf extract displayed similar phyto-chemical (Williams, 1979). The secondary metabolites are highly recorded in the sample collected from Darjeeling, when compared to Kerala collection. The phytochemicals recorded in *Dendrobium* are high in methanol extraction

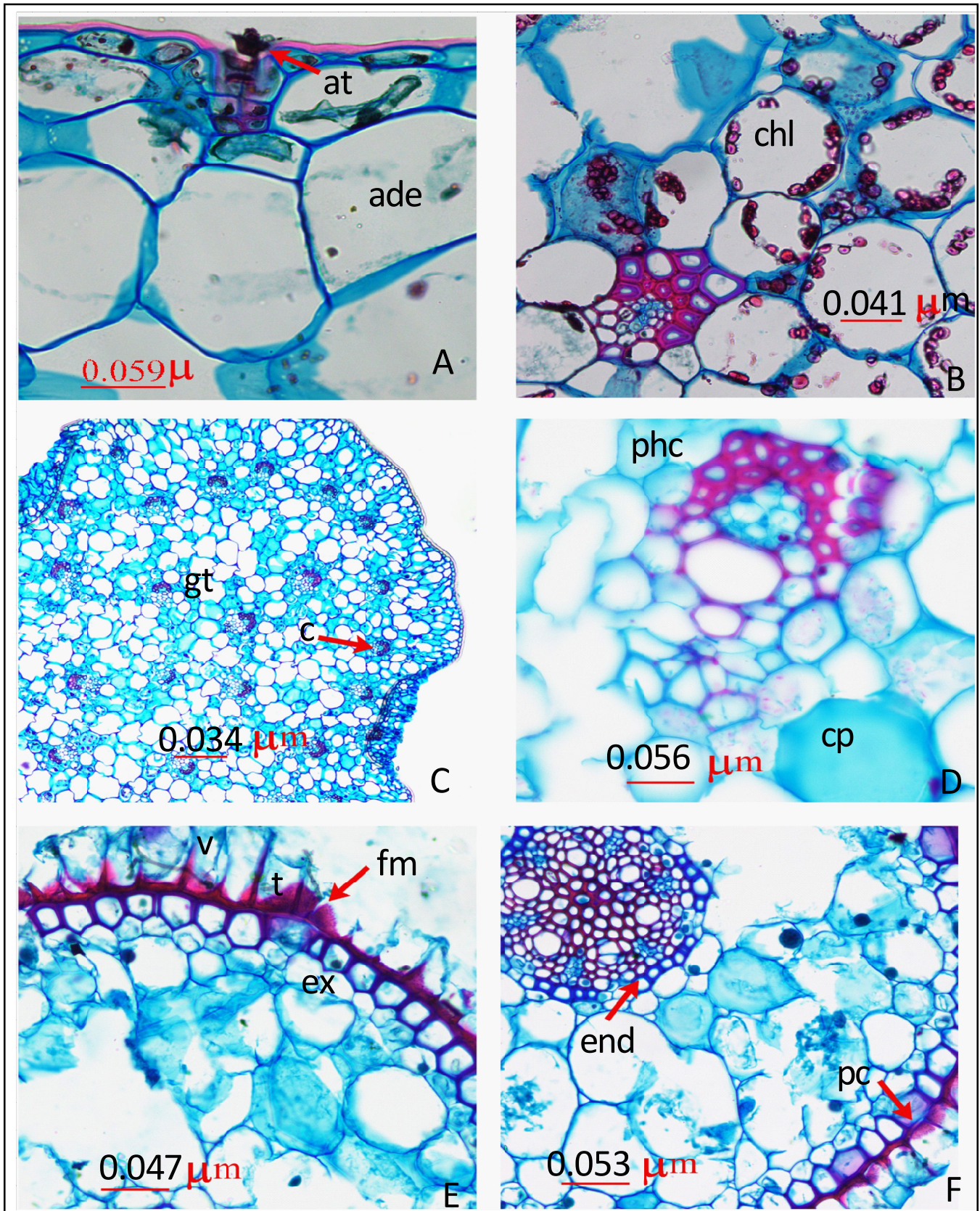


Fig. 1 : Anatomical characters of *Coelogyne flaccida*

(Rahmathulla *et al.*, 2020). The genus *Coelogyne* is rich in alkaloids (Handa, 1986). The flavonoids are well known for their antioxidant Property ( $T_6$ ).

### Conclusion :

The results of the present study of *C. flaccid* with anatomical and phytochemical implications reveal that, the Darjeeling sample collection is comparatively high from Kerala collection. It is more suitable for its habitat in Darjeeling forest due to its elevation and ecological adaptability.

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