

MICROSCOPICAL AND PRELIMINARY PHYTOCHEMICAL SCREENINGS OF *CAULOKAEMPFERIA SECUNDA* (ZINGIBERACEAE)

MD. ROFIQUL ISLAM¹ AND SELIM MEHMUD^{2*}

¹Department of Applied Biology, University of Science and Technology Meghalaya, Ri-Bhoi, Meghalaya-793101, India.

²Department of Botany, University of Science and Technology Meghalaya, Ri-Bhoi, Meghalaya-793101, India.

*Corresponding Author's Email: mehmudselim@gmail.com

The genus *Caulokaempferia* (Zingiberaceae) shows close affinities with *Kaempferia* and *Boesenbergia*. Except this genus, the microscopical features of the latter two were studied. Therefore, the present study attempted to conduct anatomy of the genus and to represent it *C. secunda* is selected. Along with anatomy, its powder microscopy is also conducted. The transverse section of petiole recorded bundle arc I, II, III, IV; midvein with arc I and III; and leaf sheath with arc I and II. The section of lamina exhibited by single-layer of epidermis with scattered oil cells and tetracytic stomata. The section of pseudostem shows scattered vascular bundles at periphery and a central mass of vascular bundles. In root, inner cortical cells are found with abundant starch grains and crystals. In powder microscopy, oil cells are observed comparatively higher in leaf powder followed by pseudostem and root. The present study also attempted to conduct a preliminary phytochemical analysis. A total of ten phytochemical analyses of leaf, pseudostem, and root samples were conducted with the extracts obtained from hexane, dichloromethane, ethyl acetate, and methanol. Nine out of ten phytochemicals were found to be present in leaf and pseudostem while roots contained seven.

Keywords: Assam, *Caulokaempferia*, Microscopy, Phytochemical, Zingiberales.

Introduction

The genus *Caulokaempferia* K. Larsen (Zingiberaceae) represents around 30 species, confined to the Himalayas to south China, Indo-China, and Thailand; among these, only three species, viz., *C. linearis* (Wall.) K. Larsen, *C. secunda* (Wall.) K. Larsen, and *C. sikkimensis* (King) Larsen, are found in India¹. Previously classified under the *Kaempferia* L. genus, *Caulokaempferia* was later recognized as a separate genus based on morphology and cytology²; it was also found to be closely related to *Boesenbergia* Kuntze. In India, species of *Caulokaempferia* are distributed in a few states of the northeastern region. The species *C. secunda* is found in

Assam, Meghalaya, and Sikkim, along with neighboring countries viz., Bangladesh and Myanmar^{1,3}. Anatomy of *Caulokaempferia* is poorly known however, anatomy of 41 species of Zingiberaceae including four species of *Kaempferia*⁴ also anatomy of Indian species under *Kaempferia* was studied⁵ but lacks anatomy of any species of *Caulokaempferia*. Furthermore, anatomy of few species of *Boesenbergia* was also conducted⁶. As the genus shows morphological similarities with *Kaempferia* and *Boesenbergia*, its comparative anatomical features will be helpful to find out the distinct anatomical features. Its phytochemical screenings were previously

not conducted. Therefore, the present study is an attempt to conduct a microscopical study of different parts of *C. secunda* (Figure 1)

along with their preliminary phytochemical screenings, e.g., alkaloids, saponin, lignin, etc., are conducted here for the first time.



Figure 1: *C. secunda*: a. Habit. b-c. Flower.

Material and Methods

The samples were collected from the Putsari (26°00'5.5"N, 092°16'00.8"E) of West Karbi Anglong, Assam, India. Samples were preserved in formalin (4%), and a voucher was submitted at Assam University Silchar Central Herbarium (AUSCH). The preserved samples were washed thoroughly for free-hand transverse sections (T.S.). The sections were double stained (safranin and fast green), passing through alcoholic grades⁷ and finally mounted by Dibutylphthalate Polystyrene Xylene (DPX) For the leaf epidermal study, leaves were treated in a 4% NaOH solution for 2–3 days, and slides were prepared by peeling both surfaces of the lamina. Fresh leaf, pseudostem, and roots were separated and washed by tap and deionized water then allowed to air dry at room temperature. Fine powders of these dried parts were prepared using a mixer grinder. For powder microscopy, relevant literature⁸ was consulted. All slides were examined under microscopes (phase contrast and fluorescence microscopes). The extracted samples were used for preliminary phytochemical

screenings to identify alkaloids (Mayer's test), flavonoids (Alkaline reagent test), tannins (Gelatin test), saponins (Froth test), terpenoids (Salkowski test), glycosides (Kellar-Kiliani Test), phenols (Ferric Chloride test), resins (Acetone-water test), coumarins (UV Fluorescence test), and proteins (Biuret test) by following standard protocol⁹.

Results and Discussion

Anatomy

T.S. of the petiole (Fig. 2 a-b):

"V"-shaped. Epidermis 1-layered, cells were ellipsoid, ovate or pentagonal, thin-walled, 21–40×11–41 μm; oil cells scattered. Ground cells parenchymatous, 27–97×29–104 μm. Bundle arcs I, II, III, and rarely IV. Arc I (181–200×148–160 μm), embedded in spongy mesophylls and in between parenchymatous cortex; metaxylem vessels ca. 27×21 μm.

T.S. of midvein (Fig. 2 c):

Shape, epidermal, and ground cells were similar to the petiole; bundle arc I and III.

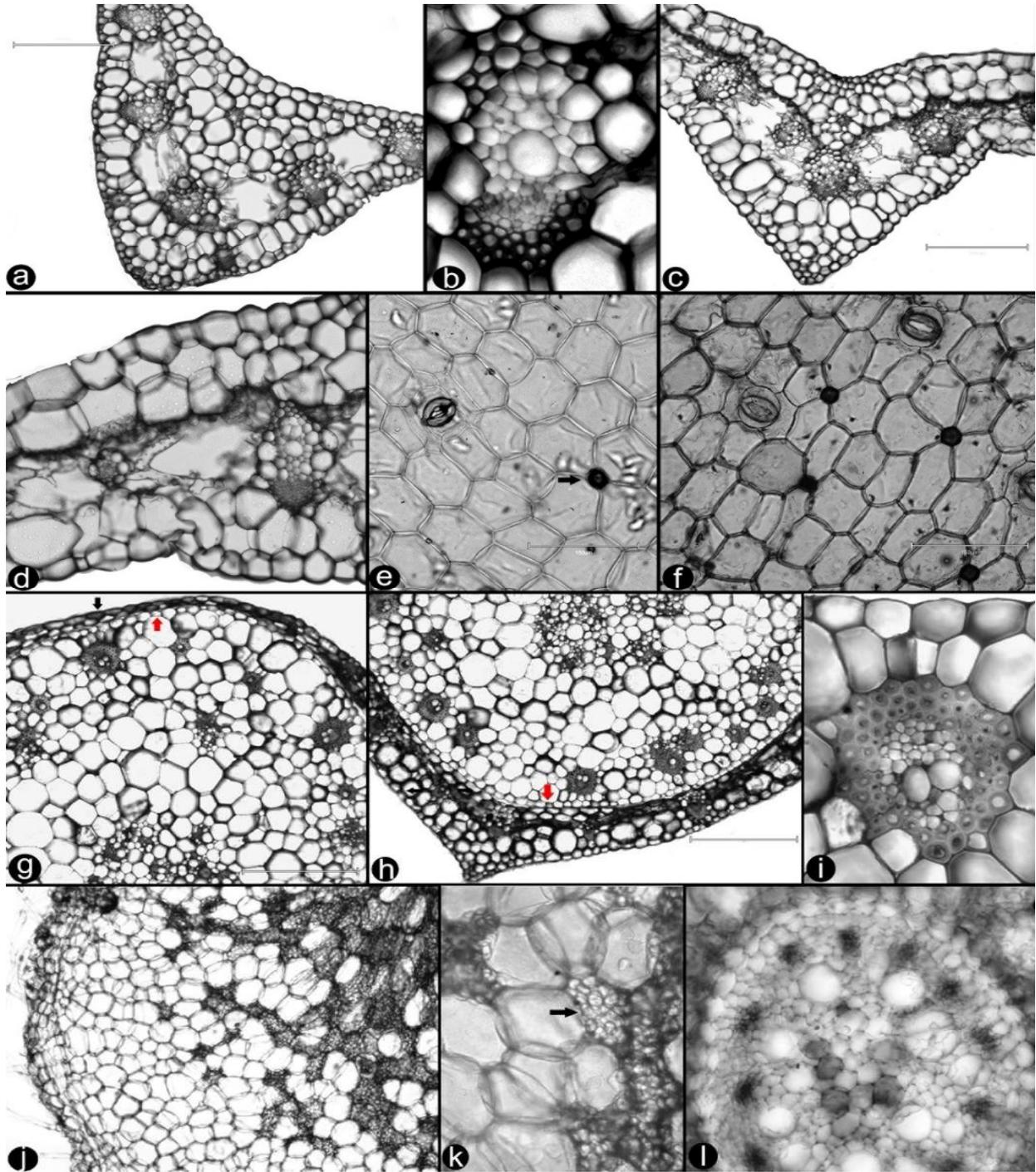
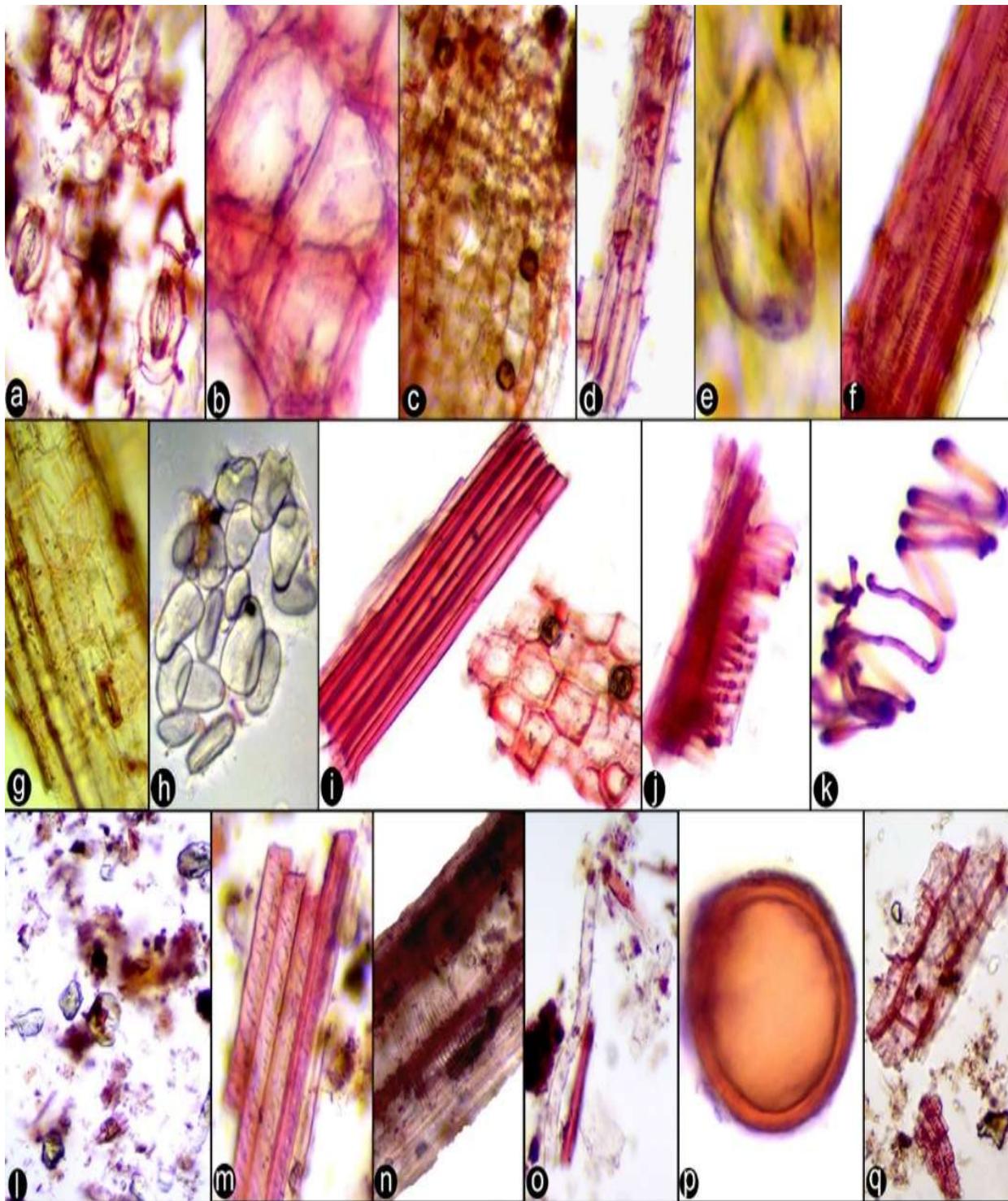


Figure 2: *C. secunda* **a.** T.S. of petiole. **b.** Vascular bundle of petiole. **c.** T.S. of midvein. **d.** T.S. of lamina. **e.** Epidermal morphology of upper surface of the lamina. **f.** Epidermal morphology of lower surface of the lamina. **g-h.** T.S. of pseudostem with leaf sheath. **i.** Vascular bundle of pseudostem. **j.** T.S. of root. **k.** Starch grains in inner cortex of root (arrow). **l.** Vascular bundle of root.



c

Figure 3: *C. secunda* : **Leaf powder:** a. Stomata and epidermal cells. b. Parenchymatous cells. c. Stomata, epidermal cells and oil cells. d. Coastal epidermal cells. e. Oil cell. f. Fiber and spiral xylem. **Pseudostem and sheath powder:** g. Epidermal cells and stomata of leaf sheath. h. Starch granules. i. Fibers and epidermal cells. j-k. Spiral xylem vessels. **Root powder:** l. Powder of root with abundant crystals. m. Fibers. n. Spiral xylem vessels. o. Root hair. p. Oil cell. q. Epidermal cells and crystal.

T.S. of lamina (Fig. 2 d):

Epidermis 1-layered. Upper epidermis cuticular, cells were rectangular, 21–100×25–70 µm; lower epidermal cells were rectangular to ovate, 36–54×28–54 µm. Mesophyll comprises 1-2 layered of palisade cells, and 1-layer of spongy cells. The bundle cap was within mesophyll or in contact with the upper epidermis. Both epidermises with oil cells and tetracytic-type of stomata, lower surface bears comparatively more stomata.

Surface view of the lamina (Fig. 2 e-f):

Epidermal cells hexagonal to polygonal in both surfaces, 59–100×57–76 µm in upper surface, and 41–76×80–98 µm in lower surface; oil cells, ca. 18 µm in diameter; stomata ca. 54×34 µm, stomatal opening 26–31×8.4–10 µm, guard cell ca. 51×14 µm, subsidiary cell ca. 48×12 µm.

T.S. of sheath and pseudostem (Fig. 2 g-h):

Sheath abaxially 'U' shaped, and adaxially 'V' shaped. Epidermis 1-layered, cells were ovate, ellipsoid, with few stomata. Bundle arcs I and II. Pseudostem oval-round; epidermal cells were ovate, ellipsoid, or pentagonal, 23–38×26–34 µm; ground cells 47–95×52–109 µm; peripheral scattered vascular bundles were 65–98×52–94 µm (Fig. 2 i); centrally a mass of vascular bundle.

T.S. of root (Fig. 2 j-l):

Circular; epidermis 1-layered, cells were ovate with unicellular hairs; cortical cells were thin-walled, angular, 30–78×48–79 µm, starch grains and crystals observed in inner cortical cells; endodermis and pericycle 1-layered, cells were 10–16×9–12 µm; vascular cylinder solitary, centrally situated, bundle embedded in sclerenchymatous cells, metaxylem ca. 25 µm, phloem strands alternately arranged with xylem; pith well developed, few were cells filled with oil.

*Powder microscopy**Leaf powder (Fig. 3 a-f):*

Sweet-scented, light green, exhibits hexangular to polyangular epidermal cells, tetracytic stomata with epidermal cells, rectangular coastal epidermal cells, oil cells, fibres, and spiral xylem vessels.

Pseudostem and sheath powder (Fig. 3 g-k):

Light brown, mild smell, exhibits epidermal cells, few tetracytic stomata, ovate to pear-shaped starch granules, oil cells, fibres, vessels, and few rectangular to irregularly shaped crystals.

Root powder (Fig. 3 l-q):

Brown, exhibits fibres, rectangular-triangular crystals, reticulate xylem vessels, parenchymatous cells, oil cells, vessels, root hairs, starch granules, and cork cells.

The numbers of oil cells are comparatively higher in leaf powder followed by pseudostem and root.

Preliminary phyto-chemical screenings:

The leaf extract was dark green, light green in pseudostem extract, and brown in root. The qualitative phytochemical screenings of these parts are presented in [Table 1](#).

This is the first anatomical study of a species under *Caulokaempferia* (*C. secunda*) conducted from India. This study will help in separation of the genus from *Kaempferia* and *Boesenbergia*. This is the first anatomical study of a species under *Caulokaempferia* (*C. secunda*) conducted from India. This study will help in separation of the genus from *Kaempferia* and *Boesenbergia*. The petiole of *C. secunda* shows I-IV bundle arcs, while a study recorded arcs I and III in *Kaempferia*⁵, and arcs I-IV in some species of *Boesenbergia* e.g., *B. rotunda* (L.) Mansfield⁶. In petiole, oil cells were recorded in the ground cells of *K. elegans*⁵, but observed in epidermal cells of *B. meghalayensis* Aishwarya & M. Sabu⁶ and *C. secunda*. The lack of bundle arc II in the mid vein of *Kaempferia*⁵ resemblance with *C. secunda*, while present in *Boesenbergia*⁶.

Table 1: Phytochemical screening of leaf, pseudostem, and root of *C. secunda*.

Parts	Extracts	Alkaloids	Flavonoids	Tannins	Saponins	Terpenoids	Glycosides	Phenols	Resins	Coumarins	Proteins
Leaf	Hexane	-	-	-	-	+	-	-	+	+	-
	DCM	+	-	-	-	+	+	+	+	+	-
	EA	-	-	-	-	+	+	++	-	+	-
	MeOH	-	+	+	-	+	-	+++	-	+	-
Pseudostem	Hexane	-	-	-	-	+	-	-	+	+	-
	DCM	-	-	-	+	+	+	+	+	+	-
	EA	+	-	-	-	-	+	+	+	+	-
	MeOH	+	+	+	-	-	+	-	-	+	-
Root	Hexane	-	-	-	-	+	-	-	+	-	-
	DCM	+	+	-	-	+	+	-	+	-	-
	EA	+	+	-	-	+	+	-	+	-	-
	MeOH	+	+	+	+	+	+	-	-	-	-

*present (+), moderately present (++), highly present (+++), absent (-)

The sheath of *C. secunda* with arcs I-II, but arcs I-IV recorded in some species of Indian *Kaempferia*⁵, whereas arcs I, II, and III in some *Boesenbergia* species⁶. The stomata in *C. secunda* was tetracytic-type, and similar type was also recorded *Boesenbergia* and *Kaempferia*¹⁰. In powder microscopy of *C. secunda*, leaf exhibits more stomata in comparison to pseudostem and sheath. The starch grains and crystals are comparatively more in root powder than pseudostem and sheath, but absent in the leaf. However, oil cells are comparatively higher in leaf powder followed by pseudostem and root. The preliminary phytochemical screening reveals occurrences of the highest phytochemicals in pseudostem followed by leaf and root. The analysis revealed the presence of nine phytochemicals in the leaf and pseudostem,

whereas seven in the root. The Dichloromethane (DCM) extract of the leaf sample had six phytochemicals, followed by five in methanol (MeOH) extract, four in Ethyl acetate (EA), and three in n-hexane extract. The DCM extract included a maximum of six phytochemicals for the pseudostem sample, followed by five in methanol, and three in n-hexane. Two phytochemicals were found in the n-hexane extract, five in the DCM, and six in the methanol extract for the root sample. Proteins were absent in all the samples. Coumarins are only absent in root samples. Hence, the preliminary phytoconstituents exhibit the presence of various bioactive substances such as alkaloids, flavonoids, terpenoids, etc. which may be source of drug discovery and employed for therapeutic purposes.

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