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Calcium Oxalate Crystals and Leaf Anatomical Characteristics of *Kaempferia galanga* L.

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ABSTRACT

Kaempferia galanga L. is a medicinal herb and an ornamental plant of Thailand. However, it is in decline due to the reducing forest area. In this report, we used a light microscope, a scanning electron microscope equipped with an energy dispersive X-ray spectrometer (EDS) to study leaf surface, leaf anatomy and crystals in the cells of K. *galanga* leaves. The leaves have a unique fragrant because there are oil cells on the leaf surface which contains a lot of essential oils. In addition, the leaf surface showed numerous stomata, trichomes and oil cells. The amphistomatic type had stomata distributed on both abaxial and adaxial epidermal layers. Tetracytic stomata types were found on the abaxial epidermis layer more than on the adaxial epidermis layer. There are unicellular trichomes on leaf veins and specific characteristics on the leaf surface. Specifically, the mesophyll cells of leaf contained calcium oxalate crystals in the cells. We found four forms of calcium oxalate crystal including prismatic, rhombic, hexagonal and octahedron. EDS spectra and X-ray maps show that the crystals were composed primarily of calcium and oxygen. This study on leaf surface, leaf anatomy and calcium oxalate crystals in the cell was a preliminary investigation that could be used for plant classification and a baseline experiment for study medicinal plants and others.

INTRODUCTION

Kaempferia galanga L. belonging to the family Zingiberaceae is a perennial medicinal herb and a local vegetable of Thailand. It is prevalent throughout tropical Asia. It found in bamboo forest and deciduous forest in Thailand.[5] It has been cultivated as an ornamental plant around the country. There are medicinal applications in leaf, rhizome and root of *K. galanga*.[2] The *K. galanga* extracts have anti-bacterial, anti-inflammatory, anti-diarrheal, analgesic, sedative, insecticidal and anthelmintic properties.[2] Its leaf contains an essential oil and its rhizome contains linoleoyl chloride, caryophyllene oxide, cubenol, caryophyllene and others.[4] Most of the leaf surface in Zingiberaceae family contains oil cells, stomata and trichomes on epidermal layer. The calcium oxalate crystals were found in the mesophyll cells in the form of clusters of varied sizes to a single large crystal in each cell.[6] The crystal shapes include rhombohedral, prismatic, tabular and rod shape. In addition, it was reported that calcium oxalate crystals were found in leaves of many plants in Thailand such as Siam cardamom, Bustard cardamom, Peperomia, and Purple allamanda.[8] In Peperomia calcium oxalate crystal were found in mesophyll cell rather than in specialized idioblasts indicating a different function.[7] In this study, we used a light microscope, a scanning electron microscope equipped with an energy dispersive X-ray spectrometer (EDS) to study leaf surface, leaf anatomy and crystals in the cells of *K. galanga* leaves. The aims of this study were to investigate the anatomical characteristics of leaves and crystals present in the leaf cells.

This study could also be used for plant identification and for other medicinal plants.

METHODOLOGY

K. galanga leaves were collected from plants that grew in a nursery at Scientific Equipment and Research Division, Kasetsart University Research and Development Institute, Bangkok, Thailand [Fig.1]. The fresh leaves were examined by a light microscope (LM), a scanning electron microscope (SEM) and an energy dispersive X-ray spectrometer (EDS).

Morphological of leaf surface under LM

The surface layer was peeled away from the *leaf* and it was strained in 1 % safranin O in distilled water. The specimens were examined under a LM (Axiostar plus, Carl Ziess, Aalen, Germany).

Anatomical characteristics of leaf under LM

The leaf samples were cut into 3x5 mm. and pre-fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.2 for overnight at 4 °C in refrigerator. The samples were post-fixed in 1% osmium tetroxide for 1 hr. They were dehydrated in acetone series, infiltrated

Figure 1. Fresh *K. galanga* plant.

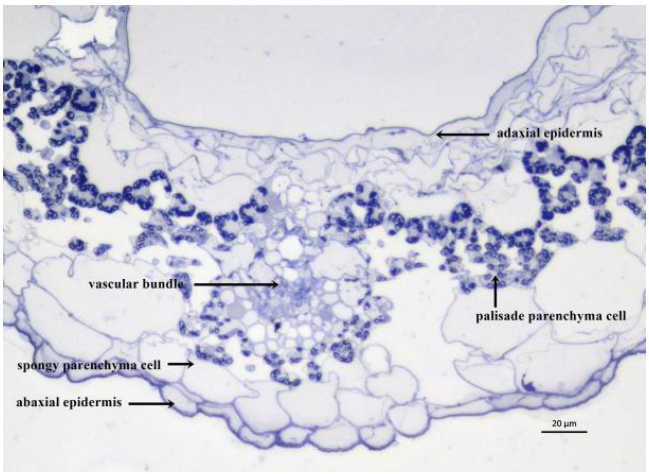


Figure 2. Transvers section of *K. galanga* leaf showing anatomical characteristics of midrib area.

and embedded in Spurr's resin. They were cut into 1µm thick with a glass knife on an ultramicrotome (UCT, Leica, Wetzlar, Germany), stained with toluidine blue O 1% solution and examined under the LM.

Morphological characteristics of leaf surface and calcium oxalate crystal analysis using SEM and EDS

Fresh leaves were observed without gold coating by low vacuum SEM (TM4000, Hitachi, Japan), which operated at 10 kV for leaf surface imaging. The leaf samples were cut into 3x5 mm. and pre-fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2 for overnight at 4 °C in refrigerator. They were dehydrated in acetone series and dried in critical point dryer (K850, Emitech, Kent, UK). The crystals in the leaf cells were examined under SEM (SU8020, Hitachi, Japan), which operated at 20 kV for energy dispersive X-ray spectrometry analysis (EDAX, USA).

RESULTS AND DISCUSSION

Leaf anatomy

The leaf anatomy of *K. galangal* is composed of epidermis layer, mesophyll layer and vascular bundle. The abaxial and adaxial epidermis layers are outer layers of leaf. The cuticle covered outside on the abaxial and adaxial epidermis layer [Figs.2-3]. There are epidermis cells, stomata, oil cells and trichomes on the leaf surface. The stomata type is amphistomatic type but the number of stomata on abaxial epidermis layer is more than adaxial epidermis layer [Figs.4-7]. The stomata have tetracytic form with 2 guard cells and 4 subsidiary cells [Fig.8]. The sizes of stomata were 40.90-62.10 µm. The oil cells were 18.02-40.70 µm and found on both abaxial and adaxial epidermis layers. The trichomes were unicellular and found in the leaf vein area [Figs. 9,10]. There were 129-1,550 µm long. The anatomical characters of leaf showed that there were a single abaxial and adaxial epidermis layer with polygonal cells in varied size. The shape of epidermis cells are rectangle, trapezoid, trapezium, pentagon and hexagon. The epidermis cell sizes were 60.4-117µm long and 41.95-83.08 µm wide on abaxial epidermis and 46.60-133 µm long and 58.45-99µm wide on adaxial epidermis (Table1). The mesophyll layer is consisted of palisade parenchyma cells, spongy parenchyma cells and vascular bundles. The leaf anatomical characters can be applied to identify the genus *Kaempferia.*

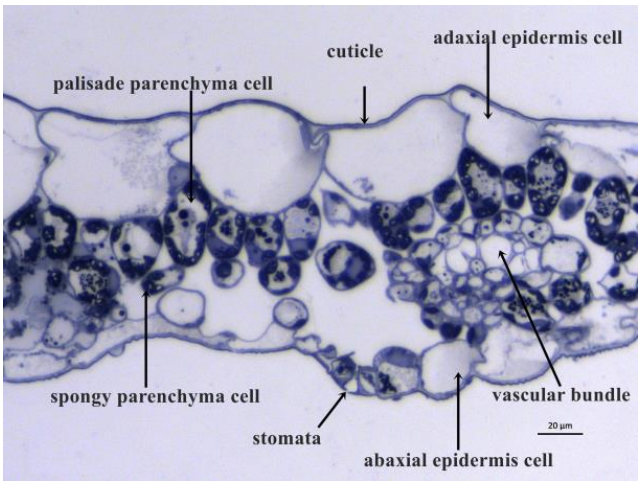


Figure 3. Transvers section of *K. galanga* leaf showing anatomical characteristics of leaf margin area.

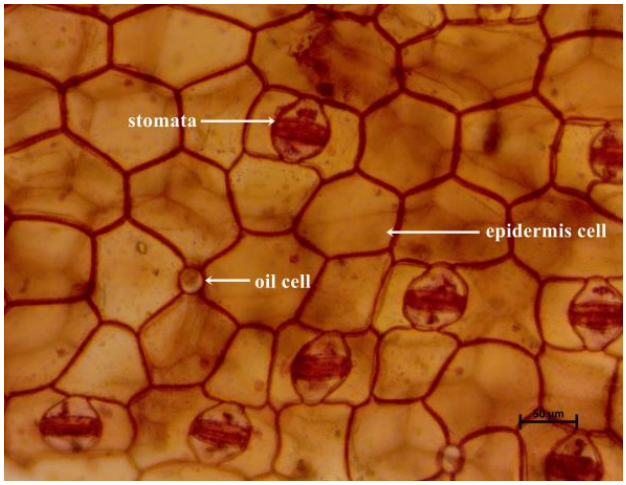


Figure 4. LM image of *K. galanga* leaf surface of abaxial showing tetracytic stomata, oil cells and epidermis cells.

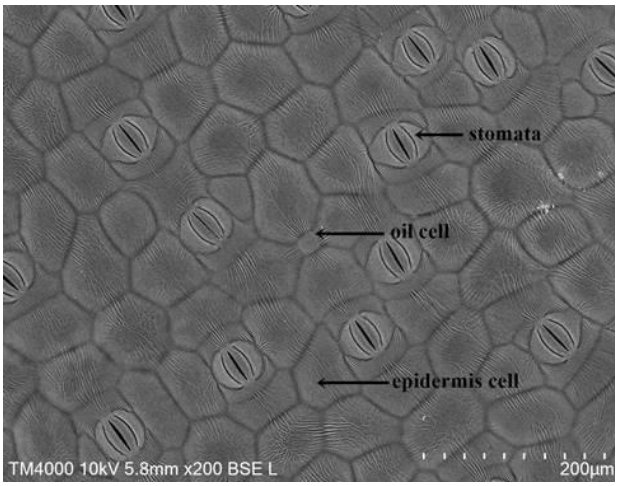


Figure 5. SEM image of abaxial epidermis of *K. galanga* leaf surface showing stomata, oil cell and epidermis cells.

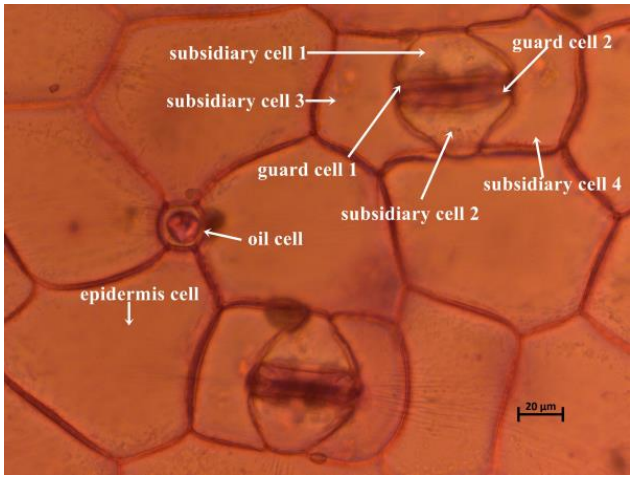


Figure 8. LM image of K. galanga leaf surface of abaxial showing tetracytic stomata, oil cells and epidermis cells. The tetracytic stomata is composed of 2 guard cells and 4 subsidiary cells.

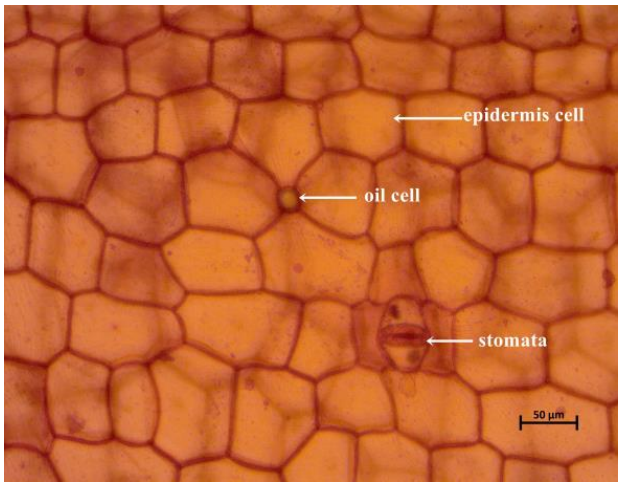


Figure 6. LM image of *K. galanga* leaf surface of adaxial showing tetracytic stomata, oil cell and epidermis cells.

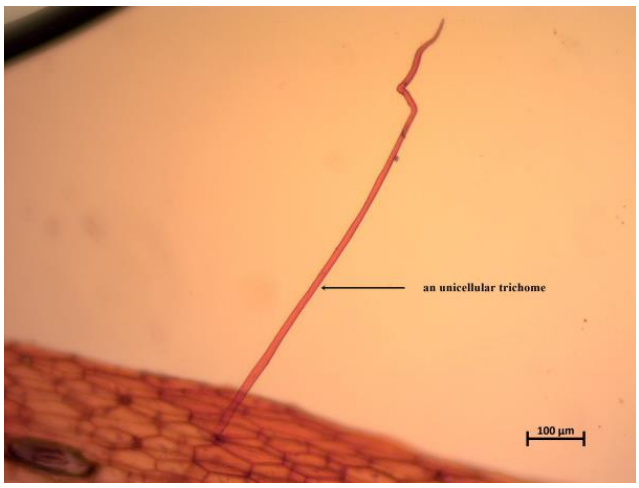


Figure 9. LM image of unicellular trichome on abaxial epidermis of *K. galanga* leaf.

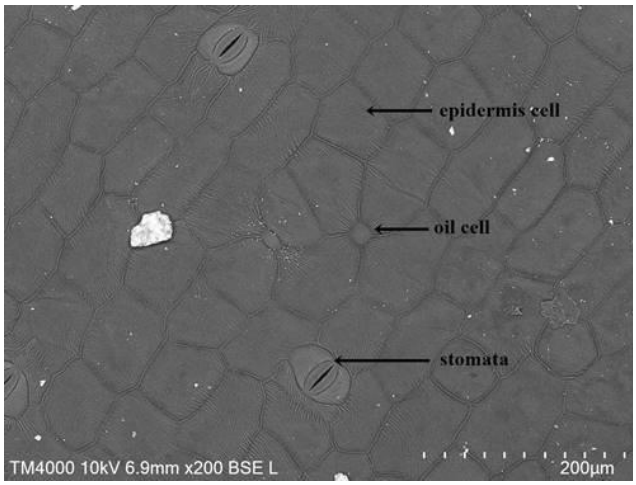


Figure 7. SEM image of adaxial epidermis of *K. galanga* leaf surface showing tetracytic stomata, oil cell and epidermis cells

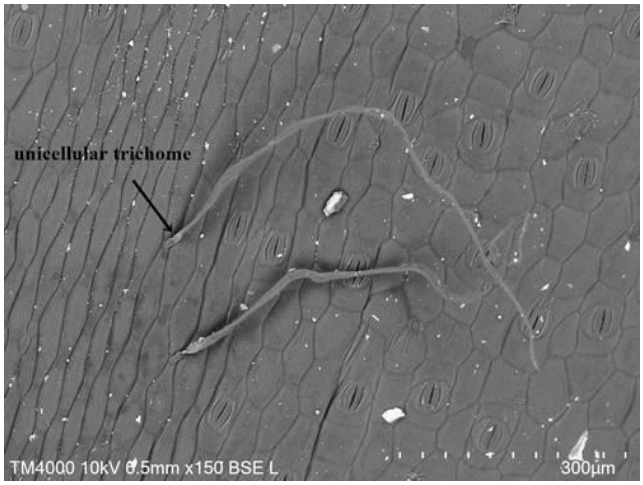


Figure 10. SEM image of unicellular trichome on abaxial epidermis of *K. galanga* leaf.

Table 1. Leaf surface and anatomical characteristics of *Kaempferia galangal* L. Leaf (n=100).

Characteristics of K. galanga leaf		Number and Cell size parameters
Upper epidermis cells	Long axis of upper epidermis cells (µm)	46.60-133.00 (85.16±19.55)
	Wide axis of upper epidermis cells (µm)	58.44-99.11 (76.78±8.94)
Lower epidermis cells	Long axis of lower epidermis cells (µm)	60.40-117.00 (88.15±12.75)
	Wide axis of lower epidermis cells (µm)	41.95-83.08 (63.87±8.28)
Stomata	Size of stomata (µm)	40.90-62.10 (50.14±5.10)
	Number of stomata on upper epidermis layer(number/µm2)	3-9 (5.10±1.42)
	Number of stomata on lower epidermis layer (number/µm2)	19-36 (26.23±4.62)
Oil cells	Size of oil cells (µm)	18.02-40.70 (25.17±3.89)
	Number of oil cells on upper epidermis layer (number/µm2)	2-6 (4.03±1.02)
	Number of oil cells on lower epidermis layer (number/µm2)	3-10 (6.63±1.97)
Trichome Unicellular	Long axis of Trichome (µm)	129.00-1550.00 (759.41±264.04)

Calcium oxalate crystal in the cell

The plant creates calcium oxalate in the cell when receives too much calcium. The mesophyll cells of *K. galanga* contain calcium oxalate crystals of 4 shapes [Fig.11]. There were prismatic, rhombic, hexagonal and octahedron shapes [Figs.12-15]. Franceschi and Nakata (2005) reported that the calcium oxalate crystals shape in plant cells is determined by the genetic mechanism of each plant. Prychid *et al.* (1999) reported that the main types of calcium oxalate crystals in monocotyledon are raphide, styloid and druses. Franceschi and Nakata (2005) reported that the main shapes of calcium oxalate crystals are prismatic, sand, raphide and druse. Paopun *et al.* (2011) reported that calcium oxalate in mesophyll cell of *Thunbergia laurifolia* Lindl. had needle-like shape. The shape and size of calcium oxalate are specific to plant species. [3] In this study, we found other shapes of calcium oxalate in the mesophyll cell of *K. galanga* leaf. EDS spectra and X-ray maps showed that the crystals were composed primarily of calcium and oxygen [Fig16]. The prismatic crystal sizes were1.87-8.70 µm wide, 4.14-10.60 µm long and1.14-2.91µm high. The rhombic crystal sizes were 8.28-17.07µm wide, 7.23-19.5µm long and 7.33-16.68µm high. The hexagonal crystal sizes were 11.59-27.53µm wide, 8.29-19.66 µm long and 1.10-2.91µm high. The octahedron crystal sizes were 0.81-5.14µm wide, 0.96-5.68µm long and 0.77-4.88µm high (Table 2). Creating calcium oxalate crystals in plant cells is a surviving mechanism of plants but it is harmful to human, especially when received too much of calcium oxalate. It can cause kidney stones. The study on leaf surface, leaf anatomy could be used to classify plants. The knowledge of calcium oxalate in plant cell can be used for the medicinal field and for human to prevent themselves from getting to much calcium oxalate.

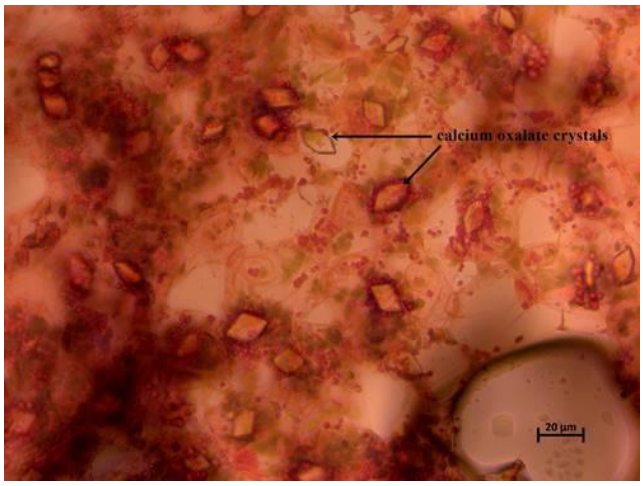


Figure 11. LM image of calcium oxalate crystals in the cells of *K. galanga* leaf.

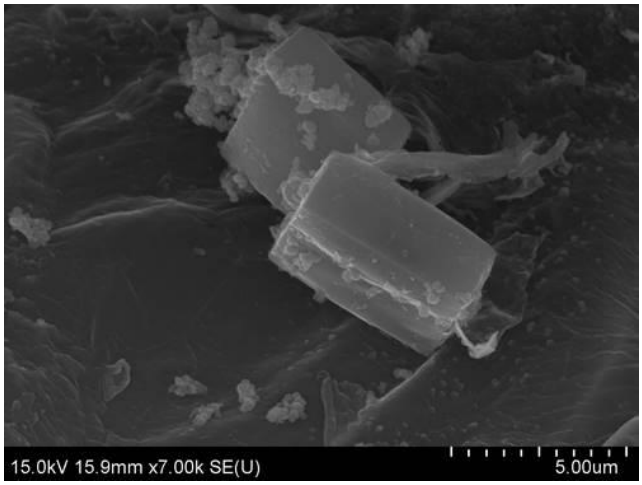


Figure 12. SEM image of prismatic shape of crystals in the cell of *K. galanga* leaf.

CONCLUSION

The leaf surface of *K. galanga* was consisted of epidermis cells, tetracytic stomata, oil cells and unicellular trichomes. The anatomy of leaf showed adaxial epidermis layer, mesophyll layer, vascular bundle and abaxial layer. The stomata were on the abaxial epidermis layer rather than adaxial epidermis layer. The mesophyll layer was consisted of palisade parenchyma cells, spongy parenchyma cells and vascular bundles. The mesophyll cells contained calcium oxalate crystals that were prismatic, rhombic, hexagonal and octahedron in varied sizes. EDS analysis confirmed that the compositions of the crystals were calcium and oxygen.

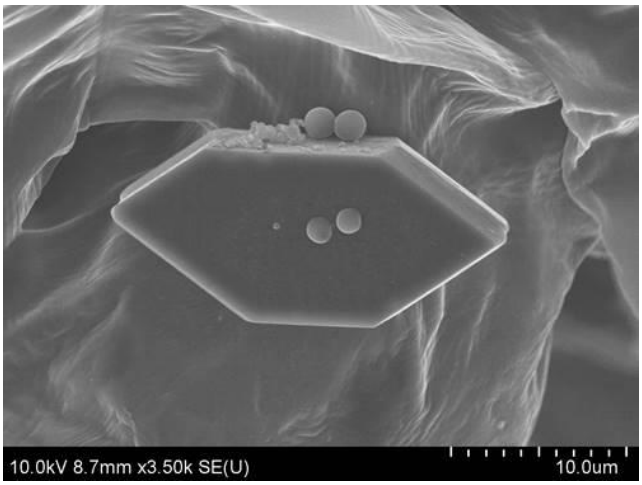


Figure 14. SEM image of hexagonal shape of calcium oxalate crystal in *K. galanga* leaf.

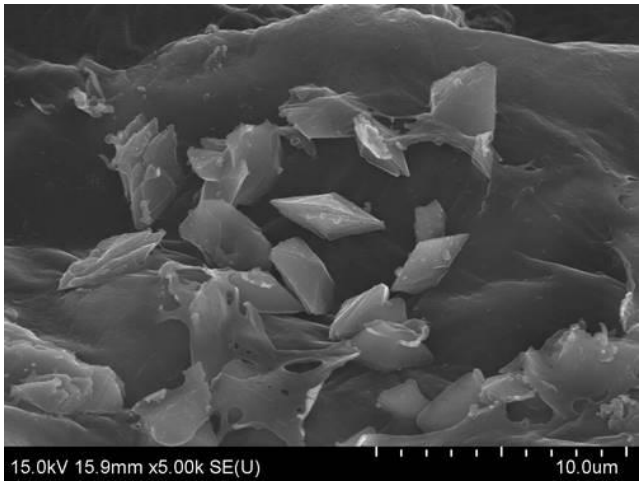


Figure 15. SEM image of a group of octahedon shape of calcium oxalate crystal in the cell of *K. galanga* leaf.

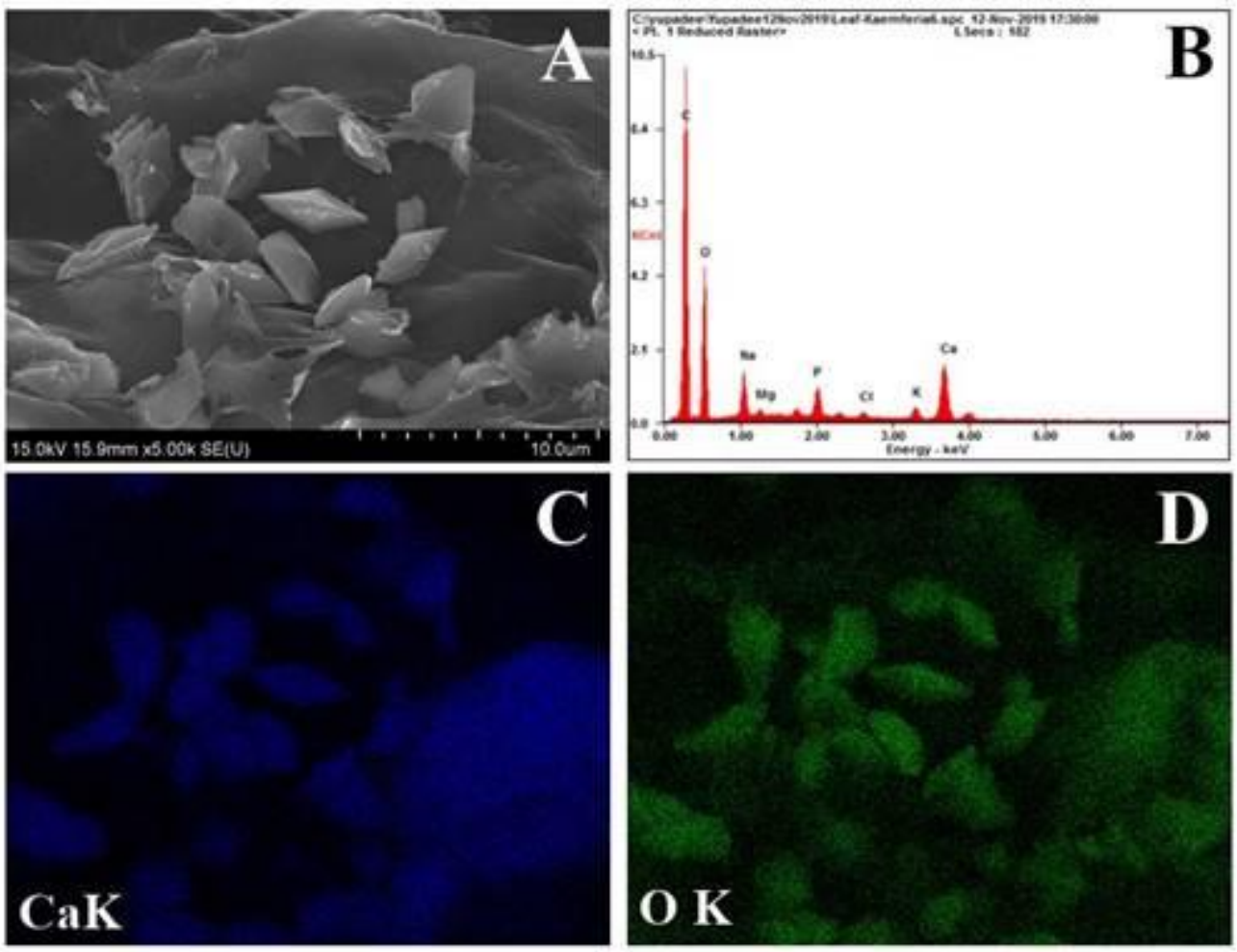
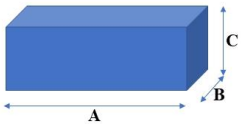
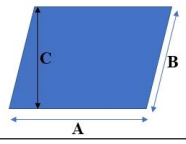
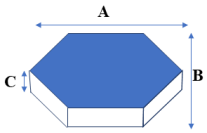
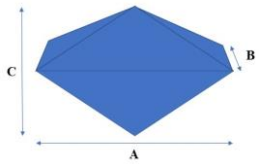


Figure 16. (A) SEM image of crystals in the cell of *K. galanga* leaf (B) EDS spectrum of crystals (C) X-ray map of Ca Kα (D) X-ray map of O Kα.

Table 2. Calcium oxalate crystal characteristics in mesophyll layer of *Kaempferia galangal* L. Leaf (n=30)

	Crystal size measurement		
Crystal form	**Length (Side A) (µm)**	**Width (Side B) (µm)**	**Height or thickness (Side C) (µm)**
Prismatic shape 	4.14-10.60(6.26±2.24)	1.87-8.70(3.26±2.13)	1.14-2.91(1.95±0.5)
Rhombic shape 	7.23-19.5 (10.99±2.09)	8.28-17.07(12.86±2.25)	7.33-16.68 (10.56±1.98)
Hexagonal shape 	8.29-19.66 (12.83±2.78)	11.59-27.53(20.32±3.63)	1.10-2.91(1.70±0.56)
Octahedron 	0.96-5.68(3.02±1.58)	0.81-5.14(2.14±1.60)	0.77-4.88 (2.10±1.35)

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