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Colocasia esculenta (L.) Schott (Wild Taro): Calcium Oxalate Crystals in Leaf and Petiole Using Light Microscopy, Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy

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ABSTRACT

Calcium oxalate (CaOX) is commonly found in Thai herbs and in wild taro. CaOX is claimed to be a cause of kidney stones in humans who consume plants that contain CaOX. The aim of this study was to investigate the presence of CaOX crystals in leaf and petiole parts of *C. esculenta* using a light microscope, a scanning electron microscope and an energy-dispersive X-ray spectrometer. Various shapes of CaOX crystal were found, including needle-like (raphide), druse, hexagonal and octahedron. They occur in the leaf blade, midrib and petiole. In terms of size, the needle-like crystals were 45-62.5 μm long and 1.42-2 μm wide. The druses were 4.01-65.10 μm in diameter. In comparison, the hexagonal crystals were 2.71-33.80 μm long and 1.01-6.61 μm wide. The octagonal crystals were 1.8-7.38 μm long and 1.92-10.50 wide. The needle crystals formed raphide clusters in the cells while hexagonal and orthorhombic crystals were individually present in the cells. Energy dispersive X-ray spectra and X-ray maps showed that the crystals consisted of calcium (Ca) and oxygen (O). The results of this study are preliminary data that could be used in the medical field and human health care.

INTRODUCTION

The wild taro is a perennial local vegetable. The botanical name is *C. esculenta* (L.) Schott. The vernacular name is bon which belongs to the family Araceae. They are found in wet places growing naturally along the canal bank, [1] wet fields, roadside ditches and weeds in cultivated areas [2]. This plant grows well in full sunlight. It can be used for fodder in Okinawa, Japan and Northern Thailand [3, 4]. In Thailand, the leaf stalk is commonly used for sour curry. [1]. Calcium oxalate (CaOX) crystals in petiole have been reported [1]. Chomlamay *et al.* (2022) reported that the *C. esculenta var. aquatilis* Hassk extract has the potential to treat cervical cancer.

The leaf anatomy consists of the epidermal layer, mesophyll layer and vascular bundle. The mesophyll is composed of palisade parenchyma cells, spongy parenchyma cells and large air spaces. Numerous raphide idioblasts appear in this layer [6] The upper and lower epidermis cells are polygonal in shape and stomata are distributed on both sides of the epidermal layer. There are 4 subsidiary cells around the stomata [7]. The petiole anatomical of *C. esculenta* L. Schott cv. Bentul is composed of epidermal, cortex and stele [8].

Various forms of CaOX in plants were found in the cells of plants. They are present in a vacuole of special cells that are called crystal idioblasts. CaOX in plants is induced through a calcium control mechanism. The function of CaOX crystals in plants is to structural support and prevent herbivorous animals [9], against insect attacks and microbial infections [10]. CaOX crystals were found in many types of cells and tissues in plants such as the spongy and palisade cells in a leaf, and cells around the vascular bundle of the stem [11]. Paopun *et al*. (2020) found that there were 4 shapes of CaOX crystals in *Kaempferia galanga* L. including prismatic, rhombic, hexagonal and octahedron. Paopun *et al*. (2022) reported that the stem and leaf of *Peperomia pellucida* L. contained 4 shapes of CaOX crystals; druses, prismatic, hexagonal and octahedron shapes. Buragohain *et al.* (2013) reported that CaOX was found in the corm of Taro. Arogundade and Adedeji (2019) found that the leaf and petiole of *C. esculentum* var. *antiquorum* and *C. esculentum* var. *esculentum* contained raphide and druse calcium oxalate crystals in the cells. Eco and Belonias (2017) found that the leaf of *C. esculenta* contained in the druse and raphide crystals. There were 2 types of raphide viz the defensive and non-defensive. The defensive raphide crystals are long (24-30 μm) and have distinct terminal papillae at one end and a thin-walled part on the other end. Most of these raphides are found in the mesophyll cells of the leaf. On the other hand, the non-defensive raphide crystals are short (12-15μm) and found in the margin of the leaf. Cha-um *et al.* (2019) found that the raphide of CaOX crystals in the petiole of *C. esculenta* Shott var. *aquatillis* could be dissolved in hot water at 85°C. Kumoro *et al*. (2014) reported that CaOX in the Taro Corm chip could be reduced by soaking taro in 10% baking soda solution for 2 hrs. and then boiling it at 90 °C. In this research, the morphology of CaOX crystals of wild taro were examined using a light microscope (LM), a scanning electron microscope and were elements analyzed with an energy dispersive X-ray spectrometer. This is a fundamental study to characterize CaOX of wild taro, which provides important information to understand sources of irritation in some people and whether the plant is safe for consumption for those who are allergic.

MATERIALS AND METHODS

Leaves and petioles of wild taro were collected from plants that grew in the natural wet field at Kasetsart University.

For light microscopy studies, fresh samples were washed with distilled water. The samples were transverse sectioned with a sliding microtome (Leica: SM2010R) at 60 µm and stained with 1% Safranin O in distilled water and 1% Fast green in 95% Ethyl alcohol. The specimens were placed on a glass slide and covered with a cover slide. The prepared slides were observed under an inverted light microscope (Zeiss: Axio, Vert. A1).

For scanning electron microscopy studies, the leaf and petiole of wild taro samples were cut into small pieces of 5x5 mm^2 and dried in a Freeze dryer (Quorum: K750X) for 32 hrs. The dried samples were coated with carbon in a carbon coater (Quorum: Q150R) for 120 seconds to increase conductivity and reduce electron charging in SEM. The samples were observed under a SEM (Hitachi: SU8020), operated at 10 kV for imaging and 20 kV for energy dispersive X-ray spectroscopy analysis (EDAX: Apollo X).

RESULTS AND DISCUSSION

The CaOX crystals appear in the cells of the leaf and petiole of wild taro. The transverse section of the wild taro leaf near the midrib consisted of the epidermis, mesophyll, air space and vascular bundle layer. There were CaOX crystals in the spongy cells of the mesophyll layer of the leaf blade and parenchyma cells near the vascular bundle of the midrib (Figure 2). There were 4 shapes of CaOX crystals including needle-like (raphide), druse, hexagonal and octahedron (Figures 2, 3 and 4). The idioblast cells contain CaOX crystals. The raphide bundle is a group of needle-like crystals in a cell. In this plant, the raphide crystals were easily observed to be long and numerous in a group of bundles as occur in idioblast cells. The idioblast cells were distributed in the mesophyll layer and vascular bundles. The raphide bundle in the idioblast cells protruded into the air space. Furthermore, there were other shapes, such as druse, hexagonal and octahedron, of CaOX crystals in the parenchyma cells. The petiole anatomical characteristics showed the epidermis, ground tissue, air space and vascular bundle. The idioblast cells contained the raphide bundle and distribute in the cortex layer of the petiole which can be clearly seen as the idioblast cells protrude into the air space. There were druse, hexagonal and octahedron crystals in the cells of the petiole as well. CaOX crystals in plants may form in monohydrate ($CaC_2O_4 \cdot H_2O$) or dihydrate ($CaC_2O_4 \cdot 2H_2O$) which is related to the crystal shape. The raphide crystal is a monohydrate and druse crystals are monohydrate or dihydrate [9]. The crystal composition was confirmed by energy-dispersive X-ray spectroscopy analysis which found calcium (Ca) and Oxygen (O). The X-ray mapping of the Ca and O positions was shown in the crystal area (Figures 5-8). This result agreed with a study by Arogundade and Adedeji (2019) who reported that the raphide and druse shapes of CaOX crystal appeared in the petiole of *C. esculentum* var. *atiquorum* and *C. esculentum* var. *esculentum*.

Figure 1. A photograph of wild taro grown in a ditch at Kasetsart University. It is about 1 m tall. Leaves and petiole are green.

Wild taro tends to grow rapidly in wet places or wetlands. It is a plant that can absorb elements such as calcium and minerals from soil and water well. When plants receive excessive amounts of calcium, it can be stored in the form of CaOX in the cells of many parts such as root, stem, leaf, petiole, flower and fruit [18]. CaOX crystals are found in many shapes such as prism, raphide, styloid, sand and druses, etc. Buragohain *et al.* (2013) found CaOX crystals in the corm of indigenous taro (*C. esculenta*) cultivars of Nagaland. Many tropical plants have been found to produce CaOX and store in their cells. Paopun *et al.* (2022) found that *Peperomia pellucida* (L.) Kunth contained prismatic, druse, hexagonal and octahedron shapes of CaOX crystals. Paopun *et al.* (2020) reported *that Kaempferia galanga* L. contained rhombic, prismatic, hexagonal and octahedron crystals in the mesophyll cell of leaves. In addition, calcium oxalate crystals are also found in the leaves of Siam cardamom, Bustard cardamom and purple allamanda [19]. Factors affecting CaOX formation include temperature, habitat, pressure and ion concentrations. The monohydrate crystal could grow at the temperature of 0-100 °C and the dihydrate crystal appears at 0-30 °C [9], resulting in the raphide crystals. CaOX crystals may be one of the causes of plant irritation [11] and stone formation in the urinary tract [20]. The wild taro had many raphide crystals in the leaf stalks, causing irritation if consumed and becoming toxic to humans. However, Kumoro *et al.* (2014) found that soaking taro in 10% baking soda for 2 hours and then boiling at 90° for 60 minutes could reduce the amount of calcium in corm chips of Taro.

Light microscopy may be a good way to study the morphology of large crystals while scanning electron microscopy offers effective characterization of smaller crystal shapes. The energy-dispersive X-ray spectroscopy could be used to identify elements in the crystals to confirm the presence of CaOX. Therefore, the use of light microscopy, scanning electron microscopy and energy-dispersive X-ray spectroscopy techniques allows for a more complete and efficient study of the CaOX crystals in wild taro.

CONCLUSIONS

The leaf and petiole of wild taro contain numerous CaOX crystals in the cells. Four types of crystal shapes of CaOX were found similarly in leaves and petioles including raphide, druse, hexagonal and octahedral shapes. Druse and raphide crystals were larger than hexagonal and octahedron crystals. The raphide crystals are in bundles in the idioblast cells which could be clearly observed through a light microscope. Druse, hexagonal and octahedron crystals distributed in the mesophyll cells of the leaves and parenchyma cells of the petioles The EDS X-ray analysis and X-ray mapping confirm that all types of crystals contain CaOX.

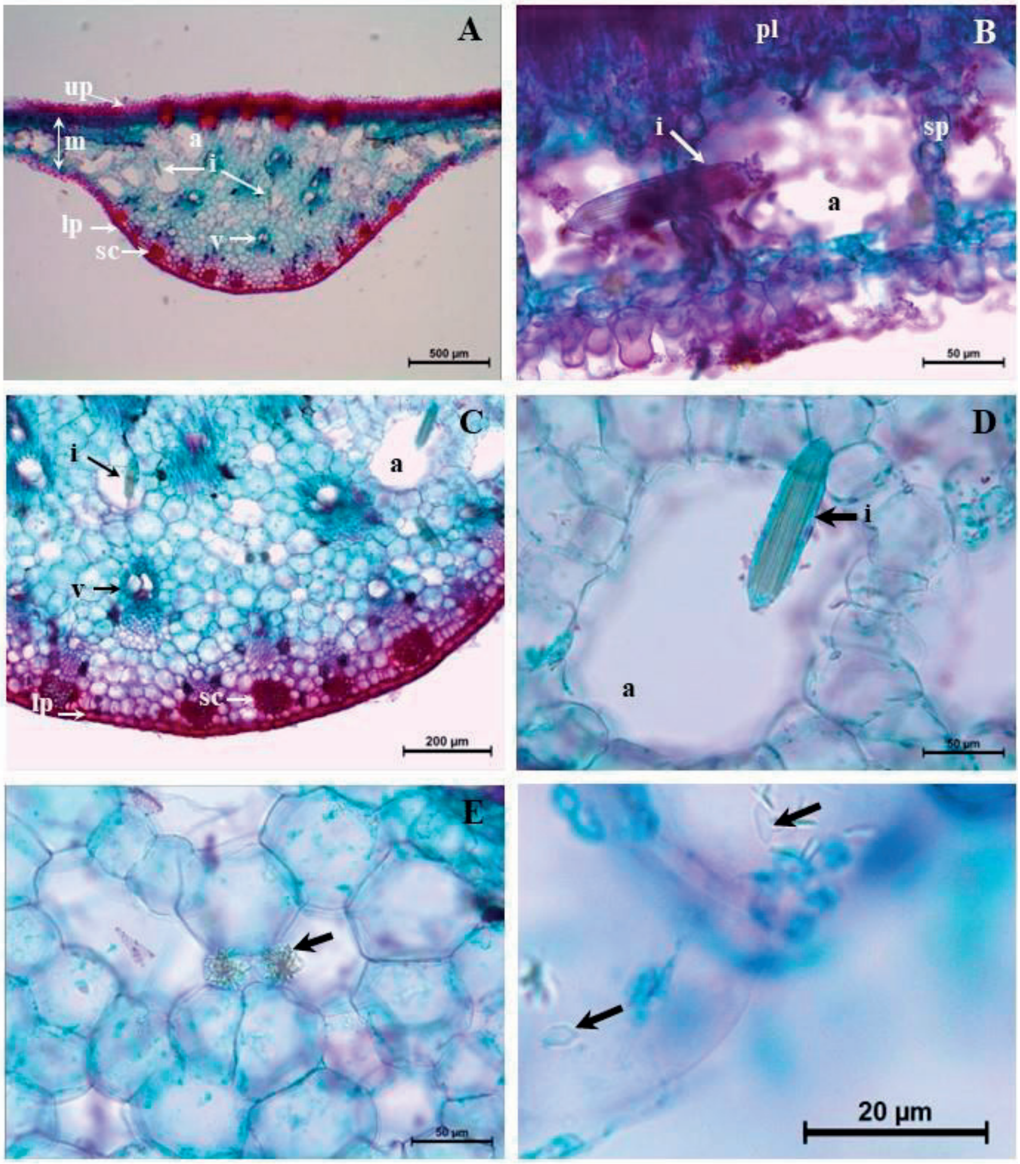
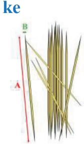
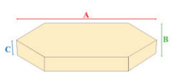
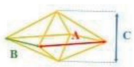


Figure 2. Light micrographs of the transverse section of wild taro leaf: A) transverse section of leaf showing idioblast in midrib (arrow), B) idioblast in the mesophyll of leaf blade (arrows), C) distribution of idioblast in midrib (arrow), D) idioblast contain a group of raphide bundle (arrow), E) groups of druse crystals (arrows), F) groups of hexagonal crystals (arrows). a= air space, i= idioblast, lp= lower epidermis, m= mesophyll layer, pl= palisade cell, sc= sclerenchyma, sp= spongy parenchyma cell, up= upper epidermis, v= vascular bundle.

Table1. Size analysis of various crystal forms in wild taro leaf and petiole (N=50).

Crystal form	Crystal size measurement (µm)			
	Diameter	Length (Side A)	Width (Side B)	Height or Thickness (Side C)
Druse 	4.01-65.10 (18.27±12.61)	-	-	-
Needle-like ke 	-	45.00-62.50 (49.41±3.26)	1.42-2.00 (1.61±0.14)	-
Hexagonal 	-	2.71-33.80 (6.75±6.05)	1.01-6.61 (2.22±1.22)	0.66-3.45 (1.38±0.71)
Octahedron 	-	1.80-7.38 (3.32±1.47)	1.92-10.50 (3.26±1.61)	3.73-15.20 (6.54±2.91)

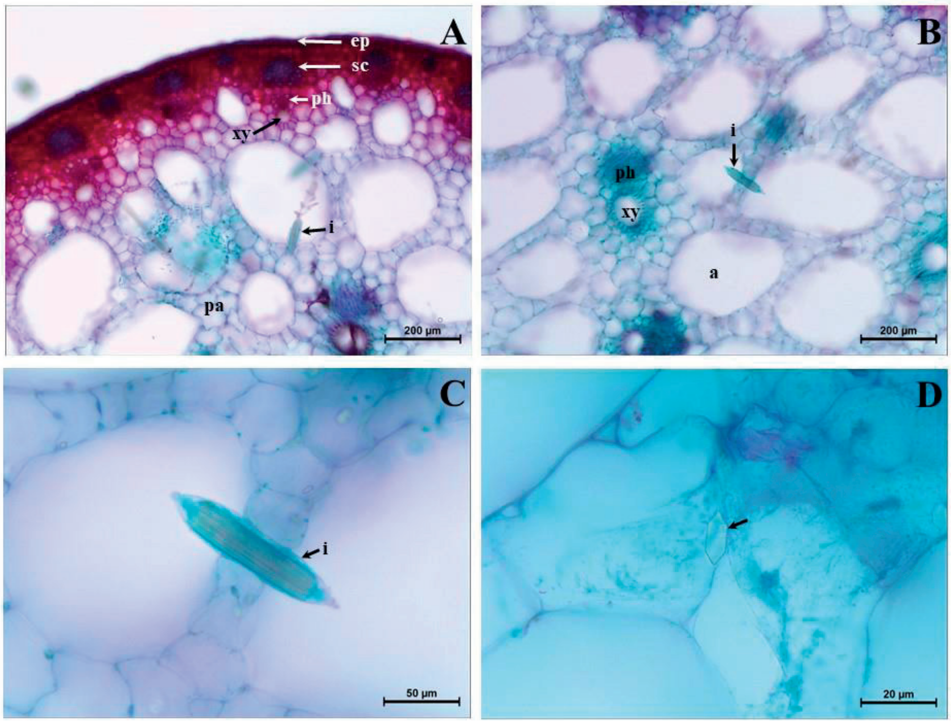


Figure 3. Light micrographs of A) transverse section of wild taro petiole showing distribution of idioblast cell (arrows)l, B) raphide crystals and idioblast cell in petiole (arrows), C) idioblast cell contains a group of raphide bundle (arrows) and D) a single crystal of hexagonal shape in parenchyma cells of ground tissue (arrow). a = air space, ep= epidermis cell, ph= phloem, sc= sclerenchyma cell, xy=xylem, i=idioblast.

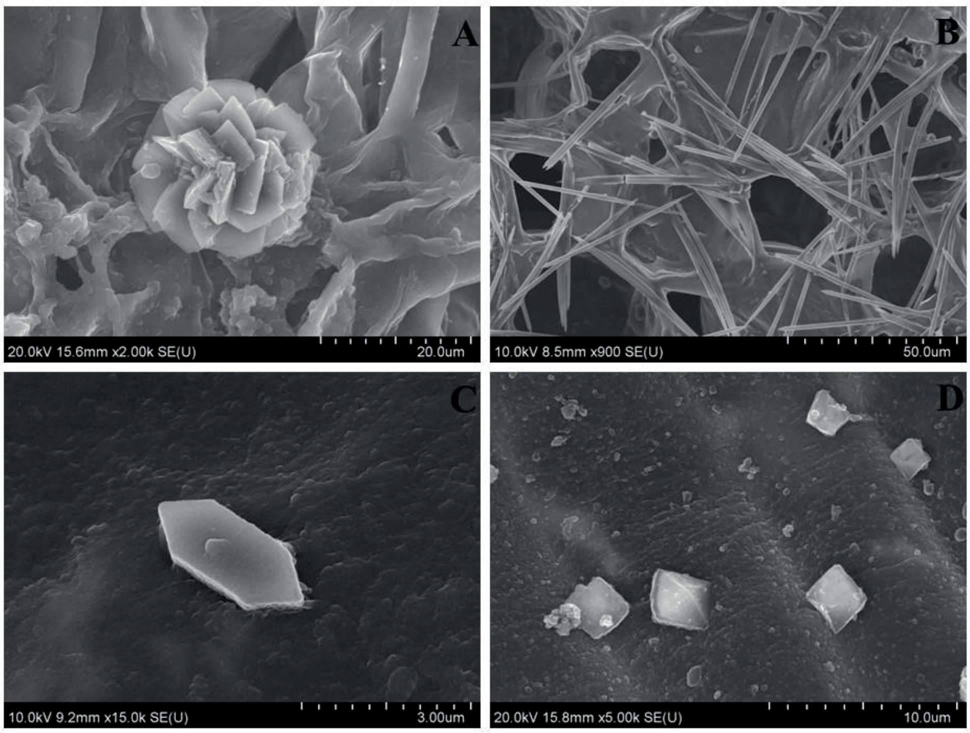


Figure 4. SEM micrographs of CaOX crystals in leaf and petiole of wild taro: A) druse shape, B) needle-like shape, C) hexagonal shape and D) octahedron shape.

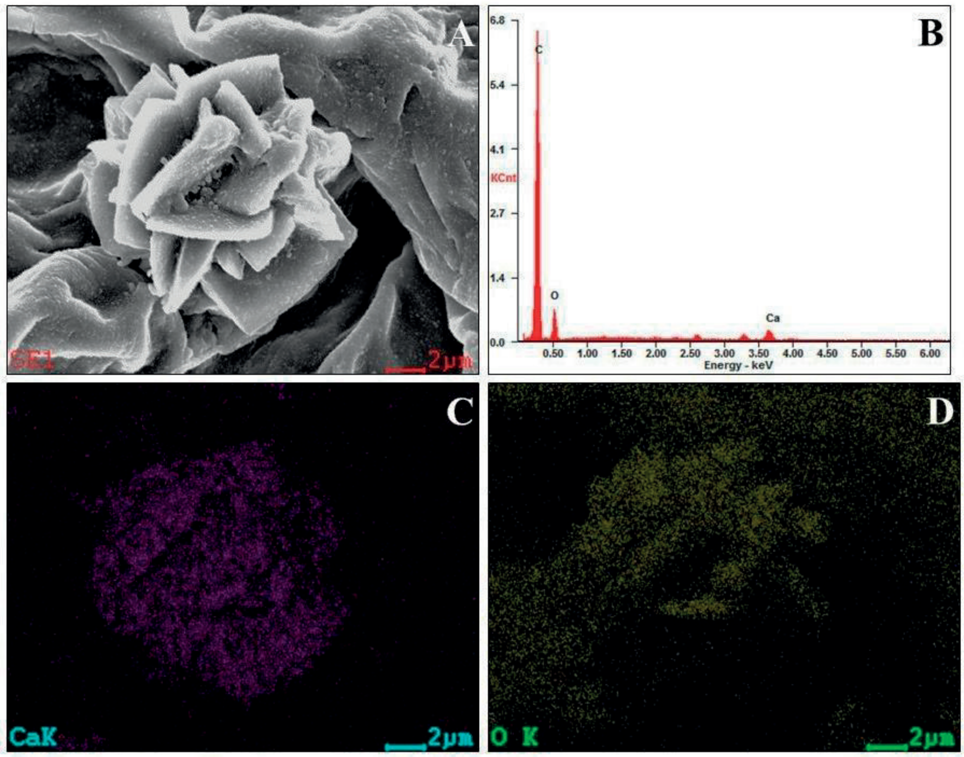


Figure 5. A) SEM micrograph of CaOX druse in petiole of wild taro, B) EDS spectrum of CaOX, C) X-ray map of CaKα and D) X-ray map of OKα.

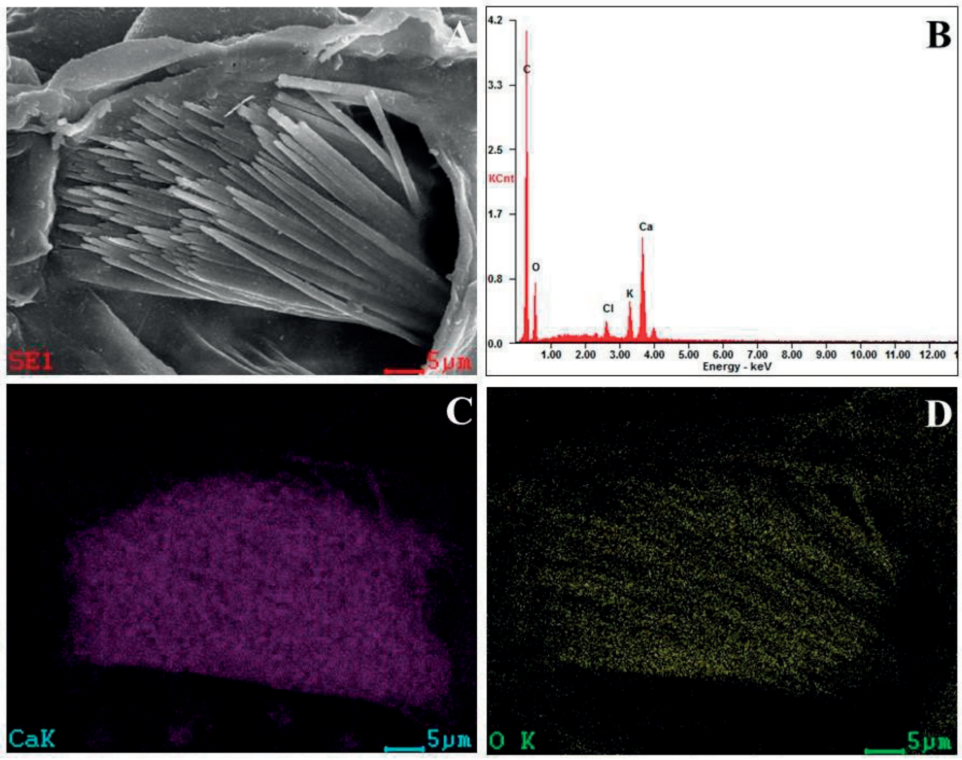


Figure 6. A) SEM micrograph of CaOX needle-like bundle in petiole of wild taro, B) EDS spectrum of CaOX, C) X-ray map of CaKα and D) X-ray map of OKα.

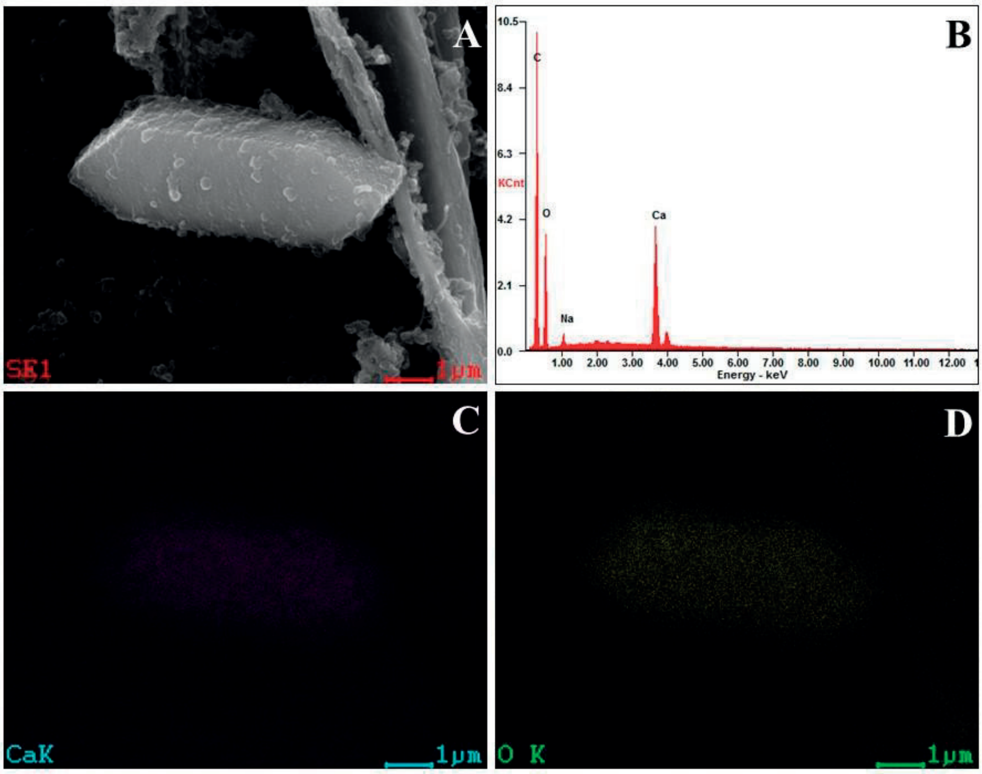


Figure 7. A) SEM micrograph of CaOX hexagonal in petiole of wild taro, B) EDS spectrum of CaOX, C) X-ray map of CaKα and D) X-ray map of OKα.

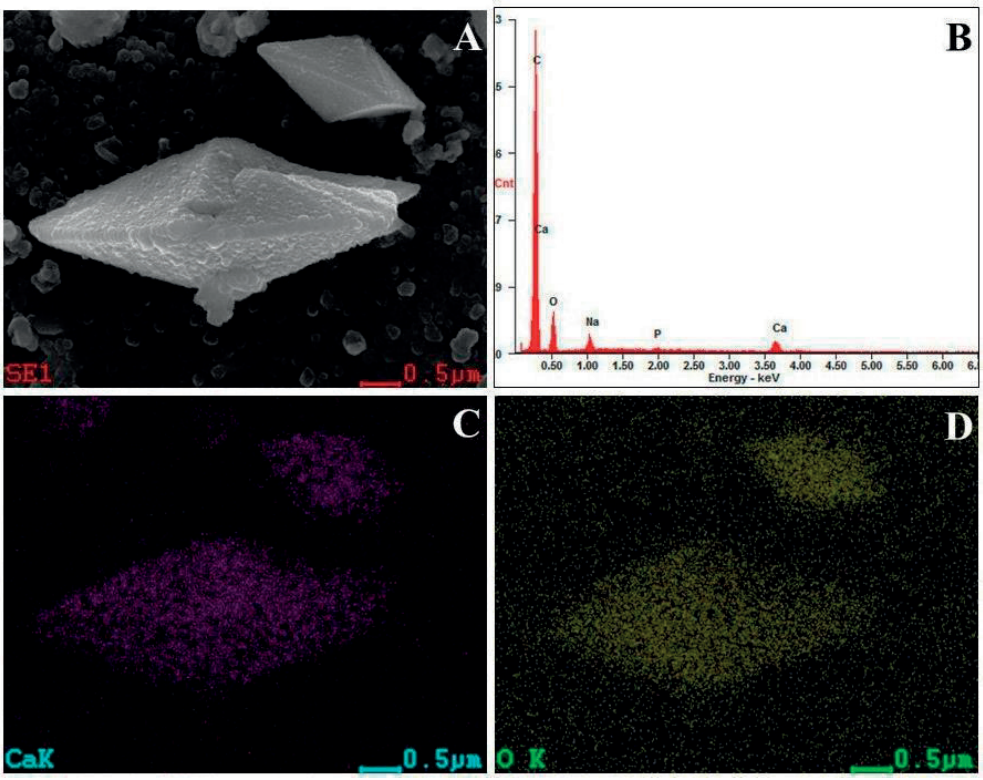


Figure 8. A) SEM micrograph of CaOX octahedron in petiole of wild taro, B) EDS spectrum of CaOX, C) X-ray map of CaKα and D) X-ray map of OKα.

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REFERENCES

[1] Y. Paisooksantivatana, K. Nakahara, Local Vegetables of Thailand: Color illustrated, Japan International Research Center for Agricultural Sciences (JIRCAS) and Kasetsart University, 79 (2009).

[2] P.C. Boyce, D. Sookchaloem, W.L.A. Hetterscheid, G. Gusman, N. Jacobsen, T. Idei, N. Van Du, Araceae, *Flora of Thailand*., Vol. 11(2), 1-221 (2012).

[3] P. Matthews, E. Takei, T. Kawahara, *Colocasia esculenta* var. *aquatilis* on Okinawa Island, Southern Japan: The distribution and possible origins of a wild diploid taro, *Man and Culture in Oceania*., Vol. 8, 19-34 (1992).

[4] N. Turreira-Garcia, A.M. Vilkaman, A. Byg, I. Theilade, Diversity, knowledge and use of leafy vegetables in Northern Thailand-maintenance and transmission of ethnobotanical knowledge during urbanisation, *Nat. Hist. Bull. Siam Soc*., Vol. 62(1), 85-105 (2017).

[5] N. Chomlamay, W. Poorahong, S. Innajak, R. Watanapokasin, Apoptosis induction associated with enhanced ER stress response and up-regulation of c-Jun/p38 MAPK proteins in human cervical cancer cells by *Colocasia esculenta* var. *aquatilis* Hassk extract, *Scientia Pharmaceutica*., Vol. 90, 45. https://doi.org/10.3390/scipharm90030045. (2022).

[6] B.D. Stein, M.S. Strauss, D.C. Scheirer, Anatomy and histochemistry of taro, *Colocasia N esculenta* (L.) Schott, leaves, International Society for Tropical Root Crops, Sixth Triennial Symposium, 21-26 February, Lima, Peru, 125-130 (1983).

[7] I. Erlinawati, E.F. Tihurua, Leaf surface comparison of three genera of Araceae in Indonesia, *Buletin Kebun Raya*., Vol. 16(2), 131-145 (2013).

[8] D.R. Wulandari, R.K. Ningrum, A. Wulansari, T.M. Ermayanti, D. Ratnadewi, Morphological and anatomical characters of diploid and tetraploid taro (*Colocasia esculenta* L. Schott) cv. Bentul grown in lathhouse, *IOP Conf. Series: Earth and Environmental Science*., 439, 012050. doi:10.1088/1755-1315/439/1/012050 (2020).

[9] V.R. Franceschi, H.T. Horner Jr., Calcium oxalate crystals in plants, *The Botanical Review*., Vol. 46(4), 361-427 (1980).

[10] K. Cha-um, S. Sangjun, K. Prawetchayadom, C. Theerawitaya, R. Tisarum S. Klomklaeng, S. Cha-um, Physiological, organic and inorganic biochemical in the leaves of elephant ear (*Colocasia esculenta* Schott var. *aquatillis*), *The Horticulture Journal*., Vol. 88(4), 499-506 (2019).

[11] S.T. Konyar, N. Ozturk, F. Dane, Occurrence, types and distribution of calcium oxalate crystals in leaves and stems of some species of poisonous plants, *Botanical Studies*., Vol. 55(1), 32. doi: 10.1186/1999-3110-55-32 (2014).

[12] Y. Paopun, D. Changchan, P. Thanomchat, Calcium oxalate crystals and leaf anatomical characteristics of *Kaempferia galanga* L., *Journal of Microscopy Society of Thailand*., Vol. 33(1), 28-33 (2020).

[13] Y. Paopun, P. Thanomchat, P. Umrung, S. Seraphin, Calcium oxalate crystals in Thai *Peperomia pellucida* (L.) Kunth stems and leaves, *Journal of Microscopy Society of Thailand*., Vol. 35(1), 26-32 (2022).

[14] J. Buragohain, T. Angami, B.U. Choudhary, P. Singh, B.P. Bhatt, A. Thirugnanavel, B.C. Deka, Quality evaluation of indigenous taro (*Colocasia esculenta* L.) cultivars of Nagaland, *Indian Journal of Hill Farming*., Vol. 26(2), 16-20 (2013).

[15] O.O. Arogundade, O. Adedeji, Taxonomic significance of the vegetative anatomy of members of genera *Colocasia* (L.) Schott and *Xanthosoma* (L.) Schott in the family Araceae, *African Journal of Plant Science*., Vol. 13(4), 92-106 (2019).

[16] K.C. Eco, B.S. Belonias, Biomineralization of calcium oxalate crystals in leaves of *Colocasia esculenta* L. Schott (Araceae) in response to herbivory and water regime, *Annals of Tropical Research*., Vol. 39(1), 54-69 (2017).

[17] A.C. Kumoro, Rr.D.A. Putri, C.S. Budiyati, D.S Retnowati, Ratnawati, Kinetics of calcium oxalate reduction in taro (*Colocasia esculenta*) corm chips during treatments using baking soda solution, *Procedia Chemistry*., Vol. 9, 102-112 (2014).

[18] V.R. Franceschi, P.A. Nakata, Calcium oxalate in plants: Formation and function, *Annual Review of Plant Biology*., Vol. 56, 41-71 (2005).

[19] Y. Paopun, P. Thanomchat, S. Seraphin, Primatic and needle-like crystals of calcium oxalate in Thai herbs, *Siriraj Medical Journal*., Vol. 68 (Suppl 1) (3), S34-S35 (2016).

[20] S.C. Noonan, G.P. Savage, Oxalate content of foods and its effect on humans, *Asia Pacific Journal of Clinical Nutrition*., Vol. 8(1), 64-74 (1999)