

Crystalline Calcium Oxalate in *Pandanus Odoratissimus*

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Abstract— *Pandanus odoratissimus* (*P. odoratissimus*) belongs to the *Pandanus* genus from Pandanaceae. The research involving this coastal species was reviewed and compiled. From the literature, the pharmacognostical and phytochemical investigations including chromatographic analysis were carried out on the leaves extract. Two phenolic compounds, four lignan type compounds plus a benzofuran derivative were published as the constituents of this plant. In addition, the biological work was also conducted. This included the investigation of antioxidative, antimicrobial, anti-inflammatory and free radical scavenging activities of the fractions. In this paper, the microscopical study utilising Confocal Laser Scanning Microscopy (CLSM) and Scanning Electron Microscopy (SEM) is presented. From the photomicrographs, both fruits and leaves of *P. odoratissimus* contained singly scattered and bundles of calcium oxalate raphides, observed as acicular crystals, having the shape of narrow, long and pointed needles. In addition, another morphological type of calcium oxalate was also observed. The styloids were detected as rectangular crystals having extensions around their four edges. Previously, the styloids were found in a different *Pandanus* species, *P. gasicus* and *Freycinetia*, another genus of Pandanaceae. In summary, this is the second report of the simultaneous occurrences of two crystalline shapes from a *Pandanus*.

Keywords— *Pandanus*, calcium oxalate, microscopy

I. INTRODUCTION

The Pandanaceae family comprises of three genera; *Pandanus*, *Freycinetia* and *Sararanga*, with *Pandanus* being the biggest. This genus contains approximately 600 species, including *Pandanus odoratissimus* (*P. odoratissimus*) [1]. The research involving this coastal species was reviewed and compiled. From the literatures, the pharmacognostical and phytochemical investigations as well as chromatographic analysis were carried out on the leaves extracts [2, 3]. Two phenolic compounds, four lignan type compounds plus a benzofuran derivative were published as the constituents of this plant [4]. In addition, the biological work was also conducted. This included the investigation of antioxidative [4], antimicrobial [5], anti-inflammatory [6] and free radical scavenging activities of the fractions [7].

Fig. 1 and 2 show *P. odoratissimus*' plant parts, comprising of the fruit head, the fruit key and the thorny leaves. Similar to *P. tectorius*, the fruit head or bunch consists of an aggregate of many tightly bunched wedge-shaped phalanges or drupes.

These drupes are also called keys, as removing a key will allow the rest to come apart easily [8]. The shape of the fruit head in this research looks ellipsoid, with overall

dimensions of 13 cm long by 10 cm diameter, while tightening 30 keys (Fig. 1).



Fig. 1. The fruit head and keys of *Pandanus odoratissimus*.



Fig. 2. Note the thorny leaves of *Pandanus odoratissimus*.

The individual keys are narrowly oblong to ovoid and 5 cm long by 3 cm wide, at widest point. The outer skin (epicarp or exocarp) is very tough and hard. Meanwhile, the internal tissue surrounding the seeds (endocarp) is brown and hard, too.

In this paper, the microscopical study of *P. odoratissimus* utilising Confocal Laser Scanning Microscopy (CLSM) and Scanning Electron Microscopy (SEM) is presented. As applied to species from Cactaceae [9] and Cannaceae [10] families, the identification of calcium oxalate biomineral could be a useful tool for plant identification and chemotaxonomy. It is anticipated that the photomicrographs of *Pandanus*' specimen could display crystalline calcium oxalate in the needle forms called raphides [11, 12].

II. METHODOLOGY

The wild *Pandanus* sample for this work was collected from its coastal habitat. Fresh materials were taken from Port Dickson, Negeri Sembilan, Malaysia. Experiments were performed with specimens collected before this study and identified by the author (SAA) in January 2007. The plant parts were carefully cleaned to remove any possible external contamination [10]. They were dried, deposited at the faculty and were given voucher specimen number (POPD200607). The *Pandanus* were kept at the Faculty of Pharmacy, UiTM Puncak Alam, for future reference.

The crystals were extracted based on methodologies, adapted from [11, 12]. The dried leaves were firstly cut into smaller pieces. Meanwhile, the epicarp of *P. odoratissimus* was sliced with a single-edge razor blade (industrial-type of carbon steel). The samples were macerated in 70% ethanol. Then, the ethanolic suspensions were agitated. The aliquots were transferred to the microscope slides and allowed to air dry. The specimens were examined by using Leica Microsystems CLSM (laser beam = 532 nm), complemented with JEOL JSM-6701F SEM. All SEM analysis was carried out using an acceleration voltage of 5 kV.

III. RESULTS & DISCUSSION

From the CLSM photomicrograph in Fig. 3, the leaves of *P. odoratissimus* contained a single calcium oxalate raphide. It was observed as an acicular crystal, having the shape of a narrow, long and pointed needle. The crystal could also stack together, forming a bundle of needles (Fig. 4).

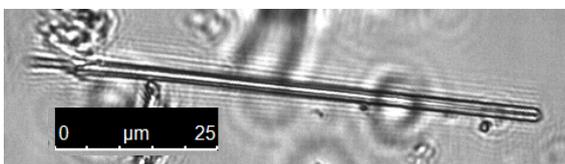


Fig. 3. The photomicrograph from *P. odoratissimus* viewed from CLSM (bar = 25 μm), showing a needle from the leaf sample.

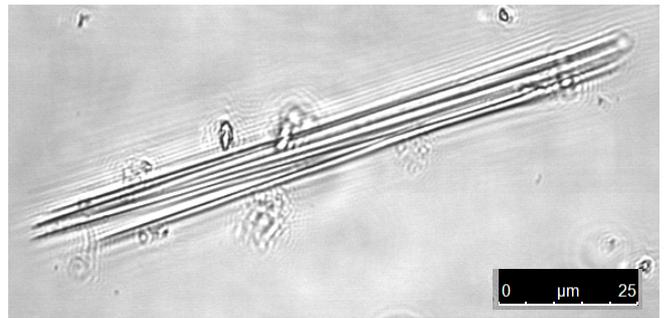


Fig. 4. The photomicrograph from *P. odoratissimus* viewed from CLSM (bar = 25 μm), showing a bundle of needles from the fruit sample.

In addition, another morphological type of calcium oxalate was also observed in the fruit of *P. odoratissimus*. The styloids were detected as rectangular crystals, some having extensions around their four edges (Fig. 5 and 6). Previously, the styloids were found in a different *Pandanus* species, *P. gasicus* and *Freycinetia* [13], another genus of Pandanaceae. Within the interior of a plant cell, the styloids tend to be solitary [13], unlike the needles that can be found in bundles.

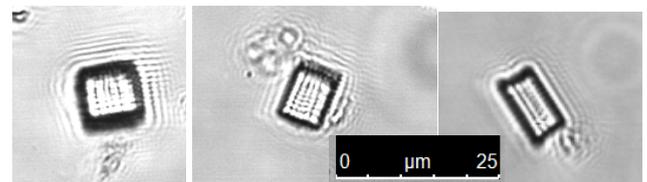


Fig. 5 The photomicrograph from *P. odoratissimus* viewed from CLSM (bar = 25 μm), showing the styloids from the fruit sample.

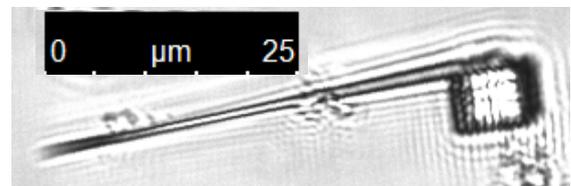
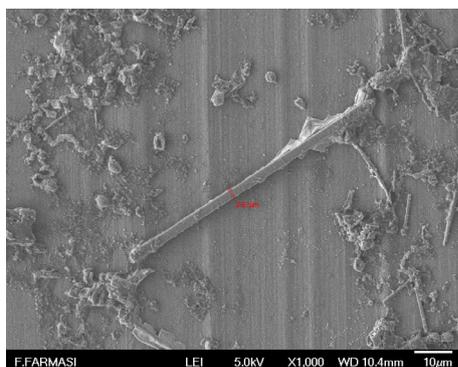
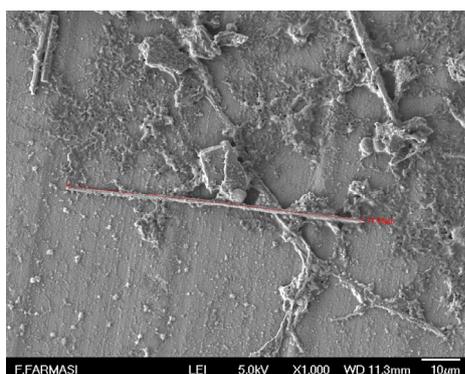


Fig. 6. The photomicrograph from *P. odoratissimus* viewed from CLSM (bar = 25 μm), showing both styloid and needle types of crystal from the fruit.

The presence of the crystalline calcium oxalate is also supported by the images, obtained from SEM (Fig. 7). The photomicrographs of *P. odoratissimus*' leaves and fruit showed a needle type of crystal. The mid width was 2.57 μm as seen in Fig. 7(a). Meanwhile, the length of the needle was found to be 77.43 μm . The summary of three identified morphological features of calcium oxalate from *P. odoratissimus* was shown in Figure 8.



(a)



(b)

Fig. 7. The photomicrograph from *P. odoratissimus* viewed from SEM (1000x objectives, bar = 10 µm), showing a needle type of crystal from the (a) leaf, with a diameter of 2.57 µm. Meanwhile, (b) the fruit sample gave a needle with a length of 77.43 µm, next to a styloid.

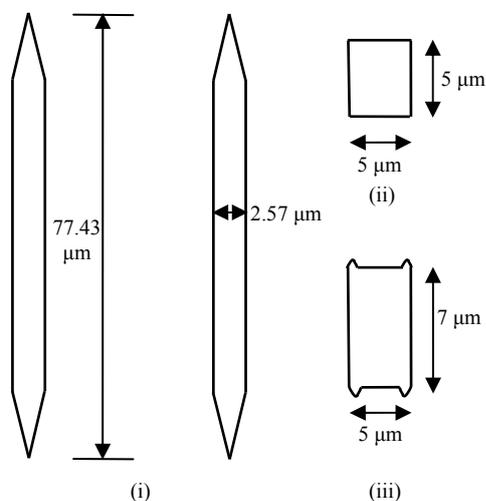


Fig. 8. Diagram of the general summary of three identified morphological features of calcium oxalate from *P. odoratissimus*, visible by CLSM and SEM, showing (i) two pointed terminations of a needle raphide, (ii) a rectangular styloid and (iii) a styloid with extensions around its four edges.

IV. CONCLUSIONS

In conclusion, the calcium oxalate needle raphides and styloids were both observed in the leaves and fruit of *Pandanus odoratissimus* by utilising micro imaging applications. Therefore, this is the second report of the simultaneous occurrences of two crystalline calcium oxalate shapes from a *Pandanus*.

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