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Styloid-Shaped Calcium Oxalate Crystals from *Piper* Species

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Abstract

This paper deals with the microscopic observation of the *Piper* species (Piperaceae family). This plant is also referred as pepper, which is widely used as a spice. From the literature search, the leaf and stem parts from a number of *Piper* were submitted to analyses under optical and scanning electron microscopy (SEM). Three crystalline categories, which consist of pure calcium, oxalate, mixtures of oxalates and sulfates, and combinations of oxalates, sulfates and silica; were viewed. These crystal rosettes and silicified crystals were described as unpublished reports. Therefore, a research is carried out in order to provide more biochemical understandings to the complex taxa of these plants. The alcoholic extract of the powdered fruits from both black and white peppers were investigated. It is aimed to record the morphological characteristics of the calcium crystals via SEM. From the photomicrographs, it was found that black pepper sample contained small intracellular crystals of calcium oxalate, particularly the styloids. Other documented Piper's crystal formations such as the cuneiform or the wedge-shaped, tabular, cubic, prismatic, raphides and druses, are absent. Unfortunately, the crystal from white pepper sample was not observed. It is concluded that the shape and size of the calcium oxalate crystals in this plant could be used in the pharmacognostical evaluation of the *Piper* species.

Keywords: oxalate, *Piper*, styloid

Introduction

The *Piper* genus from the Piperaceae family consists of about two thousand plant species. This plant is also referred as pepper, which is widely used as a spice. Both white and black pepper comes from the fruit of *P. nigrum L.* They are widely used as spices in food. In facts, Malaysia is one of the countries that actively produce and export black and white pepper to all over the world, earning 77 million USD from pepper export in 2012 (International Pepper Community, 2014). The methods of harvesting the *Piper* fruit could distinguish between the white and black pepper. The black pepper is obtained from fully-grown unripe fruits of *P. nigrum L.*, which are still green in color. It will then be sun dried, which will change the color to brownish-black. On the other hand, the white pepper is obtained from the orange to red ripe fruits. It will then be soaked in water. The skin and outer part of mesocarp are softened and then are removed mechanically. The remaining pale-fawn or grayish core will be sun dried which produce the white pepper (Steinhaus and Schieberle, 2005).

Ferreira Silva *et al*. (2014) analysed the leaf and stem parts from a number of *Piper* species. Those materials were monitored, both under optical or light microscopy and under scanning electron microscopy (SEM). Three classes of crystals, which consist of pure calcium oxalate, mixtures of oxalates and sulfates, and combinations of oxalates, sulfates and silica; were observed. These crystal rosettes and silicified crystals were described as unpublished reports. Therefore, a research is carried out in order to provide more biochemical understandings to the complex taxa of these plants. A phytochemical study was launched, in order to reveal the constituents of interest, which are the bisalkaloids (Figure 1-2), due to the variety in the stereochemistry (Tsukamoto *et al*. 2002a; 2002b).

Methodology

The research materials consisted of two fruit samples. Both black (Indian *Piper*) and white pepper (local *Piper*), were purchased from retail. The dried, powdered fruit were dissolved in absolute ethanol. Parallel to a phytochemical investigation, the alcoholic

extracts of the powdered fruits from both black and white peppers, respectively from abroad and local *Piper* samples, were investigated. It is aimed to record any shape variations of the calcium crystals via SEM. The samples were later subjected to JEOL JSM-6701F VPSEM (pressure = 120 Pascal, 5.00 kV).

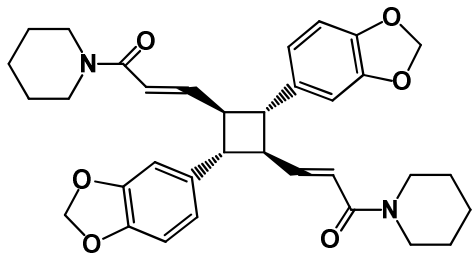

Figure 1. Dipiperamide A, the bisalkaloid from *Piper* species.

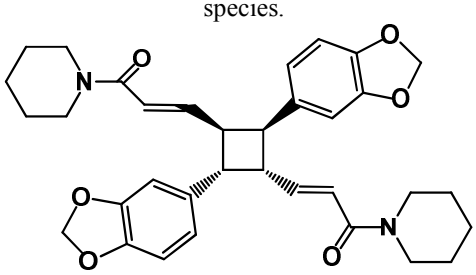

Figure 2. Dipiperamide B, the bisalkaloid from *Piper* species.

Results and Discussion

The choice of solvent extraction of the *Piper* fruits may affect the microscopic findings. This is due to the presence of alkaloids in alcoholic extract, as commented by Patel *et al*. (2011). From the photomicrograph (Figure 3), it was found that black pepper sample contained small intracellular crystals of calcium oxalate, particularly the styloids, measuring in size of 1.4 x 15.0 µm. Other documented *Piper*'s crystal formations such as the cuneiform or the wedge-shaped, tabular, cubic, prismatic, raphides and druses, are absent. Unfortunately, the crystal from white pepper sample was not observed during the experiment.

Conclusions

The SEM method is deemed to be useful in detecting the authenticity of medicinally useful *Piper* plant, as performed on *Piper betle* (Periyanayagam *et al*. 2012) and other genera of Piperaceae (Horner *et al*. 2012). It is then, concluded that the shape and size of the calcium oxalate crystals in this *Piper* sample could also be used in its pharmacognostical evaluation.

Figure 3. The photomicrograph of the black pepper sample, containing crystals of calcium oxalate, particularly the styloids (scale bar = 10 µm).

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