Microscopic Characterization as a Tool for Separation of *Stemona* Groups

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**ABSTRACT**

**Introduction:** *Stemona* plants have been traditionally used as an insecticide, scabicide and pediculocide, and for the treatment of skin and respiratory diseases. *Stemona* can be separated into two groups according to their morphological characters and bioactive components i.e. stichoneurine and protostemonine groups. Protostemonine group contains alkaloids that possess potent insecticidal activity while stichoneurine group accumulates alkaloids with antitussive activity. In Thailand, a vernacular name “Non Tai Yak” refers to the roots of different species of *Stemona*, making it confusing to discern different species. The purposes of this study are to investigate the microscopic characteristics of the roots of seven species of *Stemona* growing in Thailand and to distinguish and identify these groups of *Stemona*.

**Methods:** Cross-sectional histology of fresh root samples and powdered drug characteristics of 7 species of *Stemona* were studied under a microscope.

**Results:** The roots of *Stemona* in the stichoneurine group (*S. tuberosa* and *S. phyllantha*) contained a non-lignified large pith while the roots of protostemonine group (*S. burkillii*, *S. cochinchinensis*, *S. collinsiae*, *S. curtisii* and *S. kerrii*) had a small lignified one. The powder of stichoneurine group contained numerous thin-walled parenchyma, but only few thick-walled parenchyma and lignified fibers and vessels were present. In contrast, thick-walled parenchyma and lignified fibers and vessels were frequently found in the powdered roots of protostemonine *Stemona*. These characteristics could be used to discern between *Stemona* in the stichoneurine and protostemonine groups.

**Conclusions:** The microscopic characterizations can be used as a primary tool to categorize and separate 2 main *Stemona* groups.

**Key words:** Non Tai Yak, protostemonine, *Stemona*, Stemonaceae, stichoneurine

**INTRODUCTION**

Since ancient time, *Stemona* plants have been traditionally used as an insecticide, scabicide, pediculocide; used for treating skin and respiratory diseases, and also for killing head lice.[1-3] “Non Tai Yak” is a Thai vernacular name that refers to various species of *Stemona* in Thailand[4] and some other plants such as *Asparagus* sp. of the family Asparagaceae and *Clitoria* sp. of the family Leguminosae in some locations.[1] This plant has been used to protect plants against insect attack, the infection of fermented fish “Pla Raa” or fermented shrimp “Ka Pi” from housefly larvae. The inconsistency when providing and using the proper *Stemona* plant materials has led to the confusion in the scientific identification and in agricultural and pharmaceutical uses.[1] Recent taxonomic revision of the family Stemonaceae indicates that *Stemona* in Thailand comprises of 11 known species i.e. *S. aphylla* Craib, *S. burkillii* Prain, *S. cochinchinensis* Gagnep., *S. collinsiae* Craib, *S. curtisii* Hk. F., *S. involuta* Inthachub, *S. kerrii* Craib, *S. phyllantha* Gagnep., *S. pierrei* Gagnep., *S. rupestris* Inthachub and *S. tuberosa* Lour.[5] They can be separated into two main groups according to their morphological characters and bioactive component accumulation i.e. stichoneurine or tuberosa group and protostemonine or non-tuberosa group.[4] Stichoneurine group comprises of *S. tuberosa* and *S. phyllantha* and they are different from other *Stemona* plants because of their large and thick tuberous roots, large perianths and scented flowers.[5,6] However, the three dominant species of *Stemona* (*S. tuberosa*, *S. collinsiae* and *S. curtisii*) and some other species of *Stemona* in Thailand are still called “Non Tai Yak”, making it confusing and causing misuses of these plants.

Phytochemical investigations of *Stemona* species revealed the presence of alkaloids, stilbenoids and chromenols. The
alkaloids display a remarkable accumulation trend in *Stemona* species.[2,7] *Stemona* alkaloids constitute a unique chemical feature of the family Stemonaceae and cannot be detected in any other plant families thus far.[1,2] Classification of *Stemona* alkaloids based on biosynthetic considerations confirms three skeleton types i.e. stichoneurine- (tubero-stemonine-), protostemonine-, and croomine-type alkaloids.[10] Stichoneurine group contains stichoneurine- and croomine-type alkaloids while protostemonine group contains protostemonine-type alkaloids.[1,7] Potent insect toxicity of *Stemona* plants is attributed to the derivatives of protostemonine-type alkaloids, especially the stemonofoline derivatives,[8,9] whereas stichoneurine-type alkaloids possess only a remarkable insect repellance.[8] In contrast, stichoneurine- and croomine-type alkaloids were reported to be associated with antitussive activity.[10-14]

Herbal extracts from various *Stemona* plants have been used for over a century. *Stemona* has been developed into commercial products for bioinsecticide or antitussive drug. Since the variation of authentic *Stemona* raw materials affects their promised biological properties, the confusion stems out when *Stemona* roots as well as their powdered drug are used. Identification of these plants via morphological characteristics is limited by the presence of flowers and it cannot be determined in the form of powdered drug. Authentication of *Stemona* species using DNA-based techniques is effective but it is also expensive and time-consuming. This study presented a simpler method to identify and distinguish *Stemona* in stichoneurine or tuberosa group from protostemonine or non-tuberosa group by exploiting the dissimilarities between their microscopic characteristics.

**MATERIALS AND METHODS**

**Plant materials**

The samples of 7 *Stemona* species (*S. burkillii*, *S. cochinchinensis*, *S. collinsiae*, *S. curtissii*, *S. kerrii*, *S. phyllantha* and *S. tuberosa*) were collected from various locations in Thailand. Each sample was cultivated at National Corn and Sorghum Research Center, Nakorn-Ratchasima province in the North-East of Thailand. All root samples at the age of 4 year old were collected in August, 2009. The plant specimens were identified by Dr. Vichien Keeratinijakal, Kasetsart University, and the voucher specimens (VKS001-VKS007) were deposited at Pharmacognosy Department, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

Each fresh sample was dried at 60°C then powdered by an electronic mill. The powder was passed through a sieve no. 60 to yield fine powder. The powder of each sample was separately kept in a tightly-closed vial until used. Other portions of fresh root were used for histological inspection.

**Microscopic methods**

Cross-sectional histology of fresh root samples and powdered drug of the seven species of *Stemona* were examined under a microscope (Olympus, Japan) using mounting reagents. The characteristic tissues were photographed using a camera and drawn using a camera lucida (Olympus, Japan).

**RESULTS**

The tuberous roots of *Stemona* in stichoneurine group (*S. tuberosa* and *S. phyllantha*) were large and thick, 10-50 cm long, pale yellowish-brown in color, while the protostemonine group (*S. burkillii*, *S. cochinchinensis*, *S. collinsiae*, *S. curtissii* and *S. kerrii*) had slender pale yellowish-brown roots with varied length (4-50 cm). Cross-sectional histological characteristics of the fresh root samples of *Stemona* showed that the stichoneurine group had a larger pith compared to those in the protostemonine group. After applying aniline sulfate solution, the pith of the protostemonine *Stemona* turned yellow, making it a lignified pith, while the stichoneurine *Stemona* roots contained a non-lignified pith (Figure 1).

![Figure 1: Cross-sectional histology of fresh root samples of various Stemona species applied with aniline sulfate showing characteristic features: (A) a large non-lignified pith of stichoneurine group, or tuberosa (B) a small lignified pith of protostemonine or non-tuberosa group.](image-url)

Powders of all *Stemona* species appeared as creamish-white to creamish-yellow with a faint distinct odor and a sweet and bitter taste. The diagnostic characteristics of the powders of both groups of *Stemona* are shown in Figure 2 and are compared in Table 1. These characteristics are as follows.

1. Abundant starch granules that are simple, small, spherical to ovoid, or compound with two, three, four or occasionally up to six components.
2. Abundant parenchyma from the cortex and stele. The cells are fairly large and vary from rounded to elongated rectangular in outline with thin wall. The cells are almost filled with starch granules.
3. The lignified vessels of xylem occur in groups of interlocking cells. The vessels contain numerous bordered pits.
4. Long fibers, fragmented.
5. Thick-walled parenchyma of the xylem and medullary ray.

**DISCUSSION**

*Stemona* in stichoneurine group (*S. tuberosa* and *S. phyllantha*) had larger, thicker and longer tuberous roots than the protostemonine group (*S. burkillii*, *S. cochinchinensis*, *S. collinsiae*, *S. curtisii* and *S. kerrii*). The colors of the roots of both groups were the same pale yellowish-brown.

Cross-sectional histology of the roots of stichoneurine *Stemona* showed a non-lignified large pith containing numerous thin-walled parenchyma cells while the protostemonine *Stemona* roots had a small lignified pith with less abundant thin-walled parenchyma cells. The powdered drugs of the roots of stichoneurine group contained several thin-walled parenchyma but only few thick-walled parenchyma cells were found. The thick-walled parenchyma cells were frequently spotted in the powdered roots of protostemonine *Stemona* but rarely found in the root powders of the stichoneurine group. Numerous simple

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**TABLE 1: Comparison of powdered drug characteristics of the roots of *Stemona* spp. in stichoneurine and protostemonine groups**

<table>
<thead>
<tr>
<th><em>Stemona</em> species</th>
<th>Parenchyma</th>
<th>Amount found</th>
<th>Starch granules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vessels</td>
<td>Fibers</td>
</tr>
<tr>
<td><strong>Stichoneurine gr.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosa</em></td>
<td>numerous thin-wall, rarely thick-wall</td>
<td>few</td>
<td>few</td>
</tr>
<tr>
<td><em>S. phyllantha</em></td>
<td></td>
<td>small, numerous</td>
<td></td>
</tr>
<tr>
<td><strong>Protostemonine gr.</strong></td>
<td>moderate thin-wall and thick-wall</td>
<td>moderate</td>
<td>moderate</td>
</tr>
<tr>
<td><em>S. burkillii</em></td>
<td></td>
<td>small, numerous</td>
<td></td>
</tr>
<tr>
<td><em>S. cochinchinensis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. collinsiae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. curtisii</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>S. kerrii</em></td>
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</table>
and compound types of small starch granules were observed in both groups of *Stemona*.

**CONCLUSION**

Cross-sectional histology and powdered drug characteristics of the roots of various *Stemona* species growing in Thailand verified that the roots of the stichoneurine or tuberosa group had non-lignified larger pith containing numerous thin-walled parenchyma cells. The protostemonine *Stemona* roots had smaller lignified pith and were less abundant in thin-walled parenchyma. Thick-walled parenchyma cells were frequently found in the protostemonine *Stemona* roots, but rarely found in the roots of the stichoneurine group. The lignified fibers and vessels were frequently found in the root powders of protostemonine *Stemona* but rarely found in the stichoneurine group. These microscopic characterizations could be used as a primary tool to clearly identify groups of *Stemona*, and it could confirm their macroscopic characteristics. However, these characteristics could not distinguish each *Stemona* species. This is the first report on the utilization of microscopic characterizations of *Stemona* groups, particularly the ones growing in Thailand. The information will be of benefit to the correct identification of actual sources of *Stemona* for agricultural and pharmaceutical purposes.

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