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# Morphological Similarities and Differences between the Two Varieties of Cat's Whiskers (*Orthosiphon stamineus* Benth.) grown in Malaysia

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**Abstract:** The purple variety and the white variety of cat's whiskers (*Orthosiphon stamineus* Benth), a medicinal plant with diuretic and anti-diabetic properties, could be mistakenly considered as the same type if not carefully observed because most of their external morphological characters were similar. Present morphological studies showed that these two varieties could be identified by their floral and leaf morphology. The purple variety produced ovate shape leaves with acute apex, truncate base and purple veination. Light yellowish spots were scattered unevenly on both the adaxial and abaxial surfaces of the leaves. The white variety produced rhomboid shaped leaves without any colored spots with acuminate apex, obtuse base and light green veination. The two most distinct floral characters were the colors of corolla and calyx. The purple variety produced light purple tint at the lobes of the two-lipped corolla with maroon calyx while the petals of the white variety was totally white without purple tint at the edge of the petal lobes and green calyx. The micro-structures of leaves, anthers, stigma and pollen grains were similar for both varieties.

**Key words:** Floral morphology, leaf morphology, micro-structures, variety, corolla color

## INTRODUCTION

The genus name Orthosiphon was coined from two Latin words, Iorthos and siphon. The word Iorthos referred to straight while siphon meant tube-like or cylindrical. These two words actually referred to the straight tube-like flowers that were produced by the *Orthosiphon* spp. and this was considered as one of the main characteristics of the Labiatae or Lamiaceae family. *Orthosiphon stamineus*, one of the valuable medicinal plants of the tropics, was also based on this floral characteristic to be placed in the same family. In Malaysia, *O. stamineus* was known as 'misai kucing' which literally meant cat's whiskers, a common name used by the local communities in Europe (Burkill, 1966). *O. stamineus* had been used traditionally for the treatment of renal stones, arterioclerosis, nephritis, diabetes and rheumatism because of its anti-hypertensive, anti-inflammatory and diuretic properties (Jagarath and Ng, 2000). Various bio-active compounds were identified and isolated from this plant which contributed to these medicinal properties. Among the identified compounds were 5-hydroxy-6,7,3,4-tetramethoxyflavone and 4,5,6,7-tetramethoxyflavone (Arebi *et al*., 1993), orthosiphon, terpenoids, inositol, myo-inositol, carotenoids and saponine (Jagarath and Ng, 2000). In Malaysia, *O. staminues* was believed to consist of two varieties based on floral color which was not very distinct if not carefully observed. One of the varieties produced white flowers while the other variety produced corolla with light purplish tint at the edges of the petal lobes, hence named as the white and purple varieties, respectively. The purple variety was reported to possess higher bio-active compounds than the white variety (Lee, 2004). However, the purplish color of the corolla was so light in shade until at time it was difficult to differentiate between the two varieties. The aims of the present study were therefore to compare the similarities and differences in the vegetative and reproductive morphologies of the two varieties of *O. stamineus* and to establish a system to identify these two varieties.

## MATERIALS AND METHODS

The two varieties of *O. stamineus* were collected from two locations: the herbal garden of School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia and the Agricultural Research Station, Relau, Penang, Malaysia. The macro-morphology and micro-morphology studies were carried out on the two varieties from July 2004 until February 2005.

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The macro-morphological study included the morphology study of the leaves, stem and roots and the floral inflorescence. Ten fully expanded leaves were randomly and freshly collected from each plant for observation and measurement. Thirty plants were used for each variety in the morphological study. The shape, size, color and petiole length were the parameters used for the morphological study of the leaves while the internode length was determined for the stem. While the type of root system was used to determine the root morphology. All quantitative measurements were carried out with the aid of a measuring tape and a vernier scale (Kern Germany).

The floral structures for both varieties were compared based on the inflorescence type, flower color, the size of petal, stamens, pistil and calyx. The shape, color and size of fruits and seeds were also determined. All the measurements were determined using the vernier scale (Kern Germany). Thirty plants of each variety were used for each morphological study and 10 samples were taken randomly from each plant. The data was analyzed using student t-test at $p = 0.05$ to determine the similarities and differences between the two varieties.

The micro-structures of the leaves, the reproductive structures and the pollen grains were determined with the aids of scanning electron microscope (Leo Supra 50 BP field emission SEM). Standard procedures were followed for Scanning Electron Microscope (SEM) studies for the selected samples. The samples fixed in Formalin-Acetic acid-Alcohol (FAA) solution were infiltrated with liquid $CO_2$ and dried in a critical-point drier, sputter-coated with gold and viewed with the SEM. The size of stomata and trichomes was based on their length. The size of the epidermal glands was measured at the widest point while the size of the pollen grains was determined based on the diameter.

## RESULTS AND DISCUSSION

Both the white and purple varieties of *O. stamineus* produced petiolated dark green leaves. Two leaves were placed opposite each other at each node on the stem. However, there were some distinct differences on the leaves that could be used to differentiate the two varieties. The leaves of the purple variety were ovate in shape with acute apex, truncate base and purple veination and light yellowish spots could be seen scattered unevenly on both the adaxial and abaxial surfaces of the leaves. The white variety produced rhomboid shaped with acuminate apex, obtuse base leaves but without any colored spots and common light green veination (Fig. 1). Measurement results showed that the leaf length of the white variety (5.7 cm) was longer than the purple variety (4.5 cm) but the purple variety produced broader leaves (2.7 cm) than the white variety (2.5 cm). The leaf length and leaf width ratio for the white variety (2:1) indicated rhomboid shape while that of purple variety (3:2) indicated ovate shape (Hsuan, 1978). The leaf of the white variety also possessed longer petiole than the purple variety (Table 1).

Table 1: The external morphology of leaves and stem of the purple and white varieties of *Orthosiphon stamineus* Benth

| Characteristics | White variety | Purple variety |
|---|---|---|
| Leaf length (cm) | 5.7±0.1a | 4.5±0.1b |
| Leaf width (cm) | 2.5± 0.1a | 2.7±0.1a |
| Leaf length : leaf width | 2 : 1 | 3 : 2 |
| Petiole length (mm) | 7.1±0.2a | 6.2±0.2b |
| Internode length (cm) | 4.3±0.2a | 5.1±0.2b |

Mean values for each parameter between the two varieties followed by the same alphabets are not significantly different at $p = 0.05$, student t-test

Table 2: Comparison of the floral structures between the Purple and White varieties of *Orthosiphon stamineus*

| Parameters | Purple variety | White variety |
|---|---|---|
| Corolla color | Light purple tint at edge of white petal lobe | White petal |
| Calyx color | Maroon | Green |
| Length of corolla tube (cm) | 1.9±0.2a | 2.2±0.1b |
| Length of upper lip of corolla (cm) | 0.8±0.1a | 0.8±0.1a |
| Width of upper lip of corolla (cm) | 0.8±0.1a | 0.8±0.1a |
| Length of lower lip of corolla (cm) | 0.9±0.2a | 0.9±0.1a |
| Width of lower lip of corolla (cm) | 0.4±0.1a | 0.2±0.1b |
| Length of long stamen (cm) | 4.2±0.4a | 4.7±0.2b |
| Length of short stamen (cm) | 4.0±0a | 4.4±0.1b |
| Pistil length (cm) | 5.6±0.4a | 6.4±0.4b |
| Calyx length (cm) | 0.6±0.1a | 0.8±0b |

Mean values for each parameter between the two varieties followed by the same alphabets are not significantly different at $p = 0.05$, student t-test

External morphology of stem was found to be similar for the white and purple variety of *O. stamineus* except of the stem color. Both possessed square shaped stem but the stem is green in color for the white variety but greenish maroon for the purple variety. Another difference was that the purple variety had longer internode than the white variety (Table 1). Both varieties produced well-developed fibrous root system.

With respect to the morphology of the reproduction structures, there were similarities found in both of the *O. stamineus* varieties. Both varieties produced racemose verticilaster type of inflorescence with six flowers growing in whorls along the floral axis. The lower flowers were older and bloomed earlier than the upper ones (Fig. 2). This inflorescence type was one of the main characteristics that categorized *O. stamineus* in the Labiatae family (Hickey and King, 1997). Each flower was made up of two-lipped (bilabiate) gamopetalous corollas where the limb of the corolla divided into two lips, the upper and the lower, with the mouth gaping wide open. The upper lip consisted of four lobes fused together but

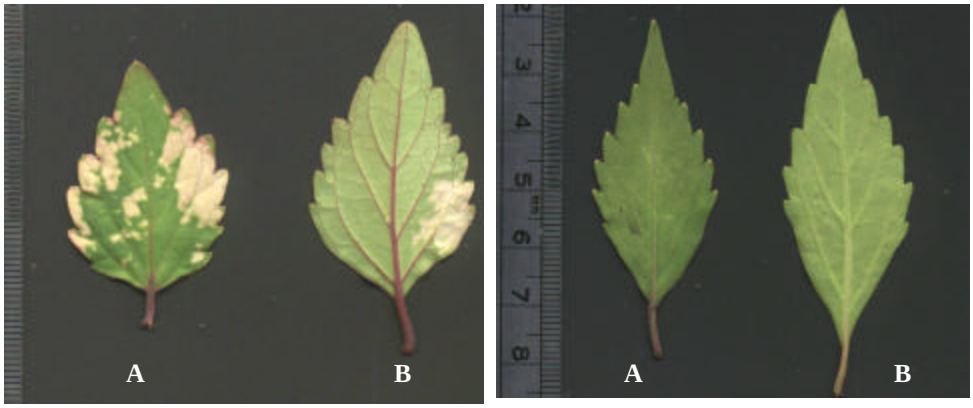


Fig. 1: Leaf morphology of purple variety (Left), ovate shape with light yellowish spots on the adaxial (A) and abaxial (B) surface and purple veination; white variety (right), romboid shape with acute apex and green veination obvious on the abaxial surface (B)

Fig. 2: Both varieties of *O. stamineus* produced racemose verticilaster type of inflorescence and the lower flowers were older and bloomed earlier than the upper ones

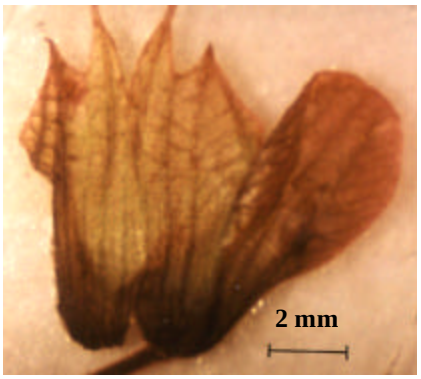


Fig. 3: The irregular gamosepalous calyx of *O. stamineus* (Purple variety) that made up of five sepals. Tthe biggest sepal was ovate shape. On each side of the big sepals, the sepal had acuminate apex while the remainder two sepals were joined to each other producing long and sharp apex

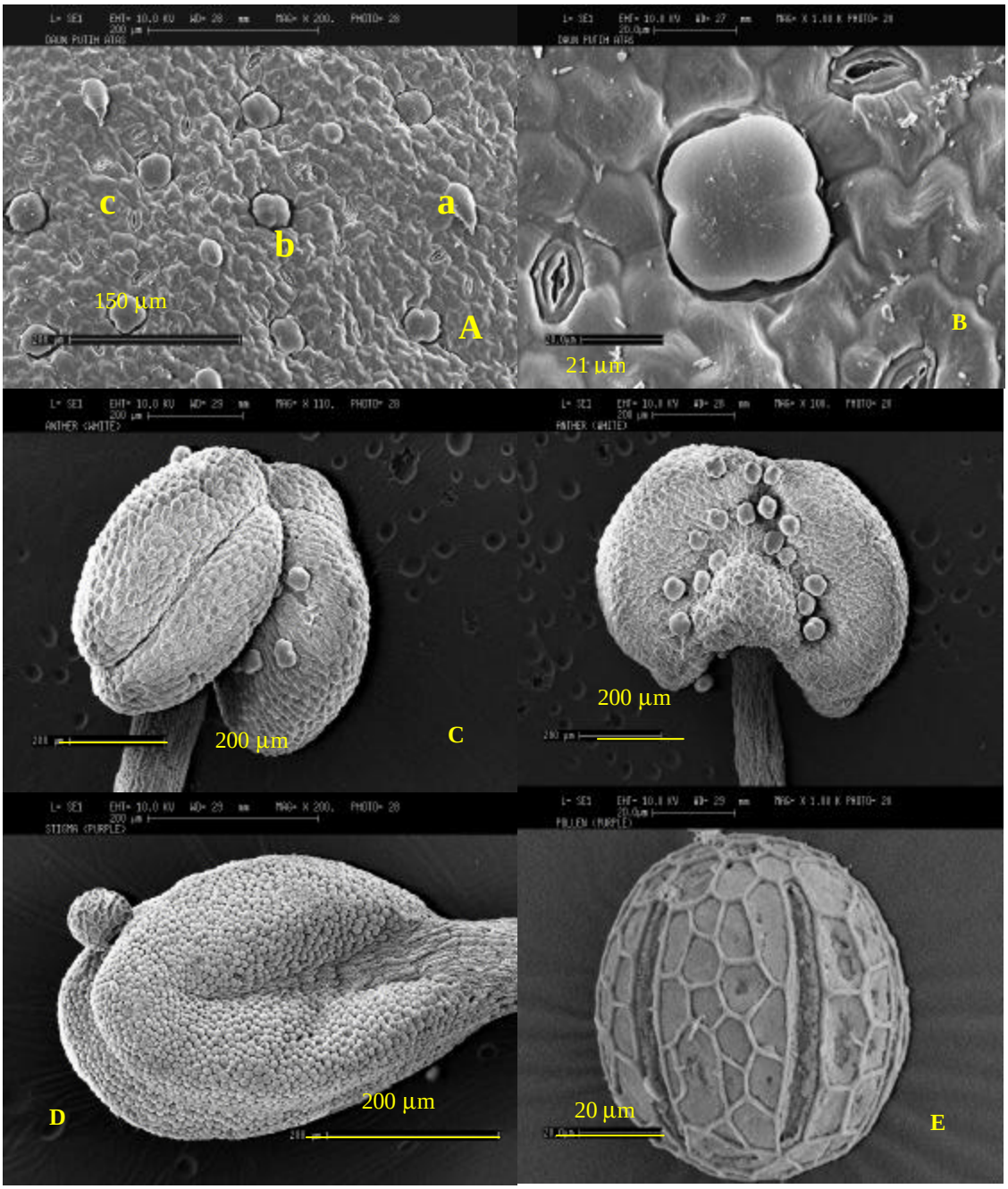


Fig. 4: The distribution of trichomes (a), epidermal glands (b) and stomata (c) on the abaxial surface of white variety of *O.stamineus* (A); The four-cell epidermal gland and stoma on the leaf lamina (B); Two lobes and four loculi of anther with globular glands on the connective part (C); Two lobes stigma (D); Spherical pollen grains with two of the six furrow like colpus and netted muri (E)

Table 3: Distribution of stomata on the adaxial and abaxial of leaves for the purple and white varieties of *O. stamineus*

| | | *Orthosiphon stamineus* | |
|---|---|---|---|
| Leaf surface | | Purple variety | White variety |
| Number of stomata per mm$^2$ | Adaxial | 56.4±4.7 | 55.1±3.9 |
| | Abaxial | 249.7±2.4 | 240.6±10.2 |
| Stomata size (μm) | Adaxial | 14.1±1.7 | 13.9±1.6 |
| | Abaxial | 14.5±1.2 | 15.1±4.0 |
| Size of epidermal gland (μm) | Adaxial | 28.3±2.7 | 30.8±2.8 |
| | Abaxial | 26.5±2.2 | 34.2±2.6 |

free at the margin while the lower lip was a one-lobed petal. Van der Pijl (1973) reported that small butterflies were responsible for pollination in *O. stamineus*. The bilabiate corolla could help in the pollination process. The lower lip acted as the landing place while the upper lip provided a restricted space for movement of the small insects and hence most of the pollen grains from the anthers were deposited onto the insect's body. The pollen grains would then be deposited on the stigma of the same flower or another flower of the inflorescence or other inflorescence during the insect's next visit.

Each flower possessed irregular gamosepalous calyx that was made up of five sepals. One of the sepals, the biggest was ovate shape. On each side of the big sepal, the sepals had acuminate apex while the remainder two sepals were joined to each other producing long and sharp apex (Fig. 3). Each flower consisted of four stamens, two long and two short, attached to the dorsal part of the corolla tube. One pistil that made up of an ovary, a style and a stigma was found in each flower.

However, differences were found in term of color and sizes on the floral structures. The obvious difference was the corolla color. In fact the varieties were named based on the corolla color. The purple variety produced light purple color at the lobes of the two-lipped white corolla while the petals of the white variety was totally white without purple tint at the edge. The purple tint could be due to the presence of antocyanin pigment accumulated in the cell sap (Stern, 2000). Tatsuzawa *et al.* (2005) recently confirmed that the purple hue in Orchidaceae was also due to the presence of anthocyanins. The calyx color was also different for both varieties. The purple variety produced maroon color calyx while the white variety had green calyx.

Only the length of the upper and lower lips of the corolla was similar for both varieties, the sizes for the other floral structures were found to be different. For instance, the white variety was having a longer corolla and calyx tubes than the purple variety. The white variety produced narrower lower lips of the corolla. The purple variety produced shorter stamens and pistil compared to the white variety (Table 2).

Seed morphology was considered as one of the important characteristic for identification of plants due to the stable and distinct seed surface features (Gunn, 1972). The seeds of the two varieties of *O. stamineus* were oval shape with hard and rough surface testa. Each fruit pod produced four seeds. The only different was the color of the mature fruits. The purple variety produced purplish red fruits while the fruit of the white variety was greenish red in color.

Scanning Electron microscopy studies indicated that the micro-structures on the leaves, anther, stigma and pollen grains for both varieties of *O. stamineus* were similar. Short pointed trichomes, epidermal glands and stomata constituted the prominent features of both the adaxial and abaxial surfaces of leaves (Fig. 4A). The short pointed trichome was the only type of trichome found in the two varieties of *O. stamineus*. Most of these trichomes were concentrated along the leaf veins. But in *Cuphea,* Amarasinghe *et al.* (1991) found that there were seven different trichome types and they were found to have distinct taxonomic value at sectional and subsectional levels. For both varieties of *O. stamineus,* the four-cell epidermal glands and stomata were distributed randomly on the leaf lamina (Fig. 4B). However, in *Clerodendrum* spp., twelve basic and 18 combination of stomatal distribution pattern were recognized (Rao and Ramayya, 1984). Olowokudejo and Pereira-Sheteolu (1988) also found that the stomata and epidermal glands were the prominent features for six species of *Ocimuum* (Lamiaceae) but the sizes of the epidermal glands varied within and between the six species. Sheahan and Cutler (1993) reported that the trichome type, stomatal type and the epidermal cell shape played an important role in distinguishing the taxa of the Zygophyllaceae. However, for both the white and purple varieties of *O. stamineus*, there was no different in the sizes of the stomata and the epidermal glands but there were differences in stomata numbers on the adaxial and abaxial of the leaves for each variety and the number of stomata was not significantly different between the two varieties (Table 3).

The morphology of anthers was found to be similar for both varieties. Each anther was consisted of two lobes and each lobe contained two loculi (pollen-sacs). Globular glands could be seen on the anther surface especially at the connective section (Fig. 4C). Both varieties of *O. stamineus* produced two-lobed stigma with rough

surface for pollen grains adhered to it (Fig. 4D). Both varieties produced spherical pollen grains with six furrow-like colpus and netted muri (Fig. 4E). According to Faegri and Johannes (1975), pollen grains with six furrow like colpus was termed as stephanocolpate. When one of the colpus came in contact with the stigma, the pollen grains would germinate (Troughton and Simpson, 1973).

In conclusion, the purple and white varieties of *O. stamineus* could be differentiated by merely based on the color of the corolla and calyx and the leaf characteristics. The purple variety produced flowers with purple tint at the lobes of the two-lipped white corolla with maroon calyx while the petals of the white variety was totally white without purple tint at the edge and green calyx. The leaves of the purple variety were ovate in shape with light yellowish spots scattered unevenly on both the adaxial and abaxial surfaces of the leaves, with acute apex, truncate base and purple veination. On the other hand, the white variety produced rhomboid shaped leaves without any colored spots with acuminate apex, obtuse base and common light green veination. The similar micro-structures of leaves, anther, stigma and pollen grains of both varieties could not be used for identification of the two varieties of *O. stamineus*.

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