

Foliar anatomy and micromorphology of *Polygonum minus* Huds. and their taxonomic implications

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Abstract

Foliar anatomical characters of *Polygonum minus* Huds. were described to determine their taxonomic significance and implications. Comprehensive micromorphological study on leaf epidermis was investigated on both adaxial and abaxial surfaces by light and scanning electron microscopy. The epidermal cells on adaxial surface are polygonal, while irregularly shaped cells were observed on the abaxial surfaces. Stomata show paracytic pattern. Reticulate venation and one layer of palisade cells with elongate-prismatic in shape were observed, as well as druse and prismatic crystals recorded. Epidermal cavities were observed in both adaxial and abaxial sides. Two trichome types were identified: peltate secretory and non-secretory trichomes (conical brushlike clustered, simple multicellular and multiseriate). We report the presence of peltate glandular secretory trichomes in *P. minus*. These findings suggest an adjustment of the taxonomic description of the genus *Polygonum* section *Persicaria*.

Keywords: Leaf crystals; Micromorphology; Oil cell; Peltate trichomes; *Polygonum minus* Huds. taxonomy; Simple trichomes.

Introduction

Polygonum L., a relatively large genus, belongs to the family *Polygonaceae* consisting of eight genera (*Aconogonon*, *Koenigia*, *Persicaria*, *Reynoutria*, *Bistorta*, *Fallopia*, *Polygonum* and *Fagopyrum*) (Haraldson, 1978). *Polygonum* sensu lato is also divided into two tribes: Polygoneae and Persicarieae. *Polygonum minus* Huds. is classified in section *Persicaria* of the genus. The taxonomic and systematic study of the genus and sections in elucidation of relationships within *Polygonum* have traditionally been based on morphological similarity of various characteristics with emphasis on a few distinct morphological features such as vegetative anatomy and trichome properties (Haraldson, 1978; Lersten and Curtis, 1992; Curtis and Lersten, 1994; Yasmin et al., 2009a, b, 2010), pollen morphology (Hong and Hedberg, 1990; Hong et al., 2005) and chromosome numbers (Fedorov, 1969). However, whatever systematic relationships and classifications have been established within the genera since the work of Steward (1930) are still confused, and *Polygonaceae* has remained a challenge for taxonomists classifying these plants (Brandbyge, 1993). The subgeneric division of the large genus *Polygonum* into unit sectional rank seems to be problematic since the first attempt to recognize different genera in *Polygonum* was made, and it is still a great matter of debate which rank should be used to

accomplish a convincing conclusion in improving tribal classification (Haraldson, 1978; Ronse Decraene and Akeroyd, 1988; Hong, 1992). *P. minus*, commonly known as kesum is a traditional herb in Malaysia (Burkill, 1966). The plant grows up to 50 cm high. The leaves are narrow, lanceolate and 5 – 7 cm long. *P. minus* leaves have a unique lemonade flavour and are widely used as a flavor ingredient in Asia, especially in Southeast Asia. As a natural source of aliphatic aldehyde, *P. minus* is believed to have great potential as a food additive and for use in perfume industries. *P. minus* extracts display cytotoxic activity against human cervical carcinoma cells and also antibacterial activity. Additionally, it has antioxidant activity and moderate anti-cancer activity (Normisah et al., 2005). The current and potential uses of kesum justify scholarly attention to this plant. Morphologically and anatomically the leaf is the most variable plant organ, and the difference such as trichomes is occasionally specific for species, genera or even families. Various anatomical characters within the leaf have been verified to be of systematic value in distinguishing diverse lineages and particularly as a practical source of potentially important systematic characteristics (Dickison, 2000; Yasmin et al., 2009a; 2009b; Kahraman and Celep, 2010). Foliar epidermal morphology such as epidermis, stomata, trichomes

Table 1. Qualitative and quantitative epidermal characteristics of *Polygonum minus* Huds. (All measurements are in μm).

Foliar characters	<i>Polygonum minus</i> Huds.	
	Adaxial	Abaxial
Type of epidermal cells	Polygonal and smooth walled	Irregular in shape with slightly undulating walls
-Length	25-55	25-50
-Width	25-60	15-30
Stomata	Paracytic	Paracytic
-Length	15-20	15-25
-Width	10-15	10-20
Palisade	One layer of palisade cells, elongate-prismatic in shape and restricted to the adaxial side of the leaf	
-Length	20-55	
-Width	5-15	
Spongy parenchyma	Nearly isodiametric	
-Length	5-10	
-Width	5-10	
Vascular bundles	Reticulate venation	
Non glandular trichomes	Conical brushlike clustered	Conical brushlike clustered
-Length	50-250	50-150
-Width	30-70	10-40
-Distribution	Higher density on leaf veins	Higher density on leaf veins
-Cell number	One to six cells	One to six cells
Glandular trichomes	Peltate trichomes	Peltate trichomes
-Length	20-30	15-25
-Width	20-25	15-25
-Distribution	Randomly distributed between the stomata	Randomly distributed between the stomata
-Cell number	Multicellular	Multicellular
Crystals	Druse and prismatic crystals	Druse and prismatic crystals

and other characters have also proved valuable in taxonomic classification and may be able to yield significant clues regarding subfamilial, tribal and generic relationships (Baranova, 1972). An extensive study on foliar anatomy of the Polygonaceae is well documented by Metcalfe and Chalk (1950). Lersten and Curtis (1992) gave comprehensive report on several anatomical characters in leaves and their distribution for 153 species of *Polygonum* sensu lato representing all sections of the genus. Ayodele and Olowokudejo (2006) made comparative studies on the leaf epidermal features of the 19 species in the family Polygonaceae in West Africa and found that epidermal characters are increasingly important source and valuable in the identification of taxa. The systematic potential of foliar anatomical features in 13 species *Polygonum* from Pakistan was described by Yasmin et al. (2009a) and found that foliar anatomical investigations are taxonomically significant and potentially in understanding the relationships between the species. Recently, Yasmin et al. (2010) investigated leaf epidermal anatomy of selected *Persicaria* revealed variation in size and shape of epidermal cells, stomata, glandular and non glandular trichomes. The study revealed some distinctive anatomical features such as five different stomatal patterns observed, the shape of epidermal cell was variable but mostly polygonal, two types of non glandular trichomes reported only in *P. barbata* (L.), *P. stagnina* (Buch.-Ham. ex Meisn.) and *P. orientalis* (L.) which serve as their distinctive characters and

glandular trichomes are peltate, capitate and spheroidal. Metcalfe and Chalk (1950) and Munshi and Javeid (1986) published extensive studies of trichomes in *Polygonum*, identifying two types: glandular and non-glandular. Haraldson (1978), working on the morphology, anatomy and taxonomy of *Polygonaceae* subfamily *Polygonoideae*, pointed out 13 trichome types in various parts of 87 species as significant characters at the generic level in her taxonomic interpretations, but she did not use trichome information as a distinguishing feature in her classification system of *Polygonum* genera. Lersten and Curtis (1992), however, found that the clustered type of non-secretory trichome was distinctive and relatively rare among dicots, suggesting the value of continued attention to trichome structures as a significant taxonomic feature. They distinguished three subtypes of non-secretory trichomes, manifested as either stellate, brushlike or pointed (conical) brush form, but it was the brushlike non-secretory trichomes that were distinctive taxonomic features. Furthermore, they found that when *Polygonum* genera had secretory trichomes, those trichomes were capitate or capitate and spheroidal. In section *Persicaria*, the two most widespread are the spheroidal epidermal glands in 21 species and cavities (epidermal and subepidermal forms) in 15 other species, while *P. glabrum* Willd. and *P. portoricensis* (Bertero) each had both features (Lersten and Curtis, 1992). Additionally, they found that the clustered type of non-secretory trichomes variants occurred

only among species in the sections *Echinocaulon*, *Tovara* and *Persicaria*. This study aims to describe the foliar anatomy and micromorphology of *Polygonum minus* with attention to its trichome structures, which we propose to be significant for its taxonomic classification.

Materials and methods

Plant material

Fresh mature leaves of *P. minus* were collected in January 2009 from Genting Highland, Pahang, Malaysia (3° 25' 22.43" N, 101° 47' 32.38" E). For the purpose of these investigations, mature leaves were regarded as vegetative leaves harvested from the third and fourth nodes of the plant from the tip. Voucher specimens are deposited in the Herbarium of the Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Malaysia (UKMB).

Scanning electron microscopy

Fresh leaves, 5-8 mm in length, were removed from the aerial part of the plant. Both abaxial and adaxial surfaces were observed under a TM-1000 Tabletop Microscope from Hitachi High-Technologies.

Light microscopy

Fixation, embedding and sectioning were performed following the method described by Johansen (1940) and Sass (1958). The sectioning was done using a sliding microtome, and sections were stained in Safranin and Alcian green and dehydrated, then mounted in Euparal. Photographs of sections were taken using a Leitz Diaphlan polarizing microscope and images were processed using Analysis Docu Software.

Results

The characteristics of leaf epidermis of *P. minus* are listed in Table 1. In *P. minus*, epidermal cells on adaxial surface are polygonal and smooth, while the cells are irregularly shaped with slightly undulating walls on the abaxial surface. Quantitative data indicate cell size in *P. minus* adaxial surface are 25-55 x 25-60 µm and in abaxial surface 25-50 x 15-30 µm. Different frequencies of stomata distribution on both leaf surfaces were noted. Paracytic stomata was recorded in *P. minus* with average stomata size varies between 15-20 x 10-15 µm on adaxial surfaces and 15-25 x 10-20 µm on abaxial surfaces. The palisade region of *P. minus* consists of one layer of elongate-prismatic shaped cells. The spongy region below the palisade appears nearly isodiametric. The arrangement of the vascular bundles pattern in *P. minus* is reticulate venation with the smaller bundles diverging from the larger. In our study, all the specimens have epidermal cavities in both adaxial and abaxial sides. Morphologically, non-glandular trichomes were sharply pointed, non-secretory, multicellular, simple, unbranched, and multiseriate with between one and six cells arranged in a group described as conical brushlike clustered

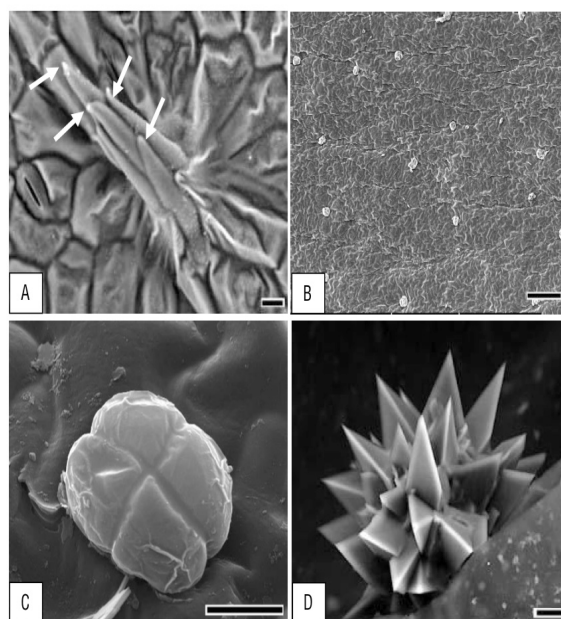


Fig 1. Micromorphology of glandular and non-glandular trichomes of *Polygonum minus*, as seen under SEM. (A) A conical brushlike clustered trichomes along the vein (arrow points to cells of a trichome ending at different levels). (B) Distribution of peltate trichomes on abaxial leaf surface. (C) A thin-walled peltate trichome with multicellular secretory head. (D) A druse crystal seen randomly on both leaf surfaces. Scale bar: 50 µm (A-B), 10 µm (C-D).

(Fig. A) by Lersten and Curtis (1992). Based on the morphology, peltate trichomes were distinguished. Peltate glandular trichomes were more abundant and densely distributed (Fig. 1B) on the abaxial than the adaxial surface. The peltate trichomes consisted of one basal epidermal cell, a wide unicellular stalk cell and a multicellular secretory head were evident on both leaf surfaces (Fig. 1C). Druse and prismatic crystals were found randomly on both leaf surfaces (Fig. 1D). The druses are compound and clustered in spherical masses whose many element crystals project from the surface to give a star-shaped appearance. Observation under light microscope revealed the distribution of conical brushlike clustered trichomes along the veins on the leaf surface. The distribution of peltate trichomes were apparently random at approximately 15-25 trichomes mm⁻² over the adaxial and abaxial surfaces. The arrangement and relative size and density of the trichomes are shown in Fig. 2A. An oil cell in the palisade layer (Fig. 2B-C) and peltate trichomes (Fig. 2D) were also detected. Cells with crystal inclusions were also observed (Fig. 2E).

Discussion

The leaf epidermis anatomical studies of *P. minus* show many typical or common foliar characters in Polygonaceae as described by Metcalfe and Chalk (1950). We observed that epidermal cells of *P. minus* are smooth-walled polygonal on

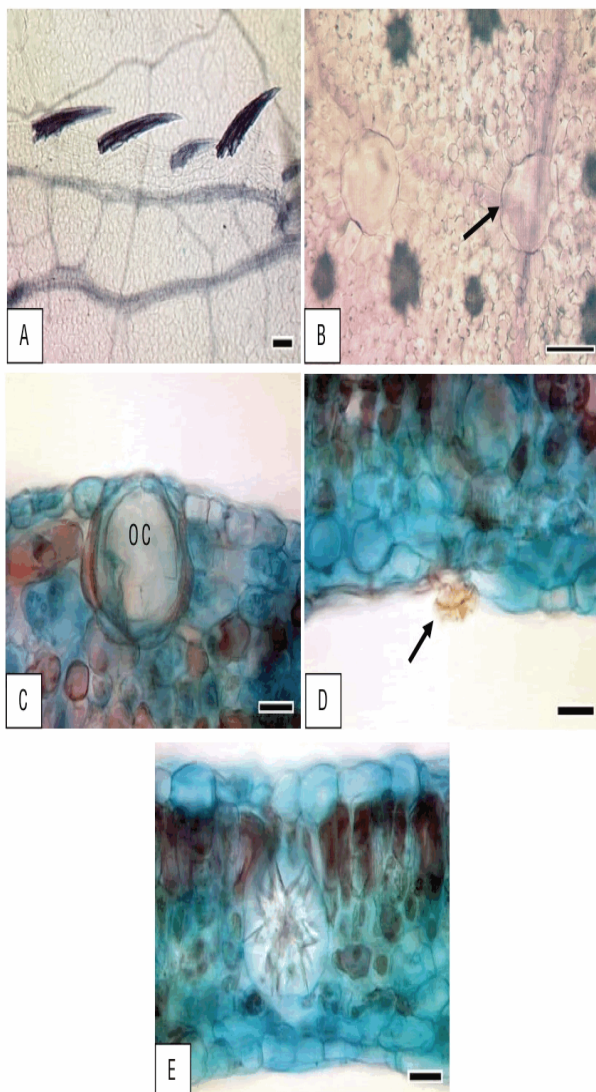


Fig 2. (A) Distribution of conical brushlike clustered trichomes along the vein of *Polygonum minus* leaf. (B) Random distribution of oil cell (arrow) on the leaf surface. (C) Transection of leaf lamina with oil cell (OC). (D) Peltate glandular trichomes (arrow) on the abaxial side of leaf surfaces. (E) Cells with crystal inclusions in leaf lamina of *P. minus*. Scale bar: 50 μ m (A-B), 20 μ m (C-E).

adaxial surface and irregularly shaped with slightly undulating walls on abaxial surface. Yasmin et al. (2010) reported irregular shaped epidermal cells only on abaxial surface of seven *Persicaria* species. Among the taxa of *Persicaria*, the shapes of epidermal cells vary and provide as a taxonomically important characters (Yasmin et al., 2010). The most common type of stomata in *Persicaria* is paracytic and it was observed on both leaf surfaces of *P. minus*. However, the stomatal pattern in

Polygonaceae cannot be used to differentiate the genera within the family (Lersten and Curtis 1992). They studied three different specimens of *P. minus* and found one specimen (110c) do not have any epidermal cavities while the others have epidermal cavities in both adaxial and abaxial sides. Our specimens have epidermal cavities in both sides. Curtis and Lersten (1994) recognised two types of internal oil cavity, epidermal cavity and subepidermal cavity complexes in leaves of 15 species of *Polygonum* section *Persicaria*. Epidermal cavities observed in *P. hydropiper* by them was similarly observed in our study on *P. minus*. Druse and prismatic crystals, well known as general occurrence in *Polygonaceae*, were observed on leaves of *P. minus*. Among the major crystal types, druses and prismatic crystals are the most frequently occurring forms in dicotyledons, often being found together in the same plant (Dickison, 2000). Druses are common in several families, including *Leguminosae*, *Juglandaceae*, *Tiliaceae* and *Polygonaceae* and therefore are likely not to be significant in a taxonomic sense (Dickison, 2000). The most widespread features revealed by this study are peltate trichomes. We found evidence that *P. minus* leaves possess peltate glandular trichomes on both adaxial and abaxial surfaces. Interestingly, the report by Lersten and Curtis (1992) on the foliar anatomy of *P. minus* noted only capitate trichomes on the epidermis of *P. minus*. Our observation of this previously unreported trichome type represents the first new discovery about the microfoliar of *P. minus* during the last 15 years of study of this plant. We found that the non-glandular trichomes were distributed on both leaf surfaces. Conical brushlike clustered trichomes that we observed in *P. minus* have been seen in section *Persicaria* in *P. lapathifolium* L., where the trichomes cohere but differ in length, thereby producing a tapered, pointed structure (Lersten and Curtis, 1992). The clustered type of the non-secretory trichomes were emphasized by Lersten and Curtis (1992) were distinctive and relatively rare among dicots, suggesting the value of continued attention to trichome structures as a significant taxonomic feature. Extensive study should be conducted to reveal the variations of non-secretory trichomes as taxonomically important and significant characters to the taxonomic schemes for the genus *Polygonum*. Given that *Polygonum* is a genus with indistinct taxonomic boundaries between its members, systematic evidence obtained from study of the features of the foliar epidermis, such as trichome morphology, is useful in providing significant distinctive characters for plant classification. The results of our study revealed a potentially important (but as yet unreported) distinctive features of *P. minus*: peltate secretory trichomes. This finding suggests that more extensive study should be done on the foliar micromorphology of the genus *Polygonum* to determine the usefulness of features of trichome morphology for making clearer taxonomic distinctions between genera.

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