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TAXONOMIC SIGNIFICANCE OF POLLEN, ANATOMICAL AND MORPHOLOGICAL CHARACTERISTICS OF RED AND GREEN LEAVES *CHRISTIA VESPERTILIONIS* (L.f.) Bakh. f. (FABACEAE)

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Abstract

Anatomy, morphology and palynology studies were conducted on green and red *Christia vespertilionis* (L.f.) Bakh.f. The objective of this study is to identify the common, variation and diagnostic features and to construct key identification of species. Anatomy study involves sectioning using sliding microtome on the petiole, lamina, midrib and leaf margin, epidermal mechanical scrapping to observe epidermis, leaf clearing for type of venation, for observation under light microscope, images are captured using Olympus DP25 camera and images analyzed using Docu Analysis software. Leaf and flower morphological features are measured for morphology study. Pollen study involves sample preparation, acetolysis techniques and observation under a light microscope. Common features obtained from this study such are the presence of crystals and muscilaginous cells, presence of additional vascular bundles in the petiole and midrib, the presence of air spaces between the cells, pattern of anticlinal cells wall, presence of glandular and papillae trichomes, number of leaf veins, pedicel characteristics, number of petals, pollen class, aperture type, pollen size, and exine pattern. Variation than can be used to distinguish green and red leaf *C. vespertilionis* are outline shape of leaf lamina, presence of swollen tracheid, type of trichome, vascular tissue arrangement in the midrib and petiole, presence and type of stomata, leaf colour, petiole characteristics, terminal leaf characteristics, abaxial and adaxial leaf surface, main leaf and calyx feature, pollen shape and exine thickness. The results have shown high variation between green and red *C. vespertilionis*. In conclusion, this study proves that green and red leafy *C. vespertilionis* are likely different species, subspecies or varieties and accordingly a status study should be done on both green and red leafy to see their taxonomic position.

Key words: *Christia vespertilionis,* Anatomy, Morphology, Palynology, Rerama.

Introduction

Christia vespertilionis (L.f.) Bach. f. known as rerama tree or *mariposa* in Spanish. This species is a shrub or herb from the family Leguminosae (synonymous Fabaceae), subfamily Papilionoidae (Barham, 1996; Langran *et al*., 2010). *Christia* Moench has 13 species (Huang & Hiroyashi, 2010) and according to IUCN Red List of Threatened Species, *C. vespertilionis* is categorized as 'least concern' or little known. This species is widespread in tropical Southeast Asia and is believed to originate from countries such as Thailand, Vietnam, Cambodia, Indonesia, China, Myanmar and is also found in Malaysia. The status of its' origin is still unknown whether it is naturally found in Malaysia or brought into Malaysia from abroad (Anon., 2012). According to Allen & Ethel (1981), *C. vespertilionis* is a species that is widely distributed in Indochina, Malaysia, and northern Australia that is widely cultivated for medicinal purposes. This species was first introduced by Joao de Loureiro in Cochinchina which was part of Vietnam in 1774 (Barham, 1996). Barham (1996) reported that this species was first cultivated at Kew in 1980 and was described in 1782 as *Hedysarum vespertilionis* L. f. Based on The International Plant Name Index (IPNI), the legal name used is *Christia vespertilionis* (L. f.) Bakh. f. (Anon., 2005). This species grows in open grasslands, roadsides, and beaches (Huang & Hiroyashi, 2010). This species is always used as a decorative landscape plant because it is easy to care for, and has a unique leaf shape (Anon., 2013).

Christia is a genus of perennial or sub-herbaceous plants that are erect or creeping, have three pinnate leaves or more, small flowers of 3-6mm consisting of racemes and panicles, petals are usually yellowish white, thin stems, erect and can reach height up to 1m (Huang & Hiroyashi, 2010). Trifoliate compound leaves, slightly purple when immature and become light green or dark green when the leaves are mature (Barham, 1996). According to Huang and Hiroyashi (2010), it usually has compound leaves and rarely compound trifoliate; petioles measuring 2-2.5 cm; covered with long, fine hair; rhombus-shaped terminal leaves or pointed rhombuses; the base of the leaf is slightly cordate while the tip of the leaf is rather wide and truncate, or slightly emarginate; lateral leaves obcordate. Leaf veins have three or four veins on each side of the midrib. Pedicle 2-4 mm; covered by short gray hairs. Semi-hyaline calyx that elongates as it matures.

This species is reported to have anticancer substances for the treatment of Neuroendocrine Tumors (NETs) and the treatment of malaria (Hofer *et al*., 2013; Nguyen-Pouplin *et al*., 2007; Dash, 2016). Studies show that the roots, leaves and

stems have active substances such as alkaloids, triterpenes and phenols (Rajandeep & Harpreet, 2017; Nguyen-Pouplin *et al*., 2007). Harian Metro (2016) reports that this species is also famous as a herbal tea drink because it is believed to treat cancer and blood-related diseases such as dengue, stroke and high blood pressure. The whole part of this plant was once used to treat tuberculosis and the effects of snake bites while the leaf part was traditionally used to restore cracked or broken bones (Huang & Hiroyashi, 2010). Rerama which has green leaves has been widely used in Malaysia as a basic ingredient in making rerama tea which is said to have medicinal potential in traditional treatment for cancer. Nevertheless, the species of rerama that is said to have anti-cancer substances was once published in the International Journal of Pharmacy and Pharmaceutical Sciences, in an article titled 'The red butterfly wing (*Christia vespertilionis*): *A promising cancer cure in Malaysia*' is a species of rerama that has coloured leaves red (Hamidun *et al*., 2015), but many still consider red rerama to be the same as green rerama. The study conducted on *C. vespertilionis* which has green leaves is still superficial and less than the study on red leaf rerama (Osman *et al*., 2017).

A study of anatomical, pollen and morphological characteristics of this green leaf rerama was done to see if there is a difference between *C. vespertilionis* with red and green leaves. Previous studies have proven that anatomical and pollen features have a high taxonomic value and are suitable for comparative studies for identification, differentiation and classification of plant species (Noraini *et al*., 2009, 2012, 2014, 2016, 2017; Firoze-Quamar *et al*., 2017; Chung *et al*., 2003; Worasitikulya *et al*., 2022). For morphology study, observations were made for a period of six months time in the field. Identification keys of green and red *C. vespertilionis* were constructed using the morphology, leaf anatomy and pollen characteristics obtained from the study.

Material and Methods

Chistia vespertilionis (L.f.) Bakh. f. the green and red heart leaves (Fig. 1) were collected from the locality as stated in (Table 1), and were grown in the nursery at Plant House, Faculty of Science and Technology, UKM 43600 Bangi, Selangor.

Table 1. List of species and samples studied.

Species	Specimen code	Locality
C. vespertilionis green leaf	SS1	1) Sungai Buloh Nursery, Selangor
	SS2	2) Evergreen Nursery, Bangi, Selangor
C. vespertilionis red leaf	SS3	1) Sungai Buloh Nursery, Selangor
	SS4	2) Evergreen Nursery, Bangi, Selangor

Specimens were compressed, dried at 60ºC for two weeks, stitched on herbarium cards, labeled and stored at the Universiti Kebangsaan Malaysia Herbarium Bangi (UKMB). The anatomical study method was carried out according to the method of Sass (1958) and Johansen (1940) with modification and description of morphological characteristics according to Huang & Hiroyashi (2010). The study involved collection and preparation of voucher specimens, slices with a sliding microtome, scraping techniques, leaf immersion and observation under a light microscope. The characteristics observed are cross-sections of petioles, leaf bones and laminae, as well as vascular and abaxial and adaxial epidermal characteristics of leaf lamina. Morphological study is performed by observing and measuring specimens with the naked eye, using a magnifying lens and a microscope. The characteristics measured refer to the study by Huang and Hiroyashi (2010) on *C. vespertilionis* red leaf which focuses on the morphological characteristics of leaves and flowers. Pollen study refers to the method of Erdtman (1969). Observation of anatomical features was performed using an Olympus DP25 camera connected to an Olympus BX53 microscope and images were processed using Cell^B software. Pollen characteristics were described according to Erdtman (1952) and Erdtman (1969), the observed characteristics were the presence of apertures for the determination of pollen class, pollen shape, total length of the pollen pole (P), diameter of the pollen equator (E), P/E ratio, exine ornamentation, opening or aperture of pollen, number of pollen (N), position of aperture (P), specific characteristics of aperture (C), shape of pollen and amb (external shape in view of pollen pole), and exine thickness (ratio of exine size to pollen equatorial length). While the exine ornamentation pattern is according to Frenguelli (2004).

Results and Discussion

The results of anatomical study has shown that druses and single crystals are present on both red and green leaves. The anatomical features of the cross-section of the leaf can be referred to (Figs. 2 and 3), while the anatomical characteristics of the cross-section of the leaf midrib can be referred to Figs. 4 and 5. Solitary crystals of square shape are present on the petioles of *C. vespertilionis* green leaves and *C. vespertilionis* red leaves. Crystals are an important taxonomic feature for comparative studies between families or higher taxon levels (Metcalfe & Chalk, 1950; Dickison, 2000). Crystals found in plants can vary in shape and location which are sometimes specific to a certain species (Noraini *et al*., 2016; Franceschi & Horner, 1980). The number of crystals present in a plant is not fixed because it depends on the season and the life cycle of the plant (Marcati & Angyalossi, 2005). The types of crystals and their position in plant tissue are often used in taxonomic classification systems, especially at the genus and species level (Prychi & Rudall, 1999; Maideen *et al*., 2013). For this study, it shows the same characteristics for green and red leaf rerama.

Secretory cells or mucilage cells are present on petioles and midrib. The results of this study support the findings by Metcalfe & Chalk (1957) on several genera from the Leguminosae (*Helimodendron* from the Papilionoidae subfamily and *Cassia* from the Cesalpinoidae). According to Melcalfe & Chalk (1979), channel cells or mucilage cells are usually found in cortex parenchyma tissue, pith parenchyma tissue, phloem tissue and pith area. The study also showed that mucilage cells were found in the parenchyma tissue of the cortex, in the pith of petiole and midrib of the green and red leaves of *C. vespertilionis.*

Fig. 1. A) *C. vespertilionis* green leaves, B) *C. vespertilionis* red leaves.

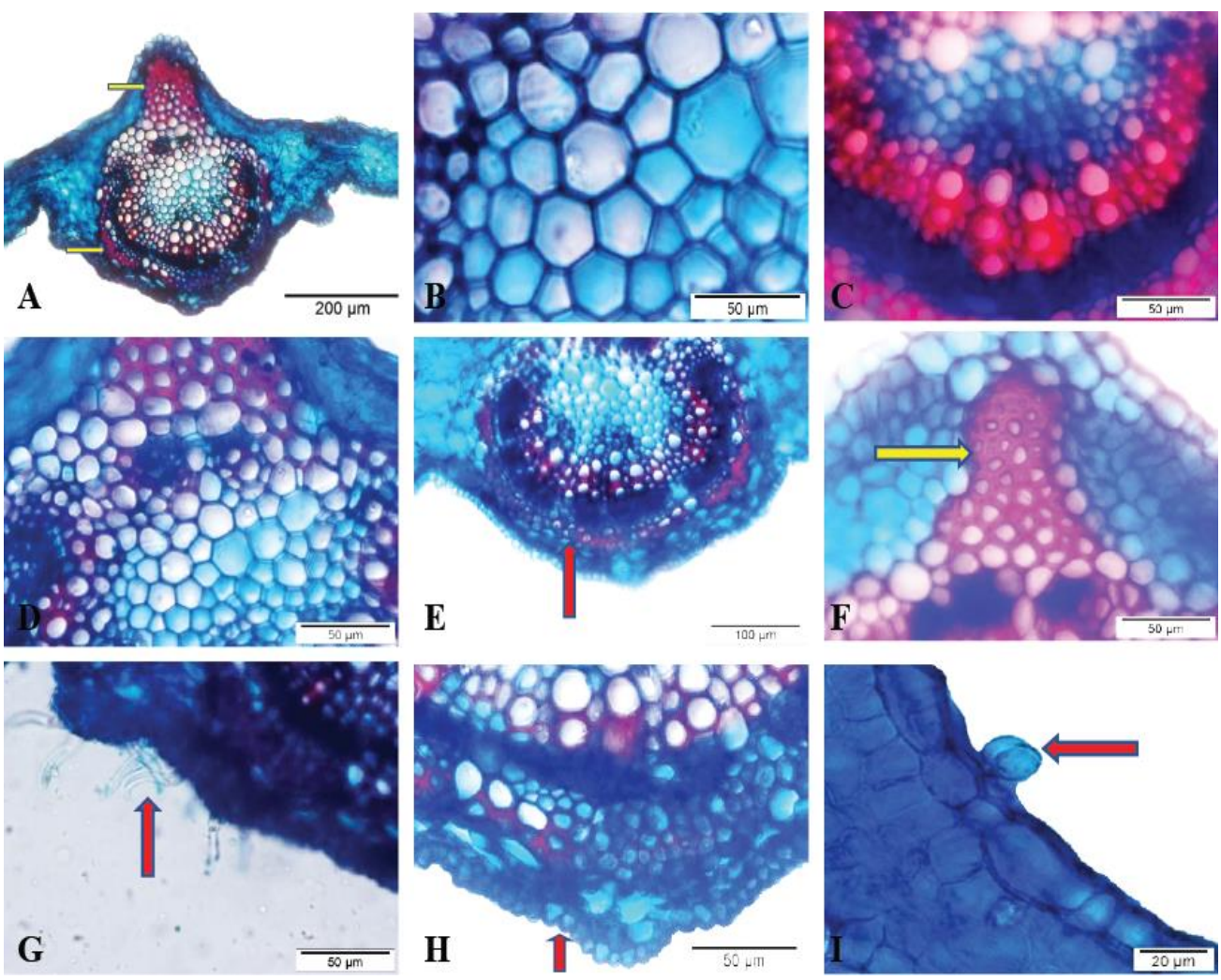

Fig. 2. Anatomical characteristics of midrib of *C. vespertilionis* green leaf: A) External shape and arrangement pattern of midrib vascular tissue, collenchyma cell layer under the adaxial and abaxial epidermis, sclerenchyma cells outside the vascular bundle (yellow arrow), B & C) Arrangement of main vascular tissue, D) Vascular tissue on abaxial side, E) Mucilage cells, druses on cortical parenchyma tissue (red arrow), F) Sclerenchyma cells and parenchyma cells on adaxial side (yellow arrow), G) Simple unicellular trichome on adaxial side (red arrow), H) Papillae trichomes on the abaxial side (red arrow), I) Capitate glandular trichomes (red arrow). Scale: A= 200"µm, B, C, D, F, G & H=50µm, E= 100µm, I=20µm.

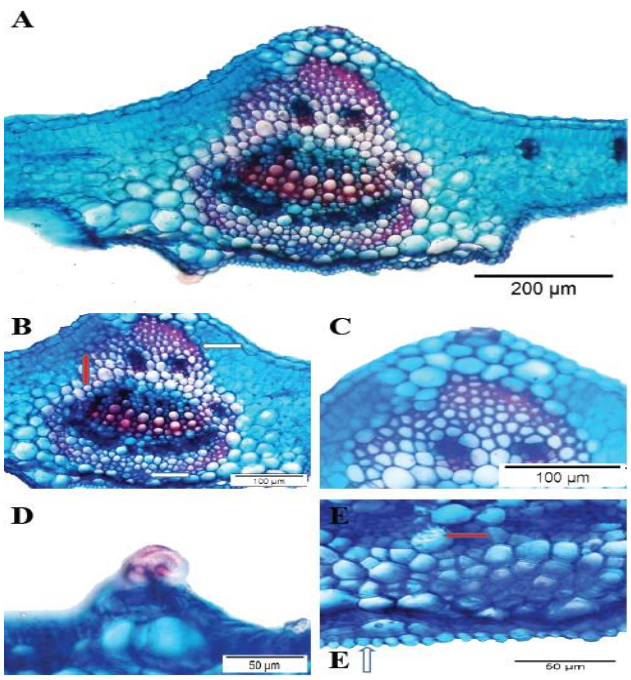

Fig. 3. Anatomical characteristics of midrib of *C. vespertilionis* red leaf: A) External shape and arrangement pattern of vascular tissue; collenchyma layer under adaxial and abaxial epidermis, B & C) Collenchyma cell layer under adaxial epidermis (red arrow), sclerenchyma cell layer (white arrow), D) Capitate glandular trichome, E) Druses in parenchyma cells (red arrow) and papillae on abaxial epidermis (white arrow), papillae present (white arrow). Scale: A= 200"µm, B & C=100µm, D & E= 50µm.

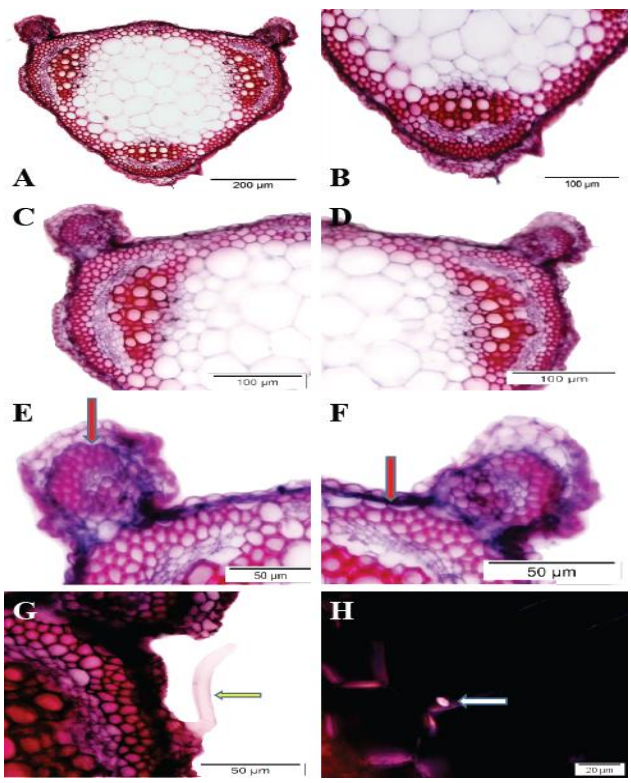

Fig. 5. Anatomical features of the petiole of *C. vespertilionis* red leaf: A) Outline shape of the petiole and arrangement pattern of vascular tissue. B, C & D) Vascular bundle, sclerenchyma cells in vascular tissue (yellow arrow), E & F) Additional vascular bundles and lobes, collenchyma cells under epidermal layer (red arrow), G) Simple trichome (yellow arrow), H) Solitary crystals (white arrow). Scale: A= 200µm, B, C & D=100µm.

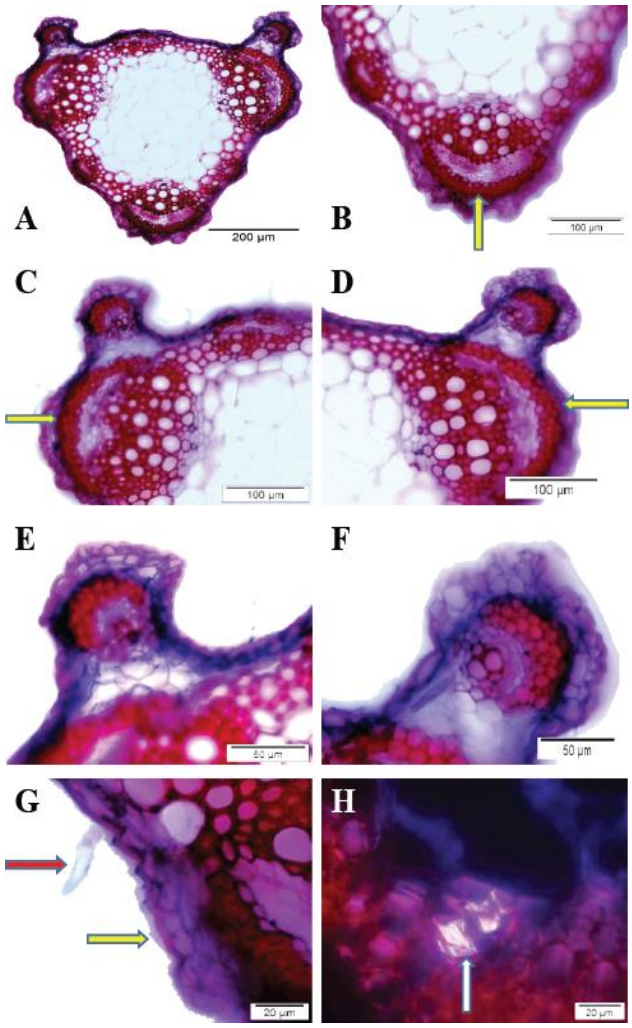

Fig. 4. Anatomical characteristics of the petiole of *C. vespertilionis* green leaves: A) External shape of the petiole and arrangement pattern of vascular tissue, mucilage cells (cm), B-D) Vascular bundle, sclerenchyma cells outside the phloem and in the vascular tissue (yellow arrow), E & F) Additional vascular bundles and lobes in the petiole, G) Multicellular simple trichome (red arrow), cuticle layer (yellow arrow), H) Solitary crystals (white arrow). Scale: A= 200 µm, B, C & D=100µm, E, F & G= 50µm, I=20µm.

Both *C. vespertilionis* green and red leaves have closed system vascular tissue and have additional vascular bundles on the lobes of the petiole. While the vascular bundle system found in the midrib shows an open system and has two additional vascular bundles on the adaxial side. The results of the study show that both have air cavities between cells. The presence of air cavities is closely related to the habitat occupied by a plant species (Cutler *et al*., 2008). *C. vespertilionis* green and red leaves have the same type of anticlinal cells wall pattern on the adaxial and abaxial surfaces which is sinuous. The similarity of the obtained characteristics between plant species proves the probability that the species have a close relationship (Noraini & Cutler, 2009). The leaf margins of both *C. vespertilionis* green and red leaves are complete. Capitate glandular trichomes (multicellular terminals), peltate glandular trichomes (multicellular terminals) and papillae present on the adaxial and abaxial epidermal surfaces of both *C. vespertilionis* green and red leaves (Figs. 2, 3, 4 & 5).

Variation of leaf anatomical characteristics of *C. vespertilionis* green and red leaves: The variation of *C. vespertilionis* leaf anatomical characteristics can be seen in the pattern of leaf veins; the presence of swollen tracheids; presence and type of trichome; in the presence, position and type of stomata; the presence of crystals; external shape of leaf margins, midrib and petioles, and pattern of vascular tissue in petioles and midrib. The list of variation of leaf anatomical features found in the study is given in (Table 1).

Leaf arrangement: According to Dilcher (1974), Badron *et al*., (2014) and Noraini *et al*., (2016), leaf structure has significant implications for plant taxonomy and phylogeny. The results of this study show the presence of the majority of open leaf vein with unbranched free ends, one and two branches, and a minority of closed leaf veins for *C. vespertilionis* green leaves. While for *C. vespertilionis* red leaves, the majority of the areolar venation are open with the free end unbranched and single-branched, the minority of the veinlets are closed. There are simple unicellular trichomes present on the central vein of *C. vespertilionis* green leaves, while for *C. vespertilionis* red leaves, there are simple unicellular trichomes present on marginal and areolar of the leaf lamina, and capitate glandular trichomes present only on the areolar of the leaf lamina. The results of the study also show that swollen tracheids are only present in *C. vespertilionis* red leaves (Fig. 6).

Trichomes: Trichomes are hair growths or projections from the epidermis (Hewson, 1988; Noraini *et al*., 2016). There are many types of trichomes that differ in form and function, including simple unicellular and multicellular trichomes; branched or unbranched, scaly trichomes and glandular or non-glandular trichomes (Rudall, 2007). Trichomes are very important in taxonomy in addition to serving to protect the plant from herbivores, heat and sunlight (Croteau, 1977; Duke 1994; Werker 1993). In this study, multicellular simple trichomes (tapering, hooked tip), unicellular simple trichomes (tapering, hooked tip) were found only in *C. vespertilionis* green leaves. *C. vespertilionis* red leaves, has unicellular simple trichomes (long, blunt end), unicellular simple trichomes (short, blunt end), multicellular simple trichomes (long, tapering, sharp-pointed end). The red *C. vespertilionis* can be distinguished from the green leaves by the presence of trichomes on the margins of the leaves.

Outline shape and vascular tissue of petiole, midrib and leaf margin: *C. vespertilionis* green and red leaves show variation in the outline shape of petiole, midrib and leaf margin. Summary and illustration of the outline shape of petioles, midribs and leaf margins is as in Table 2 while the illustratios aren in (Figs. 7-9). A summary and illustration of the pattern variation of petiole vascular tissue and midrib is as shown in (Table 2).

Presence, position and type of stomata: Stomata consist of an elliptical pore surrounded by guard cells. Changes in the shape of guard cells can affect the opening and closing of stomatal pores. Subsidiary cells are cells that surround the stomata and have a different shape and cell content to the epidermis (Noraini *et al*., 2016; Syamsurina *et al*., 2020). The results of the study show that *C. vespertilionis* green leaves, with stomata are present on both of adaxial and abaxial epidermis (amphistomatic). The stomata are of the parasitic type on the adaxial epidermis while on the abaxial epidermis the majority of the stomata are of the parasitic type, the minority being anisocytic. For *C. vespertilionis* red leaves, stomata are only present on the abaxial epidermis (hypostomatic). The stomata present on the abaxial epidermis are of the anomocytic type. Clearly the characteristics of the presence and type of stomata can be used for the differentiation and identification of *C. vespertilionis* green and red leaves. A summary of the variation of leaf anatomical characteristics of green and red *C. vespertilionis* can be referred in (Fig. 10 and Table 2).

Description of morphological features: Leaf of *C. vespertilionis* have the same shape which is obcordate (inverted triangle) and asymmetric, the same number of leaf veins, the flower pedicel has fine gray hairs, the calyx has reticulate, short and fine hairs. Has a flower with three petals, conate with larger petals joined together to form one large petal. Legume type seeds with 4-5 seeds stored in the calyx. The surface of the seed is smooth and has a reticulate texture. The seeds are green when unripe and blackish brown when ripe.

Variation in morphological characteristics of *C. vespertilionis* green and red leaves: Variation in morphological characteristics is seen in the colour, type, length and adaxial and abaxial epidermis of the leaves as well as in the shape of the terminal leaves. For flowers, variations can be identified in the length of the panicle, calyx, and petal colour (Table 3).

Pollen morphology under the light microscope

Description of pollen characteristics: *C. vespertilionis* (green leaves): tricolporate. P/E ratio: 0.9. Shape: oblate spheroid. Aperture: colpus, porate. Exine thickness: medium, 0.07-009µm. Medium size; polar view measurement (P) 36.0-37.5µm, equatorial view measurement (E) 40.0-42.1µm (Fig. 11). *C. vespertilionis* (red leaves): tricolporate. P/E ratio: 1.1. Shape: prolate spheroid. Aperture: colpus, porate. Exine thickness: small, 0.045-0051µm. Medium size; polar view measurement (P) 35.2-36.5µm, equatorial view measurement (E) 32.0-33.6µm (Fig. 12).

Table 2. Variation of leaf anatomical characteristics in *C. vespertilionis* green and red leaves.

Characteristics	*C. vespertilionis* green leaf	*C. vespertilionis* red leaf
Marginal vein	Secondary veins unbranched, branched-1 or -2. Swollen tracheids are absent	Secondary veins are unbranched, branched-1 Swollen tracheids are present
Swollen Tracheid	Absent	Present
Type of trichomes	Simple unicellular (tapering, hook end) Simple multicellular (tapering, hook end)	Simple unicellular (short, blunt end) Simple unicellular (long, blunt end) Simple multicellular (long, tapered, sharp tip)
Leaf marfin outline	Rounded, decreasing in size towards the tip of leaf margin, the leaf margin curved 30º towards the abaxial side of the leaf	Rounded, forming a knob-like structure, the leaf margin direction is straight
Midrib outline	The adaxial surface is humped inverted V-shaped and the abaxial surface is wide V-shaped	The adaxial surface is broad V-shaped convex and the abaxial surface is flat
Petiole outline	The adaxial surface is slightly convex, the abaxial surface is ¾ triangular	Adaxial surface slightly convex V-shaped very wide, abaxial surface ¾ triangular
The number and position of the sclerenchyma cell layer on the midrib	Sclerenchyma cells (2-3 layers) are present outside the main vascular tissue discontinuously and are present filling the adaxial part of the leaf midrib projecting to touch the group of colorless parenchyma cells below the adaxial epidermis	Sclerenchyma cells (2-4 layers) are present outside the vascular tissue continuously and are present filling the adaxial part of the midrib projecting to touch the group of colorless parenchyma cells under the adaxial epidermis
Arrangement of vascular tissue in midrib	The main vascular tissue is a continuous U-shaped vascular tissue	The main vascular tissue is a horizontally arranged continuous vascular tissue
Presence, position and type of stomata	Amphistomatic (stomata present on abaxial and adaxial epidermis); parasitic type on adaxial epidermis; majority parasitic, minority anisocytic on abaxial epidermis	Hypostomatic (stomata present only on the abaxial epidermis); anomocytic type in the abaxial epidermis
Presence of palisade cells on the leaf margin.	Present until the edge of the leaf margin	Present until it touches the epidermal layer at the edge of the leaf margin
The main vascular bundle of the petiole	The primary vascular tissue consists of six separate vascular bundles arranged discontinuously in a triangular shape	The primary vascular tissue consists of four separate vascular bundles arranged discontinuously in a triangular shape

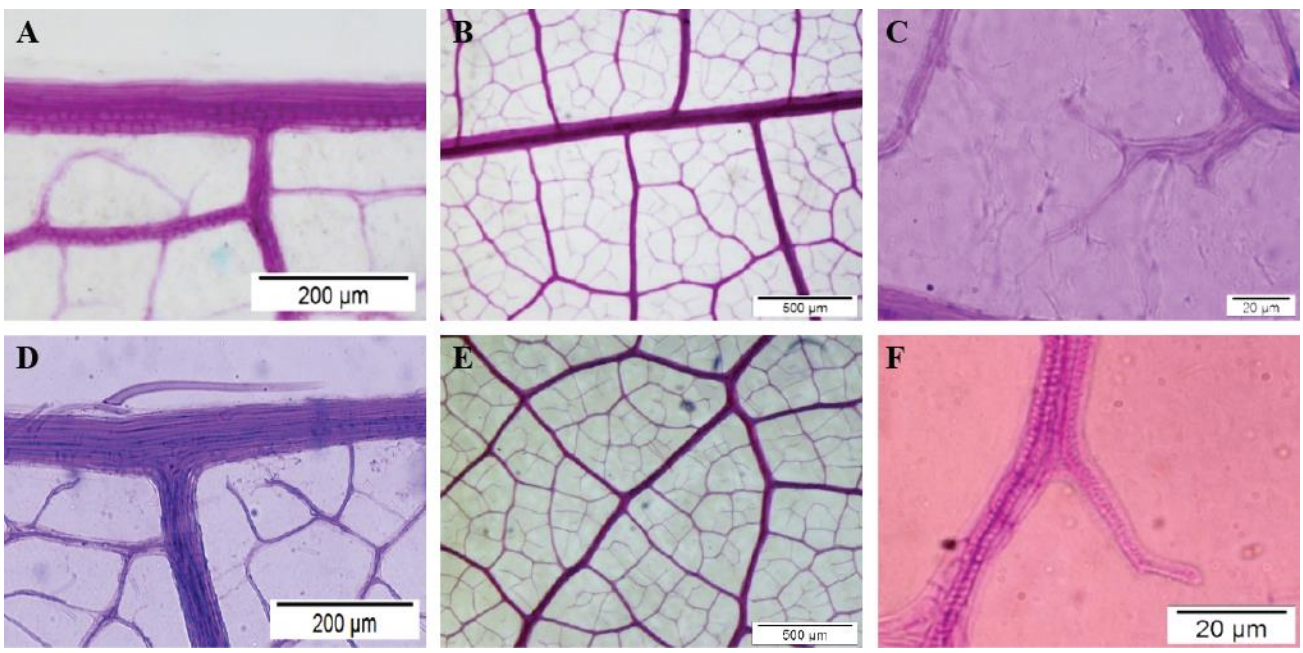

Fig. 6. Anatomical characteristics of the leaves of *C. vespertilionis* green leaves: A) Complete vein of leaf margins, B) Areolar; majority of open veinlets with free end, unbranched, branched-1 and -2, minority closed veins, C) Tracheid not swollen. *C. vespertilionis* red leaves: D) Complete vein of leaf margins, E) Areolar, majority of open veinlets, unbranched, branched-1 or -2, F) Swollen tracheids. Scale: A & D= 200µm, B & E=500µm, C & F= 20µm.

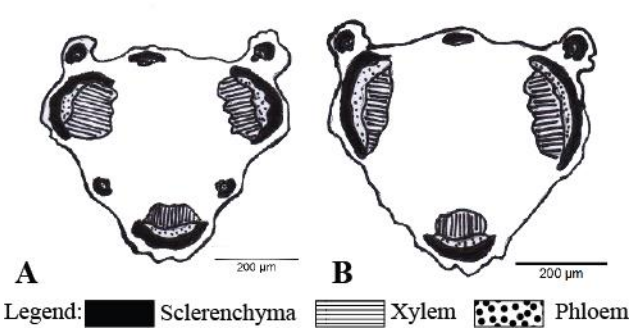

Fig. 7. Variation of vascular tissue pattern and petiole outline shape A) *C. vespertilionis* green, B) *C. vespertilionis* red. Scale: 200µm.

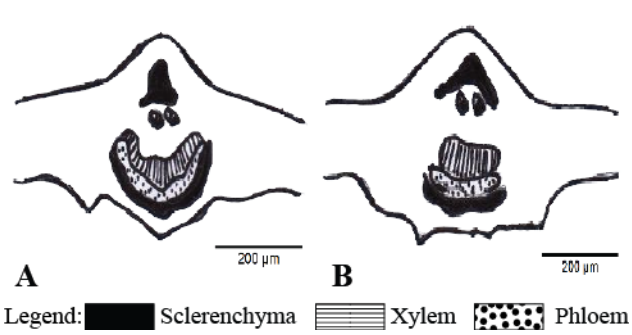

Fig. 8. Variation of vascular tissue pattern and outline shape of midrib: A) *C. vespertilionis* green, B) *C. vespertilionis* red. Scale: 200µm.

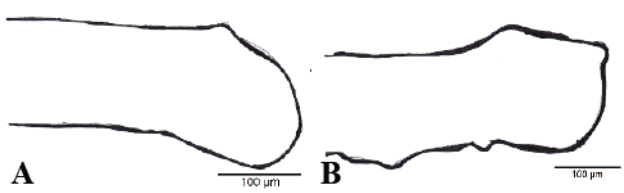

Fig. 9. Variation of leaf margins: A) *C. vespertilionis* green, B) *C. vespertilionis* red. Scale: 200µm

Variation of pollen characteristics: The results of the study on the pollen of *C. vespertilionis* found that the shape of the pollen for *C. vespertilionis* green leaves is oblate spheroidal with a P/E value of 1.1 while the red leaf pollen is prolate spheroid with a P/E value of 0.9. Both pollen of *C. vespertilionis* green and red leaves are medium in size with characteristic of colpus apertures and pores and tricolporate pollen class. A summary of the characteristic variation of pollen of *C. vespertilionis* green and red leaves is as in (Table 4).

Table 4 Variation in pollen characteristics of *C. vespertilionis* green and red leaves.

Species	Morphological characteristics
C. vespertilionis green leaves	The shape of the pollen is oblate spheroid Exine thickness 0.07µm
C. vespertilionis red leaves	Pollen shape is prolate spheroid Exine thickness 0.045µm

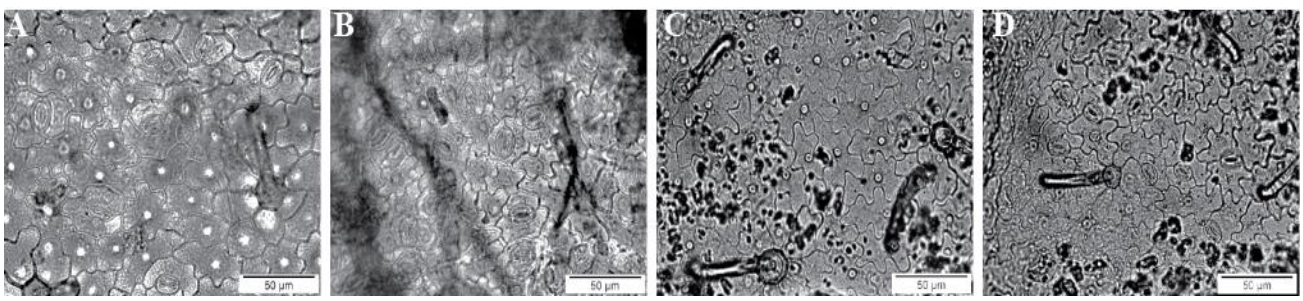

Fig. 10. Anatomical characteristics of the epidermal surface of *C. vespertilionis* green leaves: A) Adaxial epidermal surface; sinuous anticlinal wall, B) Abaxial epidermal surface, distribution of stomata; the presence of amphistomatic, parasitic stomata. Anatomical characteristics of the epidermal surface of *C. vespertilionis* red leaves: C) Adaxial epidermal surface; sinuous anticlinal wall, D) Abaxial epidermal surface, simple unicellular trichomes, D) Abaxial epidermal surface, presence of hypostomatic stomata of anomocytic type. Scale: 200µm.

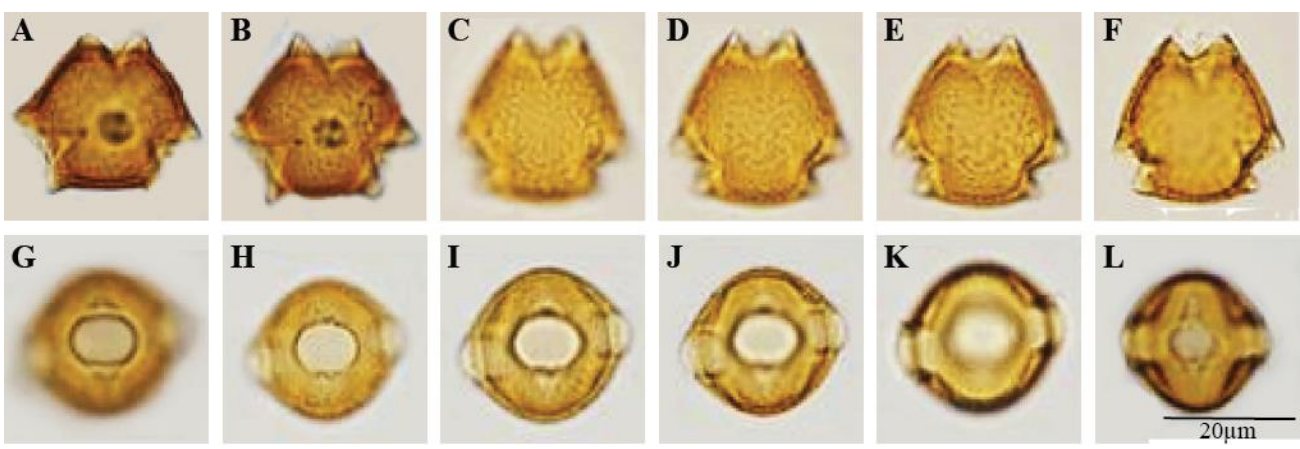

Fig. 11. Pollen morphology of *C. vespertilionis* green leaves. A- F) Polar view, G- L) Equatorial view. Scale: 20µm.

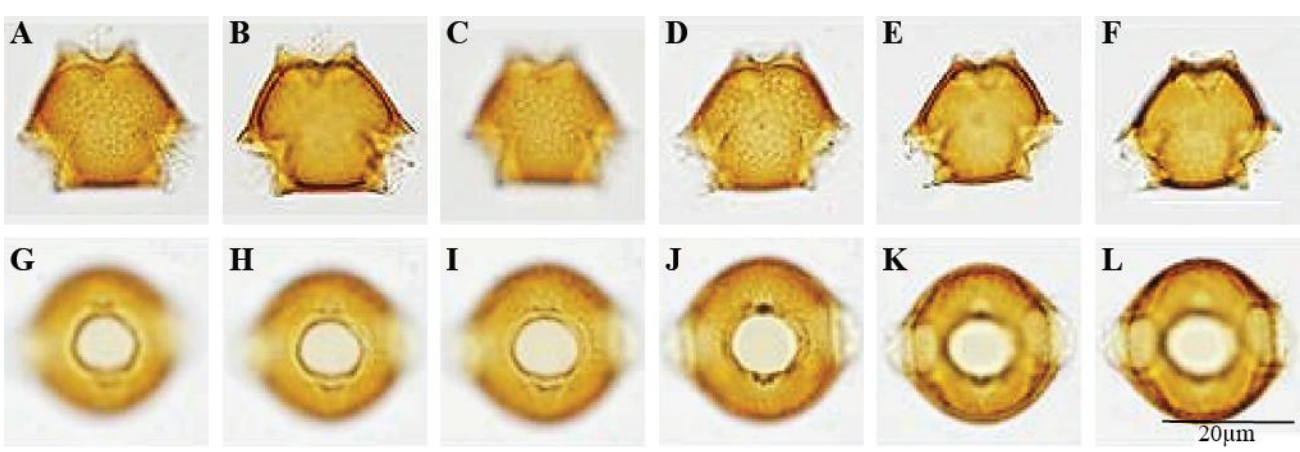

Fig. 12. Pollen morphology of *C. vespertilionis* red leaves. A- F) Polar view, G- L) Equatorial view. Scale: 20µm.

Conclusions

The results of this study have successfully proved that the anatomical, morphological and palynological characteristics of leaves can be used in the identification and differentiation of both *C. vespertilionis* green and red leaves. Similar characteristics obtained from this study are the presence of crystals, the presence of mucilage cells, the vascular bundle system and the presence of additional vascular bundles in the petioles and midrib, the presence of air spaces between cells on the leaf lamina, the pattern of anticlinal walls on the epidermal surface of the leaf, the venation of the leaf margin, presence of glandular trichomes and papillae, main leaf shape, number of leaf veins, pedicel characteristics, calyx and number of petals, seed characteristics, pollen class, aperture type, pollen size, and also the exine pattern.

The variation characteristics that can be used to distinguish *C. vespertilionis* green and red leaves are the type of leaf margin, the presence of swollen tracheids, the type of trichomes present, the outline shape of the leaf margin, midrib and petiole, the number and position of sclerenchyma cells in the midrib, tissue arrangement vasculature in midrib, presence, position and type of stomata, presence of palisade cells in leaf margin, main vascular bundle in petiole, maximum height of *C. vespertilionis* green and red leaves, leaf colour, leaf type, petiole length and surface, leaf shape and characteristics of abaxial and adaxial leaf surfaces, main leaf features, length of flower panicles, calyx features, petal colour, pollen shape and exine thickness. The species dichotomy key was constructed based on diagnostic anatomical and morphological characteristics of leaves and pollen characteristics obtained from the study:

1. The external shape of the leaf edge is like a knob and straight; the outline shape of the midrib, the axil is convex, wide V-shaped and flat abaxially; hypostomatic stomata; simple unicellular (long or short, blunt tip) and multicellular (long, sharp tip) trichomes; swollen tracheids; pollen shape prolate spheroid; exine thickness 0.07µm; leaves unifolia; petiole 1.8-2.3cm glabrous; the base of the leaf is rounded or rounded and the apex is truncate; leaf base cordate, tip broad and emarginate; pedicel 2.0-10.0cm; purplish white flowers ..... *C. vespertilionis* red leaves
2. The outline shape of the leaf margin tapering and curves 30º in the abaxial direction; the external shape of the adaxial midrib humped inverted V-shape and abaxial broad V-shape; amphistomatic stomata; multicellular and unicellular simple trichomes (tapering, hooked tip); tracheids not swollen; pollen shape oblate spheroid; exine thickness 0.05 µm; trifolia compound leaves; petiole 2.0-2.5cm glabrous; leaf base cuneate or nearly rounded, tip truncate; leaf base cordate, tip broad, truncate and slightly emarginate; pedicel 5.0-15.0cm; yellowish white flowers ..... *C. vespertilionis* green leaf

The results of the study show more variations or differences in characteristics than similarities between C. vespertilionis green and red leaves. The conclusion is clear from the results of the study of anatomical, morphological and pollen characteristics on *C. vespertilionis* green and red leaves showing that both are different species or subspecies. Therefore, it is suggested that a molecular DNA study be carried out to further confirm this finding. A taxonomic study for the status of both *C. vespertilionis* green and red leaves needs to be done to determine the taxonomic position of both.

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