LEAF ANATOMY OF YOUNGIA JAPONICA (L.) DC. (ASTERACEAE) FROM PAHANG, MALAYSIA

Zainab Sholehah Abdul Rashid, Che Nurul Aini Che Amri*, Rozilawati Shahari

Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia, 25200, Kuantan, Pahang, Malaysia.

*Corresponding Author Email: chenuralainicheamri@iium.edu.my

This is an open access article distributed under the Creative Commons Attribution License CC BY 4.0, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ARTICLE DETAILS
Article History:
Received 15 April 2022
Accepted 17 May 2022
Available online 20 May 2022

ABSTRACT
Leaf anatomical and micromorphological studies on Youngia japonica (Asteraceae family) were accomplished. In the North America, this wild plant is consumed as salad as a healthy diet. The whole plant is edible and has been consumed in past years especially during famine. The Chinese community has been using this plant to perform blood cleansing, to cure sore throat, diarrhoea, mastitis, shingles, sprains, and bruises. Since there is yet any comprehensive study on the taxonomic study of Y. japonica in Kuantan, this study aims to investigate and examine its leaf anatomical and micromorphological characteristics that can be used as a complete guide for its identification and additional data for its species classification. The methods involved in the leaf anatomy included a cross sectioning method on the petiole, midrib, lamina, and margin using a sliding microtome, clearing of venation using basic fuchsin, and epidermal peel methods using Jeffrey solution. The micromorphology of the leaf was observed under a scanning electron microscopy (SEM). The result of the characteristics of leaf anatomical and micromorphological studies for Y. japonica found the type of vascular bundle at petiole which was an opened system, noncontinuous ring of separated vascular bundle arranged in arc-shaped with additional vascular bundles, there were presence of mucilage cells, incomplete marginal venation, anomocytic stomata at leaf epidermis, granules and crust of wax, and multicellular trichomes with different ended and capitate glandular trichome. In conclusion, the findings have shown that the anatomical and micromorphological characteristics have their taxonomic value and can be useful in the identification, differentiation, and classification of the plant at the species level.

KEYWORDS
Leaf Anatomy, Leaf Micromorphology, Youngia Japonica, Asteraceae, Medicinal Plant

1. INTRODUCTION
Asteraceae plants are commonly known as annual or perennial herbs, subshrubs or shrubs, climbers (Mikania subspecies) or lianas, small but rarely large trees, epiphytes (Gongroystylus species), and rarely aquatic (Sclerolepis subspecies) as recorded (Heywood et al., 2015). A group researchers mentioned that the order of Asterales is inherited by the Asteraceae family and categorised into three common subfamilies: Barnadesioideae, Cichorioideae, and Asteroideae. Y. japonica lies under the subfamily (Katinas et al., 2014; Cichorioideae, 2022). According to Barros as cited in, Asteraceae family consists of 25,000 species including 1,100 genera around the world excluding Antarctica (Milan et al., 2006).

Y. japonica is native in Asia-Temperate, Asia-Tropical (Peninsular Malaysia), Australasia, and later introduced to the Africa, Europe and Northern America (Cichorioideae portal, 2022). The name of Y. japonica is also synonym with Crepis japonica (L) Benth., but the Y. japonica is accepted in the International Plant Names Index (IPNI). Wakhidah recorded that the name of Y. japonica has been validated by de Candolle in 1838 (Wakidah, 2022). However, there are still more than 50 names that are synonym to Y. japonica such as Hieracium sp. Y. japonica is commonly known as oriental hawksbeard and has been used by the Chinese community in their traditional medicine. A group researcher also reported that the majority of Asteraceae species have medicinal value in treating various diseases (Koc et al., 2014).

Babcock and Stebbins reported that the morphology has been documented in 1838. It is annual plant can grow from 8–90 cm high (Babcock and Stebbins, 1937). The leaf has short or long winged or narrowly winged petiole. Its base leaf is broader, smooth or glabrous surface, and sometimes has lightly or densely puberulent. There are about 1–6 stems, that are rigidly upright, thin, vigorous, terete, and branched from the middle or the base of the plant. The flower has many-headed cymose corymbiform. It has about 2–10 lliform peduncle with 15 mm long. The head of flower is erect with tiny 10–20 flowers. The corolla is yellow, the anther is dark green, and the style is yellow. The pappus is white and soft.

Based on the extract of Y. japonica was able to induce the action of cytotoxin towards the cancer cell and caused death of the cell (Oi et al., 2004). A group researchers mentioned that Y. japonica has been used in the Chinese traditional medicine especially in the native area to treat atopy, pyrexia, and detoxification (Munira et al., 2018). Duke and Ayensu as cited in that this plant can be used to treat snakebites (Munira et al., 2018). A group researchers found out that the essential oil of this plant contains larvicidal activity that can treat bites of Aedes albopictus, an Asian tiger mosquito (Xin et al., 2015). Thus, this study is conducted to investigate the anatomical and micromorphological characteristics of the plant and eventually able to produce a set of data that can be added in the taxonomical study of the species.

2. MATERIALS AND METHODS
Three replicates of Y. japonica were collected from Bandar Indera Mahkota, Kuantan, Pahang. Voucher specimens of the fresh leaves were

deposited at the Herbarium Room, International Islamic University Malaysia (IIUM) Kuantan. The fresh leaves were fixed in 3:1 AA solutions (70% ethanol: 30% acetic acid) (Juhari et al., 2017). Its petiole and midribbs were sectioned transversely in a range of thickness (15–30 µm) by using a sliding microtome, LEICA SM2010R. The knife microtome was lubricated before use by applying 50% alcohol with a soft brush. Then, the sections were stained with diluted Safranin and Alcian blue. The sections were dehydrated by a series of 50%, 70%, 95%, and 100% ethanol solutions and later were mounted on microscope slides using Euparal (Cutler et al., 2007). The images were captured using a three-CCD (3CCD) camera attached to a microscope (Leitz Diplan; United Kingdom) and an imaging software (Cell®B). For the leaf epidermal peel method, the adaxial and abaxial epidermises of the fresh leaves were scraped by using a sharp blade. After getting a small, thin, and clear surface, the leaf sample was immersed in Jeffrey solution for a few minutes and was stained with Safranin. Then, the leaf sample was placed on a slide and covered with a cover slip and was observed under the light microscope. For the micromorphology method, the lamina of the leaves was cut into squares (1.0 cm²) and was affixed to aluminium stubs with double-sided adhesive tapes. The specimens were then coated with a thin film of gold and were examined under a scanning electron microscope (EVO®50; Carl Zeiss AG) (Cutler et al., 2007).

3. RESULTS AND DISCUSSION

3.1 Cross Section of Petiole

External outline of petiole: The surface of adaxial is convex and the surface of abaxial is ⅔ circle. The whole shape is round (Figure 1 A). Epidermis cell H: W: The ratio of height and width of epidermal cell is 1:1. Vascular tissue: Opened system, noncontinuous ring of separated vascular bundle arranged in arc-shaped with four additional vascular bundles (Figure 1 A). Schlerenchyma cells: Nil. Mucilage cells: Mucilage cells present at the parenchyma cortex (Figure 1 F & G). Crystal: Nil. Parenchyma cortex 6-13 layers pf parenchyma cortex (Figure 1 A). Collenchyma cells: 1-2 layers (Figure 1 A). Trichome: Simple multicellular trichome (long, pointed-end) (Figure 2 I), simple multicellular trichome (long, flattened end) (Figure 2 J), simple multicellular trichome (long, blunt-end) (Figure 2 L), simple multicellular trichome (short, pointed-end) (Figure 2 J) and simple multicellular trichome (short, blunt-end) (Figure 2 L).

3.2 Cross Section of Midrib

External outline of midrib: The surface of adaxial is convex and the surface of abaxial is U-shaped (Figure 1 B). Vascular tissue: Opened system, one continuous ring of vascular bundle (Figure 1 B). Schlerenchyma cells: Nil. Collenchyma cells: 1-2 layers (Figure 1 B). Mucilage cells: Mucilage cells present at the parenchyma cortex (Figure 1 F). Crystal: Nil. Trichome: Simple multicellular trichome (long, flattened end) (Figure 2 H), simple multicellular trichome (short, blunt end) (Figure 2 L) and simple multicellular trichome (short, pointed-end) (Figure 2 J).

3.3 Cross Section of Lamina

Cuticular layer: Thin layer (Figure 1 C). Adaxial epidermis H: W: The ratio of height and width of adaxial epidermal cell is 1:2 (Figure 1 C). Abaxial epidermis H: W: The ratio of height and width of abaxial epidermal cell is 1:1/1:2 (Figure 1 C). Chlorechyma: The palisade cells occupied 1/3 width of lamina and the spongy cells have 5-6 layers that occupied 2/3 width of lamina (Figure 1 C). Collenchyma: Nil. Vascular bundle: Vascular bundles present along the lamina. Parenchyma cells: Present around main vascular bundle of the lamina. Schlerenchyma cell: Nil. Crystal: Nil. Trichome: Simple multicellular trichome (long, pointed-end) (Figure 2 G), simple multicellular trichome (short, flattened end) (Figure 2 K) and simple multicellular trichome (short, blunt-end) (Figure 2 L) present on the adaxial and abaxial epidermal.

3.4 Cross Section of Margin

Outline: Curved-downward with rounded blunt end (Figure 1 D). Trichome: Simple multicellular trichome (short, blunt end) (Figure 2 L). Vascular bundle: Nil.

3.5 Peeling

Adaxial anticlinal wall: Sinuous (Figure 1 H). Abaxial anticlinal wall: Sinuous (Figure 1 I). Stomata: Amphistomatic, homostomatic, anomocytic on the adaxial and abaxial epidermis surface (Figure 1 J). Size of stomata: width (1.135 µm – 19.97 µm), height (17.78 µm – 30.57 µm). Trichomes: Simple multicellular (long, pointed-end) (Figure 2 G) presented on the adaxial epidermal. Simple multicellular trichome [long flattened end (Figure 2 H) and simple multicellular trichome (short, blunt-end) (Figure 2 L)] presented on the abaxial epidermal.

3.6 Clearing

Marginal vein: Incomplete (Figure 2 A). Laminal vein: majority open with branched (Figure 2 B). Tracheid end: Non-swollen-ended (Figure 2 C). Trichomes: Simple multicellular trichome (short, flattened end) (Figure 2 K) and simple multicellular trichome (short, pointed-end) (Figure 2 J).

3.7 Micromorphology

Wax: Granules and crust of wax present on adaxial and abaxial of epidermal cells (Figure 2 D & E). Cuticular ornamental adaxial and abaxial: Slightly distinguishable, anticlinal wall raised into ridges (Figure 2 D & E). Stomata: Amphistomatic, paraficial stomata, subsidiary cell is undistinguishable, the edges of stomata are unclear and sunken, elliptical shape, size of stomata: width: (3.39 µm – 6.28 µm), height: (15.26 µm – 18.38 µm) (Figure 2 F). Trichome: Capitate glandular trichome [multicellular, short stalk, unicellular head] (Figure 2 M) present on adaxial and abaxial epidermal. Simple multicellular trichome (short, blunt end) (Figure 2 L) present only on the abaxial epidermal.

There was only one layer of collenchyma and one or two parenchyma layers in the leaf. The unusual info about the midrib of the leaf was the aerial cavity present on the adaxial epidermis. Since there was only one layer of collenchyma cells below the aerial cavity, the midrib was unable to resist the environmental challenge especially during heavy rainfall that caused the midrib to crack and eventually broken. This finding shows that the function of collenchyma is undeniably supportive to the adaxial and abaxial epidermises, especially during the changes of environmental surrounding. Study that has been conducted here showed there were one to two layers of collenchyma in the petiole and midrib of Y. japonica towards the adaxial and abaxial epidermises. However, there was no aerial cavity observed but the collenchyma layers were sufficient for the plant to withstand the changes in its surrounding.

3.1.1 Mucilage Cell

Other characteristics that can be used to identify the plant species are mucilage cell. Bahmani et al. stated that the Asteraceae family has the highest potential in treating gastrointestinal tract disorders than other families at Urmia, Iran. Since the mucilage canal is different from the parenchyma cell in terms of the chemical compositions, thus, the mucilage cell has become the common characteristic that can be observed in Asteraceae (Bahmani et al., 2014). According to Johansen as cited in Bombo et al., methylene blue is used as reagent to identify the presence of the mucilage (Bombo et al., 2017). Alldona bokeriiana (Asteraceae family) was found to contain the mucilage substances during the histochemical screening study. Another species, A. grandiflora showed higher production of essential oil from its leaves, roots, and underground stems. Earlier research by Merwe et al. documented that the mucilage cells also store water in the epidermal wall (Merwe et al., 1994). Since mucilage is hydrophilic (attracted to water), the cell can discharge the water if the parenchyma cells dry out. This study has found the existence of mucilage cells on the parenchyma cortex of the midrib. This finding explains the appearance of the Y. japonica leaf that looked turgid and fresh, due to the presence of the mucilage cells.

3.1.2 Leaf Margin

According to Talip et al., the leaf margin is one of the significant characteristics in leaf anatomical study especially among dixyloedons plants (Talip et al., 2019). The margin area refers to the last vascular bundles along the lamina until the shape of the leaf’s edge. The leaf margin could be rounded, pointed end, and straight or curved downwards. However, the authors reminded to place the slice of the leaf properly as the shape of the leaf margin could be changed due to carelessness while placing the sample on the slide. Mason and Donovan observed the morphology of leaf margin, reduced teeth/variable entire, and regular large teeth/variable reduced teeth to evaluate the species-level traits of Helianthus genus (Asteraceae family) by using the leaf economics spectrum (LES) (Mason and Donovan, 2015). The authors also found that the entire margin leaf has lower water efficiency than the large teeth margin. This finding showed that the types of margin influence the endurance of the plant towards the environmental surrounding. In this study, the shape of Y. japonica was found to be curved-downward with rounded-blunt end. The leaf margin also contained simple multicellular trichome (short, blunt-end). Hence, these characteristics can be useful in the species identification.

3.1.3 Stomata

Y. japonica in this study was found to have anomocytic stomata at the surface of its adaxial and abaxial epidermises. The presence of stomata on both epidermises showed that Y. japonica is an amphistomantic plant. The shape of stomata was elliptical as observed. Hayashi and Gloria also listed stomata in the plant characteristics during their observation on A. tenufolia and A. kuntiana (Asteraceae family) (Hayashi and Gloria, 2014). The stomata presented on both surface of epidermises. Also, the stem of Aldona has stomata on the outer surfaces and it has hydathodes on the margin of the leaves. Even though the hydathodes look similar to the stomata pores, they are different. Ghimire et al. reported that the characteristics of stomata are vital and can be utilised to classify the plants according to their genera (Ghimire et al., 2014). This statement is supported by when they studied the stem and leaf structural diversity among 52 species in Lychnophorinae (Asteraceae)
family) in Brazil (Lusa et al., 2018). The location of stomata on leaf epidermis showed the different types of species that they studied. A group researchers added that the anisocytic stomata is mostly presented in Ebenaceae, Malvaceae, Myrtaceae, Rhiophoraceae and Sapotaceae families (Talip et al., 2017). Some of the identified stomata were anisocytic, cyclocytic, diacytic, paracytic, and tetracytic. Tavakkoli et al. also investigated the size of stomata in the Hieracium genus (Asteraceae family) in their research (Tavakkoli et al., 2017). This findings from the previous studies show that the size of stomata can also contribute to the data of taxonomic study.

3.14 Anticlinal Wall and Periclinal Wall

According to the shape of anticlinal wall of Aldama genuse (Asteraceae family) was also used to distinguish species (Filartiga et al., 2016). According to a study, there were seven types of anticlinal wall: straight, straight to curved, curved, curve-to-wavy, sinuous and wavy to sinusoid (Talip et al., 2019). The types of anticlinal wall can be varied as the species observation may be different among the researchers and their evaluations in future studies. Sosa et al. also mentioned a few species of Chrysothemus genus (Asteraceae family) in Argentina (Sosa et al., 2013). According to them, the C. flexusa, C. lithospermifolia, and C. propinqua have straight to straight-anticlinal wall for the adaxial epidermis and sinuous for the abaxial epidermis. Meanwhile, C. cognata, C. platensis, and C. verbascomifolia have sub-straight-anticlinal wall on its abaxial epidermis. Based on these species, it can be proven that the type of anticlinal wall is important characteristic in determining a plant in taxonomic study. In this study, the anticlinal wall of Y. japonica has sinuous type on both adaxial and abaxial epidermises. This result is aligned with Y. japonica feature documented (Choi et al., 2020). These findings show that even a slightest different can be added as additional data in Asteraceae family.

3.15 Venation

Another characteristic that can be useful to identify a plant species is venation. The anatomical part that is observed include the marginal vein, laminar vein, and tracheid end. In this study, the anatomical parts of Y. japonica observed were incomplete marginal vein, majority closed, minority open with branched of laminar vein, and non-swollen ended of tracheid end. As stated from Filartiga et al mentioned that the venation patterns including the primary vein, secondary vein, areoles, and minor veins were necessary to identify the plant. A lot of previous studies show that the 17 species of Aldama family (Asteraceae family) that they studied in Brazil (Filartiga et al., 2016). Kaderete and Bohley also conducted a research of Tephrorosiris genus (Asteraceae-Senecioceae family) to study the leaf venation and found out that a previous research on the leaf venation of Tephrorosiris was inaccurate (Kaderete and Bohley, 2020).

Maitra in his research of two Anaphalis genus (Asteraceae family) also investigated the venation of the species (Maitra, 2019). Anaphalis margaritacea (L.) Benth. & Hook.F. and A. trilinervis (Sims) Simx eases the perfect-acrodromous and palmate-acrodromous venations, respectively. He recorded that the differences can also be seen from the venation such as the primary, secondary, tertiary, and quaternary veins. Although the characteristic did not show the marginal vein, laminar vein, and tracheid end to be compared with this study, the result observed in that study showed that the pattern of the leaf itself can be a significant characteristic to differentiate the plants.

3.16 Epicuticular Wax

Epicuticular wax is one of the micromorphological characteristics in classifying a species. Struwig et al. recorded the importance of Boehavria L. and Conncarpus Standl. (Nyctaginaceae family) that live in arid condition and possess epicuticular striae and waxes to enhance the running water on the surface of the leaves and at the same time can reduce water loss and avoid solute diffusion from the inner leaves to the outer surface (Struwig et al., 2021). Talip et al. stated that the wax of epidermis in various shapes, contain chemical properties, and interact directly with the surrounding (Talip et al., 2019). The presence of wax on the outer surface of the epidermis helps the surface to become waterproof and able to conserve water for the plant. Small particles are unable to penetrate the epidermis due to the presence of wax. The wax has its own significant values as the one found in the Durio genus (Malvaceae family). Some of the identified waxes that are commonly observed under SEM are acicular, block, crust, crustus, film layer, flake, granules, sheet, and verrucate. The findings of this study showed two types of epicuticular wax in Y. japonica which were granules and crust that exist on the adaxial and abaxial epidermises. Meanwhile, the cuticular ornament of adaxial and abaxial were slightly distinguishable with the anticlinal walls raised into ridges and periclinal wall sunken. 3.17 Trichomes

Since trichomes can be used to identify a plant, this characteristic is significant in the taxonomical study (Struwig et al., 2011). Previous study has analysed the trichomes of six taxa of Melastoma genus (Melastomataceae family) and found that some of the trichome characteristics were different among the taxa. Talip, Yunus, Talip and Cutler, and Aladdin et al. also agreed that the types of trichomes can be used to differentiate the variety, species, and genus of a plant (Yunus et al., 2020; Talip, 2006; Yunus et al., 2022; Talip and Cutler, 2009; Aladdin et al., 2016). Commonly, the types of trichomes do not change promptly between the species from the same family. Ruydi et al. reported that an essential oil that has been found from a capitate glandular can be commercialised in perfume industry (Ruydi et al., 2013) Jusuf as cited by Tavakkoli et al. stated that the glandular trichome are useful in differentiating the taxonomy of Hieracium (Asteraceae family) as specific to their species level (Tavakkoli et al., 2017). For example, H. prenanthoides and H. umbellate can be recognised due to the presence of glandular trichome and absence of glandular trichome, respectively. The findings in this study showed there were a variation of trichomes that exist in Y. japonica which were simple multicellular trichome (long, pointed-end), simple multicellular trichome (long, flattened end), simple multicellular trichome (long, blunt-end), simple multicellular trichome (short, flattened end), simple multicellular trichome (short, pointed-end), simple multicellular trichome (short, blunt end), and capitulate glandular trichome (multicellular, short stalk, unicellular head).

4. Conclusion

Since Y. japonica is well known in traditional and modern medicines through its medicinal value, the systematic study of the anatomical and micromorphological characteristics is significant in identifying this species. The results of this study showed that the pattern of its vascular bundle, the thickness of its parenchyma and colenchyma layers, its mucilage cells, leaf margin, stomata, epicuticular wax, and trichome can be utilised as additional data in identifying the Y. japonica of the Asteraceae.

Acknowledgement

We are thankful to the Department of Plant Science, Kulliyah of Science, IIUM for providing the equipment and other physical support for this research. Authors contribution including Roziawai as confirming the species and help out the morphology of the species. Che Nural Ain provided the idea and the theory of the experiment. Zainab Shoelah carried out the research, wrote and revised the article together with Che Nural Ain. Special appreciation is dedicated to the Ministry of Education Malaysia for funding [research grant: FRGS19-085-0694 (FRGS/1/2019/STG03/UIAM/03/2)] this meaningful research.

References


Wakhidah, A.Z., 2019. Karakterisasi variasi morfologi youngia japonica (l) DC. (asteraceae) dari pulau sumatera, Indonesia, international standard number of botany, 42 (1), Pp. 1321338

