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LEAF ANATOMY AND MICROMORPHOLOGY OF POTENTIAL MEDICINAL WEED *Ruellia repens* L. (ACANTHACEAE) FROM TASIK CHINI, PAHANG

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Abstract

Weeds are commonly known as unwanted plants that grow in wild and human-controlled areas such as farms, gardens, and fields. However, these undesirable plants might benefit humans, especially as a traditional medicine in some rural areas. *Ruellia repens* belongs to the family Acanthaceae, one of the common weeds in Peninsular Malaysia. This species is believed to become a potential medicinal weed due to its several potent chemical constituents that might be useful in the pharmaceutical industry. But, a lack of research attempts is reported in the taxonomic study of this species. By keeping this point, the present study was carried out to provide the leaf anatomical and micromorphological characteristics of *R. repens* obtained from Tasik Chini, Pahang. This detailed information will be helpful for the botanist and pharmacologist to avoid misidentification of the species. Several methods were involved such as cross-sectioning on petiole, midrib, lamina and marginal parts, leaf epidermal peeling, leaf clearing, observation under a light microscope and observation under a scanning electron microscope. Results revealed some important leaf anatomical and micromorphological structures such as petiole, midrib and marginal outlines, presence and type of cystoliths, presence and type of trichomes, type of stomata, and presence and types of waxes. The leaf anatomical and micromorphological structures in *R. repens* are taxonomic significance and might be used as additional data to identify and classify the species. Also, the present study might be a good starting point that leads to extensive future works on the medicinal weeds, especially from Tasik Chini, Pahang.

INTRODUCTION

The observations and knowledge on the significance of medicinal plants are currently increasing worldwide. The traditional uses of medicinal plants were employed centuries ago, especially in people's health care systems. Also, 25% of prescribed modern medicines are reported to contain one or more ingredients derived directly or indirectly from the plants [1, 2] thus, giving evidence on the importance of medicinal plants to humankind. Furthermore, various types of plants are observed to be used by people from rural areas, especially in their traditional healing and treatment [3]. These plant materials served as an alternative way to treat ailments whenever modern medicines are costly, difficult to obtain, or unavailability of a modern drug to treat the diseases [4]. Additionally, the ethnobotanical review that has

been conducted recently on the family Acanthaceae has proved that many contributions of the knowledge of medicinal plants in the treatment of diseases were gathered from indigenous people, Malay villagers, and local market traders [5].

Medicinal plants including weeds are reported to comprise medicinal values and can be potentially used for the production of new drugs and medicines. Weed in general refers to the wild plant that is commonly considered as a nuisance in a garden, lawn or other agricultural areas [6]. In simple words, weed is a type of plant that grows in an area where it is not wanted since it limits the growth of other plants by blocking light or using up nutrients from the soil. Even researchers stressed that the occurrence of weeds is problematic both in agricultural and non-agricultural areas which later on resulted in economic losses [7]. Moreover, it is also mentioned that weeds give a serious problem for agriculturists since they tremendously reduce the productivity of agricultural trends [8]. Because of the negative impacts of the weeds on agricultural trends, most agriculturists get rid of these plants without realizing their medicinal value and benefits. Yet, in some exceptional cases, weeds are considered "beneficial plants or herbs" because of their edibility, source of food and herbal medicine [6, 7]. Indeed, a total of 25 species from medicinal weeds belong to two plant families of the Acanthaceae and Asteraceae are enumerated in a survey of medicinal weeds in Chonburi, Thailand [9]. Not only that, it is estimated around 65 plants belong to 35 families have been considered as medicinal weeds and used in Ayurvedic and Traditional physicians in Sri Lanka [6]. However, less known uses of medicinal weeds are reported, specifically for medicinal weeds in Peninsular Malaysia.

The family Acanthaceae is one of the dicotyledonous flowering plants belong to the order Lamiales. The Acanthaceae mainly occurs as herbaceous and shrubs but are sometimes found as climbers or lianas [10, 11]. Recently, the systematic treatment of Acanthaceae is divided into four subfamilies which are Acanthoideae, Nelsonioideae, Thunbergioideae and Avicennioideae, with seven tribes occurring under the subfamily of Acanthoideae which are Acantheae, Ruellieae, Justicieae, Barlerieae, Andrographideae, Whitfieldieae and Nemacanthus [12]. Previous studies described that the Acanthaceae is a large and diverse pantropical family consisting of approximately 240 genera and 3250 species in the world [13]. Even, it is documented on about 29 genera and 158 species of the Acanthaceae in Flora of Malaya [14]. From that number, a species namely *Ruellia repens* is one of the potential medicinal weeds belong to the Acanthaceae that occurred in Peninsular Malaysia.

Ruellia repens or synonymously known as *Dipteracanthus repens* (L.) Hassk falls under the subfamily of the Acanthoideae and Ruellieae tribes. Ruellieae is a taxonomically and ecologically diverse tribe in the Acanthaceae [15]. This species occurred under the genus *Ruellia* by which the genus is commonly known as Ruellias or Wild Petunias [16]. There are several vernacular or common names of *R. repens* recorded according to their localities including akar keremak, deras malam and rumput halnyor in Malaysia, plinten, remah and sekatan in Indonesia, whereas chaa hom and daa maeng in Thailand [17]. *R. repens* is widely distributed in India, Burma (Myanmar), Indo-China, southern China, Taiwan, Thailand and throughout the Malesian region except for New Guinea [17]. This species can be found in moderately shaded areas, often grassy localities, in roadsides [18], brushwood, and sometimes in abandoned fields [17]. *R. repens* is an erect and herb 15-50 cm tall. Its leaves are opposite arrangement, the leaf blade is ovate to linear-lanceolate or ovate-lanceolate, and the subentire margin shows the occurrence of cystoliths. Besides, the flowers are axillary, solitary, and corolla occurred as white or pink to light purple [17,18].

Apart from being a popular ornamental plant, many of the Acanthaceae species have been used in folk medicinal practices because of their medicinal properties. The applications of *R. repens* in traditional medicine thereby are also comparable to those of several other Acanthaceae species. Also, it shows the recognition of uses of the whole parts of the genus *Ruellia* to treat bladder stones and bronchitis [19]. Furthermore, many species in the genus *Ruellia* are reported to contain several important chemical constituents such as glycosides, flavonoids and alkaloids [2]. The leaf infusion of *R. repens* is used as a natural antiurolithiatic agent against kidney stones in Malaysia. Besides, its pounded leaves are reported to be used externally as a poultice to treat sore legs, ulcers and cuts [20]. Also, it can be used as a cooling agent and as an anodyne to relieve toothache [17]. Indeed, the involvement of *R. repens* amongst indigenous people is also reported whereby its powder of dried leaves is mixed in warm water and consumed by indigenous people from Semang tribe to remove kidney stones [21]. Also, it is stated that bruised plants of *R. repens* are fried with eggs and taken internally to treat cough in China [17].

Other than possessing several important chemical constituents and being used in folk medicinal practices, *R. repens* is also popular being a "host plant" to certain butterfly species. These butterflies play an important role in the food chain of an ecosystem and also act as pollinators for the production of plants [22]. The flowers and butterflies possessed colorful and amazing aesthetic values which create a very relaxing and eye-catching scenery of a place. Some of the butterfly species from the families of Nymphalidae, Lycaenidae and Pieridae are reported to ovipositing eggs on larval host plants of Acanthaceae species [22, 23]. They lay their eggs either under the leaves or on top of the leaves, but sometimes also on the branches of the plants [22]. Not only *R. repens*, several other Acanthaceae species are also observed to be host plants for butterflies such as *Barleria prionitis* and *Hemigraphis reptans* [23], thus proving how important plants including weeds towards the

ecosystem. However, a lack of attempt to record the importance of medicinal weeds from the Acanthaceae has been reported, specifically for Peninsular Malaysia. In addition, a very scarce report is observed on the taxonomy and pharmacology parts of *R. repens*, especially species from Tasik Chini, Pahang. Instead, both parts are very crucial for the researchers and society to have a correct identification of *R. repens* as well to produce natural medicinal drugs for medicinal purposes. This research thereby deals with the leaf anatomy and micromorphology approaches that have been conducted on *R. repens* from Tasik Chini, Pahang. The informative data obtained from the leaf anatomical and micromorphological analyses are very significant and possess taxonomic values for the identification and classification of *R. repens*, especially species from Tasik Chini, Pahang.

MATERIALS AND METHODS

This foliar study was conducted on one of the potential medicinal weeds from the family Acanthaceae namely *R. repens* as shown in Figure 1. Fresh specimens of *R. repens* were collected from Tasik Chini, Pahang. Three to four plants replicates were used throughout this research. For herbarium preparation, the collected samples that were composed of vegetative and reproductive parts were organized properly on the newspaper and layered by cardboards. Samples were pressed by a wooden presser and appropriately tied. The stack of plant specimens was labeled and placed into an oven for two weeks duration at 55°C. After the drying process, the samples were taken out from the oven and affixed to herbarium paper by using a specific needle and thread. These samples were labeled properly with a herbarium label at the lower right-hand corner of the sheet [24, 25]. The voucher specimens were then deposited at

Figure 1. A & B) Photographs of plant samples *R. repens* obtained from Tasik Chini, Pahang.

Universiti Kebangsaan Malaysia Herbarium (UKMB) for future reference. The leaves specimens were fixed in AA Solution (30% acetic acid: 70% Ethanol in a ratio of 1:3).

For the leaf anatomical investigation, several methods were involved such as cross-sectioning of petiole, midrib, lamina and marginal parts. These parts of specimens were sectioned in a range of thickness (10-30 µm) by using a sliding microtome that was composed of a fixed sample holder known as polystyrene [26], followed by a staining process with Safranin and Alcian blue, and dehydration in a series of alcohol. The epidermal peeling method was prepared manually by scrapping off the underside of leaf surfaces by using a razor blade followed by a staining process with Safranin and dehydration in a series of alcohol. The slides were mounted with Euparal and covered by coverslips. The leaf clearing method was conducted by immersion of leaf lamina and margin into the Basic Fuchsin solution before being placed into an oven that set up at 60°C for a few days. The cleared leaf was undergone a dehydration process in a series of alcohol and mounted in Canada balsam. All the anatomical slides were kept in an oven for two weeks duration at about 60°C for drying purposes. Anatomical images were captured using a video (3CCD) camera attached to a Leitz Diaplan microscope using Cell^B software. Suitable modifications in terms of fixation and embedding followed the standard method of anatomical investigations [27, 28]. The images obtained were analyzed according to the anatomical descriptions and discussed conscientiously.

For the leaf micromorphological investigation, the specimens were obtained from dried samples of the herbarium from UKMB. Samples of the lamina were cut in a size of about 1 cm^2 and mounted with gold by using a

sputter-coated machine. The micromorphological images were captured by using a Scanning Electron Microscope Zeiss Model Evo 50. The images obtained were analyzed according to the micromorphological descriptions and discussed conscientiously.

RESULTS

Figure 2 shows the characteristics of leaf anatomy and micromorphology of *R. repens*. The descriptions of the leaf anatomical and micromorphological characteristics for *R. repens* are summarized as below:

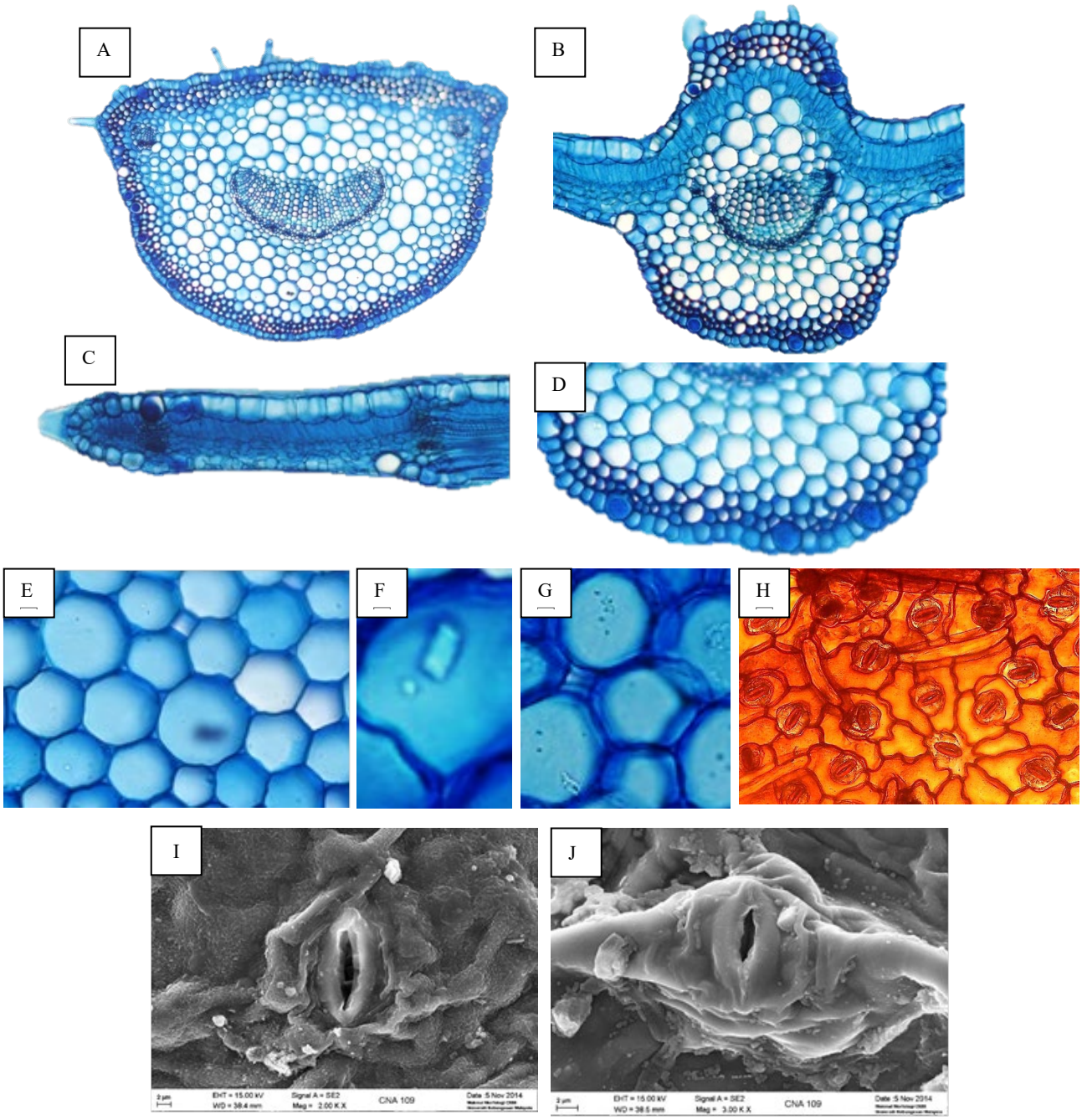


Figure 2. The leaf anatomical and micromorphological characteristics of *R. repens*: A) Cross-section of petiole part, B) Cross-section of midrib part, C) Cross-section of marginal part, D) Collenchyma cells underneath the abaxial epidermis, E) Mucilage cells present in parenchyma cortex, F) Prismatic calcium oxalate crystal, G) Druses calcium oxalate crystals, H) Sinuous anticlinal walls and diacytic stomata, I) Crustose cuticular waxes, J) Granules cuticular waxes. Scales: A) 500 µm, B&C) 200 µm, D) 100 µm, E, F, G & H) 50 µm, I&J) 2 µm.

Petiole

Adaxial outline: convex with ear-like shaped appears at the edge of adaxial epidermis. Abaxial outline: U-shaped. Vascular bundles: One main vascular bundle (opened system with continuous rings of vascular bundles) with two additional vascular bundles situated at the left and right side of the main vascular bundle. Parenchyma cells: ca. 5-7 layers of parenchyma cells. Sclerenchyma cells: Clusters of sclerenchyma cells present ensheathing the phloem tissue of main and additional vascular bundles. Collenchyma cells: ca. 2-4 layers of collenchyma cells present underneath the epidermis cells. Mucilage cells: Present in the parenchyma cortex. Calcium oxalate crystal: Prismatic crystals present in parenchyma cortex. Cystolith cells: Solitary elongated cystolith with round-shaped present in the epidermis of abaxial and adaxial. Trichomes: Simple unicellular (short, blunt end), simple multicellular (long, blunt end) and simple multicellular (long, pointed end) trichomes present on epidermis of petiole.

Midrib

Adaxial outline: hump with ½ rectangular shaped. Abaxial outline: U-shaped. Vascular bundles: One main vascular bundle (opened system with continuous rings of vascular bundles) with one additional vascular bundle situated at the left side of the main vascular bundle. Parenchyma cells: ca. 3-5 layers of parenchyma cells. Sclerenchyma cells: Clusters of sclerenchyma cells present ensheathing the phloem tissue of main and additional vascular bundles. Collenchyma cells: ca. 2-4 layers of collenchyma cells present underneath the epidermis cells. Mucilage cells: Present in the parenchyma cortex. Cystolith cells: Solitary elongated with round-shaped present in the epidermis of abaxial and adaxial. Trichomes: Peltate glandular (terminal multicellular) and simple multicellular (long, blunt end) trichomes present on epidermis of midrib.

Leaf Margin

Outline: Slightly tapered end and the size decreases gradually towards the edge of margin.

Leaf Lamina

Cuticular layer: Relatively thin. Adaxial epidermis: one layer with height: width ratio – 1:1. Abaxial epidermis: one layer with height: width ratio – 1:1. Chlorenchyma cells: mesophyll palisade cells; present as one layer equal to 1/2 part of the leaf lamina thickness, spongy mesophyll cells; 2-3 layers of spongy mesophyll cells. Vascular bundles: simple vascular bundles. Calcium oxalate crystals: Druses present in spongy mesophyll cells. Cystolith cells: Solitary elongated with round-shaped cystoliths present in epidermis of abaxial and adaxial. Trichomes: Simple unicellular (short, pointed end, cone-shaped) and simple multicellular (long, pointed end) trichomes present on epidermis lamina.

Leaf Epidermis

Adaxial anticlinal walls: Sinuous. Abaxial anticlinal walls: Sinuous. Stomata: Homostomatic, amphistomatic and diacytic type of stomata. Cystolith cells: Solitary elongated with blunt end and solitary elongated with one end pointed present on epidermis of abaxial and adaxial. Trichomes: Peltate glandular (terminal multicellular) trichome present on epidermis of abaxial and adaxial.

Leaf Epidermis SEM

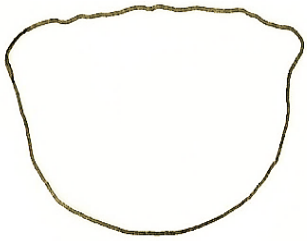
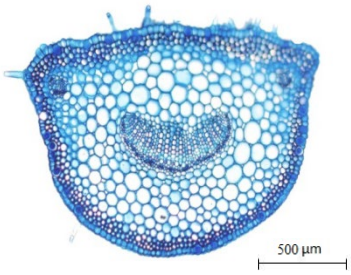
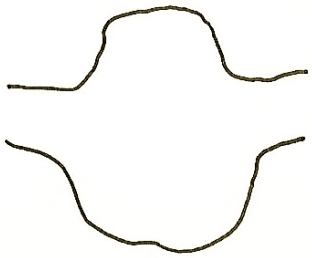
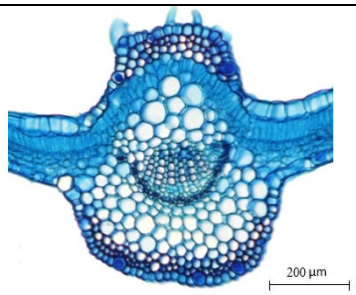
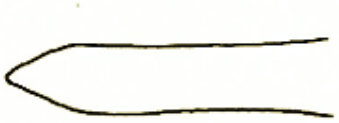
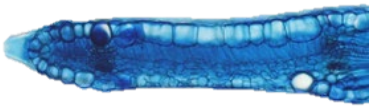
Epicuticular waxes: Crustose and granules present on epidermis of abaxial and adaxial. Cuticular ornamentation: Abaxial and adaxial epidermis undifferentiated, anticlinal and periclinal walls undifferentiated. Stomata: Amphistomatic, superficial, elliptic-shaped, stomata type undifferentiated. Stomata size range: H x W (12.74 – 19.17 µm) x (6.11 – 9.72 µm). Cystolith cells: Present of cystolith cells on epidermis of adaxial and abaxial. Trichomes: Simple multicellular (long, pointed end, echinate ornamentation) trichome present on epidermis of adaxial and abaxial, and peltate glandular trichome present on epidermis of abaxial.

DISCUSSION

Morphologically and anatomically the leaf is the most variable plant organ, and thus various anatomical and micromorphological characters within the leaf have been verified to be of systematic value including the leaf of *R. repens*. The petiole anatomy feature is one of the valuable parameters used in the identification and classification of many plant families, genera and species [29, 30]. Recently, the interest and research on petiole anatomy as a complementary tool in plant taxonomy has been increasing, especially to provide evidence on plant classification [31]. In fact, the significance of petiole anatomy in plant classification has proven to be useful as in 30 species of Asteraceae that showed the presence of eight types of petiole outlines within the species [32]. The petiole outlines criterion has also proven to be useful in the delineation of plant species as in the identification of some species in the genus *Coelostegia* (Bombacaceae) [33]. Also, the significance of petiole outlines in the classification of plant species has been observed on the genus *Microcos* (Tiliaceae) [34]. Besides, various authors have agreed that the variations in vascular structure and the combination of petiole outline could be very useful in the classification of the genus *Parashorea* (Dipterocarpaceae) [35]. The findings of this foliar study hence revealed several important anatomical characteristics including leaf petiole, midrib and marginal outlines of *R. repens*. For easy reference, the illustrations and descriptions of each outline are shown in Table 1. The adaxial petiole outline shows slightly convex with an ear-like shape appearing at the edge of the adaxial epidermis, whereby the abaxial outline is U-shaped. The adaxial midrib outline shows a hump with a half rectangular shape, whereas the abaxial is U-shaped. The leaf margin of *R. repens* has a slightly tapered end and the size decreases gradually towards

the edge of the margin, hence supported by the findings of the taxonomic significance in leaf margin of genus *Alpinia* [36].

Table 1. The illustrations of petiole, midrib, and marginal outlines of *R. repens*.

Parts	Descriptions	Illustrations	Figures
Petiole	Adaxial outline: Slightly convex with ear-like shaped appears at the edge of adaxial epidermis Abaxial outline: U-shaped		
Midrib	Adaxial outline: Hump with ½ rectangular shaped Abaxial outline: U-shaped		
Marginal	Marginal outline: Slightly tapered end and the size decreases gradually towards edge of margin		

More anatomical features are claimed to be significant in the systematic study of plants. For instance, the petiole anatomy can be used for grouping genera and the identification of species [37]. In fact, the number of vascular bundles, shape, arrangement and the petiole outline are reported to be consistent in angiosperms and could serve as useful clues to identify and classify the plants [34, 38]. Previously, the pattern of vascular tissue on the petiole part is declared to be significant in the classification of *Shorea* (Dipterocarpaceae) species [39]. Even it is reported the significance of vascular tissue arrangement in the identification of *Dipterocarpus* (Dipterocarpaceae) [40]. The findings of the present study hence revealed the vascular bundle pattern in the petiole and midrib parts of *R. repens*. Both the main vascular bundles and additional vascular bundles of petiole and midrib showed opened system with continuous rings of vascular bundles. The importance of vascular bundle arrangement has also been supported by research conducted on families of Malvaceae [34] and Annonaceae [41], whereby the vascular bundle arrangement can be used in delimiting these two plant families.

Although the physiological function of calcium oxalate crystals has not yet fully been discussed, this component plays an important role especially in calcium metabolism and the regulation of ionic equilibrium [42]. Besides, the presence of crystals in higher plants is usually related to the physical protection, calcium storage, the removal of oxalate from the metabolic system as well as regulation of light during photosynthesis whenever the plants grow under shade [43]. It has been also suggested that the occurrence of calcium oxalate crystals in plants is related to the level of calcium concentration in the surrounding medium [42]. This present study also reported on two types of calcium oxalate crystals that occurred either in the parenchyma cortex or spongy mesophyll cells of *R. repens*. Two types of calcium oxalate crystals are prismatic (Figure 2F) and druses (Figure 2G) types. The calcium oxalate crystals can be found in solitary or clusters. Solitary crystals in plants occurred in

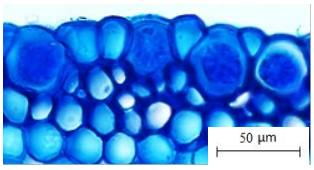
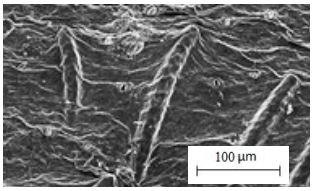
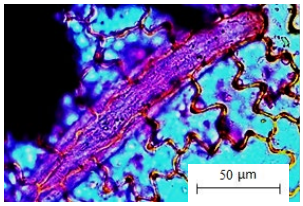
various shapes such as rhombus, cube, square, rod-shaped, acicular or needle-shaped and styloid, whereby cluster crystals appeared as druses [44]. It is also mentioned that the compact and spherical aggregates of angular crystals are known as druse, whereas the aggregations of needle-like crystals as raphides [45]. This cluster of needle-shaped of calcium oxalate crystals or raphides usually can be found in monocotyledons plants [44]. The occurrence of calcium oxalate crystals as in acicular or prismatic either in cortex or pith is reported in some species from the genus *Chionanthus* and *Olea* from the family Oleaceae, thus proving that this character might be important to delineate the plant genus or taxa [46]. Moreover, the types and patterns of crystals are proven to be valuable characters as in the genus *Prunus* (Rosaceae subfamily Prunoideae) since these characters are useful to recognize the genus [47].

The leaf epidermal characteristics can be used as diagnostic features in the identification of plants as they possessed various informative data for the taxonomical study. Many distinct patterns of stomata have been found in the epidermis of different plants [48], and therefore might be considered as important clues in systematic study. Also, there are several important anatomical characteristics recognized in the genus *Alpinia* (Zingiberaceae) including anticlinal walls and stomata types [36]. Even various morphological characters of foliar epidermal are proven to be useful in taxonomic classification and might be able to provide significant clues regarding the subfamilial, tribal and generic relationships [49]. A foliar study conducted on *Thunbergia laurifolia* Lindl. (Acanthaceae) revealed some important anatomical features of the species such as the presence of stomata on lower leaf surface only (hypostomatic) and the stomatal complexes consist of two subsidiary cells surrounding the guard cells or known as diacytic stomata [50]. Furthermore, it is also reported that the anatomical feature of the stomatal pattern in *Polygonum minus* Huds. (Polygonaceae), whereby the stomata have shown a paracytic pattern, thus might be an important feature to recognize the species [51]. This present leaf anatomical investigation of *R. repens* thereby reported on the presence of homostomatic, amphistomatic and diacytic types of stomata. Homostomatic refers to the condition where the stomata type occurs as in one type only, whereas the amphistomatic is the existence of stomata on both epidermal surfaces (abaxial and adaxial surfaces). The stomata type possesses taxonomic value since it can help to classify the plants according to their ranks, especially at family, genus or species level. To date, ten stomata types are classified according to their subsidiary cells. The stomata types include anomocytic, polocytic, diacytic, amphidiacytic, paracytic, anisocytic, diallelocytic, tetracytic, staurocytic and cyclocytic [44]. The presence of amphistomatic in *R. repens* has also been supported by previous research findings of hypostomatic and amphistomatic leaves among selected Acanthaceae species [52] and hypostomatic leaves in Rubiaceae [53]. Furthermore, the presence of hypostomatic and diacytic stomata has been reported in 22 species of Acanthaceae [54]. This character hence is very useful and might be a good criterion to delineate plant taxa, or can be used to classify plants up to their genus level.

Besides, the pattern of anticlinal walls and epidermal cells also served as an important criterion in the systematic study, especially for the identification and classification of plants. The epidermis of dicotyledonous plants has mostly occurred in polygonal-shaped cells epidermis, whereby the epidermis of monocotyledonous plants occurred as parallel elongated shaped [44]. Also, the existence of irregular or polygonal shapes of leaf epidermal cells is recorded in selected species from Schisandraceae [55]. Apart from that, there are various patterns of anticlinal walls occurred in plants such as straight, straight to curved, curved, curved to wavy, wavy, wavy to sinuous and sinuous [44]. Even the value of a sinuous wall is also believed to increase the tensile strength of the epidermis. A more secure attachment between the epidermal and mesophyll cells has been depicted from the sinuous shape of *Dryopteris thelypteris* L. (Thelypteridaceae) [56]. The evidence on the taxonomic importance of the patterns of anticlinal walls is portrayed in previous research on *Melastoma* (Melastomataceae) species [57]. The systematic significance in patterns of anticlinal walls has also been depicted by research findings on 23 species of Schisandraceae by which the species studied composed of straight, sinuolate, sinuous or sinuate patterns of anticlinal walls [55]. The significant patterns of anticlinal walls hence supported the findings of the present research on the presence of sinuous anticlinal walls on both abaxial and adaxial surfaces of *R. repens*, thus might act as a criterion to characterize the species.

Cystolith cell is one of the most recognizable anatomical features of the Acanthaceae, except in the tribe of Acantheae [58], subfamilies of Thunbergioideae and Nelsonioideae [59] and Avicennioideae [60]. Cystolith or also known as calcium carbonate is reported to be present in several other plant families such as Cannabaceae, Urticaceae and Moraceae [15, 61]. Cystolith is originated from Greek words in which cyst referred to “cavity” and lith referred to “stone” [62]. The occurrence of cystolith cells is usually in the vegetative parts of the plants such as roots, stems and leaves. Cystolith acts as a repository of calcium, especially for metabolic and growth requirements under certain conditions [63]. Besides, cystolith can serve as temporary storage for calcium by which will be remobilized during the calcium starvation in the plants [64]. It has been stated that the occurrence of cystoliths in the vegetative parts of the Acanthaceae is considered as taxonomic important to characterize the plant family [54]. Thereby, this present study revealed three types of cystolith cells that occurred in *R. repens* hence supported by previous research findings on the existence of cystolith cells in the tribe of Rueliaeae [15]. For convenient purposes, cystolith cells of *R. repens* have been illustrated into recognizable types as in Table 2 to avoid confusion and complex descriptions.

Table 2. The illustrations of cystolith types present in *R. repens*.

Type of cystoliths	Illustrations	Figures
Solitary elongated with round-shaped		
Solitary elongated with blunt end		
Solitary elongated with one end pointed		

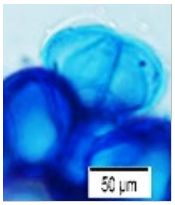
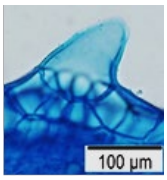
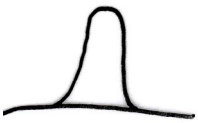
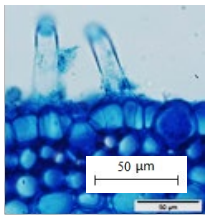
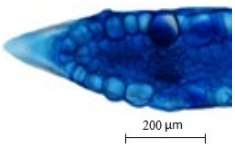
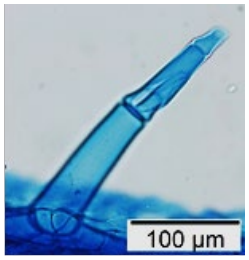
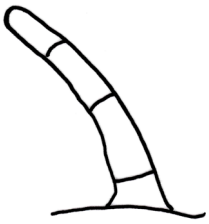
Three types of cystolith cells occurred are solitary elongated with round-shaped, solitary elongated with a blunt end and solitary elongated with one end pointed. These findings are also supported by recent research findings on the cystoliths type of 41 taxa belonging to Acanthaceae in Peninsular Malaysia [65].

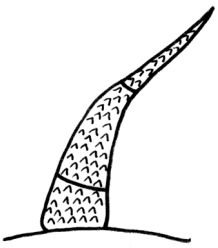
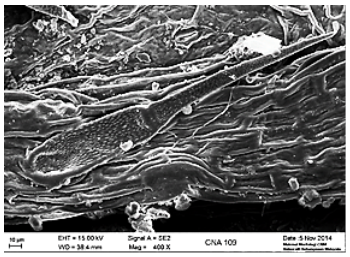
Trichomes are defined as unicellular or multicellular appendages that originate from the epidermal cells and develop outwards on the surfaces of plant organs [66]. Several functions of trichomes are discussed such as reducing insect movements, mechanical abrasion and leaf wetness, increasing light reflectance, reduce water loss and pollinator attraction [67]. The hairs or epidermal outgrowths are claimed to possess ecophysiological significance, especially for the mangrove species [68]. It has been mentioned that the taxonomic and phylogenetic significance of trichomes has been long recognized by a number of researchers [69]. In fact, the trichomes morphology is proven to have systematic significance as in some petals of the Acanthaceae species [70]. The finding on trichomes types in *T. laurifolia* Lindl. (Acanthaceae) also revealed such valuable data by which two kinds of trichomes present in that species are sessile glandular trichomes with a panduriform head and unicellular non-glandular trichomes, thus proving that trichome is one of the important characteristics in the identification of plants species [50]. This present study thereby revealed seven types of trichomes in *R. repens* which consists of both glandular and non-glandular trichomes. For easy reference and better understanding, the illustrations of each trichome type are shown in Table 3. Seven types of trichomes present are peltate glandular (terminal multicellular), simple unicellular (short, pointed end), simple unicellular (short, blunt end), simple unicellular (short, pointed end, cone-shaped), simple multicellular (long, pointed end), simple multicellular (long, blunt end) and simple multicellular (long, pointed end, echinate ornamentation). The trichomes morphology is hence considered to have taxonomic values in the identification and classification of species.

The epidermal investigation that involved observation under a scanning electron microscope in plants is believed to reveal several important epidermal characteristics which later on, might be very helpful to better understand the phylogenetic and physiological activity of the plants. For instance, cuticular waxes which have various shapes and chemical constituents are important for the interaction of plants with the environment [44]. In addition, the occurrence of waxes on the epidermal leaves helps the leaves to be waterproof thus preventing small particles to attach to the leaves' epidermis. Even the upper cuticle layers of some mangrove species showed a range in thickness, and many mangrove species showed the upper epidermis cuticle is thicker than the lower epidermis cuticle [68]. Furthermore, the systematic significance of cuticular waxes is proven to be important as in the genus *Durio* (Malvaceae) [44]. The foliar study in *R. repens*, therefore, revealed two types of cuticular waxes that are present on the epidermis of adaxial and abaxial which are crustose and granules. The importance of cuticular waxes is also portrayed in other several genera studied, whereby it is suggested that the cuticular character

might be useful if the character is studied carefully since it helps to recognize the plant taxa in the family Lauraceae such as the genera of *Alseodaphne*, *Nothaphoebe* and *Dehaasia* [71]. In fact, ten types of common cuticular waxes are found in plants including crustose and granules waxes [44].

Table 3. The illustrations of trichomes types present in *R. repens*.

Type of trichomes	Illustrations	Figures
Peltate glandular (terminal multicellular)		
Simple unicellular (short, pointed end)		
Simple unicellular (short, blunt end)		
Simple unicellular (short, pointed end, cone-shaped)		
Simple multicellular (long, pointed end)		
Simple multicellular (long, blunt end)		

Simple multicellular (long, pointed end, echinate ornamentation)		

CONCLUSION

Less known taxonomic information of medicinal weeds is recently reported, especially the medicinal weeds from Peninsular Malaysia. Certainly, the taxonomic and advantages of medicinal weeds should be studied thoroughly. The agriculturists should pay serious attention to identify and promote the beneficial weeds and their medicinal uses to the community. The current and potential uses of *R. repens* are expected to give scholars more attention to the application and benefits of this plant, especially in the pharmacological area. Despite that, this present research revealed several important features of the leaf anatomical and micromorphological of *R. repens*, therefore expected to provide useful information for the botanists and pharmacologists. The anatomical and micromorphological features are petiole, midrib and marginal outlines, types of vascular bundle arrangements, the presence and types of calcium oxalate crystals, the presence and type of stomata, the pattern on anticlinal walls, the presence and types of cystolith cells, the presence and types of trichomes and types of cuticular waxes. The various important leaf anatomical and micromorphological features of *R. repens* may believe to provide informative data in the taxonomic study and might lead to further investigations of *R. repens*, especially for the pharmaceutical benefits. Also, the data obtained can be used as additional data to support the taxonomic study of *R. repens*, specifically for the identification and classification of plants either at the genus or species level. In fact, the present research is hoped to be one of the good efforts to prove the significance of medicinal weeds towards humankind and the ecosystem.

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CONFLICTS OF INTEREST

The authors declared no conflict of interest in this publication of the manuscript.

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