

Morphological, Anatomical and Phytochemical Studies of *Sphagneticola calendulacea* (L.) Pruski

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Abstract

Sphagneticola calendulacea (L.) Pruski growing wild in Mandalay area, was collected from January, 2019 to April, 2019. The microscopical, macroscopical characters and phytochemical constituents of this species were mentioned in this paper. The morphology, encompassing both the vegetative and reproductive parts, and anatomy, covering only the leaves parts of this species were studied, described and discussed. The characters observed in this species revealed that they can be used as potential diagnostic characters for botanical identification of the species. The anatomical characters observed in this study can be used in identification of this species. The preliminary phytochemical screening leaves of the species respond positive test for alkaloids, glycoside, phenolic compounds, polyphenols, reducing sugars, saponins, steroids, tannins and lipophenol respectively, whereas negative test for flavonoids and terpenes.

Keyword: *Sphagneticola calendulacea* (L.) Pruski, microscopical, macroscopical, phytochemical

Introduction

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years. Human have been depended, for their maintainance, the diversity of plant resources for foods, clothes, shelters and medicine. Many plant parts, such as roots, leaves, barks, seeds, fruits and flowers are used in traditional medicine. Most of the people use traditional medicine for the treatment of diseases.

In the present study, microscopical, macroscopical characteristics and phytochemical studies on *Sphagneticola calendulacea* (L.) Pruski have been thoroughly investigated. *Sphagneticola calendulacea* (L.) Pruski, Naykyakalay in Myanmar, belongs to the family Asteraceae and is growing wild and widely distributed in Myanmar.

Plant anatomy is the study of the internal structure of plants. Plant anatomy is now frequently investigated at the cellular level, and often involves the sectioning of tissue and microscopy (Esau, 1965). Therefore, the present study is focused on the morphology and taxonomy of the plants as well as the anatomy of the vegetative parts of *Sphagneticola calendulacea* (L.) Pruski.

Plants contain hundreds of different chemical constituents that interact in complex ways, although it is very useful to know that a plant contains certain active constituents (Chevallier, 1996). Some of these important compounds include alkaloid, glucosides, saponin, tannins, steroids, flavonoids, etc. These compounds are generally presence in the roots, stems, barks, leaves, seeds etc. Therefore, the present study is focused on the phytochemical test of *Sphagneticola calendulacea* (L.) Pruski. Because of the various medicinal use of *Sphagneticola calendulacea* (L.) Pruski phytochemical analysis of the plant, along with the observations of microscopical and macroscopical characters, had been undertaken.

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Therefore, the aims and objectives of the study are to reveal microscopical and macroscopical characteristics of *Sphagneticola calendulacea* (L.) Pruski, to investigate phytochemical constituents of the same species and to contribute the morphological, anatomical and phytochemical constituents information of *Sphagneticola calendulacea* (L.) Pruski.

Materials and Methods

Morphological studies of *Sphagneticola calendulacea* (L.) Pruski

Collection and identification of *Sphagneticola calendulacea* (L.) Pruski

In this study, the specimens were collected from Mandalay Area during January 2019 to April, 2019. The vegetative and floral parts of the collected specimens were identified at Department of Botany, Yadanabon University with the help of literature of Hooker (1875), Lawrence (1951) and Cronquist (1981).

Anatomical studies of *Sphagneticola calendulacea* (L.) Pruski

For the anatomical study, free hand sections of the fresh parts of the leaves (Lamina, midrib and petiole). The leaves of this species were cut into thin sections by using razor blade. Of these, the thin sections were cleared with a few tiny pieces of chloral hydrate crystals and washed with distilled water and then stained with safranin solution and temporarily mounted by dilute glycerine solution and then observed under a light microscope. For the maceration the fresh parts of the leaves were cut into pieces measuring about 3.0 mm in length. These pieces were introduced into the test tube along with an equal volume of acetic acid and hydrogen peroxide solution and boiling them. The macerated materials were taken up from the test tube, place on the glass slide gently squashed and one drop of safranin stain was added on the pieces. Then, these slides were mounted in dilute glycerine and studied. The sizes of macerated elements were measured by equipping with the help of an ocular micrometer and calculated the size of the cells by the method according to Kokate (2000).

Preliminary Phytochemical Examination of the Leaves of *Sphagneticola calendulacea* (L.) Pruski

Phytochemical test were carried out to detect the presence of organic constituents in the leaves of *Sphagneticola calendulacea* (L.) Pruski. According to the results, the leaves of *Sphagneticola calendulacea* (L.) Pruski extract consists of alkaloid, flavonoid, glycoside, saponin, reducing sugar, polyphenol, phenolic compound, tannins, terpenes, lipophenol and steroids respectively.

Results

Sphagneticola calendulacea (L.) Pruski Syn. Corn. 222. 1832.

Local Name : Negya-gale

English Name : Wedelia

Flowering and fruiting almost throughout the years.

Perennial procumbent herbs, about 0.5m high; stems and branches terete. Leaves simple, opposite and decussate, exstipulate, subsessile or petiolate; blades elliptic-lanceolate or oblanceolate, petioles very short. Heads terminal or axillary solitary, heterogamous, yellow; peduncles slender. Involucre campanulate; involucre bracts 2-seriate, elliptic-lanceolate. Receptacles convex, paleaceous. Paleae of the ray-florets lanceolate or elliptic-lanceolate. Ray-florets (outer flowers) 8 to 13 per whorl (series), uniseriate, pistillate, fertile, zygomorphic, disc-florets (inner flowers) 3- to 4-seriate, opening in acropetal succession, bisexual, fertile, actinomorphic. Pappus minute, 3- to 4-seriate. Corolla of ray-florets ligulate, the ligules spreading, the tube short, glabrous, pale yellow; limbs 3-toothed, corolla of disc-florets tubular, the tube

limbs 5-toothed, filiform. Stamens 5, epipetalous, exerted before anthesis, inserted after anthesis; filaments filiform, short; anthers syngenesious, ditheous, introrse. Ovary 3-to 4-angled, laterally compressed, cuneate-oblong, clothed with glandular hairs and multicellular nonglandular hairs, unilocular, with one basal ovule; ovules oblongoid, white; style filiform, exerted, stylar arms exerted, linear, yellow, recurved, papillose at the stigmatic surface.



Figure 1. *Sphagneticola calendulacea* (L.) Pruski

The Leaves of *Sphagneticola calendulacea* (L.) Pruski

Lamina

Studied of laminae of species *Sphagneticola calendulacea* (L.) Pruski were 200.0 - 250.0 μ thick, typically dorsiventral, venation reticulate. Distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System :Composed of epidermal cells, guard cells of the stomata and non-glandular trichomes. In surface view, the epidermal cells of both surfaces parenchymatous, irregularly arranged; upper epidermal cells deeply wavy, cell walls thick, the cells; stomata anomocytic, the guard cells, in upper epidermis stomatal number 2 per field in high power, stomatal index 4; lower epidermal cells deeply wavy, cell walls thick, the cells, stomata anomocytic, the guard cells, in lower epidermis stomatal number 13 per field in high power, stomatal index 19; non-glandular trichomes multicellular. In transverse section; both adaxial and abaxial epidermis 1-layered, compact, rectangular or barrel-shaped, anticlinal walls straight; non- glandular trichomes multicellular, cuticle smooth and thin.

Ground Tissue System : Mesophyll differentiated into palisade and spongy parenchyma; palisade parenchyma adaxial 1-layered, the cells vertically elongated, compact, cell walls thin, straight, chloroplast abundant; spongy parenchyma 5- to 7-layered, compact, chloroplasts abundant, intercellular spaces small.

Vascular Tissue System : Vascular bundles collateral type, rounded or oval, small, embedded in the mesophyll, xylem on the adaxial side and phloem on the abaxial side; bundle sheath 1-layered, parenchymatous, barrel-shaped, phloem 3- to 6-layered, composed of sieve-tubes, companion cells and parenchyma; xylem cells 1- to 3-rows, 2- to 3-celled in each row, protoxylem; metaxylem, composed of vessel elements, tracheids, fibers and parenchyma; vessel elements thick-walled, lateral walls straight or wavy, end walls transverse or oblique, perforation plates simple, thickening annual or spiral; tracheids thick-walled, lateral walls straight or wavy, end walls transverse or tapering, thickening reticulate or spiral; fibers thick-walled, lateral walls straight or wavy, end walls acute, lumen narrow; xylem parenchyma, rectangular, thin-walled.

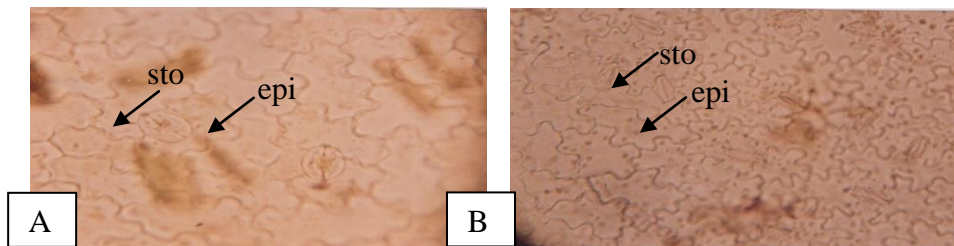


Figure 2. Surface View of a Lamina

A. Adaxial epidermal cells B. Abaxial epidermal cells

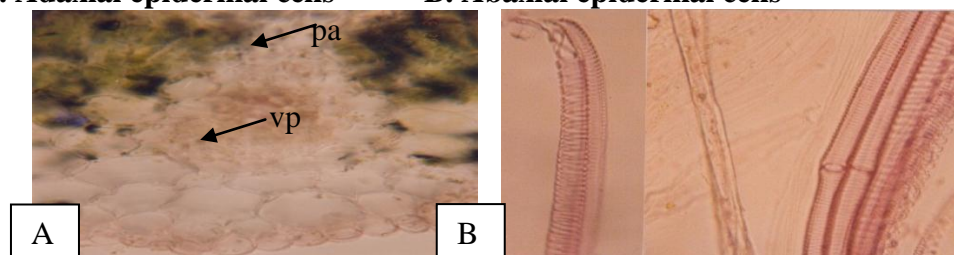


Figure 3. Transverse section of a Lamina

A. Adaxial and Abaxial Epidermal Cells, Mesophyll Cell and the Vascular Bundle, B. Macerated Component Cells of a Lamina

Midrib

In transverse section, midrib studied are semicircular at the abaxial side and crescent-shaped at the adaxial side. Distinguishable into dermal, ground and vascular tissue systems.

Dermal Tissue System: Composed of epidermal cells and non-glandular trichomes. In surface view, the upper epidermal cells parenchymatous, elongated lengthwise, anticlinal walls straight and smooth; nonglandular trichomes, elongated, multicellular; lower epidermal cells as seen in upper epidermis. In transverse section, the upper epidermis 1-layered, compact, parenchymatous, cell walls thin, oval or barrel-shaped, cuticle smooth and thin; non-glandular trichomes, multicellular; lower epidermis as seen in upper epidermis.

Ground Tissue System Composed of outer collenchymatous cells and inner parenchymatous cells as the main mass of ground tissue. Outer collenchymatous layers below the adaxial and above the abaxial epidermises; those at the adaxial side, 1- to 2-layered, polygonal; those at the abaxial side, 2- to 3-layered, the layers 50.0 - 70.0 μ thick, polygonal; inner parenchymatous layers below the adaxial and above the abaxial collenchymatous layers; those at the adaxial side, 6- to 8-layered, rounded or oval or polygonal, intercellular spaces small or large; those at the abaxial side, 3- to 7-layered, rounded, oval or polygonal, intercellular spaces small or large, resin canals near the vascular bundle.

Vascular Tissue System : Vascular bundles collateral type, arranged an arc of 3 separated bundles open towards the adaxial side of the midrib: middle bundle large, peripheral bundles small, bundle-caps sclerenchymatous, crescent-shaped strands at the outer boundary of the phloem and xylem groups, 3- to 5-layered, polygonal; phloem on the abaxial side and xylem on the adaxial side; phloem composed of sieve-tubes, companion cells and parenchyma cells, phloem cells 4- to 5-layered, polygonal; xylem arranged in 1- to 6-rows, 1- to 4-celled in each row; protoxylem, polygonal, composed of vessel elements, tracheids, fibers and parenchyma cells; vessel elements, cell walls thick, lateral walls straight or wavy, end walls transverse or oblique, perforation plates simple, thickening reticulate or spiral; tracheids, cell walls thick,

lateral walls straight or wavy, end walls oblique or transverse or tapering, thickening reticulate or spiral, fibers, cell walls thick, lumen narrow, lateral walls straight or wavy, end walls acute; xylem parenchyma rectangular, thin-walled.

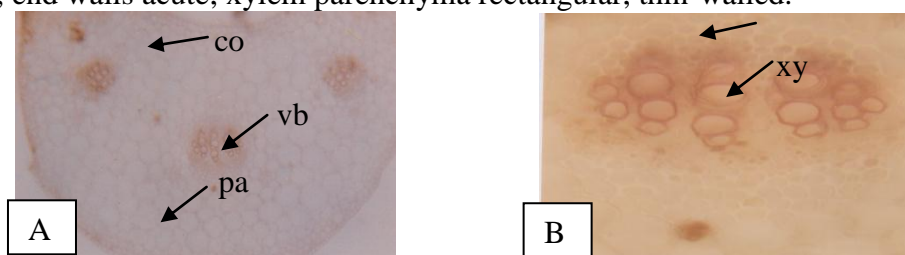


Figure 4. Transverse section of a Midrib

A. Adaxial and Abaxial Epidermises

B. Vascular Bundle



Figure 5. Macerated Component Cells of a Midrib

A. Vessel Element

B. Vessel Element and Tracheids

Table 1. The Results of Phytochemical Tests for Leaves of *Sphagneticola calendulacea* (L.) Pruski

No.	Tests	Extracts	Reagents	Observation	Results
1.	Alkaloids	1 % HCl	Wagner's reagent Dragendroff's reagent	Reddish brown ppt Orange ppt	+ +
2.	Flavonoids	EtOH	Magnesium, conc:HCl	Pink solution	-
3.	Glycosides	Distilled water	10% Lead acetate	White ppt	+
4.	Phenolic compounds	Distilled water	5 dps 10 % FeCl ₃	Blue black solution	+
5.	Polyphenols	EtOH	1% FeCl ₃ , 1% K ₃ [Fe(CN) ₆]	Greenish blue solution	+
6.	Reducing Sugars	Distilled water	Benedict's solution	Orange red ppt	+
7.	Saponins	Distilled water	Distilled water	Forth	+
8.	Steroids	EtOH	2 dps acetic anhydride, 2 dps conc:H ₂ SO ₄	Green solution	+
9.	Tannins	Distilled water	10% FeCl ₃ , dilH ₂ SO ₄	Yellowish brown ppt	+
10.	Terpenes	EtOH	2 dps CHCl ₃ , 2dps acetic anhydride 2dps conc:H ₂ SO ₄	Pale red solution	-
11.	Lipophenol	Distilled water	4 dps NaOH, KOH	Deep solution	+

(+) = presence of constituents, (-) absence of constituents

Discussion and Conclusion

In the present study, observations microscopical, macroscopical characters and phytochemical screening of *Sphagneticola calendulacea* (L.) Pruski, belonging to the family Asteraceae were made.

In present work, *Sphagneticola calendulacea* (L.) Pruski are perennial procumbent herbs. The leaves of this species are simple, exstipulate, opposite and decusate. The inflorescences of *Sphagneticola calendulacea* (L.) Pruski studied are

axillary or terminal and solitary head. There are marginal flowers (ray-florets) and inner flower (disc florets) in a same head or capitulum. In this study, the stamens of this species are 5, epipetalous and syngenesious anthers. Inferior ovary is unilocular with single anatropous ovule on the basal placenta.

Internal structure of leaves observed in this research are dorsiventral, anamocytic type of stomata and non-glandular trichomes occur on both surfaces. In transverse section, palisade tissue are 1- layered. In transverse section of midribs and petioles, composed of the outer collenchymatous cells at the adaxial and abaxial sides, and the inner parenchymatous cells as the main mass of ground tissue. A simple is of separated bundles which accompanied by bundle caps on the abaxial and adaxial sides. In ground tissue, the lamina of the mesophyll cell are differentiated into palisade and spongy parenchyma; palisade parenchyma adaxial 1-layered. The shape of midribs was semicircular at the abaxial side and crecent-shaped at the adaxial side. The ground tissue was composed of outer collenchymatous cells and inner parenchymatous cells as the main mass. The vascular bundles are circular in outline with collateral type.

In the present study, phytochemical test of leaves extracts of *Sphagneticola calendulacea* (L.) Pruski are detected the presence of Alkaloids, Glycosides, Phenolic compounds, Polyphenols, Reducing sugars, Saponins, Steroids, Tannins and Lipophenol but those of the Flavonoids and Terpenes are absence.

It is hoped that these information reported in the present study can be used for their proper utilization near future and supported to identify the source plant of the useful drugs. In addition to above mentioned morphological, microscopical and phytochemical informations can be contributed. For further researcher, pharmacological activities should be carried out.

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