

Morphological, Phytochemical and Anatomical Studies on Leaves of *Asystasia gangetica* (L.) T. Anders.

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Abstract

The aims of the present study are to study the morphological, phytochemical and anatomical characters on leaves of *Asystasia gangetica* (L.)T. Anders., family Acanthaceae. The plants were collected from Monywa Township during November 2018 and August 2019. The phytochemical study was done by the method of Harborne (1984) and anatomical study carried out by free hand section. The powdered leaves were observed the presence or absence of alkaloids, flavonoids, phenol, saponins, lipophilic compounds, carbohydrates, reducing sugar, glycosides and tannins except the cyanogenic glycosides. The petiole was palanoconvex and midrib semicircular in outline. The vascular bundles of petioles and midribs were crescent-shaped with 2-4 subsidiary bundles. The lamina was rounded in outline. Uniseriate multicellular and glandular trichomes, diacytic stomata, cystoliths were found on both surfaces of petiole, midrib and lamina, except the stomata in abaxial surface of midrib. The present study will provide morphological, phytochemical and anatomical characters and will support for identification and classification of this plant from other species and standardization of crude drug of leaves of *Asystasia gangetica* (L.)T. Anders.

Key words - *Asystasia gangetica*, phytochemical study, anatomical study, Acanthaceae.

Introduction

Plants are an important source of therapeutic remedies for various ailments. Plant based drugs have been used worldwide in traditional medicines for treatment of various diseases. The increased interest in plant-derived drugs is mainly because of the wide spread belief that ‘herbal medicine’ is safer than synthetic drugs which possess side effects. Hence, there is need to screen medicinal plants for biological activity (Sivaraj *et al.* 2013).

Acanthaceae is an important large family containing biologically active compounds namely: alkaloids, terpenoids, polyphenols and phytosterols. Of about 196 species recorded, a meager number of 25 species is considered as economically important. *A. gangetica* (L.) T. Anders is also marked as economical plant (Suseela 2005).

Asystasia comprises about 50 species, and is distributed in tropics of the old world. *A. gangetica* (L.) T. Anders is a perennial herb, with usually ascending, quadrangular stem, often rooting at the lower nodes. It is herbaceous groundcover that grows from 300-600 mm in height. It has oval-shaped leaves with white-cream coloured flower with purple markings and capsule is a club-shaped. The plant is used in ethnomedicine for heart pains, stomach pains, rheumatism, vermifuge and anthelmintic (Hamid *et al.* 2011). *Asystasia* means inconsistency and it relates to the fact that the corolla is slightly regular which is an unusual characteristic in the Acanthaceae family. The word ‘*gangetica*’ is derived from Ganges River in India and it is locally used a potherb and leafy vegetable in times of food scarcity (Hanafi 2015).

The *A. gangetica* (L.) T. Anders. is commonly found in this study area and almost in anywhere of Myanmar. It is used traditionally for many diseases and also

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used as a source of food. It has many medicinal properties and used for various ailments; like rheumatism, diabetes, especially urinary diseases. Moreover, previous work is lacking with reference to proposed objectives of the study. Thus, it is carried out to reveal morphological, phytochemical and anatomical characters of leaves in this study.

The aims and objectives of this research are to study the morphological characters, to reveal the phytochemical constituents, to show the anatomical characters and sensory characters of powdered leaves, to support the identification of this plant and standardization of crude drugs in traditional medicines.

Materials and Methods

The plant samples were collected from Monywa Township to study morphological, phytochemical and anatomical characters. This research was carried out during November 2018 and August 2019. It was noted their morphological characters and identified by using the literatures of Backer (1965), Hooker (1885) and Dassanayake (1998). Myanmar name is described by Hundley & Chit Ko Ko (1961) and Kress *et al.* (2003).

Preparation of Plant materials

The collected leaves were cleaned thoroughly and air-dried in shady place for about two weeks to get stable dried products. The dried leaves were powdered, weighted and stored in air-tight containers to study sensory microscopical characters and phytochemical constituents.

Phytochemical study

The powdered leaves were extracted with 1% diluted hydrochloric acid (HCl), 95% ethanol (EtOH) and distilled water (H₂O), and tested for the presence or absence of alkaloids, flavonoids, phenols, saponins, lipophilic compounds, glycosides, tannins, reducing sugars, carbohydrates and cyanogenic glycosides by the method of Harborne (1984).

Anatomical Study

The leaves were cut by using a new razor blade to obtain thin sections. These sections were killed and fixed in 70% ethyl alcohol solution. Then, about five sections were placed on slide. Chloralhydrate solution drops onto the sections about 10 minutes and washed by water. They were stained with standard saffranin solution for 10 minutes and washed by water. The stained sections were mounted. The cells were studied and measured the distinct characters and recorded by using microscope. The photomicrographic figures were taken with digital camera.

Sensory characters of powdered leaves

The powders were sieved with No.40 and No.60 sieve plates respectively. The resulting powdered samples were observed their color, taste, odour and texture of sensory characters and recorded.

Microscopical Studies of powdered Leaves

A few powdered samples were placed on the glass slide and added three drops of chloralhydrate solution to moisten the powder and also act as a clearing agent. Then, a few drops of safranin solution were added to stain the lignified cell walls. The slide was covered with cover slip and viewed under the light microscope. The microscopical characters were observed and recorded. Then, photomicrographic figures were also prepared by the use of a microscope with digital camera.

Results

Morphological Study

Asystasia gangetica (L.) T. Anders., Enum. Pl. Zeyl. 235-236. 1860

- Family - Acanthaceae
 English name - Chinese Violet
 Myanmar name - Kyauk- hkwe (white)

Perennial erect or straggling herbs; stems and branches quadrangular. Leaves simple, opposite and decussate, exstipulate, petiolate; blades broadly ovate, glabrous on upper surface and puberulous on lower. Inflorescences terminal racemes. Flowers campanulate, white or violet; bracts small; bracteoles minute; pedicels pubescent. Calyx 5-partite, persistent; tubes very short, glabrous; lobes linear-lanceolate, ciliate. Corolla 5-lobed, campanulate; tubes long, glandular pilulose without; lobes obovate. Stamens 4, didynamous; filaments filiform, glabrous; anthers oblong, glabrous. Ovary superior, oblongoid, bilocular, one - two ovules in each locule, axile placentae, pubescent; style filiform, pubescent; stigma bilobed. Loculicidal capsule, 4-seeded.

Table 1. Phytochemical Tests on Leaves of *A. gangetica* (L.) T. Anders.

No	Test	Extract	Test reagent	Observation	Result
1	Alkaloids	1%HCl	Dragendroff reagent	orange color	+
2	Flavonoids	EtOH	Conc: HCl + Mg	Dark green color	+
3	Phenols	H ₂ O	1% FeCl ₃ + 1% K ₃ Fe(CN) ₆ sol:	Black color	+
4	Saponins	H ₂ O	Distilled water (or) Na HCO ₃ solution	frothing	+
5	Glycosides	H ₂ O	10% lead acetate solution	white ppt	+
6	Lipophilic compounds	H ₂ O	KOH solution	Yellowish brown colour	+
7	Tannins	H ₂ O	1% Gelatin + 1% FeCl ₃ solution	Pale yellow color	+
8	Reducing Sugars	H ₂ O	Benedict's solution	White ppt	+
9	Carbohydrates	H ₂ O	Benedict's solution + Conc: H ₂ SO ₄	Pale brown ppt	+
10	Cyanogenic glycosides	H ₂ O	Conc: H ₂ SO ₄	No color change	-

+ = present - = absent ppt. = precipitation

This table shows the results on leaves of phytochemical study. In the present study, alkaloids, flavonoids, phenols, saponins, glycosides, lipophilic compounds, tannins, reducing sugar and carbohydrates are found in leaves but cyanogenic glycosides are not in leaves.

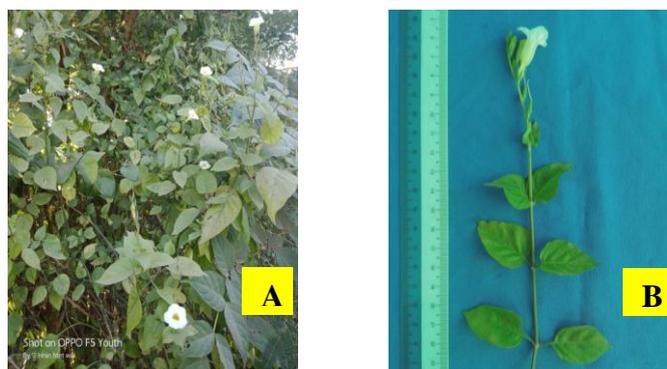


Figure 1. A. Natural habit of *Asystasia gangetica* (L.) T. Anders.
 B. Inflorescence of *Asystasia gangetica* (L.) T. Anders.

Anatomical Study of Petiole

In surface view, the epidermal cells are composed of parenchymatous, rectangular or polygonal-shaped; multicellular uniseriate and glandular trichomes, cystoliths, diacytic stomata are present on both surfaces and more present on adaxial surface.

The transverse sections of petiole are planoconvex with abaxial side convex and adxial side flat. The epidermal cells in both sides are one-layered, barrel-shaped; multicellular uniseriate and glandular trichomes more present in adaxial side. In adaxial side, collenchymatous cells are 3-5-layered, polygonal-shaped; chlorenchymatous cells 2-3-layered, polygonal-shaped; parenchymatous cells 5-7-layered, polygonal or spherical-shaped. In abaxial side, collenchymatous cells are 4-6-layered, polygonal-shaped; chlorenchymatous cells absent; parenchymatous cells 6-9- layered, polygonal-shaped, intercellular spaces small. The vascular bundle are single crescent-shaped bundle in the middle and 2-4 subsidiary bundles at the cortex, collateral type; phloem 3-4-layered, thin-walled, polygonal-shaped; xylem 22-25 rows, each 2-6-layered, polygonal-shaped.

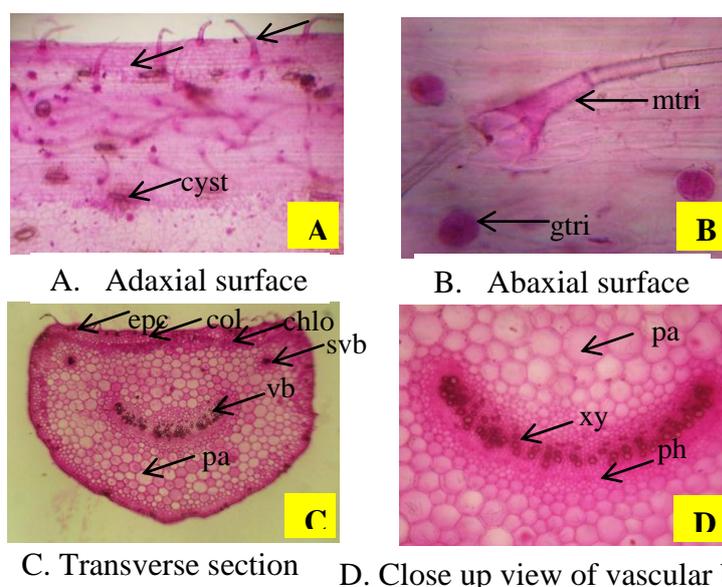


Figure 2. Anatomical structures of petiole of *A. gangetica* (L.) T. Anders.

chlo = chlorenchyma, col = collenchyma, cyst = cystoliths, epc = epidermal cell, gtri = glandular trichome, mtri = multicellular trichome, Pa = parenchyma
ph = phloem, svb = subsidiary vascular bundle, vb = vascular bundle, xy = xylem

Anatomical Study of Midrib

In surface view, the epidermal cells of both sides are parenchymatous, rectangular or barrel-shaped, thin-walled; multicellular uniseriate trichomes and cystoliths on both surfaces. The diacytic stomata are present only on upper surface.

The transverse sections of midrib are semicircular in outline with rounded abaxial side and 2 pyramidal projections in adxial side. The epidermal cells of both sides are one-layered, barrel-shaped; multicellular uniseriate and glandular trichomes present on both surfaces. In adaxial side, collenchymatous cells are 2-5-layered, polygonal-shaped; chlorenchymatous cells 1-3-layered, polygonal-shaped; parenchymatous cells 4-7-layered, polygonal-shaped. In abaxial side, collenchymatous cells 2-3-layered, polygonal-shaped; chlorenchymatous absent; parenchymatous cells 4-7-layered, spherical or polygonal-shaped. The Vascular bundle

are single crescent-shaped in middle and 2-4 subsidiary bundles in cortex, collateral type; phloem 2-3-layered, thin-walled, polygonal-shaped; xylem 20-22 rows, each 2-5-layered, polygonal-shaped.

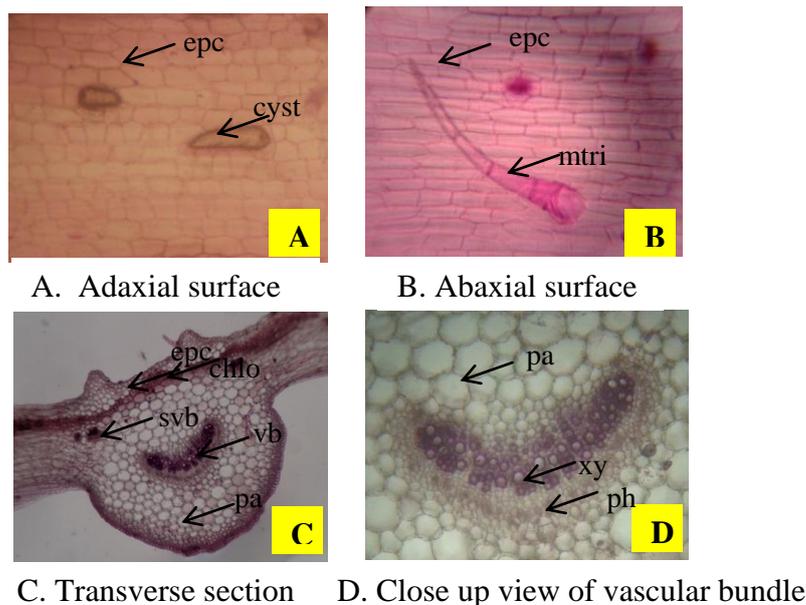


Figure 3. Anatomical structures of midrib of *A. gangetica* (L.) T. Anders.

chlo = chlorenchyma, cyst = cystoliths, epc = epidermal cell, gtri = glandular trichome, mtri = multicellular trichome, pa = parenchyma, ph = phloem, svb = subsidiary vascular bundle, vb = vascular bundle, xy = xylem

Anatomical Study of Lamina

In surface view, the epidermal cells of both surfaces are similar in shape, parenchymatous, rectangular-shaped; adaxial epidermal cells distinctly wavy; stomata more abundant on abaxial surfaces, multicellular uniseriate and glandular trichomes and cystoliths are present on both surfaces, trichomes more abundant on adaxial surface.

The transverse sections of lamina are dorsiventrally flattened. Both adaxial and abaxial epidermal cells are one-layered, rectangular or barrel-shaped; multicellular uniseriate and glandular trichomes present on both surfaces. Mesophyll cells are differentiated into palisade and spongy parenchyma; palisade at adaxial side, one-layered, vertically elongated; spongy parenchyma at abaxial side, 3-5-layered, irregular-shaped; intercellular spaces large. The vascular bundles embedded in ground tissue, collateral type, surrounded by one-layered bundle sheath cells; the xylem towards adaxial side and phloem on abaxial side.

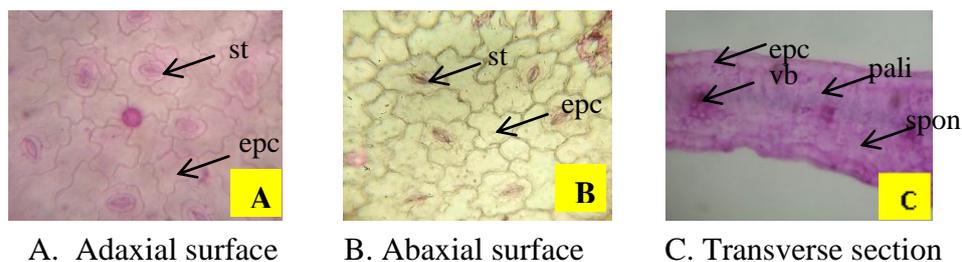


Figure 4. Anatomical structures of lamina of *A. gangetica* (L.) T. Anders.

epc = epidermal cell, epi = epidermis, pali = palisade cell, spon = spongy cell, st = stomata, vb = vascular bundle,

Sensory Characters of Powdered Leaves

The color of the powdered leaves is pale brown, slightly bitter and salty in taste and non-aromatic in odor. The texture is fibrous in nature.

Microscopical characters of powdered leaves

The microscopical characters show the fragments of vessels, tracheids and fibres. The vessels are pitted, annual and spiral thickened with perforation plates. The fibres are long. The fragments of trichomes, epidermal cells, stomata, mesophyll cells, calcium oxalate crystals and cystoliths are also found in the powdered leaves of *A. gangetica* (L.) T. Anders.

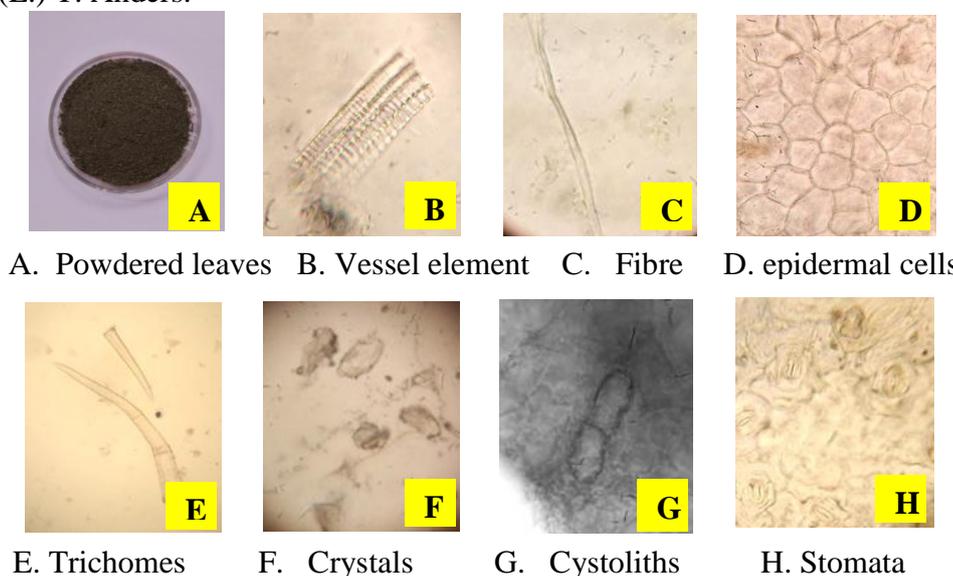


Figure 5. Powdered Characters of leaves of *A. gangetica* (L.) T. Anders.

Discussion and Conclusion

The *Asystasia gangetica* (L.) T. Anders is a perennial herbaceous plant, stems quadrangular, leaves opposite and decussate, inflorescences terminal racemes, flowers white or violet, bracts small, calyx persistent and corolla campanulate, stamens didynamous. Ovaries were superior, pubescent and capsule loculicidal. These characters were similar to those of Hooker (1885), Backer (1965), Dassanayake (1998) and Hamid *et al.* (2011). This plant was commonly found in this study area as cultivated plant for their medicinal uses. Heywood (1978) and Kress *et al.* (2003) stated that several genera of family Acanthaceae are grown as house plants and this family was distributed throughout Myanmar.

In this study, it is used in traditional medicine in diabetes, gonorrhoea and urethral discharges by practitioners, and urinary disorder and kidney diseases by local people. Tilloo *et al.* (2012) revealed that the plant is a folk remedy for treating diabetes mellitus in parts of South India and are highly effective in asthma. Hanafi (2015) stated that the leaves are eaten as a vegetable and used as a herbal remedy in traditional African medicine. The leaves are used in many parts of Nigeria for asthma. The present data were mostly similar to those of Tilloo *et al.* (2012), Hanafi (2015). But, the leaves were not used in asthma in the present study.

In this study, the leaves contained all the studied groups of compounds except cyanogenic glycosides. Suseela (2005) stated that it contains saponin, tannin, phenols, flavonoids, carbohydrates and alkaloids. Gopal *et al.* (2013) revealed that it contains flavonoids, alkaloids, tannins and glycosides. Sivaraj *et al.* (2013) showed that it contains alkaloids, carbohydrates, tannins, saponins and flavonoids. Hamid *et al.*

(2011) revealed that it contained glycosides, flavonoids and reducing sugars. Jose *et.al* (2018) showed it contains carbohydrates, alkaloids, tannins, saponins, flavonoids. According to above literatures the flavonoids were present in all. The present study was agreed with all literatures. The lipophilic compound and cyanogenic glycosides were not observed in these literatures. The lipophilic compounds gave yellowish brown ring in this study. Thus, it was concluded that the extraction of active compound is also depended on the kind of solvents and extraction methods.

In the present study, the transverse section of petiole was planoconvex-shaped in outline. In surface view, the epidermal cells are parenchymatous, rectangular, multicellular uniseriate and glandular trichomes, cystoliths and diacytic stomata were present on both surfaces. In transverse section, both adaxial and abaxial epidermal cells are one-layered, barrel-shaped, multicellular and glandular trichomes on both surfaces. These results were mostly similar to those of Suseela *et.al* (2005) and Metcalfe and Chalk (1950).

In ground tissues composed of outer collenchymatous, middle chlorenchymatous and inner parenchymatous cells in order; the cells were polygonal or spherical in shape. The Chlorenchyma cells were absent in abaxial side. In vascular tissue, it is composed of single crescent-shaped vascular bundle and 2-4 subsidiary bundles, and collateral type. The present data were similar to those of Suseela *et.al* (2005), Metcalfe and Chalk (1950) except the number of subsidiary cells. Suseela *et.al* (2005) showed that the vascular bundle consists of two traces vascular bundles.

In the present study, the transverse sections of midribs were semicircular in outline with 2 pyramidal projections in adaxial side. The epidermal cells in surface view were parenchyma, rectangular or barrel-shaped, the uniseriate multicellular and glandular trichomes and cystoliths were present on both surfaces. The stomata were present on the upper surface only. In transverse section, the epidermal cells of both surfaces one-layered, barrel-shaped. Uniseriate multicellular and glandular trichomes present on both surfaces. The present data were similar to those of Suseela *et.al* (2005) and the family characters of Metcalfe and Chalk (1950).

The ground tissue composed of outer collenchyma, middle chlorenchyma and inner parenchyma cells. The cells were mostly polygonal-shaped and the intercellular space small. The Vascular bundle composed of crescent-shaped bundle in the middle and 2-4 subsidiary bundles in the cortex. The bundles were collateral type. The vascular bundle shape and types of this research were similar to those of Suseela *et.al* (2005), Metcalfe and Chalk (1950).

In this study, the transverse section of lamina was dorsiventral flattened. The epidermal cells of surface view were parenchymatous; stomata, trichomes, cystoliths were present on both surfaces. In transverse section, epidermal cells were one layered, rectangular or barrel shaped and trichomes were present. One-layered palisade cells were vertically elongated and spongy 3-5-layered, irregular-shaped. The bundles were embedded in ground tissue, collateral type. These results were similar to those of Metcalfe and Chalk (1950) and Suceela *et.al* (2005). But, the spongy were 3-5 layered in this study while Suceela *et.al* (2005) exhibited 6-spongy cells.

The microscopical characters of powdered leaves show the fragments of vessels, tracheids and fibres. The fragments of trichomes, epidermal cells, stomata, mesophyll cells, calcium oxalate crystals and cystoliths were also found in the powdered leaves. The present data were similar to those of Metcalfe and Chalk (1950) and Suceela *et.al* (2005).

The most of the anatomical characters of the petiole, midrib and lamina, and powdered characters were found to be basically similar in structures described by Metcalfe and Chalk (1950) and Suceela *et.al* (2005) except a few quantitative characters.

The present study was focused on characterization of morphological, phytochemical and anatomical characters of leaves of *A. gangetica*. There was no enough scientific information on the chemical composition and anatomical characters of this plant. Therefore, the present research was undertaken to screen the morphological, phytochemical and anatomical analysis. Thus, it was concluded that the morphological and anatomical characterization from the present study will help in identification and classification of this plant from other species and Preliminary phytochemical studies will indicate qualitative chemical profile of this plant.

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