

A study on morphological, anatomical and phytochemical tests of *Aesculus assamica* Griffith. leaves and its antioxidant activity

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Abstract

Aesculus assamica Griffith. is locally known as Ye-myaw in Myanmar and this plant belongs to the family Hippocastanaceae had been studied. It was collected from Lo Kha Lo village, Pha Saung Township, Kayah State. The morphological characters of this plant were studied for classification and identification. *Aesculus assamica* Griffith. is tree with thyrsus cylindric inflorescence and pale yellow flower. Fruits are capsule, depressed globose and hilum dark brown. In the anatomical study, the leaves of *Aesculus assamica* Griffith. were investigated. The anticlinal walls of upper surface of lamina are wavier than the lower one. Anomocytic type of stomata is present on both surfaces but more abundant stomata on lower surface. Circular-shaped of vascular bundle, closed collateral types are present in midrib and petiole. In the diagnostic characters, the odour of leaves is pungent. In the preliminary phytochemical tests, *Aesculus assamica* Griffith. leaves were found to be abundant alkaloid and saponin. Antioxidant activity, total phenolic contents and total flavonoid contents of investigations on the leaves of *Aesculus assamica* Griffith. were tested. All of these extracts of *Aesculus assamica* Griffith. leaves have the antioxidant activity.

Keywords: thyrsus cylindric inflorescence, anomocytic stomata, phytochemical

Introduction

Medicinal plants are potential sources of new drugs and hold a great value for developing pharmaceutical products, phytomedicines and dietary supplements. The World Health Organization (WHO) has listed 21000 plants, which are used for medicinal purpose around the world. Most of Myanmar people widely used plant extract as the folk medicine and they depend on traditional medicine. The family Hippocastanaceae consists of one genus and about 2 species (Kress *et.al*; 2003). Among them, the selected plant *Aesculus assamica* Griffith. is locally known as Ye-myaw and Horse Chestnut in English. This plant is widely distributed in Kayah State. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk (Miller *et.al.*, 2000). The pounded seeds of this plant are applied to analgesic, goiter, ulcers and swellings. The crude extracts and pure compounds from *Aesculus* spp. seeds are intended to be studied on antioxidant and anticancer activities (Khare, 2007).

In this research, the morphological characters of the whole plant of *Aesculus assamica* Griffith. have been studied for verification and standardization of this plant. The anatomical and diagnostic characters of *Aesculus assamica* Griffith. leaves have been revealed. Phytochemical test, antioxidant activity, total phenolic contents and total flavonoid contents in the leaves of *Aesculus assamica* Griffith. have been substantially investigated.

The aim of the research work is to study the morphology of collected plant *Aesculus assamica* Griffith. and to assess the anatomical and diagnostic characters of *Aesculus assamica* Griffith. leaves. In this paper, the objectives are to ascertain the phytochemical test of powdered leaves of *Aesculus assamica* Griffith. and to investigate the antioxidant activity, total phenolic contents and total flavonoid contents in various crude extracts of *Aesculus assamica* Griffith. leaves.

Materials and Methods

Morphological studies

The specimen of *Aesculus assamica* Griffith. was collected from Lo Kha Lo village, Phar Saung Township, Kayah State, at latitudes 18° 49' 25.13" N and longitudes 97° 8' 52.75" E and 4000 ft above the sea level (Figure- 1), from February to May 2019. This plant was classified and identified by using Backer (1965), Dassanayake (1983) and Heywood (2007). The habit, inflorescence, leaves, flowers and fruits of this specimen was collected and recorded by using digital camera (Sony DSC-TX 66).

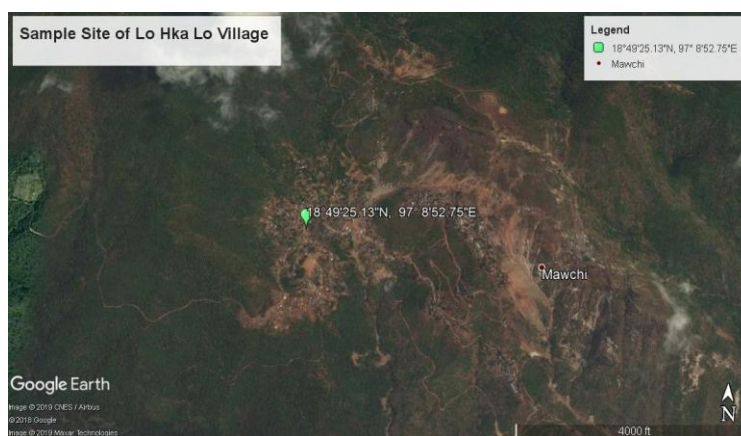


Figure (1). Sample site of Lo Hka Lo Village area

Microscopical studies

In anatomical study, *Aesculus assamica* Griffith. was performed by free hand sectioning according to the methods of Metcalfe and Chalk (1950), Esau (1953), Pandey (1999), Trease and Evans (2002) at Botany Department, Dagon University. The following reagents were used to examine the section cutting and powdered samples.

-Chloral hydrate solution B.P as clearing reagents.

-Solution of phloroglucinol B.P followed by concentrated hydrochloric acid for testing lignin.

Preliminary phytochemical investigation

Preliminary phytochemical investigation on leaves of *Aesculus assamica* Griffith. was carried out to examine the plant constituents. The methods of Marini Bettolo, *et al.*, (1981), Quality Control Methods for medicinal plant materials (1998) and Trease and Evans (2002) were applied for investigation of phytochemical studies at Botany Department, Dagon University. The results were as shown in Table (2).



Figure (2). Preliminary phytochemical investigation on leaves of *Aesculus assamica* Griffith.

Antioxidant activity assay

Antioxidant activity was evaluated by the 1, 1-Di-phenyl-2-Picrylhydrazyl (DPPH) assay in accordance with the method of Blois (1958) at Chonbuk national university, Korea. The results were as shown in Figure (29).

Total phenolic contents and total flavonoid contents assay

Total phenolic contents and total flavonoid contents assay were done according to the method described by Dewanto *et.al* (2002) at Chonbuk national university, Korea. The results were as shown in Figure (30 and 31).

Results

Morphological characters of *Aesculus assamica* Griffith.

Scientific name	-	<i>Aesculus assamica</i> Griffith.
Family	-	Hippocastanaceae
Local name	-	Ye-myaw
English name	-	Horse Chestnut
Specimens examined	-	Lo Kha Lo village, Phar Saung Township, Kayah State

Deciduous trees, erect, 10.0 -12.0 m high, with rough brown bark. Leaves alternate, palmate compound, the lamina 5-7 foliate, leaflet blades oblong-lanceolate to oblong-oblongeolate, 13.2 -40.0 cm long and 4.1 - 14.9 cm wide, the bases cuneate, the margins crenulate, the tips acuminate, both surfaces puberulent, petioles long and slender, 6.7 - 33.0 cm long and 0.15 -0.4 cm wide, exstipulate. Inflorescence axillary, thyrses, the peduncle long; ebracteate; pedicellate; ebracteolate. Flowers pale yellow, 2.5 - 3.0 cm long and 2.0 - 2.3 cm in diameter, complete, bisexual, irregular, zygomorphic, tetra-merous, cyclic, hypogynous; sepals (2), synsepalous, tubular with unequal and imbricate lobes; petals 4, apopetalous, unequal, two spatulate to oblong and two oblong- obovate or obovate, 9.0 - 11.0 cm long and 4.0 - 6.5 cm wide, pale yellow with bright yellow spots, both side glabrous; stamens 6, apostamenous, exerted, the filaments filiform, the anthers ditheous, introrse, dorsifixed, longitudinally dehiscent; ovary superior, globose, tricapellary, syncarpous, trilocular, the placentation axile, many ovules in each locule in T.S, the style long and slender, the stigma oblique, subcapitate. Fruits capsule, depressed globose, 6.5-7.5 cm long and 6.0-7.0 cm wide, brown, hilum(dark brown); seeds depressed globose, 5.5 - 6.5 cm long and 5.0 - 6.0 cm wide, brown. Flowering and fruiting from February to May (Figure 3 - 12).



Figure (3) A- Habit, B- Inflorescence, Figure (4) Inflorescence Figure (5) Ventral view of leaf C- Bark



Figure (6) Dorsal view of leaf Figure (7) Close up view of flower Figure (8) Open view of Calyx

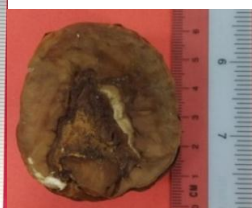


Figure (9) Close up view of Corolla Figure (10) T.S of Ovary Figure (11) Fruit Figure (12) Seed of Corolla

Microscopical characters of *Aesculus assamica* Griffith. leaves

Lamina

In surface view of lamina, the epidermal cells of upper and lower surface are polygonal shaped, thin-walled and parenchymatous cells. The anticlinal wall of upper epidermal cells are thin and wavier than the lower epidermal cells. Anomocytic types of stomata are present on both surfaces but they are abundantly found on the lower surface as shown in Figure (13 and 14).

In transverse section, the upper epidermis of cuticle layer is thicker than the lower surface. The upper and lower epidermal cells are barrel-shaped. Trichomes are present on both surfaces. Mesophyll consists of one layer of palisade parenchyma and two to five layers of spongy mesophyll cells. Vascular bundles are embedded in mesophyll cells. Bundles are collateral and closed type as shown in Figure (15).

Midrib

In surface view of midrib, the epidermal cells of both surfaces are parenchymatous, thick-walled, and polygonal to rectangular shaped. Unicellular uniseriate trichomes, simple and stellate trichomes are present on both surfaces as shown in Figure (16).

In transverse section, the epidermal cells are rounded to oval shaped parenchymatous cells. Below the epidermis, angular collenchyma and parenchyma layers are present. Vascular bundles are more or less circular in outline, composed of two bundles, adaxial bundle slightly flattened rounded or very widely elliptical in shape. Vascular bundles are collateral and closed type as shown in Figure (17).

Petiolule

In surface view, the epidermal cells of both surfaces are thin-walled parenchymatous cells, polygonal shaped. Simple and unicellular uniseriate trichomes are present as shown in Figure (18).

In transverse section, petioles are circular-shaped in outline, winged petiolule. Single layer of epidermal cells are barrel-shaped in both surfaces. Vascular bundles are more or less penta-angular or circular shaped with wavy margin and closed collateral type as shown in Figure (19).

Petioles

In surface view, epidermal cells are thin-walled, polygonal in shaped and elongated along the axis of parenchymatous cells. Simple, stellate trichomes and unicellular uniseriate trichomes are present as shown in Figure (20).

In transverse section, petioles are circular-shaped in outline. Single layer of epidermal cells are barrel-shaped in both surfaces. The cortex consists of three to six layers of angular collenchymatous cells and three to five layers of parenchymatous cells. Vascular bundles are circular shaped, collateral and closed type as shown in Figure (21).

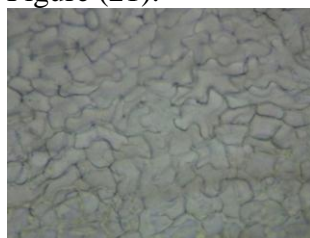


Figure (13) Surface view section of upper epidermis

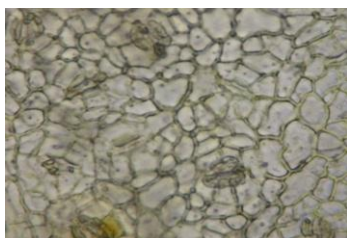


Figure (14) Surface view of lower epidermis

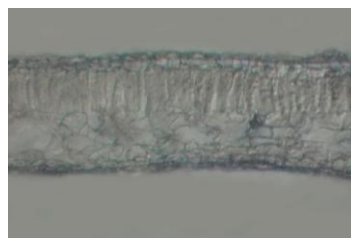


Figure (15) Transverse of lamina

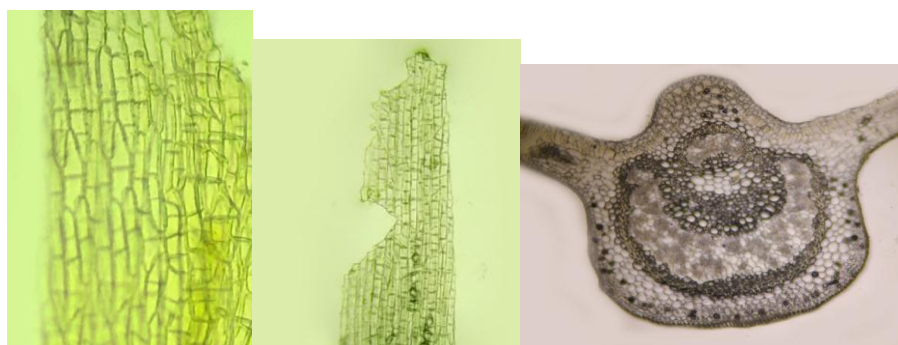


Figure (16) Upper surface and lower surface of midrib

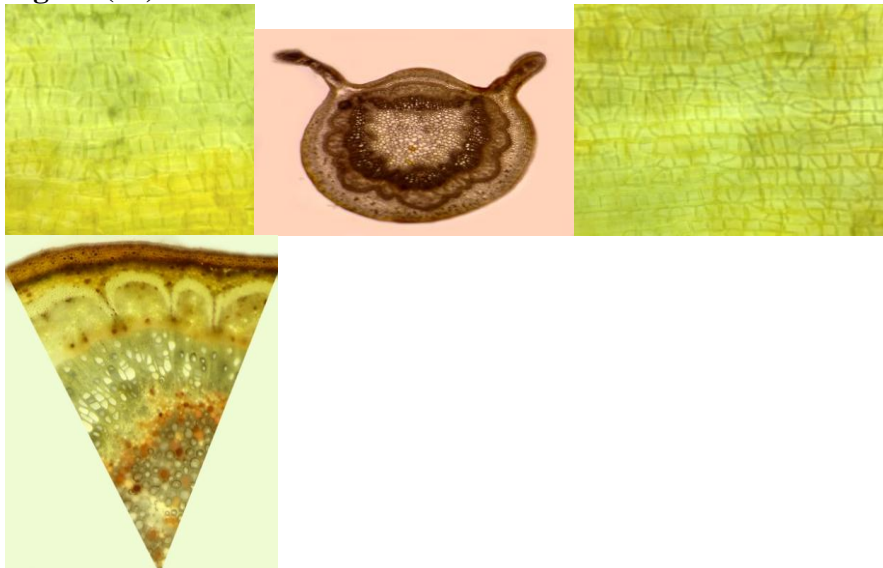
Figure (17) Transverse section of midrib

Figure (18) Surface view of petiolule Figure (19) Transverse section of petiolule Figure (20) Surface view of petiole Figure (21) Transverse section of petiole

Diagnostic characters of powdered leaves of *Aesculus assamica* Griffith.

The powdered leaves of *Aesculus assamica* Griffith. were green colour and pungent in odour. It was also hot and slightly bitter in taste and granular in texture. It consists of fragment of pieces of epidermal cells, unicellular uniseriate trichome, simple trichome, stellate trichome, pitted vessel and phloem fiber as shown in Figures (23 - 28). The sensory characters of powdered leaves are as shown in Table (1) and Figure (22).

Table (1) Sensory characters of powdered leaves of *Aesculus assamica* Griffith.

Sample Sensory characters	Leaves
Colour	Dark green
Odour	Pungent
Taste	Hot and slightly Bitter
Texture	Granular



Figure (22) Powdered leaves of *Aesculus assamica* Griffith.

Diagnostic characters of powdered leaves of *Aesculus assamica* Griffith

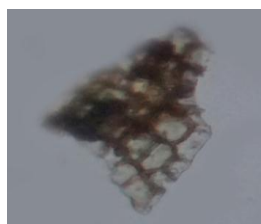


Figure (23) Fragment of trichome



Figure (24) Unicellular uniseriate parenchyma cells



Figure (25) Simple trichome



Figure (26) Stellate trichome



Figure (27) Pitted vessel

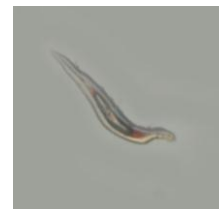


Figure (28) Phloem fiber

Qualitative analysis of powdered leaves from *Aesculus assamica* Griffith.

In preliminary phytochemical test, the presence or absence of alkaloid, α -amino acids, carbohydrate, starch, reducing sugar, glycoside, phenolic compound, saponin, tannin and flavonoid, were observed in the leaves. Starch was absent. The results were shown in Table (2).

Table (2) The qualitative analysis of powdered leaves from *Aesculus assamica* Griffith.

No	Test	Extract	Test Reagents	Observation	<i>Aesculus assamica</i> Griffith.
1	Alkaloid	1% HCL	(1) Mayer's Reagent (2) Wagner's Reagent (3) Dragendroff's Reagent (4) Hager's reagent	White ppt Brown ppt Orange ppt Yellow ppt	+ + + +
2	α -amino acids	H ₂ O	Ninhydrin solution	Light purple colour	+
3	Carbohydrate	H ₂ O	10% α -naphthol+conc-H ₂ SO ₄	Red ring	+
4	Starch	H ₂ O	I ₂ KI solution	Blue color	-
5	Reducing sugar	H ₂ O	Benedict's solution	Brick red ppts	+
6	Glycoside	H ₂ O	10% lead acetate solution	White ppts	+
7	Phenolic compound	H ₂ O	5% Ferric chloride solution	Deep blue color	+
8	Saponin	H ₂ O	Distilled water	Frothing	+
9	Tanin	H ₂ O	1% Gelatin & 10% NaCL solution	Deep blue color	+
10	Flavonoid	EtOH	(1)Mg turning (2)Conc HCL acid	Pink color	+

(+) = present, (-) = absence, ppt = precipitate, conc = concentrated

Antioxidant activity assay

Aqueous extract, 50% ethanolic, 95% ethanolic, 50% methanolic, 95% methanolic extract of leaves of *Aesculus assamica* Griffith. were tested on antioxidant activity. The various crude extracts of leaves *Aesculus assamica* Griffith. showed antioxidant activity. The results were shown in Figure (29).

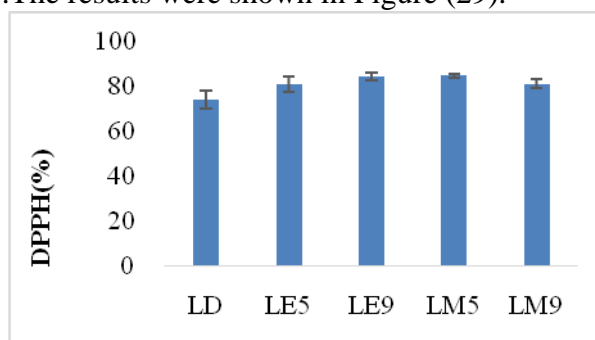


Figure (29) Antioxidant or DPPH activity of various crude extracts of *Aesculus assamica* Griffith. leaves

Total Phenolic Contents and Total Flavonoid Contents

The results of total phenolic contents and total flavonoid contents in various crude extracts of leaves *Aesculus assamica* Griffith. were shown in Figure (30 and 31).

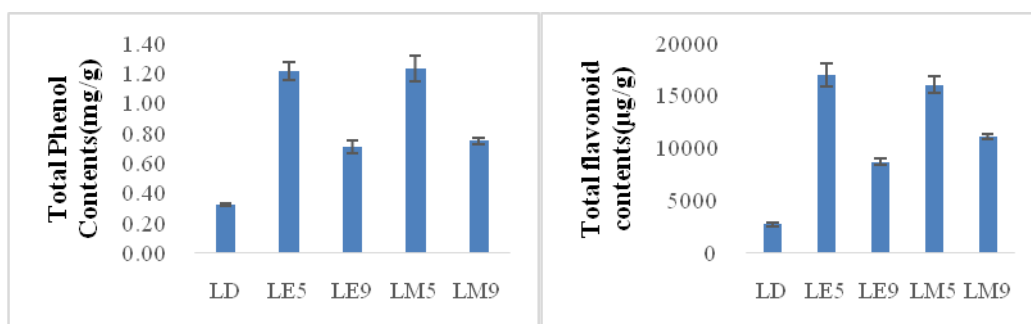


Figure (30) Total phenolic contents of various crude extracts of *Aesculus assamica* Griffith. leaves **Figure (31) Total flavonoid contents of various crude extracts of *Aesculus assamica* Griffith. leaves**

Discussion and Conclusion

In the present research, the plants are tree; stems with brown bark. The leaves are palmately compound, inflorescences are axillary, thyrse and the flowers are bisexual, zygomorphic, tetramerous and pale yellow with bright yellow spot. The calyxes are (2), fused, tubular with unequal and the corollas are 4, apopetalous, unequal, pale yellow with bright yellow spots. The stamens are 6, apostamenous, the filaments long, exerted and the ovary is tricarpeal, syncarpous, axile placentation, the style slender. The fruits are depressed globose with hilum dark brown. The seeds are brown. These characters are in agreement with those mentioned by Dassanayake (1983) and Heywood (2007).

In the microscopical studies, the anticlinal walls of upper surface of epidermal cell are wavier than the lower one. Anomocytic type of stomata is abundant on lower surface. In petiolule, vascular bundles are more or less penta-angular or circular shaped with wavy margin. Circular-shaped of vascular bundle, closed collateral types are present in midrib and petiole. These characters are in agreement with Esau (1953), Metcalfe and Chalk (1950) and Pandey (1999).

In the diagnostic characters, fragment of pieces of epidermal cells, unicellular uniseriate trichome, simple trichome, stellate trichome, pitted vessel and phloem fiber are present. The combination of sensory and microscopical characters would assist the identification of powdered drug of *Aesculus assamica* Griffith. In this study, investigation of preliminary phytochemical test on the leaves of *Aesculus assamica* Griffith. showed that alkaloid, α -amino acid, carbohydrate, glycoside, phenolic compound, saponin, tannin, flavonoid and reducing sugar are present but starch was not observed. Alkaloid and saponin were especially abundant in the leaves of this plant.

The various solvents extracts of *Aesculus assamica* Griffith. leaves showed the DPPH activity. Among them, 50% methanolic extract of leaves showed the best activity than the other and aqueous extract was least activity. The different solvents extracts of this plants showed total phenolic contents and total flavonoid contents. The 50% methanolic extract of leaves has high phenolic contents and also the 50% ethanolic extract of leaves has high flavonoid contents. According to these results, these extracts of leaves of *Aesculus assamica* Griffith. have the antioxidant activity. Moreover, further research is needed to evaluate the potential of the traditional medicinal uses and the bio activities of this plant such as anticancer activities should be conducted.

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