

Pharmacognostic Studies on the Leaf of *Dregea volubilis* (L.f) Benth. ex. Hook. f.

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Abstract

The sample, *Dregea volubilis* (L.f) Benth. ex. Hook. f., is a climbing shrub and member of the family Apocynaceae. The preliminary phytochemical analysis on its leaves was shown as the presence of alkaloids, flavonoids, phenols, saponins and tannins presented in 1% HCl, EtOH and water extract. In powder microscopy, it was observed that annular vessels, spiral vessels, fragments of paracytic stomata, trichome, prismatic calcium oxalate crystal, cluster crystal, rosette crystal, fiber and fragments of palisade parenchyma and spongy parenchyma cells. The physicochemical results showed the total ash was 14.20%, acid insoluble ash 11.27% and water soluble ash 4.03%. Therefore, the results based on the morphological, phytochemical, powder characteristics and physicochemical properties can serve as useful parameters for the proper identification of the drug and will validate the use of plant in traditional medicine.

Key words: Phytochemical, Physicochemical

Introduction

Over the past few decades, the use of traditional medicines had rapidly gained in popularity around the world and it was estimated that almost 80% of the world's population relied on traditional medicines, particularly in developing countries that had limited access to essential medicines (Farnsworth *et al.*, 1985).

Standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of an herbal drug (Patra *et al.*, 2010). The authentication of herbal drugs and identification of adulterants from genuine medicinal herbs are essential for both pharmaceutical companies as well as public health and to ensure reproducible quality of herbal medicine (Straus, 2002).

Nowadays multiple drug resistance (MDR) has emerged due to the unsystematic use of commercial antimicrobial medication commonly used in the treatment of disease. Along with this issue, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity and allergic reactions. This situation forced scientists to search for new antibacterial substances (Rocio and Rion, 1989).

In the last few years, there had been an exponential growth in the field of herbal medicine and these drugs were gaining popularity both in developing and developed countries because of their origin and less side effects.

The aims and objectives of the present study are to know knowledge on morphological characters and to investigate the some medicinally important phytochemical constituents and physicochemical constants in *Dregea volubilis* (L.f) Benth. ex. Hook. f.

Materials and Methods

Plant Collection and Identification

The fresh leaves of *Dregea volubilis* (L.f) Benth. ex. Hook. f. were collected from Chanmyawadi Quarter in Monywa Township during the flowering period in

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2019. After the collection, the vegetative and floral parts of fresh specimens were studied, measured and identified by using literatures (Dassanayake, 1998).

Preparation of plant extract for phytochemical screening

Collected leaf material was washed under running tap water to eradicate dust and microbes. The leaf samples were then air dried under shade at room temperature for two weeks. The leaf material was crushed well into fine powder in an electronic grinder and sieved through mesh number 60 to get uniform powder and then kept into air tight polyethylene bags for further use and stored at room temperature.

Phytochemical study

The air dried samples were tested for alkaloids, flavonoids, phenols, saponins, glycosides, lipophilic compounds, tannins, reducing sugars, carbohydrates and cyanogenic glycosides using the residue obtained from various solvent system.

Physicochemical constituents

Ash value

- 1. Determination of Total Ash:** Weigh accurately 2gm of the air – dried crude drug in a crucible and incinerate at a temperature not exceeding 450° until free from carbon, cool and weigh.
- 2. Determination of Acid Insoluble Ash:** Boil the ash with 25ml hydrochloric acid for 5 minutes; collect the insoluble matter on an ash less filter paper. Wash with hot water, ignite, cool in desiccators and weigh.
- 3. Determination of Water Soluble Ash:** Boil the ash, for 5 minutes with 25ml of water, collect the insoluble matter in a crucible (or) on an ash less filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450°. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash.

Extractive values

The extractive values of crude drugs were determined in water and alcohol. Five grams of powder was taken in a 250 ml stoppered conical flask. 100 ml of the respective solvent was added to the 250 ml stoppered conical flask and was allowed to macerate for 24 hr with the aid of mechanical shaker for 6 h. it was then filtered and 25 ml of the filtrate was taken in a crucible. It was evaporated to dryness in an oven at 105 °C and weighed it again.

Extractive value = (weight of extracted residue/weight of sample) x 100

Results

Morphological Studies

Scientific Name - *Dregea volubilis* (L.f) Benth. ex. Hook. f. (Figure 1)

Family - Apocynaceae

Sub Family - Asclepiadaceae

Myanmar name - Gwe-dauk-nwe

English name - Cotton milk plant; Green milk weed climber

Perennial high-climbing suffrutescent vines; latex watery. Stems and branches terete. Leaves simple, opposite and decussate, petiolate; blades broadly ovate-acuminate, entire along the margin, acuminate at the apex. Inflorescences axillary, umbelliform cyme. Flower bisexual, actinomorphic, pedicellate, green in colour. Calyx 5, green. Corolla 5, lobes triangular-ovate. Corona staminal; coronal scales 5; fleshy tips incumbent on the anther, column very short. Ovaries 2, superior; styles 2 united above. Stigma globose head, or dome-shaped. Fruits follicle. Seeds compressed.

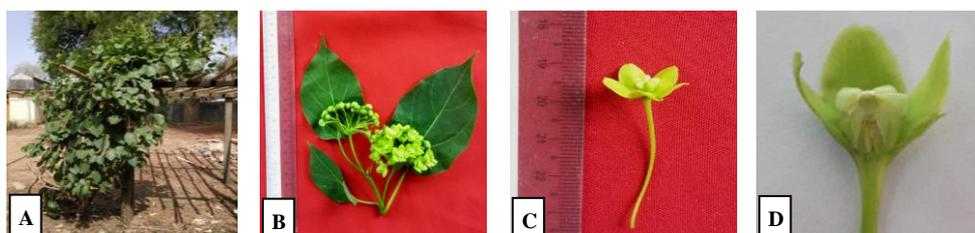


Figure 1. Plant morphology of *Dregea volubilis* (L.f) Benth. ex. Hook. f.

A. Habit B. Inflorescence C. Flower D. L.S of Flower

Phytochemical Tests

In the present investigation, the detailed pharmacognostic account of *Dregea volubilis* is given which includes macroscopic and microscopic characters, which will be helpful for the correct botanical identification of the drug. Phytochemical tests were carried out of 1% HCl extractive for alkaloids, on ethanol extractive for flavonoids and water extractives for phenols, saponins, glycosides, lipohelic compounds, tannins reducing sugars, carbohydrates and cyanogenic glycosides. Results are tabulated in Table 1 and Figure 2.

Table 1. Phytochemical test on *Dregea volubilis* (L.f) Benth. ex. Hook. f.

No.	Test	Extract	Test Reagents	Observation	Results
1.	Alkaloids	1% HCl	Dragendroff reagent	Orange ppt	+
2.	Flavonoids	EtOH	HCl (con:) + Mg	Greenish brown	+
3.	Phenol	H ₂ O	1% FeCl ₃ solution + 1% K ₃ Fe(CN) ₆	Pale green ppt	+
4.	Saponins	H ₂ O	Distilled water	Frothing	+
5.	Glycosides	H ₂ O	10% lead acetate solution	White ppt	+
6.	Lipohelic Compounds	H ₂ O	KOH solution	Yellowish brown	+
7.	Tannins	H ₂ O	1% Gelatin + 1% FeCl ₃ solution	Reddish brown ring and white ppt	+
8.	Reducing Sugars	H ₂ O	Benedict's solution	Pale green ppt	+
9.	Carbohydrates	H ₂ O	Benedict's solution + H ₂ SO ₄ (con:)	Greenish brown	+
10.	Cyanogenic glycosides	H ₂ O	H ₂ SO ₄ (con:) + Sodium Picrate solution	No colour change, no ppt	-

+ = Present con: = Concentrated - = Absent ppt= Precipitate

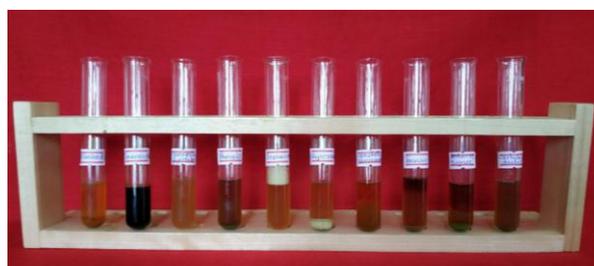


Figure 2. Phytochemical tests of *Dregea volubilis* (L.f) Benth. ex. Hook. f.

Powder microscopy

Dregea volubilis leaves powder has fragments of both epidermis along with anomocytic type of stomata, fragments of upper epidermis with cuticle and mesophyll cells, simple multicellular trichomes, fragments of lamina with a row of palisade cells underneath, phloem fibers, scalariform, spiral vessel and cluster and rosette crystal and prismatic crystal (Figure 3).

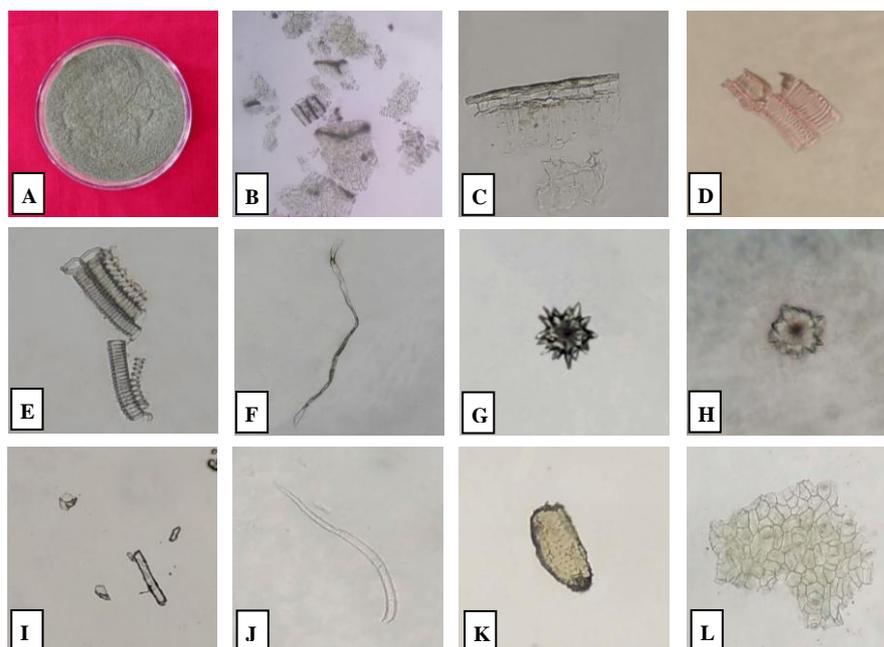


Figure 3. Powder microscopy of *Dregea volubilis* (L.f) Benth. ex. Hook. f.

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|----------------------------------|----------------------------------|
| A. Powder of leaves | B. Fragments of powder leaves |
| C. Epidermic with palisade cells | D. Scalariform and spiral vessel |
| E. Spiral vessel | F. Fiber |
| G. Cluster crystal | H. Rosette crystal |
| I. Prismatic crystal | J. Trichome |
| K. Pitted vessel | L. Parenchyma cell with stomata |

The texture of powder leaves was fine powder and the odour was pleasant aromatic. The colour was yellowish green and the taste was slightly bitter. The results were shown in Table 2.

Table 2. Organoleptic characters of *Dregea volubilis*

No.	Characteristics	Findings
1.	Texture	Fine powder
2.	Odour	Pleasant aromatic
3.	Colour	Yellowish green
4.	Taste	Slightly bitter

Physicochemical parameters

The preliminary physicochemical parameters showed the total ash 14.20%, acid insoluble ash 11.27%, water soluble ash 4.03%, water soluble extracted value, acetone soluble and ethanol soluble extracted value were 8.73%, 0.50% and 2.75% respectively as shown in Table 3.

Table 3. Physicochemical constituents of *Dregea volubilis*

No:	Parameters	Value (%)
1.	Total ash	14.20
2.	Acid insoluble ash	11.27
3.	Water soluble ash	4.03
4.	Water soluble extracted value	8.75
5.	Acetone soluble extracted value	0.50
6.	Ethanol soluble extracted value	2.75

Discussion and Conclusion

Dregea volubilis (L.f) Benth. ex. Hook. f.(Gwe dauk nwe), is widely distributed in the tropical regions in Myanmar. The morphological, anatomical characters and phytochemical constituents are important and useful in identification and in effective use for medicinal value.

Thupurani *et al* (2018) stated the presence of different types of secondary metabolites such as alkaloids, phenols, flavonoids, tannins and saponins in the *Dregea volubilis* leaf fractions of ethanol and aqueous extract. Similarly, Madathupatti *et al.*, (2012) reported that flavonoids were present in ethanol extract and alkaloids, saponins and tannin were not present in aqueous extract.

Sanda Phyo (2018) stated that alkaloids were present in extracted with 1% HCl and glycosides, phenolic compounds, reducing sugars, tannins and saponins were present in water extract. Flavonoid, and polyphenol were present in the leaves of *Dregea volubilis* extract with ethanol.

In the present study, the preliminary phytochemical analysis showed the presence of alkaloid with the extract with 1% HCl, flavonoids were present in ethanol extract; phenols, saponins, glycosides, lipophilic compounds, tannins, reducing sugars and carbohydrate were present in water extract. Cyanogenic glycosides were not present in water extract. Therefore, the phytochemical analysis of the leaves may be useful in developing new specialized drug with more efficiency.

The observations of the study on the powder of leaf can serve as useful parameters for the proper identification of the drug. The dried sample in the powdered form gives characteristic features of the drug under a microscope after proper treatments. The microscopical examination of epidermal trichome and calcium oxalate crystals is extremely valuable for authentication of crude drugs (Rabinarayan *et al.*, 2018).

In the present study, leaves powder showed that annular vessels, spiral vessels, fragments of paracytic stomata, fragments of spongy parenchyma, trichomes, prismatic crystal of calcium oxalate, rosette crystal, fragments of palisade parenchyma and spongy parenchyma cells.

Total ash involved the oxidation of the component of the product. A high ash value is an indicative of contamination, substitution and adulteration. The value of total ash was found to be 18.00% in the leaves of *Dregea volubilis* (Prabhu *et al.*, 2012). In the present study, the total ash was 14.20%.

The present study of pharmacognostical evaluation on the leaves of *Dregea volubilis* has laid down standard parameters for proper identification, authentication and for distinguishing the material from its adulterants and substitutes. The study also contributes to the documentation of the nutritional composition on the leaves of *Dregea volubilis* which are consumed as a vegetable.

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