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Pharmacognostic study of the leaf of *Ageratina adenophora*

Nungshioba Jamir, Muslek Uddin Mazumder, P. Khazeo, K. Nusalu Puro, Rosamund Jyrwa, Lalzikpuii Sailo*

Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Zemabawk 796017, Mizoram, India

Corresponding author: zikpuii08@gmail.com

Ageratina adenophora **(Asteraceae), commonly known as Crofton weed or sticky snakeroot, leaves are used for various health conditions in traditional medicines. They are used for treatment such as wound, itching, measles, skin diseases, uterine bleeding and also acts as antibacterial and astringent activity. Macroscopic and quantitative microscopic methods were applied to determine the diagnostic features for the identification and standardization of the fresh leaf samples of** *A. adenophora***. Macroscopically, the leaves were found to have an opposite trowel-shaped serration that are 6–10 cm long by 3–6 cm in width. They have a mild characteristic smell and bitter in taste. They are dark-green in colour, while the stems are reddish in colour. The flowers are creamy white coloured followed by a small brown seed with a white feathery parachute. The light and electron microscope images of cross-section of leaf revealed useful diagnostic features. They have anomocytic type of stomata. The stomatal number, stomatal index, vein islet and vein termination number were determined. In the transverse section of the leaf, collenchyma cells, parenchyma cells, vascular bundles, trichomes, palisade cells, upper and lower epidermis were observed. These findings are helpful in authentication of** *A. adenophora***, as well as in laying down its pharmacopoeial standards.**

Keywords: Microscopy, Macroscopy, Crofton leaf, transverse section, anomocytic stomata.

INTRODUCTION

New drug discoveries through natural products have proved to be very vital and effective approach (Ravinder *et al.,* 2018). Over decades, different cultures all over the world have learned to use natural products as traditional medicine to treat ailments and diseases and identified natural product have the ability to give new drugs (Kunle *et al*., 2012). India provides huge biodiversity in flora and fauna which have important therapeutic values. Though with the advancement in pharmaceutical technology in modern medicine, huge number of people still uses traditional medicine from various sources which includes plants and animals (Balamurugan *et al*., 2009).

Huge amount of natural products having medicinal value or therapeutic potential are not yet discovered or scientifically undertaken. Hence, this makes pharmacognostic studies important (Dhanbal *et al*., 2005). Due to the rise of demand in use of natural products as medicine, there is an increased use of adulterated and spurious drugs or inferior quality drugs. Therefore, purity and quality assessment becomes an essential factor and standardization becomes vital for the safety of the herbal drugs. The present study is carried out with the objective to identify and provide a pharmagonostic standard of the plant *Ageratina adenophora* (Ravinder *et al*., 2018).

A. adenophora commonly known as crofton weed, sticky snakeroot, Mexican devil, cat weed or eupatory, is a perennial herbaceous plant which is native to Mexico. It is a very aggressive invasive plant species known in most part the world. It belongs to the family Asteraceae (Compositae) (Auld, 1970). Its synonyms are *Ageratina trapezoidea* (Kunth), *Eupatorium adenophorum* Spreng., *E. glandulosum* Kunth, non Michx., *E. pasdadense* Parish, *E. trapezoideum* Kunth (Muniappan *et al*., 2014).

MATERIALS AND METHODS

Collection and authentication of the plant

The whole plant of the plant *A. adenophora* were collected from the campus of Regional Institute of Paramedical and Nursing Sciences, Aizawl, Mizoram, India. The plant was authenticated by a taxonomist at the Botanical Survey of India, Eastern Regional Centre, Shillong. A voucher specimen was deposited in the Department of Pharmacy, RIPANS, with reference no: BSI/ERC/TECH/ Plant Iden./2018/136.

Macroscopic analysis

Macroscopic analysis was done by studying the shape and size, colour, odour, taste, surface, characters, texture, the apex, margin, and base (Kokate *et al*., 2005).

Microscopic analysis

Transverse section of leaf

Microscopic analysis of the plant *A. adenophora* was carried out by cutting the transverse section of the leaf including the lamina and the midrib which was treated with phloroglucinol hydrochloride solution. It was mounted with glycerine on a glass-slide and observed under projection microscope at 10X. The images were captured and its diagnostic characteristic features were recorded.

Quantitative microscopy

Quantitative analysis of leaf microscopy was performed to determine the stomatal index, stomatal number, vein termination number and vein islet number (Kokate, 1994).

Determination of stomatal number

Upper and lower epidermal layers of the leaf was freshly prepared for the analysis. The specimen peels were prepared manually using forceps which was mounted on a glass slide with glycerin (Sumitra *et al.,* 2009). The prepared sample was observed under projection microscope (Primo Star, Zeiss) at 10X. The number of stomata present in 600 µm square were counted and recorded in four different fields. The mean value was taken.

Determination of stomatal index

Upper and lower epidermal layers of the leaf was freshly prepared for the analysis. The specimen peels were prepared manually using forceps which was mounted on a glass slide with glycerin (Sumitra *et al.,* 2009). The prepared sample slide was observed under Projection microscope at 10X. The number of stomata and epidermal cells present were counted in four different fields. Then, the mean value was taken and stomatal index (I) was calculated by using the formula,

I = S X 100/E+S

Determination of vein-islet and vein-termination number

Fresh leaves were boiled in chloral hydrate solution in a clean beaker on water bath for 3-5 hours. Then, the sample was mounted on a glass-slide and observed under Projection microscope at 10X. The numbers of vein-islet and vein-termination present were counted and recorded in four different fields. An area of 60x60 mm square were considered for observations and the mean value was calculated to find the result.

RESULTS

Pharmacognostic studies of the plant *A. adenophora* were performed and the following results were found.

Macroscopic character

Habitat: *A.adenophora* Linn is a perennial herbaceous plant which grows to a height of 1-3 m in height. It is erect and has a stem of woody root-stock.

Leaves: The plant was found to have opposite trowel -shaped serrated leaves that are 6-10 cm in length and 3-6 cm in width. The leaves are dark green in colour which is smooth in texture and the stems were reddish in colour (Figure 1). It has a mild characteristic smell and is bitter in taste.

Figure 1: Leaf of *Ageratina adenophora*.

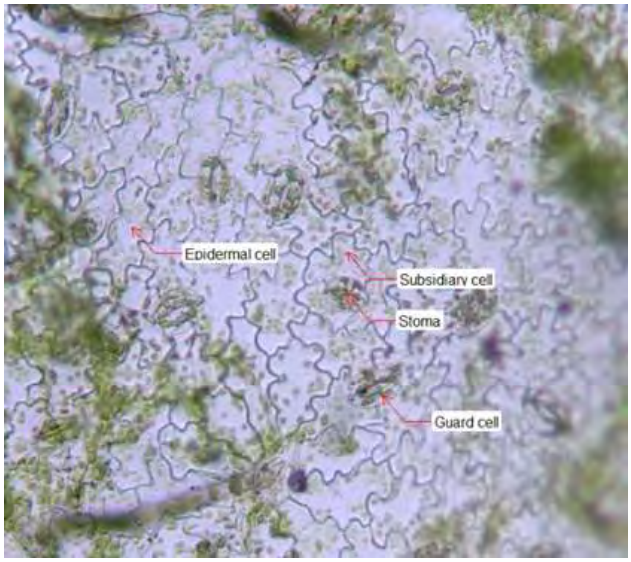

Figure 2: Anomocytic type of stomata was observed in the leaves of *A. adenophora*.

Microscopic characters

Figure 2 shows the tissue structure of the leaf of *A. adenophora* indicating anomocytic type of stomata. Transverse section of leaf in Figure 3 showed the presence of vascular bundles (xylem and phloem), parenchyma cells, collenchyma cells, trichome, palisade cells, upper and lower epidermis.

Quantitative microscopy of the leaf

The quantitative microscopic characters of the leaf of *A. adenophora* was determined and results were shown in Table 1.

DISCUSSION

The results obtained from the study will be useful in finding out the genuine potential of *A. adenophora* as a source of crude drug. It can also be used as a reliable source for detecting adulterations. This simple but reliable standard will be helpful in the identification and selection of the raw material for drug production. The plant was found to have opposite trowel-shaped serrated leaves that are 6-10 cm in length and 3-6 cm in width. The leaves are dark green in colour which is smooth in texture and the stems were reddish in colour as shown in Figure 1. It has a mild characteristic smell and is bitter in taste.

Table 1: Quantitative microscopic characters of the leaf of *A. adenophora.*

Parameters	Range
Stomatal number (lower epidermis)	3.25±0.95
Stomatal number (upper epidermis)	1.1±0.25
Stomatal index (lower epidermis)	37.5±0.85
Stomatal index (upper epidermis)	16.66±1.02
Vein-islet number	3±1
Vein-termination number	3.25±0.5

The microscopic study revealed the presence of epidermal cell, subsidiary cell, stoma and guard cell (Figure 2). It was also found out the leaf contains anomocytic type of stomata. The transverse section of leaf observed under projection microscope showed the presence of vascular bundles (xylem and phloem), parenchyma cells, collenchyma cells, trichome, palisade cells, upper and lower epidermis (Figure 3). Under the quantitative microscopic characters of the leaf of *A. adenophora* (Table 1). It was recorded and found the stomatal number (lower epidermis) to be under the range 3.25±0.95; but the stomatal number (upper epidermis) was found under the range 1.1±0.25. Stomatal index (lower epidermis) was found under the range 3.75±0.85, while stomatal index (upper epidermis) was found under the range 16.66±1.02. Vein-islet number was found under the range 3±1 and vein-termination number a was found under the range 3.25±0.5.

The result of the study serves as a valuable source of information and also provide suitable standards for identification of this plant material for future investigations.

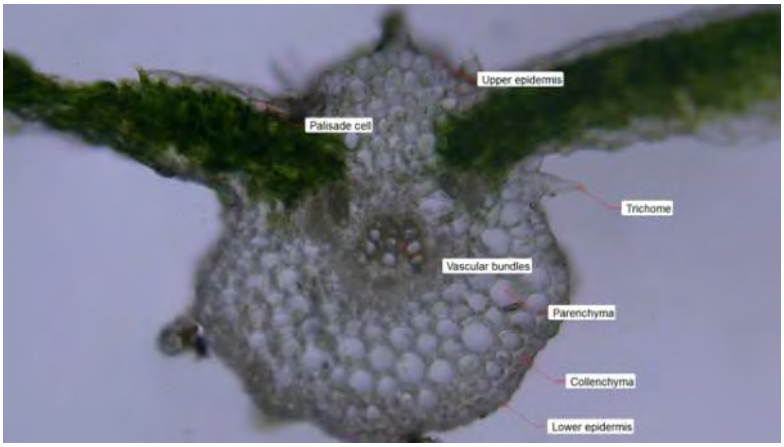

Figure 3: Transverse section of leaf showed the presence of vascular bundles (xylem and phloem), parenchyma cells, collenchyma cells, trichome, palisade cells, upper and lower epidermis.

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