ORIGINAL PAPER

https://dx.doi.org/10.4314/njpr.v16i2.2

Nig. J. Pharm. Res. 2020, 16 (2) pp 107- 115
ISSN 0189-8434 **e-ISSN 2635-3555** **Available online at http://www.nigjpharmres.com**

Evaluation of Pharmacognostic Characteristics of the Leaf of *Ageratum houstonianum* Mill. Compositae

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: It is a well-known fact that therapeutic efficacy and safety of medicinal plants depend on the quality and quantity of chemical constituents and that the misuse of medicinal plants starts with wrong identification.

Objectives: The aim of this study is to establish some pharmacognostic standards for *Ageratum houstonianum* Mill (Compositae) which has high medicinal values, with a view to determining the proper identification and other quality control parameters of the plant.

Method: The fresh leaf and powdered leaves were subjected to macroscopy and microscopy using standard procedures. In addition, some physicochemical evaluations such as moisture content, ash and soluble extractives were carried out on the powdered leaves.

Results: The result shows that the leaf is simple, pinnate, diamond shaped, serrated margin, acute apex and asymetrical base. Microscopically, the leaf is amphistomatic in nature with anomocytic and anisocytic stomata at the adaxial and anomocytic and diacytic at the abaxial surface. Non-glandular uniseriate trichomes are present on both surfaces. The transverse section across the midrib shows epidermis, collenchyma, palisade cells and a few rolls of phloem around the xylem vessels. The stomata numbers and stomata indices for the upper and lower surface, respectively are1.4 and 10.2 and 2.9 and 15.6. Moisture content, total ash, acid insoluble ash, water soluble ash, sulphated ash, water soluble and alcohol soluble extractives yields 10.3%, 15.2%, 0.8%, 20.2%, 14.4% and 5.1%, respectively.

Conclusion: The findings in this study are useful for establishing standards suitable for official monographs on *Ageratum houstonianum* proper identification and quality control.

Keywords: Pharmacognostic standards, *Ageratum houstonianum*, Quality control

INTRODUCTION

The plant *Ageratum houstonianum* Mill., commonly called Flossflower, blue billy goat, blue weed, pussy foot or Mexican pain brush belongs to the family Compositae and it is native to Central America and South Eastern Mexico (Shin *et al*., 2017; Srinivas *et al*., 2012). Folklorically, the juice is employed for wound healing, skin ulcers, pains and tumours (Mohammadi, 2017; Wiedenfield and Andrade-Cetto, 2001). Some of the reported biological activities include antimicrobial (Tennyson, 2011) antibacterial, antifungal (Kumar, 2014; Pandey *et al*., 1983), antidiabetic (Srinivas *et al*., 2012), wound healing (Shin *et al*., 2017), insecticidal and ovipositional deterrent (Tennyson *et al*., 2012). Compounds isolated

from this plant are alkaloids such as heliohoustine and lycopsamine (Srinivas *et al*., 2012; Wiedenfield and Andrade-Cetto, 2001), flavones such as agehoustines A, B. C and D, eupalestin and agecorynin (Zeeshan *et al*., 2012; Quijano *et al*., 1982, Quijano *et al*., 1985). While the pharmacognostic study on *Ageratum conyzoides*, a co-generic species has been reported (Kaur *et al*., 2018; Santos *et al*., 2016), there is no study on the microscopy of the leaf of *A. houstonianum.* The only reported study close to it was carried out on the stem, petiole, node, cypselar and pollen (Das and Mukherjee, 2013) and this has necessitated this study. There is an increasing interest in herbal medicines although there are still some challenges facing its complete acceptance which may be due to lack of proper documentation as well as inappropriate quality control and standardization processes. It is a fact that therapeutic efficacy of medicinal plants depends on the quality and quantity of its chemical constituents. The misuse of medicine or natural products starts with wrong identification; hence it is essential to lay down pharmacognostic specifications for medicinal plants being used as drugs (Prasad *et al*., 2012).

METHODOLOGY

Material and Method

Collection and Identification of the leaves of *Ageratum houstonianum*

The leaves of *Ageratum houstonianum* were collected in July, 2019 from the premises of College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, authenticated at the herbarium of the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Bayelsa State, with an herbarium specimen voucher number NDUP-2020-01

Preparation of the leaves of *Ageratum houstonianum*

Fresh leaves of *A. houstonianum* were dried in the oven at 35° C and thereafter powdered. This was used for the determination of moisture content, total ash, sulphated ash, acid insoluble ash, water soluble ash, water and alcohol soluble extractives and powdered microscopy (British Pharmacopoiea, 1973). Fresh leaf was used for the microscopic evaluation of the surface and transverse sections (Pandya *et al.*, 2010, Kokate, 1986).

Macroscopic Evaluation

The macroscopic characters of the leaf such as the size, shape, apex, margin, base, texture, colour and were observed. Measurements were also carried out using line ruler as described by Evans (2009).

Microscopic Evaluation

The microscopic evaluation of the leaf of *A. houstonianum* was carried out using standard procedure (Pandya *et al.*, 2010, Kokate, 1986). The surface preparation of the upper and lower surfaces of the fresh leaf was made, the transverse sections across the mid rib were also made and the coarse powder was cleared using chloral hydrate. Each of the sections and the powder were viewed under microscope at x40, x100 and x400 magnifications after staining with glycerol and safranin respectively and mounting, pictures taken using Sony still Digital camera.

Quantitative leaf microscopy

Quantitative leaf microscopy to determine stomata number and stomata index was carried out using standard methods under the microscope with the aid of micrometers and digital camera (Pandya *et al.*, 2010, Kokate, 1986).

Physicochemical parameters

Exactly 1g of powdered sample was subjected to physicochemical analysis such as moisture content using loss on drying method, total ash, sulphated ash, acid insoluble ash and water soluble ash using a Muffer's furnace at 600 °C, water and alcohol soluble extractives were also carried out by employing standard methods (British Pharmacopoiea, 1973). This was carried out in ten replicates.

RESULTS

Table 1: Macroscopical parameters of *Ageratum houstonianum* leaf

Parameter	Description
Duration	Deciduous
Habit	Erect, herbaceous
Lamina	
Composition	Simple, pinnate, opposite
Shape	Diamond shaped
Margin	Serrate
Apex	Acute
Base	Asymmetrical. Symmetrical
Surface	
Colour	Green
Hair	Glabrous/sparsely pubescent
Dimension	
Length	2.0 cm – 6.2 cm
Breadth	1.0 cm – 3.0 cm

Table 2: Quantitative microscopy of *Ageratum houstonianum* leaf

Parameter	Mean	Standard deviation	Minimum value	Medium value	Maximum value
Stomata number					
Upper surface	1.4	0.5	1.0	1.0	2.0
Lower surface	2.9	0.9	2.0	3.0	4.0
Stomata Index (%)					
Upper surface	10.2	3.5	6.7	8.7	16.7
Lower surface	15.6	4.1	10.5	15.8	22.2

Table 3: Physicochemical parameters of *Ageratum houstonianum* leaf

Parameter	% Mean	Standard Error of mean	% Minimum value	% Medium value	% Maximum value
Moisture content	10.3	1.1	8.2	10.7	11.7
Total ash	15.2	0.1	14.9	15.1	15.6
Acid insoluble ash	0.8	0.02	0.7	0.8	1.0
Water soluble ash	8.2	0.1	7.6	8.2	8.7
Sulphated ash	20.3	0.1	19.9	20.2	20.4
Water soluble extractive	14.4	1.4	8.8	14.5	24.2
Alcohol soluble extractive	5.1	0.1	4.3	5.3	5.6

DISCUSSION

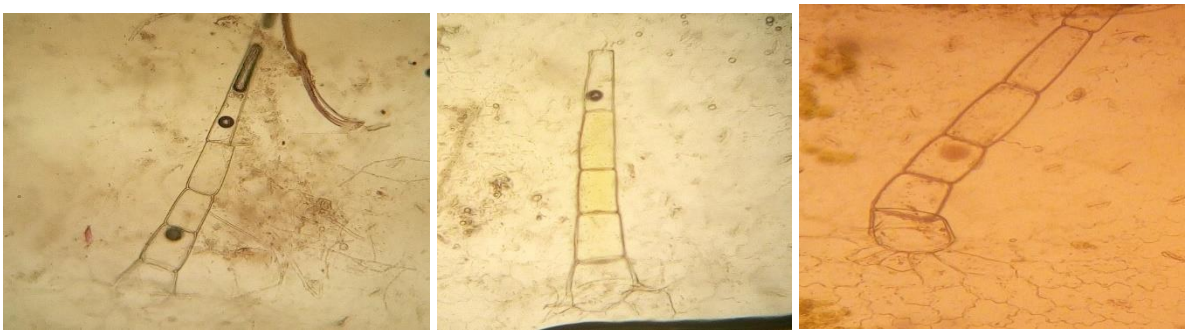
Though the use of herbal products is on the increase globally, one of the impediments to its acceptance is lack of quality control profile. Efficacy and safety are partly dependent on quality of these preparations.

The macroscopy of *A. houstonianum* shows that the leaf is simple, pinnate, diamond shaped, serrated margin, acute apex and asymetrical base (Table 1, Fig. 1).

a b c

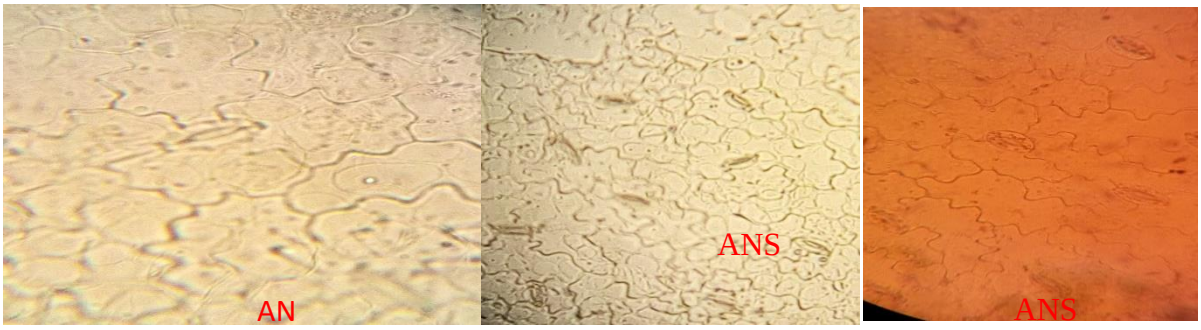
Fig. 1: a-*Ageratum houstonianum* growing in its habitat on Wilberforce Island, Bayelsa State, Nigeria, b-aerial part showing the purple flower and c-upper and lower surface of the leaf.

lmportant diagnostic features that may be of use in identifying and determining adulteration/substitution of *A. houstonianum* as observed are wavy epidermal cells, non-glandular uniseriate trichomes, anisocytic and anomocytic stomata on the upper surface (Fig. 2) while the lower surface in addition to all the above also included a third type of stoma; diacytic together with anomocytic stomata, anisocytic type of stoma is only present on the upper surface (Fig. 3), more numerous stomata are also present in the lower surface which is obvious from the stomata number and S.I (3, 16%, lower surface) (Table 2). The key standard in this plant is the presence of three types of stomata; anomocytic which is usually found in the Compositae family (Santos *et al*., 2016), anisocytic and diacytic.



X400

a b c

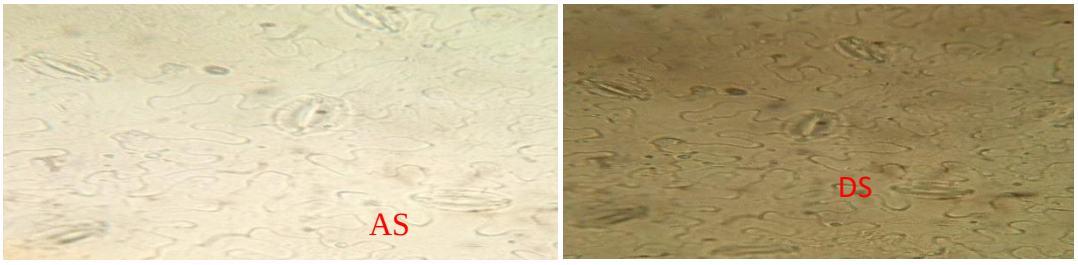


X400 X 100

d e f

Fig. 2: a-f: Upper epidermal cells of *Ageratum houstonianum* showing non glandular uniserrate trichomes arising from the epidermal cells; trichome base (a-c); wavy epidermal cells with anomocytic (AN) and anisocytic (ANS) stomata (d-f).

Anomocytic and anisocytic stomata have been reported in the leaf of *A. conyzoides while* other studies however have either reported only anomocytic or diacytic stomata (Kaur *et al*., 2018; *Janarthanan, 2016;* Santos *et al*., 2016). In our study, we have found three instead of the maximum number of two types in the leaf of *A. conyzoides.* Also, multicellular non-glandular trichomes (Kaur *et al*., 2018; Santos *et al.*, 2016) found in our study are also present in *A. conyzoides* while biseriate covering trichomes also reported in *A. conyzoides* (*Janarthanan, 2016) are absent in A. houstonianum, rather, our study shows the presence of uniseriate trichomes.*

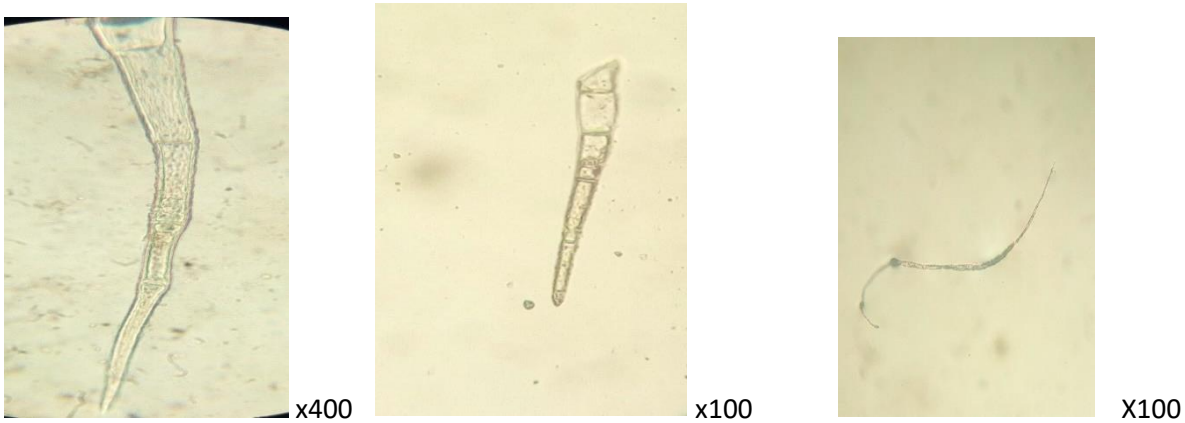


X400

a b

X100 c d e

Fig 3: a-e: Lower epidermal cells of *Ageratum houstonianum* showing wavy epidermal cells with anomocytic (AS) and diacytic (DS) stomata (a-b), non-glandular uniseriate trichome arising from the veins (c-e) and epidermal cells (a-b).

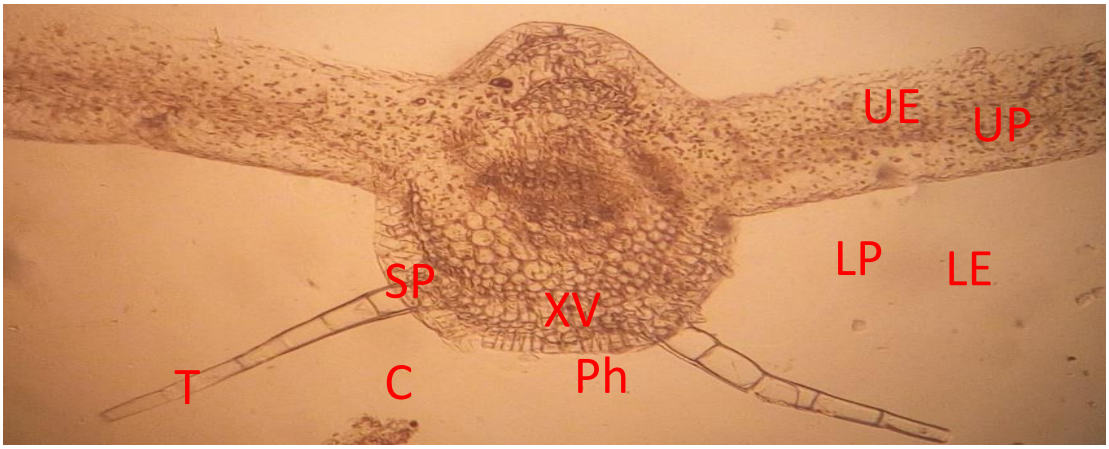


x400 x100 X100

a b c

Fig 4: a-c: Microscopy of the powdered Plant of *Ageratum houstonianum* showing broken non-glandular uniseriate trichomes(a-b); fibre (c).

The transverse section through the midrib of its leaf showed vascular bundles which are conjoint, collateral and closed (Fig. 5). Covering trichomes which occur as multicellular and uniseriate are attached to the lower epidermis unlike the glandular trichomes have been reported with the transverse section of *A. conyzoides* (*Janarthanan, 2016*). Two layers of thick walled collenchyma cells were found attached to the lower epidermis. Spongy cells are irregular, thick walled and loosely attached without permitting intercellular spaces (Fig. 5). Some of these features were also found in the powdered leaf (Fig. 4).



X 400

Fig.5: The transverse section of *Ageratum houstonianum* showing non-glandular, uniseriate trichomes (T), lower epidermis (LE), upper epidermis (UE), spongy mesophyll (SP), collenchyma (C), xylem vessels (XV), phloem (Ph), upper (UP) and lower (LP) epidermis

The water-soluble extractive value (14%) was approximately thrice that of the alcohol soluble extractive value (5%). Water soluble extractive was also reported to be higher in *A. conyzoides* (Kaur *et al*., 2018). The extractive values are useful in the estimation of specific constituents soluble in a particular solvent, they are primarily needful for estimation of exhausted or adulterated drugs, it determines the quality as well as purity of drugs (Chandel *et al*., 2011).

The ash values obtained include: total ash value 15%, water soluble ash value 8% which was higher than the acid insoluble ash value (0.8%) while sulphated ash value was 20% (Table 3). Ash value is used to determine the quality and purity of crude drugs (Prasad *et al*., 2012); it determines the level of inorganic composition and other impurities present in the drug (Wallis, 2005). The purpose of ashing therefore, is to remove all traces of organic matter (Setia and Goyal, 2010), total ash is a measure of the level of foreign inorganic impurity and adulteration with sand, earth, that is, the degree of care taken in drug preparation (Bigoniya *et al*., 2012). The European and African pharmacopeia stipulate that total ash should not be more than 15 and 12%, respectively and acid insoluble ash should not exceed 1.5% (Bigoniya *et al*., 2012). The results obtained from this study are within the normal range.

In all the reports on *A. conyzoides the acid insoluble ash < water soluble ash (*Kaur *et al*., 2018; Santos *et al*., 2016; *Janarthanan, 2016). This is similar to the findings in our study on A. houstonianum*.

The moisture content obtained was 10.273% (Table 3) which falls within the recommended range of 8-14% stipulated for vegetable drugs (Adelekan, 2000; Abere and Onwukaeme, 2012). If it exceeds this value, the drug will become prone to microbial attack if not stored well (Bigoniya *et al*., 2012). Limits of moisture content should be set for all herbal materials because an excess of water in herbal materials will encourage microbial growth and deterioration following hydrolysis and hence chemical conversion may take place which will eventually influence bioactivity. Moisture content evaluation therefore, helps to ensure that adequate measures are put in place to ensure proper storage of herbal materials with high moisture content.

CONCLUSION

The findings have provided information on the pharmacognostic parameters of the leaf of *A. houstonianum* which can be used for its identification and quality control. The information can be incorporated into the Nigerian Herbal pharmacopoeia and the West African Herbal Pharmacopoeia. This makes the plant beneficial for future pharmacological activities.

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Conflict of Interest: None declared

Received: February 4, 2020

Accepted: October 27, 2020