Original Research Article

PHARMACOGNOSTIC AND TAXONOMIC STUDIES OF THE LEAF OF *Ageratum houstonianum* Mill. (COMPOSITAE)

ABSTRACT

The aim of this study was to employ the pharmacognostic and taxonomic parameters to evaluate the leaves of *Ageratum houstonianum.* The leaves were collected, identified, air dried, pulverized and subjected to microscopy, micromeritics, chemomicroscopy, fluorescence, soluble-extractive values, moisture content and ash values using standard procedures.The result obtained from the microscopy showed an irregular epidermal cell shape and a sinuous anticlinal wall pattern, anomocytic type of stomata, double armed trichome and amphistomatic stomatal distribution on both the abaxial and adaxial surfaces. The stomatal index for the abaxial surface was 11.6% and for the adaxial surface was 25.6%.The micromeritic evaluation of the powdered leaf revealed a Carr's index of 25.23%, Hausner's ratio of 1.33 and an angle of repose of 38.8^{o} indicating a poor flow. The result for chemomicroscopy showed the presence of oils, protein, lignin, mucilage and calcium oxalate crystals. For moisture content, the result was 12.7%$^{w}/_{w,}$ the Total ash value was 14%$^{w}/_{w,}$ Acid-insoluble ash value was 0.7%$^{w}/_{w,}$ water-soluble ash value was 7.7%$^{w}/_{w,}$ methanol-soluble extractive value was 17%$^{w}/_{w,}$ ethanol-soluble extractive value was 16%$^{w}/_{w}$ and the water soluble extractive value was 21%$^{w}/_{w}$. The data obtained from the pharmacognostic and taxonomic studies provided information for the identity, quality and purity of *Ageratum houstonianum*.

KEYWORDS: ***Ageratum houstonianum*, Amphistomatic, Pharmacognostic, Taxonomic**

Introduction

Ageratum houstonianum Mill. Family:Compositae is a short lived herbaceous plant growing up to 1m tall, with glandular flower heads. Its stems are round and full, green and densely covered by soft hairs. The leaves are simple and oppositely borne on stalks 0.5cm – 3cm long, bright green, soft, hairy and slightly aromatic. The fluffy mauve, blue, pinkish or white flower heads are arranged in dense clusters at the tips of the branches. Cotyledons are round with a short stalk. Inflorescense are terminal, compound umbel and the flower heads are compact. Each flower head (5-8mm across) has numerous tiny tubular flowers that are surrounded by two or three rows of greenish coloured bracts (Funke *et al.*,2009)[1].The essential oils and extracts derived from the aerial parts of the plant exhibited antifungal, antimicrobial and mosquitocidal activities. The predominant constituent of this drug is procene-1 and procene-2, hitherto, potential application of isolated procene and ageratum essential oil is insecticidal (Sharma and Sharma, 1995) [2]. Methanol extract of the plant has wound healing property (Chah *et al.,* 2006) [3]. The phytochemicals constituents include alkaloids, flavanoids, terpenoids, tannins and saponins.Essential oils have application in folk medicine, food preservation and as feed additives. The essential oils of *A. houstonianum* are said to contain three major constituents, procene-1, procene-2 and beta-caryophyllene. Sesquiphellandrene and caryophyllene epoxide have been obtain from the leaves of the plant extract (Ekundayo *et al.*, 1988)[4].A total of 21 polyoxygenated flavonoids have been reported from the specie which include; Scutellarein-

5,6,7,1- tetrahydroxy flavones, quercetin, kaemferol, eupulestin etc. (Adebayo *et al*., 2010) [5]. Some alkaloids found in *A. houstonianum* include:lycopsamine, echinatin, caffeic acid, phytolfumaric acid, sesamine, aurantiamide acetate etc (Bronjendro, 2013)[6]. *A.houstonianum* is toxic to grazing animals causing liver lesions due to the presence of pyrrolizidone alkaloids (Couet *et al*., 2003)[7].

Scientific Classificationof *A.houstonianum* [8]

Kingdom	-	Plantae
Clade	-	Tracheophytes
Order	-	Asterales
Family	-	Compositae
Genus	-	*Ageratum*
Species	-	*A. houstonianum*
Synonym	-	*Cerelia houstoniana*(mill)Kuntze
Common name	-	Floss flower

Figure 1 ***Ageratum houstonianum***

Source: Field Data (2021)

MATERIALS AND METHODS

Collection, Identification and Preparation of plant material

The leaves of the plant were collected from University of Uyo Town campus, Akwa Ibom State in January 2021. It was identified by Dr. Imoh I. Johnny of the department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo,Nigeria and the sample deposited in the University of Uyo Pharmacy Herbarium with the voucher specimen number UUPH 10(a) for reference purpose. The fresh leaves of the plant were air-dried, pulverized and packed in a well labeled dry container.

Anatomical Studies

Microscopic Evaluation of Leaf

The standard median portion of the well expanded matured leaf was obtained. Microscopical examinations of the transverse section was made, the Epidermis of both adaxial and abaxial surfaces were also made by placing the leaf on a glass slide. The samples were irrigated with water and scraped gently with a sharp razor blade till loose cells from the epidermis were washed away with water and the desired epidermis was reached. The epidermal peels were further cleared with sodium hypochlorite and rinsed gently with water. The epidermal peels were stained with aqueous solution of safranin-O for (five) 5 minutes and 10% glycerol. The stained samples were mounted on a binocular microscope. Photomicrographs were taken from good preparations using the Olympus CX21 binocular microscope fitted with an MD500 amscope microscope eyepiece camera. Measurements were done at ×10 while ×40 for photomicrographs [9].

Quantitative Microscopy of the Leaf

Quantitative microscopy parameters such as leaf constant studies namely stomatal length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, epidermal cell thickness were carried out using standard procedures.
All measurements were made using a calibrated ocular micrometer and 10 microscopic fields chosen at random were used and data presented as mean ± Standard Error of Mean (SEM).

Stomatal Index Determination

The stomatal index (S.I) was determined according to Metcalfe and Chalk [9, 10,11].

The sample (quantitative microscopy) was placed under the microscope and the stomatal index was determined using the formula;

$$S.I=\frac{S}{E+S}\ X100$$

Where S = Number of stomata per unit area
E = Number of epidermal cells in the same area

Evaluation of Powders
Micromeritic Analysis
The flow property was determined using standard methods [12]. Which constitutes;
Bulk Density and Tapped Density
The weight of 10 g of dried powdered leaf was weighed into 100 ml measuring cylinder and the volume occupied was noted as the bulk volume (Vb). The cylinder was gently tapped repeatedly to obtain a constant volume noted as the tapped volume (Vt). Bulk density was calculated using the formula below;

$B\rho = M / Vb$

$Tv = M / Tv$

Where Bρ = Bulk density
M = Mass of powder
Bv = Bulk volume of powder
Tρ = Tapped density

Tv = Tapped volume

Hausner's Ratio and Carr's index

Hausner's ratio a function of interparticle friction was calculated using the formula

Hausner's ratio = Tp/Bp

While *Carr's index* = Tp - Bp/Tp × 100

Where; Tp = Tapped density

Bp = Bulk density.

Angle of repose(θ) = Tan^{-1} (Heap height of powder / Radius of heap base)

Chemomicroscopic Analysis of Leaf Powder

Powdered leaf was examined for its chemomicroscopic properties namely mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals using standard procedures [13].

Fluorescence Analysis of Leaf Powders

The fluorescent analysis of dried leaf powder was carried out using standard method [14].

Physico-chemical Evaluation of Leaf Powders

The physicochemical parameters such as moisture content, ash values (total ash, acid-insoluble ash and water-soluble ash values), soluble extractive values such as ethanol, methanol and water-soluble extractive values were performed according to the official method prescribed by the WHO guidelines on quality control methods for medicinal plant materials [10,15,16].

RESULTS

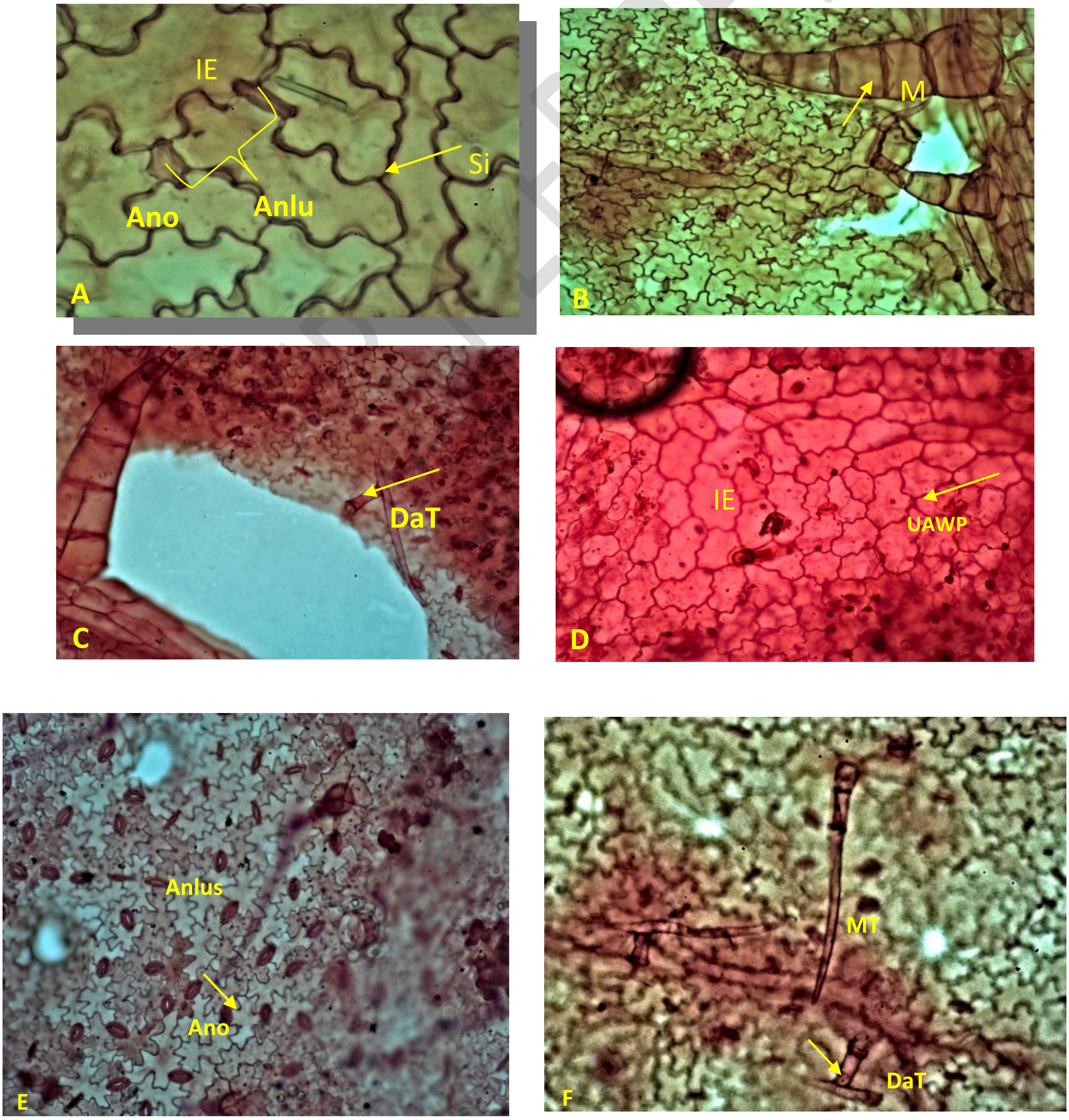
The results for the anatomical studies, micromeritic properties, chemomicroscopy, fluorescence properties, soluble extractive values, moisture content and ash values of the leaf are represented in Tables 1- 5 and the adaxial, abaxial, transverse section and powder analysis of the leaf were as represented in Figures 2 (A-J) respectively.

Table 1: Results of Microscopy of Abaxial and Adaxial surfaces of *Ageratum houstonianum* leaf

Parameters	Abaxial surface	Adaxial surface
Epidermal cell shape	Irregular	Irregular
Anticlinal wall pattern	Sinuous	Sinuous
Stomatal morphology type	Anomocytic	Anomocytic
Stomatal distribution	Amphistomatic	Amphistomatic
Stomatal length (µm)	20.39(22.37±0.38)24.00	21.35(22.18±0.32)23.99
Stomatal width (µm)	7.32(7.77±0.16)8.82	12.26(13.57±0.27)15.04
Stomata Index	11.6%	25.6%
Stomatal number	18(20.80±0.68)23	50(52.80±0.68)56
Epidermal cell number	162(170±1.85)175	148(156.30±1.63)162
Thickness (µm)	2.31(2.86±0.15)3.46	2.45(3.24±0.15)3.84
Type of trichome	Multicellular armed trichome	Multicellular trichome
Length of trichome (µm)	60.52(67.60±7.49)96.68	64.44(98.69±7.24)132.36
Width of trichome (µm)	8.88(10.60±0.43)13.28	12.82(17.52±1.24)22.32
Length of epidermal (µm)	44.11(57.23±4.04)84.31	72.75(77.90±1.34)85.27
Width of epidermal layer(µm)	14.43(25.66±2.02)34.70	32.99(40.43±2.37)54.66

Values are represented as mean of ten replicates (10) ±SEM (Standard Error of Mean)

Microscopy of *Ageratum houstonianum* Powdered leaf



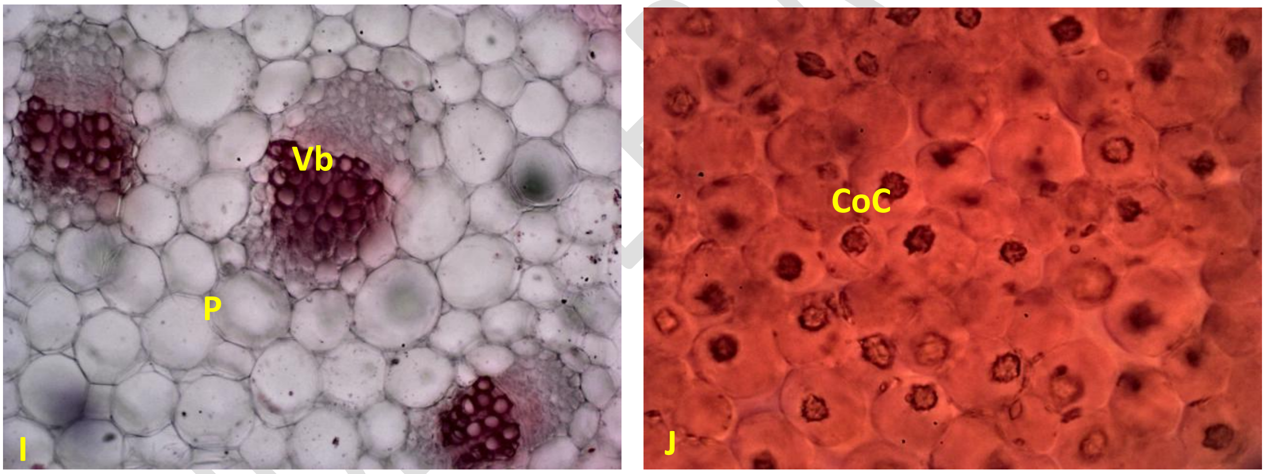


Figure 2: A: Ano (Anomocytic) Abaxial surface, Anlu (Anomalous stomata) Abaxial surface, IE (Irregular epidermal cell) Abaxial surface ×40,**B:**MT (Multicellular trichome)×10**C**: DaT (Double arm Trichome)×10, **D**: IE (Irregular Trichome), UAWP (Undulate anticlinal wall pattern)×10, **E:**Anlus (Anomalous stomata), Ano (Anomocytic stomata)×4, **F:**DaT (Double arm Trichome), MT (Multicellullar Trichome)×10, **G:**Vb (Vascular bundles)×4, **H:**C (Collenchyma), P (Parenchyma), E (Epidermis) ×10, **I:**Vb (Vascular bundles), P (Parenchyma)×40, **J:** CoC (Calcium oxalate crystals)×10.

Table 2: Micromeritic Properties Of *A.houstonianum* Powdered leaf

Parameters	Results
Bulk Volume (ml)	43±0.35
Tapped Volume (ml)	32.16±0.20
Bulk Density (g/ml)	0.23±0.00
Tapped Density (g/ml)	0.31±0.00
Hausner’s Ratio	1.33±0.01

Diameter (cm)	7.35±0.06
Angle of Repose (^{o})	38.8
Carr's Index (%)	25.23±1.14
Height of heap (cm)	2.56±0.04
Flow Time (sec)	15.81±0.88
Flow Rate (g/sec)	0.63

Values are represented as mean of three replicates (3) ±SEM (Standard Error of Mean)

Table 3: Chemomicroscopy of *A. houstonianum* Powdered leaf

Parameters	Inference
Lignin	+
Calcium oxalate	+
Starch	+
Oils	+
Cellulose	+
Mucilage	+

Table 4. Flourescence Analysis of *A. houstonianum* powdered leaf

Extract	Ordinary light	UV-365nm	UV-254nm
Water	Green	Grey	Grey
Methanol	Green	Orange	Grey
Ethanol	Green	Orange	Grey
Dichloromethane	Green	Red	White
N-hexane	Yellow	Pink	Grey
Ethyl acetate	Green	Orange	Brown

Table 5: Result of Water-extractive value, Methanol-extractive value, Ethanol-extractive value, Moisture content, Total ash value, Water-Soluble ash value, and Acid-Insoluble ash value of *Ageratum houstonianum*

PARAMETER	WEIGHT (g)	PERCENTAGE ($\%^{w}/_{w}$)
Moisture content	0.38±0.00	12.70±0.00
Total ash value	0.42±0.00	14.00±0.00
Acid-insoluble ash value	0.02±0.00	0.70±0.00
Water-soluble ash value	0.23±0.00	7.70±0.00
Water-soluble extractive value	0.21±0.01	21.00±0.01
Methanol-soluble extractive value	0.17±0.00	17.00±0.00
Ethanol-soluble extractive value	0.16±0.00	16.00±0.00

Values are represented as mean of five (5) replicates ±SEM

DISCUSSION

The results obtained from the microscopy of *A. houstonianum* in figures 2(A to J) showed an irregular epidermal wall shape with a sinuous anticlinal wall pattern on the abaxial and adaxial surfaces, the stomatal distribution was found to be amphistomatic on both surfaces, the type of stomata was found to be anomocytic on both surfaces, both surfaces showed double-armed trichome and the stomatal index for abaxial surface was 11.6% and 25.6% for the adaxial surface.The micromeritic properties in table 2 showed the flow characteristics of the powdered leaf. Carr's index was 25.23% which indicates a poor flow, Hausner's ratio was 1.33 indicating a poor flow and the angle of repose was 38.8^{o} indicating a poor flow property.Mbah *et al*.,2012 [12] used this parameters in evaluating the flow properties of *Bridelia ferruginea*. Chemomicroscopy evaluation in table 3 indicated the presence of proteins, oils, lignin, calcium oxalate crystals and mucilage.The fluorescence analysis in table 4 of the powdered drug treated with methanol, ethanol, ethylacetate, n-hexane, dichloromethane and water was observed in ordinary light, short UV light (254nm) and long UV light (365nm). It showed different colour changes as a result of the chemical interactions between the solvents and the phytochemicals in the leaf.

Table 5 showed the results for the moisture content, water-soluble extractive value, methanol-soluble extractive value, ethanol-soluble extractive value, total ash value, acid-insoluble ash value and water-soluble ash value of the powdered leaf of *A.houstonianum*. The moisture content was $12.70\%^{w}/_{w}$ which is within the recommended range of $8\text{-}14\%^{w}/_{w}$ for vegetable drugs according to the African pharmacopoeia, 1986 [10]. This shows that the plant has a long shelf life and better stability against microbial degradation. The ash values are one of the criteria for judging the identity and purity of crude drugs. The African pharmacopoeia limits of ash value for crude drugs states that a lesser amount shows that there is less solubility of the ash in water while a higher value indicates a high solubility of the ash in water. The total ash value was $14\%^{w}/_{w}$ which is within the recommended range as stated in the European pharmacopoeia 2007 [17]. The acid-insoluble ash value was $0.70\%^{w}/_{w}$ which is within the normal range as stated in the European pharmacopoeia 2007 (not exceeding $2\%\ ^{w}/_{w}$ [16].The water-soluble ash value was $7.70\%\ ^{w}/_{w}$ .The determination of water- soluble ash value of a particular crude drug helps in the detection of the amount of ash materials that are soluble in water.

The extractive values are indicative weights of the extractable chemical constituents of crude drugs under the different solvent environments. The methanol-soluble extractive value was

17.00% $^{w}/_{w}$, the ethanol-soluble extractive value was 16.00% $^{w}/_{w}$ and the water-soluble extractive value was 21.00$^{w}/_{w}$.

Conclusion

The result collected from the pharmacognostic studies provided information about the identity, quality and purity of *Ageratum houstonianum*.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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