PHARMACOGNOSTIC AND TAXONOMIC STUDIES OF LEAF AND STEM OF *Launaea taraxacifolia* (WILLD) AMIN EX C.JEFFREY **(COMPOSITAE)**

ABSTRACT

Launaea taraxacifolia from the family Compositae is used in the treatment of diabetes mellitus, hypertention, cancer, malaria, bacterial infections and arthritis. The aim of this study was to evaluate pharmacognostic and taxonomic parameters such as microscopy, micromeritics, chemomicroscopy, fluorescence, soluble-extractive values and ash values of the leaves and stems of this plant. The plant was collected, identified, air-dried, weighed and subjected to evaluation using standard procedures. The results showed that, from microscopy : the stomatal distribution was amphistomatic and the stomatal type were anisocytic and anomocytic on the abaxial surface and anomocytic on adaxial surface with T-pieces on the stomata. The stomatal index was 18.7% on the abaxial surface and 11.9% on the adaxial surface. The stomatal number on the abaxial surface was 13.2 (15.6±0.55) 18.1 and 18 (26.5±2.08) 36 for adaxial surface. Results of the micromeritics properties of the powdered leaf and stem samples were bulk volume of 38.67±0.17 and 51.33±0.33, tapped volume of 29.83±0.17 and 34.0±0.00, bulk density of 0.26±0.00 and 0.20±0.00, tapped density of 0.34±0.00 and 0.29±0.00, angle of repose of 36° and 43°, Carr's index of 22.7% and 33.78%, Hausner's ratio of 1.30±0.01 and 1.15±0.01. The micromeritics indicated that the powder had a fair flow and for chemomicroscopy the powder contained lignin, mucilage, oil and protein. The fluorescence properties determined revealed different colours under different ultraviolet lights. The water-soluble, methanol-soluble, ethanol-soluble extractive values were 35% $^{w}/_{w}$, 14% $^{w}/_{w}$ and 16%$^{w}/_{w}$ for leaf and 18% $^{w}/_{w}$, 8% $^{w}/_{w}$, and 9% $^{w}/_{w}$ for stem respectively. The moisture content of the leaf and stem were 16% $^{w}/_{w}$ and 11% $^{w}/_{w}$. Total, acid-insoluble and water- soluble ash values were 17.7% $^{w}/_{w}$, 1.3% $^{w}/_{w}$ and 8.3% $^{w}/_{w}$ for leaf and 6.3% $^{w}/_{w}$, 0.3% $^{w}/_{w}$ and 4.7% $^{w}/_{w}$ for the stem respectively. This data obtained from the pharmacognostic and taxonomic studies provide information for the identity, purity and quality of *L. taraxacifolia.*

KEYWORDS: Amphistomatic, *Launaea taraxacifolia,* micromeritics, pharmacognostic**,** T-pieces

Introduction

Launaea taraxacifolia (willd) Amin ex C.Jeffrey, Family: Compositae, is a perennial plant producing a rosette of basal leaves and erect stems growing up to 1.3 metres tall from a woody rhizome, the plant can spread by means of underground rhizome. A widely used leaf crop in Africa which including Ghana, Senegal, Benin and Nigeria, where it is well known and domesticated. The Perennial herb is up to 150cm tall, with creeping system and erect stem. It is propagated by division of rhizomes. The roots are cut in pieces of about 10cm length and these are planted horizontally and entirely covered with soil. [1]. It is a herb with basil rosette of leaves and erect stems to 1.3m high from a woody rhizome from Senegal to Nigeria and dispersed to

Sudan and Ethiopia. The leaves are eaten fresh as a salad or cooked. It is propagated by division of rhizomes and the light seeds are dispersed by air. It strives better in the tropical region and its habitat: Mesophytic, the flowers are yellow. The plant is used for treatment against vomiting, tooth ache and diabetes. The boiled leaves are applied to the head of a newly born baby if the bones are not well knitted together. A decoction of the leaves is used to treat wounds. The leaves, mixed with fine ash, are rubbed onto the sores of yaws [1].Research over the years has revealed that *Launaea taraxacifolia* possesses important pharmacological properties which include antioxidant, antimalarial, arthritic/anti-inflammable, antibacterial, cardioprotective and DNA protecting activity [2]. The phytochemical screening revealed the presence of cardiac glycosides, terpenoids, tannins, saponins, flavonoids and steroids in the leaves. The lettuce also contains nutrients which include leucoanthocyanins phenolic acids, ascorbic acid, lycopene, and β-Carotene. The results of the study indicated that *L.taraxacifolia* leaves are potential sources of useful nutrients and could be used to fulfill the growing demands of plant based food for Ghanians. [3]. The most abundant chemical components found in the leaf is palmitic acid methyl ester, and phytol [4]. A long chain alcohol, I-hexacosanol, has also been discovered to be present in the leaves [5].

Phylogeny of *Launaea taraxacifolia* (Scientific Classification) [6].

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Asterales
Family:	*compositae*
Genus:	*Launaea*
Specie:	*L.taraxacifolia* (willd) Amin ex C.Jeffrey
Common Name:	African lettuce, wild lettuce
Local Name:	Yoruba: Eto yanrin Igbo: Ugu

Figure 1: *Launaea taraxacifolia* in it's natural habitat
Source: Field data (2021)

MATERIALS AND METHOD

Collection, Identification and Preparation of the Plant Materials

Plant sample was collected from the field around Faculty of Pharmacy, Town campus, University of Uyo in January, 2021. The plant was identified by Dr. Imoh I. Johnny, Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, with herbarium identification number: UUPH 10(p). The fresh plant materials were air-dried, pulverized and packed in dry containers, well labelled and used when needed.

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 [7].

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 [8, 9, 10]. The sample was placed in 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

Micromeritics

The flow property was determined using standard methods [11] 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;

$$B\rho = \frac{M}{Vb}$$

Where;

$$T\rho = \frac{M}{Vt}$$

Where $B\rho$ = Bulk density
M = Mass of powder
Vb = Bulk volume of powder
$T\rho$ = Tapped density

Vt = tapped volume

Interparticulate porosity was also calculated using the formula below;

IP = $\frac{\rho T - \rho B}{\rho T * \rho B}$

Hausner's Ratio and Carr's index

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

$$Hausner's\ ratio = \frac{T\rho}{B\rho}$$

While Carr's Index was measured as

$$Carr's\ index = \frac{T\rho - B\rho}{T\rho} \times 100$$

Where; $T\rho$ = Tapped density
$B\rho$ = Bulk density.

Angle of repose

$$\theta = Tan^{-1}(\frac{Heap\ height\ of\ powder}{Radius\ of\ heap\ base})$$

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 [12].

Fluorescence Analysis of Leaf Powders

The fluorescent analysis of dried leaf powder was carried out using standard method **[**13, 14**].**

Physico-chemical Evaluation of Leaf Powders

The physicochemical parameters such as moisture content, ash values (total ash, acid-insoluble ash, water-soluble ash), 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 [9,15,16].

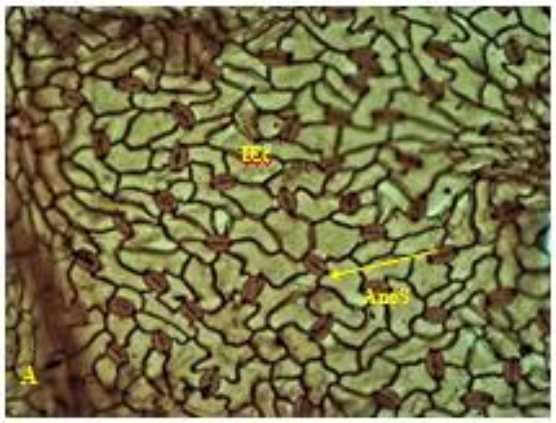
RESULTS

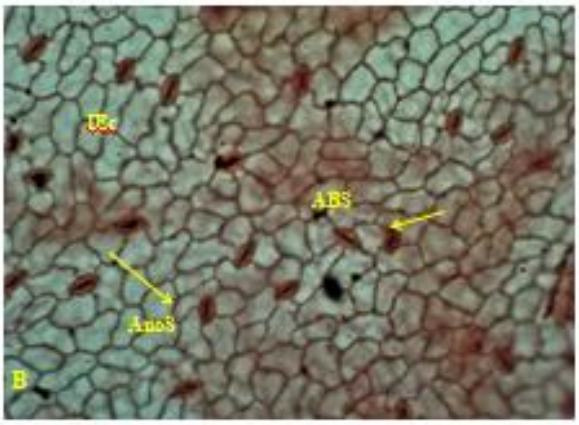
1: Results

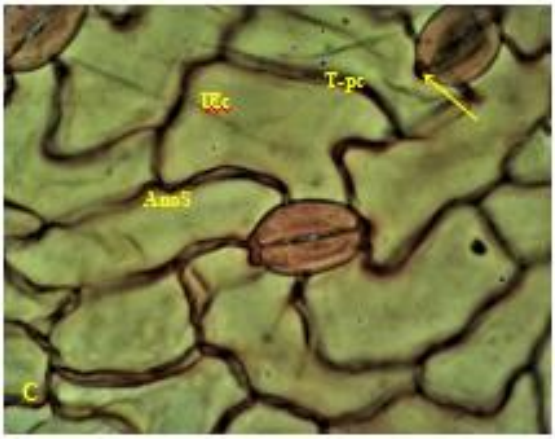
Table 1: Result for the microscopic features of *Launaea taraxacifolia* and Standard Error of Mean (SEM).

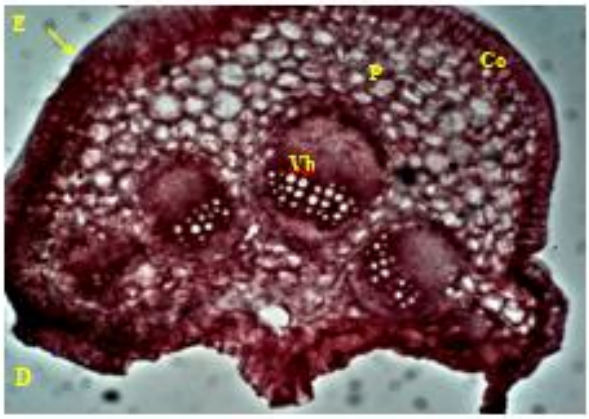
LEAF SURFACE	ABAXIAL	ADAXIAL
Stomatal morphology type	Anisocytic and Anomocytic	Anomocytic
Stomatal distribution	Amphistomatic	Amphistomatic
Stomatal length (um)	22.02 (25.4 ± 0.87) 30.19	23.84 (26.1± 0.53) 28.48
Stomatal number	13.15 (15.6 ± 0.55) 18.11	18 (26.50 ± 2.08) 36
Epidermal number	159(162.1± 5.75) 204	184 (196.9 ± 2.64) 207
Stomatal index (%)	18.7%	11.9
Length of guard cell (um)	10.88 (13.16 ± 0.64) 18.27	14.43 (17.55 ±0.50) 19.67
Width of guard cell (um)	5.47 (6.98± 0.24) 7.81	5.21(6.32±0.66) 7.52
Length of epidermal layer (um)	33.93 (48.77 ± 3.70) 67.20	34.27 (48.60 ± 2.11) 55.69
Width of epidermal layer (um)	16.09 (23.13 ± 2.32) 39.06	16.62 (21.95 ± 1.42) 26.44
Thickness of wall of epidermal (um)	3.36 (4.00 ± 0.22) 4.96	3.37(4.07±0.22) 4.96

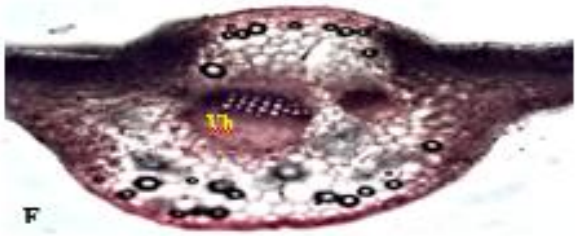
Values are represented as mean of ten replicates (10)±SEM (Standard error of mean)











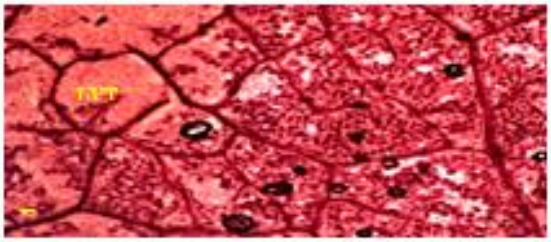


Figure 2: **(A): IEc; Irregular epidermal cell and undulate Anticlinal Wall Pattern, AnoS; Anomocytic stomata Abaxial surface × 10: (B): IEc ; Irregular epidermal cell, ABS; Abnormal stomata and AnoS Anomocytic stomata adaxial × 10; (C): T-Pc; T-pieces ×40 (D): Transverse section of petiole; Vb Vascular bundles, P; Parenchyma and Co; Collenchyma ×4; (E): Transverse section of leaf; Vb, Vascular bundle x4, (F); Venation; LVT , Linear vein termination and QE; quadrangular areole × 4, (G); Leaf powder: IEc Irregular epidermal cell, (H): Leaf powder: AnoS; Anomocytic and AniS; Anisocytic stomata x10**

Table 2: Micromeritic properties of *Launaea taraxacifolia* leaf and stem powders

Micromeritic Parameters	Leaf Powder	Stem Powder
Bulk volume (ml)	38.67± 0.17	51.33 ± 0.33
Tapped volume (ml)	29.83± 0.17	34.0 ± 0.00
Bulk density (g/ml)	0.26 ± 0.00	0.20 ± 0.00
Tapped density (g/ml)	0.34 ± 0.00	0.29 ± 0.00
Hausner's Ratio	1.30± 0.01	1.15 ± 0.01
Carr's Index (%)	22.7± 0.44	33.78± 0.45
Diameter of Heap (cm)	7.00± 0.04	7.46 ± 0.02
Height of Heap (cm)	2.57 ± 0.33	3.50 ± 0.06
Flow time (sec)	15.09± 0.52	59.66 ±1.76
Angle of repose	36^{o}	43^{o}
Flow rate (g/s)	0.66	0.17

Table 3: Chemomicroscopy of *Launaea taraxacifolia* Leaf Powder

Constituents	Quanlitative Test	Observation	Inference
Mucilage	Rutheniumred, view under microscope	Sample stains pink	Mucilage present
Lignin	Phloroglucinol + conc. HCL	Sample stains red	Lignin present
Starch	N/50 iodine	Sample did not stain blue black	Starch absent
Oils	Sudan iv, view under microscope	Sample stains pink	Oil present
Calcium oxalate crystals	Sample cleared &viewed under microscope	Calcium oxalate crystal not seen	Calcium oxalate absent
Protein	Picric acid (1%) + million's regent	Sample stains yellow strand	Protein present

Table 4: Chemomicroscopy of *Launaea taraxacifolia* stem Powder

Constituents	Qualitative Test	Observation	Inference
Mucillage	Ruthenium red, view under microscope	Sample stains pink	Mucilage present
Starch	Phloroglucinol + conc. HCL	Sample stains red	Lignin present
Oil	N/50 iodine sudan IV, view under microscope	Sample did not stain blue black. Sample did not stain pink	Starch absent oil absent
Calcium oxalate crystal	Sample cleared and viewed under microscope	Calcium oxalate crystal not seen	Calcium oxalate absent
Protein	Picric acid (1%) + million's regent	Sample stains yellow strand	Protein present

Table 5: Fluorescence properties of *Launaea taraxacifolia* Leaf powder

Extracts	Sample	Physical Observations Colour	Uv – 365nm Colour	Uv – 254 Colour
n-Hexane	Leaf	Green	Orange	Brown
	Stem	White	Pink	White
DCM	Leaf	Green	Orange	Brown
	Stem	White	Orange	White
Ethyl Acetate	Leaf	Light green	Pink	Purple
	Stem	White	Pink	Purple
Ethanol	Leaf	Light green	Pink	Purple
	Stem	White	Pink	Purple
Methanol	Leaf	Light green	Orange	Purple
	Stem	White	Pink	Purple
Water	Leaf	Light gray	Gray	Purple
	Stem	Light gray	Gray	Purple

Table 6: Physicochemical constants of leaf

Parameter	Weight(S)	Percentage (% $^{W}/_{W}$)
Moisture content	0.48	16
Total ash	0.53	17.7
Acid-Insoluble Ash value	0.04	1.3
Water- soluble ash value	0.25	8.3
Extractive Values		
Water-soluble extractive value	0.35	35
Methanol- soluble extractive value	0.14	14
Ethanol- soluble extractive value	0.16	16

Table 7: Physicochemical constants of Stem

Parameter	Weight(S)	Percentage (% $^{W}/_{W}$)
Moisture content	0.33	11
Total ash	0.19	6.3
Acid-insoluble Ash value	0.01	0.3
Water- soluble ash value	0.14	4.7
Extractive Values		
Water-soluble extractive value	0.18	18
Methanol- soluble extractive value	0.08	8
Ethanol- soluble extractive value	0.09	9

Discussion

Amphistomatic stomatal distribution was recorded for the leaf epidermal surface in *L. taraxacifolia* with anomocytic and anisocytic stomata on the abaxial surface while anomocytic stomata only on the adaxial surface (Figure 2). The presence of T- pieces in the various stomata is a diagnostic feature in this plant. The epidermal cell shapes were irregular with undulate anticlinal wall pattern. The stomatal index on the abaxial surface was 18.7 % and 11.9 % on the adaxial surfaces (Table 1). Johnny *et al*. [17] reported on stomatal index as a distinctive feature in *Cola millenii* as stomatal index is not affected by factors such as age of plant, size of leaf, environmental factors and is used in plants identification.

For venation studies, the vein termination was linear while the areole was quadrangular (Figure 2H). Therefore, these features can be used as diagnostic characters to aid in the identification of *L. taraxacifolia* as Johnny *et al* [17] reported on the uniqueness of venation properties in the differentiation of various Monkey Kola studied.

The micromeritics properties showed flow properties as well as interparticulate resistance between these powders and this information predicts the stability and solubility of crude drugs. Carr's index was 22.7% for the leaf and 33.8% for the stem which indicate that the flow properties were passable and very poor respectively as shown in Table 2. The Hausner's ratio was 1.30 and 1.51 for the leaf and stem respectively indicating a poor flow. The angle of repose for the leaf and stem were 36^{o} and 43^{o} respectively indicating a passable flow [18]. The micromeritic properties is employed in the formulation of powdered herbal drug, in order to determine it suitability for formulation into solid dosage form. Chemomicroscopy analysis of the leaf and stem of the plant recorded presence of lignin, mucilage, protein, and oil on the leaf but absence of oil in the stem (Table 4). Fluorescence analysis of the powdered leaf and stem extracts viewed in ordinary light, 254nm and 365nm of the UV lights were distinctive and reproducible as shown in Tables 5. The extractive value which is an important quality control parameter for herbal drugs was recorded as the water-extractive value was found to be $35\%^{w}/_{w}$, methanol-extractive value was $14\%^{w}/_{w}$ and ethanol-soluble extractive value was $16\%^{w}/_{w}$ for leaf. The stem had an extractive value for water to be $18\%^{w}/_{w}$, methanol of $8\%^{w}/_{w}$, ethanol of $9\%^{w}/_{w}$ as shown in Tables 6 and 7. These indicate that water is the most suitable solvent for extraction of constituents of this plant. The moisture content for the leaf and stem were $16\%^{w}/_{w}$ and $11\%^{w}/_{w}$ as shown in Tables 8 and 9. The acceptable limit for moisture content ranges between 8 – $14\%^{w}/_{w}$ which signified that the value of stem is within the recommended range for vegetable drug [9] and that of the leaf exceeded the limit. This is an indication that the powdered leaf cannot be stored for a long period of time, which can result in the breakdown of important constituents by hydrolytic reactions or enzymatic activities and may encourage the growth of yeast and fungi during storage. The Total ash value of *L. taraxacifolia* leaf is $17.7\%^{w}/_{w}$ and stem is $6.3\%^{w}/_{w}$ and this is within the limit for the stem as indicated in the European Pharmacopeia, (not exceeding 14%) [19], but that of the leaf exceeded the limit. Moreover, ash values are a useful indicator of the purity of any drug and give information relative to adulteration with inorganic matter. The acid insoluble ash value of *L. taraxacifolia* leaf and stem were $1.3\%^{w}/_{w}$ and $0.3\%^{w}/_{w}$ respectively and these are within the accepted limit of the European Pharmacopeia (not exceeding $2\%^{w}/_{w}$) [19]. The water soluble ash value for leaf and stem are $8.3\%^{w}/_{w}$ and $4.7\%^{w}/_{w}$. However, the African Pharmacopoeia [9] limit of ash value for crude vegetable drugs states that a lesser amount shows that there is less solubility of the ash in water while a higher value indicates a higher solubility of the ash in water. Hence, the determination of the water-soluble ash value of a particular crude drug helps in the detection of the amount of ash materials that are soluble in water.

Conclusion

The result obtained from the pharmacognostic and taxonomic studies provide information about the identity, quality and purity of *L. taraxacifolia.* Foliar description in the study can be helpful tool in resolving taxonomic discrepancies and identification. The data collectively might be used to provide information for further studies on *L. taraxacifolia* leaf and stem.

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