Standardization of *Aneilema lanceolatum* as a potential medicinal plant

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Abstract
Aneilema lanceolatum is a weed plant commonly found in farm lands and bushes around tropical African countries. The plant is utilized as livestock feeds and in traditional medicine in northern Nigeria for the treatment and management of pains, infectious diseases and as sexual stimulant. The present study was aimed at establishing microscopic, physicochemical and phytochemical constituents of the plant. The aerial parts of the plant was collected in May, 2021 from Kubanni, Sabon-gari local government area of Kaduna state and were identified and authenticated at the Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria-Nigeria and a voucher number ABU0533 was assigned. The physicochemical parameters, microscopic evaluation and qualitative and quantitative screening of the plant were carried out using standard procedures. The stomatal index of the upper epidermis (18.60±1.91) is lower than the lower epidermis (22.10±2.03) with palisade ratio of 10.07±0.90. The microscopic of the stem and leaf revealed presence of epidermis, xylem and phloem. Moisture content of the powdered plants was 1.23±0.05% with water and alcohol extractives values of 20.40±0.03% and 12.40±0.05% respectively. The total ash, water-soluble and acid-insoluble ash values are 5.52±0.02, 2.05±0.02% and 2.62±0.01% respectively. The microscopic characters revealed presence of wavy and polygonal shapes of epidermal cells with anomocytic type of stomata and unicellular non-glandular trichomes. The qualitative and quantitative phytochemical studies revealed presence of saponins (10.61%), alkaloids (0.98%), terpenoids (0.10%) and flavonoids (4.08%). The pharmacognostic parameters of the aerial parts of *A. lanceolatum* were established.
Keywords: Standardization; *Aneilema lancolatum*; Aerial part; Physicochemical; Microscopic; Phytochemical

Introduction

Aneilema lanceolatum belonging to the Commelinaceae family is a vivacious species of plant with a fusiform tuberous root system. The plant is a native of tropical Africa, and widely distributed in Nigeria, Mali, Burkina Faso, Ghana, Benin, Togo, Cameroon, Chad, Central African Republic and Sudan (Burkhill, 1985; Faden, 1991; Ikpe and Akpabio, 2013).

A. lanceolatum commonly called Day Flower in English and Kayar-galma in Hausa is used as feeds for livestock and various parts of the plants are in the treatment of erectile dysfunction and infertility, while the whole parts of the plant above the ground are used in the management of pain. The juice of the plant is used in treatment of sores. It is also used as anti-microbial agent and as an anti-tussive agent (Ikpe and Akabio, 2013).

This plant is utilized in northern Nigeria by traditional medical practitioners for the treatment and management of skin diseases, pain and as a sexual stimulant. However, little or no studies have been reported on the

pharmacognostic studies of the plant. Therefore, the present study is aimed at establishing the microscopic, physicochemical and phytochemical parameters of *A. lanceolatum.*

Materials and Methods

Plant material

The aerial parts of *A. lanceolatum* consisting of the leaves, stem, flowers and seeds of were collected during the dry season in the early morning hours from Kubanni, Sabon-gari local government area of Kaduna state in May, 2021 and were identified and authenticated at the Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria-Nigeria and a voucher number ABU0533 was assigned

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Plant Preparation

The leaves and stem of the plant were fixed in Formalin: Acetic acid: Alcohol (FAA) for microscopy, while the whole part of the plant was air dried at room temperature. The dried samples were pulverized and stored in a container for further use.

Physicochemical and Microscopic Studies

The moisture contents, ash values, water soluble and acid insoluble ash values of the pulverized plant material were evaluated according to the WHO, (2011) guidelines.

The stomatal number, stomatal index, palisade ratios, vein-islet and vein termination numbers were of the leaf was determined. Transverse section of the leaf and stem were made, cleared and observed under microscope.

Phytochemical Screening

The powdered sample 300g was weighed and transferred into a container and macerated for 72 hours using methanol. The filtrate was collected and evaporated to dryness.

Qualitative Phytochemical Screening

Test for phenolic compound (Ferric chloride test): 2m**l** of the crude solution of the extract was added to few drops of 10% ferric chloride solution (light yellow). A green blackish color indicates the presence tannins (Evans, 2009)

*Test for saponins (Frothing test***):** 3ml of the crude solution of the extract was mixed with 10ml of distilled water in a test tube. The test tube was stopped and shaken vigorously for about 5 minutes and observed for honey comb froth, which is indicative of the presence of saponins (Sofowora, 2008).

Test for Alkaloids:

*A. Mayer's reagent**:*** Drops of Mayer's reagent was added to a portion of the extract solution in a test tube and observed for yellowish precipitate indicative of the presence of alkaloids (Sofowora, 2008)

B. Dragendorff's **reagent:** 2ml of acidic solution in the second test tube was neutralized with 10% ammonia solution. Dragendorff's reagent was added and turbidity or precipitate was observed which was indicative of presence of alkaloids (Sofowora, 2008).

Test for steroids and triterpenes

A. Lieberman- Burchard's ***test:*** 2ml of crude extract was mixed with 1ml of acetic anhydride followed by the addition of 1ml of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration indicative of steroids (Sofowora, 2008)

B. Salkowski's test: 2ml of crude extract was mixed with concentrated sulphuric acid carefully so that the formed a lower layer and the interface were observed for a reddish-brown color indicative of steroid ring (Sofowora, 2008).

Test for flavonoids
A. Shinoda test): 2ml of crude extract was added metal magnesium and 6 drops of concentrated hydrochloric acid were added. The solution when red is indicative of flavonols and orange for flavones (Evans, 2009)

*B. Sodium hydroxide test***:** few drops of sodium hydroxide added to 5ml of the ectract and the reaction will be observed (Evans, 2009)

Quantitative Phytochemical Screening
These analyses were carried out as described by Mshelia *et al*., (2016).

Determination of Alkaloids: Total alkaloid content was determined by the alkaline precipitation gravimetric method as described by Harborne, (1973). 20 g of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4h at 28^{O}C, filtered and the filtrate collected was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of conc. aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was collected in a weighed filter paper, washed with 1% ammonia solution and dried in oven at 80^{O}C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

Determination of Flavonoids: This was determined according to the method of Harborne (1973). 5 g of the powdered plant was boiled in 50 ml of 2M HCl solution for 30 min under reflux. It was allowed to cool and then filtered through whatman No 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

Determination of Saponin: 20 g of the powdered plant material was transferred into a conical flask and 100 cm^3 of 20 % aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Determination of Terpenoids 2 g of the powdered plant was weighed and soaked in 50 ml of 95% ethanol for 24 h in a conical flask. The extract was filtered and the filtrate extracted with petroleum ether (60-800C) and concentrated to dryness. The dried ether extract was treated as total terpenoids.

Results



Figure 1: Picture of *Aneilema lanceolatum* in Ahmadu Bello University Zaria- Nigeria Main Campus Taken on 12th July, 2021

Microscopic Studies of A. lanceolatum

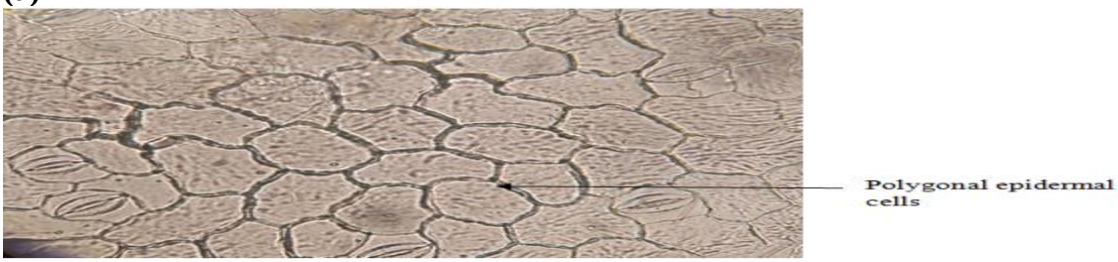
The microscopic studies indicate the presence of anormocytic type of stomata with various epidermal cells that are polygonal and wavy in shape. The stomata are elliptical in shape and have straight walls and the trichomes are unicellular and non-glandular. Transverse section of the leaf shows presence of epidermis, palisade tissue, xylem and phloem. The transverse section of the stem revealed the presence of epidermis, endodermis, xylem, phloem and pith.

Table 1: Microscopic Studies of *Aneilema lanceolatum*

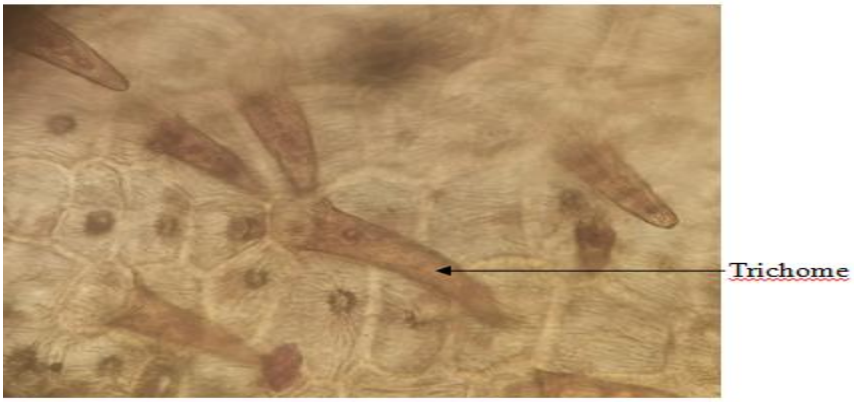
s/no	Characters	Features
1	Epidermal shape	Polygonal and Wavy
2	Anticlinical wall pattern	Straight
3	Stomata shape	Elliptic
4	Stomata type	Anomocytic
6	Trichome type	Unicellular and Non-glandular
7	Upper stomatal index (%)	18.60 ± 1.91
8	Lower stomatal index (%)	22.10 ±2.03
9	Palisade ratio (%)	10.07 ± 0.90



(a)

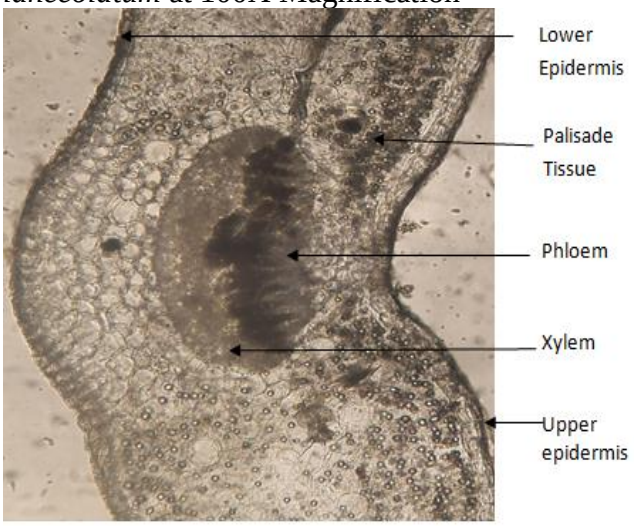


(b)

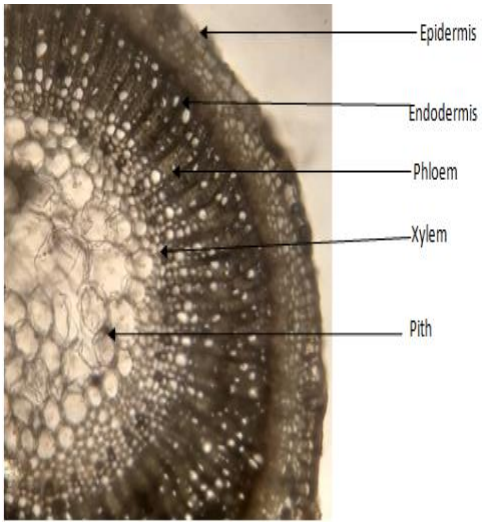


(c)

Figure II(a)-(c): Photomicrograph of Epidermal cells, Stomata and Trichomes of *Aneilema lanceolatum* at 100X Magnification



(a)



(b)

Figure 2:Tranverse Sections (TS) of *Aneilema lanceolatum* (a) Leaf (b) Stem at 100X Magnifications

Physicochemical Studies of Aneilema lanceolatum

The physicochemical studies of the powdered plant was carried out. The moisture content was found to be 1.23 ± 0.05 %, total ash 5.52 ± 0.02 %, acid-insoluble ash value 2.62 ± 0.01 %, water soluble ash value 2.05 ± 0.02 %, water extractive value 20.40 ± 0.03 % and methanol extractive value of 12.40 ± 0.05 %.

Table 2: Physicochemical Studies of *Aneilema Lanceolatum*

S/N	Physicochemical parameters	Value % (w/w)
1	Moisture content	1.23±0.05
2	Total ash	5.52±0.02
3	Acid-insoluble ash value	2.62±0.01
4	Water soluble ash value	2.05±0.02
5	Methanol extractive value	12.40±0.05
6	Water extractive value	20.40±0.03

Phytochemical Studies of *Aneilema lanceolatum*

The qualitative phytochemical studies of the methanol extracts revealed presence of saponins, flavonoids, terpenoids and alkaloids. The powdered plant material was found to be rich in saponins and flavonoids with alkaloids and terpenoids in low quantities.

Table 3: Phytochemical studies of *Aneilema lanceolatum*

Constituents	**Chemical test**	**Inference**
Saponins	Frothing	+
Flavonoids	Shinoda's	+
	NOH	+
Terpenoids	Salkowski's	+
	Liebermann – Burchard's	+
Alkaloids	Mayer's	+
	Dragendorff's	+
	Wagner's	+

Table 4: Quantitative Phytochemical Studies of *Aneilema lanceolatum* Powdered Plant

s/no	**Phytochemicals**	**Quantity (%)**
1	Alkaloids	0.98
2	Saponins	10.61
3	Terpenoids	0.10
4	Flavonoids	4.08

Discussion

Standardization and authentication of medicinal plant using microscopy, physicochemical and phytochemistry is an important tool in drug discovery. Microscopical features such as epidermal cells, type and arrangement, number and size of stomata, and shape of trichomes are important in the identification and classification of plants. The microscopy of the leaf of *A. lanceolatum* showed abundance of anomocytic stomata on the lower surface (22.10 ± 2.03) than the upper surface (18.60 ± 1.91), which is a distinctive features to many plants including plants of the commelianaceae family. However, species such as *A. umbrosum, A. aequinoctiale, C. diffusa* and *C. benghalensis* were reported to have hexacytic, paracytic and tetracytic types of stomata and less stomatal index when compared with *A. lanceolatum*. The polygonal shape of the epidermal cells with straight anticlinal walls is also a characteristic features of the *A. lanceolatum* family, but the presence of wavy epidermal cells on the upper surface of the plant is in contrast to the rectangular epidermal cells reported in *A. aequinoctiale*. Trichomes are also considered characteristic features of the commelianaceae. The presence of unicellular non-glandular trichomes in the lower surface of the leaf of *A. lanceolatum* is similar to the type of trichomes observed in *A. aequinoctiale*. The microscopical features of the transverse sections of both the leaf and stem classifies the plant as a dicot (Timothy *et al.*, 2000; Tehseen *et al*., 2010; Tripathe *et al*., 2012; Oladipo and Ayo-Ayinde, 2014; Ekeke and Agobua, 2018).

The moisture content of the powdered plant (1.23±0.05 % w/w) falls within the WHO, 2011 acceptable limit, indicating less likelihood of microbial attack. The total Ash (5.52±0.02 % w/w) also falls within the acceptable limit as stated in the European pharmacopoeia of a maximum of 14 % (European pharmacopoeia, 2007). The water-soluble ash is (2.05±0.02 %w/w) and the acid insoluble ash (2.62±0.01 % w/w) are parameters used in determining the purity and quality of the powdered plant. The methanol extractive value is (12.40±0.05 % w/w) and the water extractive value was found to be (20.40 % w/w).

The qualitative and quantitative phytochemical screening of the methanol extract of *A. lanceolatum* revealed the presence of alkaloids, saponins, flavonoids and terpenoids, similar to that of various researchers on the species of *Aneilema* including *A. aequinoctiale, A. beniniense, A. paludosum* and *A. unbrosum* (Ogbebor, and Edeogba 2008). The presence of these secondary metabolites can be said to be responsible for their medicinal properties. The presence of saponins and flavonoids in high amount in the plant (10.61 % and 4.08%) may be responsible for antimicrobial and anti-inflammatory activities of the plant as reported in literatures (Kubmarawa, *et al*., 2007; Rathee *et al*., 2008; Boorgi *et al*., 2008). Although, alkaloids (0.98 %) and terpenoids (0.10 %) were found in low quantity, they may also be responsible for the medicinal properties of the plant as Taejoo *et al*., (2020) reported that alkaloids and terpenoids have anti-inflammatory activities.

Conclusion

The present study was able to establish the microscopic, physicochemical and phytochemical parameters of *A. lanceolatum*.

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Conflict of Interest

The authors have declared no competing interest in the present research.

Ethical approval

This article does not contain any studies on animal or human subject.

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