

## Research Article

# Pharmacognostic Evaluation of the Leaves of *Secamone afzelii* (Schult) K Schum (Asclepiadaceae)

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### Abstract

**Purpose:** Establishment of the pharmacognostic profile of the leaves of *Secamone afzelii* (Schult) K. Schum, known for its antimicrobial, antioxidant and free radical scavenging properties, will assist in standardization, quality assurance, purity and sample identification.

**Methods:** Evaluation of the fresh, powdered and anatomical sections of the leaves were carried out to determine the macromorphological, micromorphological, chemomicroscopic, numerical (palisade ratio, stomata number, stomata index, vein-islet number and veinlet termination number, moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol and water soluble extractive values) and phytochemical profiles.

**Results:** Macro- and microscopical studies indicated the presence of pinnately compound leaf, an entire margin with lanceolate shape, acute base, acuminate apex and reticulate venation. Epidermal walls were straight with numerous calcium oxalate crystals. Stomata arrangement was paracytic, with numerous unicellular uniseriate covering trichomes on both surfaces. Chemomicroscopic characters present included lignin, cellulose, mucilage, suberin and cutin, while phytochemical evaluation revealed the presence of alkaloids, tannins, cardiac glycosides and saponins. The findings also included numerical and quantitative leaf microscopy.

**Conclusion:** These findings could serve as a basis for proper identification, collection and investigation of *Secamone afzelii*.

**Keywords:** *Secamone afzelii*, Pharmacognostic evaluation, Sample identification.

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## INTRODUCTION

The family Asclepiadaceae is of the order Rubiales, formerly called Gentianales. The family consists of about 130 genera and 2,000 species distributed all over the world. Some of them are tropical and subtropical shrubs, often twining, or perennial herbs. The latex cells usually contain a latex rich in triterpenes. Other constituents include: cyanogenetic glycosides, saponins, tannins and cyclitols [1].

*Secamone afzelii*, known in three Nigerian languages as arilu, ailu or alu in Yoruba, utunta (Ibo) and Ewuonkwonegie (Bini) [2] is a familiar creeping woody climber found on fences and on trees and grows to a very long length of about 2-3 cm. It is a creeping woody climber with pinnately compound leaves. It is often seen as a nuisance to other plants or crops because of its domineering spread wherever it grows.

*Secamone afzelii* is used in traditional medicine for stomach problems, diabetes, colic, dysentery and also for kidney problems. The whole plant boiled with rice is used as purgative for children. The decoction of the entire plant is prescribed for cough, catarrhal conditions and as galactagogue. For the treatment of gonorrhoea, the whole plant is crushed with fresh palm nuts and oil [2]. Its synonym, *Secamone myrtifolia* is claimed to be used for the management of cough, sore throat, backache, purge and galactagogue [3]. The root is said to be poisonous but is used, more the less, by the Zulu medicine man as a remedy for spinal disease [4]. The antioxidant activity of a methanolic extract of *Secamone afzelii* stems was tested using the Diphenyl-1-picrylhydrazyl (DPPH) assay and the active compound was identified as  $\alpha$ -tocopherol. High Performance Liquid Chromatography (HPLC) determination showed that 0.12% w (sol.)/w  $\alpha$ -tocopherol was present in the plant. The total extract also showed effective free radical scavenging activity in the assay for non-enzymatic lipid peroxidation in liposomes with an  $IC_{50}$  value of 90  $\mu$ g (sol.)/ml, with  $\alpha$ -tocopherol isolated

from the plant having an  $IC_{50}$  of 15  $\mu$ g (sol.)/ml in the same system, thus demonstrating the presence of other antioxidants [5].

The extracts of *Secamone afzelii* have also been shown to have anti-inflammatory and antioxidant properties due to flavonoids, triterpenoids, diterpenoids and caffeic acid derivatives [6]. The plant also possesses antibacterial and antifungal activities.

Some drugs of plant origin in conventional medical practice are not pure compounds but direct extracts or plant materials that have been suitably prepared and standardized [7]. The parameters obtained from the pharmacognostic evaluation of medicinal plants have been used in their identification and differentiation of the various species. The Apocynaceae family is closely allied to the Asclepiadaceae family [1]. Establishment of the pharmacognostic profile of the leaves of *Secamone afzelii* will assist in standardization, which can guarantee quality, purity and identification of samples.

## EXPERIMENTAL

### Collection, identification and preparation of plant material

Fresh leaves of *Secamone afzelii* were collected in Ugbowo area of Benin City, Nigeria. Identification and confirmation were done by Usang Felix of Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria, where a voucher specimen was deposited with the number, FIH 107158. The fresh leaves were air-dried, powdered in an electric mill (Moulinex), and then stored in a dry and well-sealed bottle.

### Macroscopy

The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste [1,8].

## Microscopy

The outer epidermal membranous layer (in fragments) were cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence as well as absence of the epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution) was observed. The transverse sections of the fresh leaves through the lamina and the midrib as well as a small quantity of the powdered leaves were also cleared, mounted and observed [9].

## Chemomicroscopic examination

Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques [1].

## Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins, glycosides and alkaloids using standard techniques [1,10-12].

## Quantitative investigation

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein-islet number and veinlet termination number were carried out on epidermal strips. Other parameters determined for the powdered leaves were moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol (90 % ethanol) and water soluble extractive values [13].

## Statistical analysis

The data obtained were expressed as mean  $\pm$  SEM (standard error of mean), and n represents the number of replicates in an experiment.

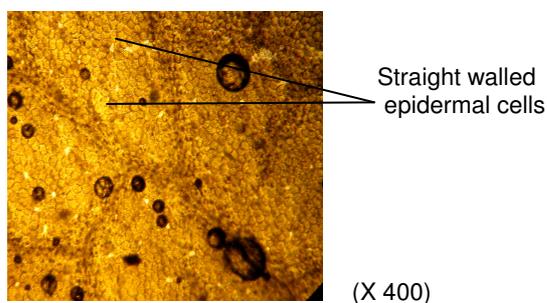
## RESULTS

### Macroscopic description of the leaves of *S. afzelii*

The leaf was dark green in the upper surface and light green in the lower surface. It had a stipule and a short petiole. Pulvinus was absent. It was pinnately compound, with a lanceolate shape. The margin was entire, apex was acuminate, base acute and venation was reticulate. Average leaf size was  $4.4 \pm 0.8$  cm (length) and  $2.4 \pm 0.7$  cm (breadth). The fresh leaf exudes a white gummy substance when cut, odourless and slightly acrid in taste.

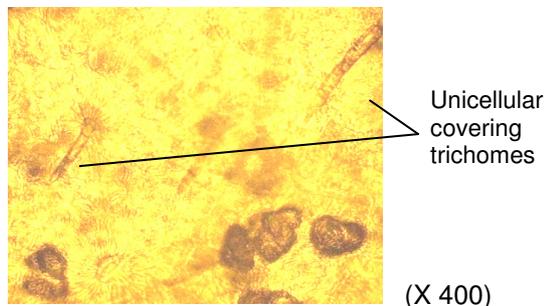
### Microscopic description of the leaves of *S. afzelii*

Micromorphological features revealed that anticlinal walls were thick and straight. Stomata were present in both lower and upper epidermi. The stoma was surrounded by two epidermal cells whose axis was parallel to the axis of the guard cells (Paracytic arrangement) (Fig 1). There were numerous uniseriate covering trichomes present on both surfaces, mostly unicellular (Fig 2) and multicellular (Fig 3).

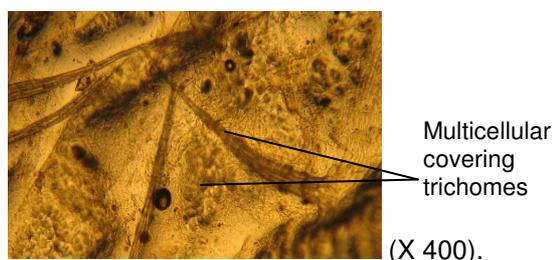


**Fig 1:** Photomicrograph of *S. afzelii* with straight walled epidermal cells and paracytic stomata arrangement

A transverse section of the leaf across the mid-rib showed an upper and lower epidermis consisting of cells of similar sizes (Fig 4). There were uniseriate covering trichomes on both surfaces. It had a bifacial



**Fig 2:** Photomicrograph of *S. afzelii* with unicellular covering trichomes.



**Fig 3:** Photomicrograph of *S. afzelii* with multicellular covering trichomes



**Fig 4:** Photomicrograph of the transverse section of the leaf of *S. afzelii* with dorsiventral arrangement

surface i.e. there were two different surfaces with different identities, hence dorsiventral. The mesophyll consisted of a palisade and the spongy mesophyll, embedding a crystal sheath. There were crystal clusters of calcium oxalate in the spongy mesophyll. The mid-rib bundle was surrounded by a zone of collenchyma on both surfaces. The phloem vessels embedded the xylem vessels.

Chemomicroscopic examination of the leaves revealed the presence of lignin, starch,

mucilage, calcium oxalate crystals and cellulose.

### Phytochemical analysis of the leaves of *S. afzelii*

Phytochemical evaluation (Table 1) revealed the presence of alkaloids, tannins, cardiac glycosides and saponins.

**Table 1:** Preliminary phytochemical screening of *Secamone afzelii* leaves

Secondary metabolites	<i>Secamone afzelii</i>
<b>Alkaloids</b>	
Dragendorff's reagent	+
Wagner's reagent	+
Mayer's reagent	+
1% picric acid reagent	+
<b>Anthracene derivatives</b>	
Combined anthraquinones	-
Free anthraquinones	-
<b>Tannins</b>	
Phenazone test	+
Iron complex test	+
Formaldehyde test	+
Modified iron complex test	+
<b>Saponin glycosides</b>	
Frothing test	+
Haemolysis test	+
<b>Cardiac glycosides</b>	
Keller killiani test	+
Kedde test	+
<b>Cyanogenetic glycosides</b>	
Sodium picrate test paper	-

**Key:** - = absent; + = present

### Quantitative microscopy of the leaves of *S. afzelii*

The results of the quantitative microscopy of the leaves of *S. afzelii* are presented in Table 2. The average number of palisade cells

beneath each epidermal cell in 5 determinations was  $4.70 \pm 0.27$ . The stomatal number was  $13.60 \pm 0.23$  (upper surface) and  $12.95 \pm 0.21$  (lower surface), while stomatal index was  $32.05 \pm 0.32$  (upper surface) and  $31.15 \pm 0.40$  (lower surface). The vein-islet number was  $1.20 \pm 0.12$  and the veinlet termination number  $2.15 \pm 0.15$ ).

**Table 2:** Quantitative leaf microscopy of *Secamone afzelii*

Parameter	Range	Mean $\pm$ SEM
Palisade ratio*	4.00 – 5.50	$4.70 \pm 0.27$
Stomata number Upper surface†	12.00 – 15.00	$13.60 \pm 0.23$
Stomata number Lower surface†	12.00 – 15.00	$12.95 \pm 0.21$
Stomata index Upper surface†	29.26 – 34.15	$32.05 \pm 0.32$
Stomata index Lower surface†	29.27 – 34.15	$31.15 \pm 0.40$
Vein islet number*	1.00 – 1.25	$1.20 \pm 0.12$
Veinlet termination number*	1.75 – 2.50	$2.15 \pm 0.15$

\* $n = 5$ ; † $n = 20$

### Numerical data of the leaves of *S. afzelii*

The moisture content of *S. afzelii* which falls within the Pharmacopoeial limit, the ash values as well as the amount of constituents which are extractable by methanol and water under specified conditions are presented in Table 3.

**Table 3:** Numerical data of leaves of *Secamone afzelii*

Parameter	Mean $\pm$ SEM (%w/w)
Moisture content*	$9.00 \pm 0.23$
Total ash*	$9.08 \pm 0.26$
Acid-insoluble ash†	$6.10 \pm 0.20$
Water-soluble ash†	$2.40 \pm 0.13$
Alcohol-soluble extractive*	$2.74 \pm 0.01$
Water-soluble extractive*	$4.27 \pm 0.02$

\* $n = 30$ ; † $n = 15$

## DISCUSSION

*Secamone afzelii* is a plant that has been confused with other species due to their relative similarities. Also, species of the Apocynaceae family, which is closely related to the Asclepiadaceae family, have been confused with *Secamone afzelii*. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant.

The macro- and micro-morphological features of the leaf described distinguishes it from other members of the genera. The exudes from the fresh leaves when cut, which is white in colour and gummy, has a unique and characteristic taste. The straight-walled epidermal cells, paracytic stomata arrangement, presence of unicellular and multicellular covering trichomes and dorsiventral arrangement of the transverse section of the leaf are diagnostic.

The Pharmacological activities of a given plant are associated with the type and nature of secondary plant metabolites present. The need for phytochemical screening has become imperative, since many plants accumulate biologically active chemicals in their tissues. Phytochemical evaluation of *S. afzelii* revealed the presence of alkaloids, tannins, cardiac glycosides and saponins. Typical alkaloids often have marked pharmacological effects when administered to man and other animals, thus their presence is of particular interest [1]. The pharmacological effectiveness of glycosides is dependent on the aglycones, but the sugars render the compounds more soluble and increase the power of fixation of the glycosides. Flavonoids and Caffeic acid, are polyphenols present in *S. afzelii* and have been reported to be responsible for its anti-inflammatory and antioxidant activities [6].

Chemomicroscopy, numerical data and quantitative leaf microscopy are parameters that are unique to the plant and are required for correct identification. The results of

quantitative microscopy of the leaves of *S. afzelii* can be used to distinguish between *S. afzelii* and closely related species not easily characterized by general microscopy. The stomatal number  $13.60 \pm 0.23$  (upper surface) and  $12.95 \pm 0.21$  (lower surface), though may be useless for distinguishing *S. afzelii* from closely related species, compared to the stomatal index, the ratio between the number of stomata on the two surfaces is of diagnostic importance. The average number of palisade cells beneath each epidermal cell (Palisade ratio), the vein-islet number and veinlet termination number determinations can also be used to distinguish *S. afzelii* from closely related plants.

The numerical data were determined to assist in establishing the identity of crude drugs. Not only is the purchase of drugs which contain excess water uneconomical, but also in conjunction with suitable temperature, moisture will lead to the activation of enzymes and given suitable conditions, to the proliferation of living organisms [1]. As most vegetable drugs contain all the essential food requirements for moulds, insects and mites, deterioration can be very rapid once infestation has taken place. The moisture content of *S. afzelii* obtained in the determination of quantitative standards met the pharmacopoeial limits of water content for vegetable drugs, which is between 8–14 % [9]. From the foregoing, the plant material can be conveniently stored at room temperature without the deterioration of its active constituents.

When vegetable drugs are incinerated, they leave ash, which varies within fairly wide limits in many drugs and is therefore of little value for purposes of evaluation. In other cases, the total ash value is of importance and indicates to some extent the amount of care taken in the preparation of the drug [1]. The total ash usually consists of carbonates, phosphates, silicates and silica. In the determination of total ash values, the carbon must be removed at as low a temperature ( $450^{\circ}\text{C}$ ) as possible because alkali chlorides,

which may be volatile at high temperatures, would otherwise be lost. When the total ash was treated with dilute hydrochloric acid, the percentage of acid-insoluble ash was determined. This usually consists mainly of silica, as most of the natural ash is soluble leaving the silica as acid-insoluble ash which represents most of the ash from the contaminating soil [1,8,14]. A high acid-insoluble ash in drugs such as senna, cloves, liquorice, valerian and tragacanth indicates contamination with earthly material. Senna leaf, which may be used directly as the powdered drug, is required to have a low acid-insoluble ash (2.5 %) while hyoscyamus, which unavoidably attracts grit onto its sticky trichomes is allowed a higher value (12.0 %). *S. afzelii* with numerous trichomes and acid-insoluble ash values of  $6.10 \pm 0.20$  % may therefore be used directly as powdered drug.

The water-soluble ash is used to detect the presence of foreign material(s) substituted for the genuine drug. Such substitution results in the lowering of the water soluble ash value. In many cases, as in ginger, a minimum percentage of water-soluble ash is demanded, this being designed to detect the presence of foreign material e. g. exhausted ginger. Water-soluble ash values for *S. afzelii* below 2.40 could mean adulteration with foreign material(s).

The parameters obtained in the pharmacognostic investigations of *Mitracarpus scaber* Zucc (Rubiaceae) [15], *Dissotis rotundifolia* Triana (Melastomataceae) [16], leaves and stems of *Viburnum erubescens* Wall (Caprifoliaceae) [17], all known to possess similar antimicrobial properties with *Secamone afzelii*, now serve as the basis for the proper identification, collection and investigations of these medicinal plants. These findings from *S. afzelii* should be suitable for inclusion in the pharmacopoeia of medicinal plants.

## CONCLUSION

*Secamone afzelii* is currently being used in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, some pharmacognostic parameters and standards must be established.

These parameters, which are being reported for the first time, could be useful in the preparation of the herbal section of the proposed Nigerian Pharmacopoeia. Any crude drug which is claimed to be *Secamone afzelii* but whose characters significantly deviate from the accepted standards above would then be rejected as being contaminated, adulterated or substituted.

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