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RESEARCH ARTICLE

Evaluation of pharmacognostic and phytochemical profile of *Spigelia anthelmia* linn leaves

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Abstract

The medicinal credence of Spigelia anthelmia suggests the plant as an important herbal remedy in the treatment of asthma and helminthiasis. A valid pharmacognostic assessment is done to give a standard and quality identification of the medicinal plant. A fresh leaf and a powdered form were evaluated. The foliar micro-morphology (qualitative and quantitative), microscopy, chemo-microscopy, and phytochemical composition were assessed using standard methods. The result indicates that for micromorphology, the cells are rectangular to polygonal with very large cells for both abaxial and adaxial epidermis. It has a straight anticlinal wall. stomata type is anomocytic for both the epidermis which means the leaf is amphistomatic. Trichomes present on the abaxial and adaxial are uniseriate and non-glandular, while they are absent on the adaxial epidermis. The stomata index for abaxial is (10.42%), while for adaxial is (5.88%). The cell length for abaxial (84.55 µm) and adaxial is (81.92 µm), while the cell width for abaxial is (58.15 µm) and for adaxial is (57.85 µm). The cell density is higher on the abaxial with (43.0 µm) and lesser on the adaxial with (32.0 µm). Mean Stomata number on the lower epidermis is (5), while on the adaxial is (2). The macroscopic features identified shows that the leaves are opposite, simple and entire, having a cylindrical shape with horny texture, the bark is coarse and the surface is smooth with a spot of brown nodules. The trunk is simple, without thorns but hairy. The leaf epidermis is straight with numerous starch grains and the crystals of calcium oxalate are present on the abaxial epidermis and absent on the adaxial. It has a granular fracture surface; its color is greyish green with a faint characteristic odor. The chemo-microscopic analysis shows that Starch, calcium carbonate crystals and cellulose are present while lignin, fats, calcium oxalate crystals, and mucilage were absent. The phytochemical screening shows that flavonoid, alkaloid, saponin, phenols, and tannin are present except, cardiac glycoside and anthraquinone. The foliar micro-morphology, the macroscopy and phytochemical composition of S. anthelmia have provided proper information for its identification and authentication which can enable it to be included in the official pharmacopeia of Nigerian medicinal plants.

Keywords: Micromorphology, phytochemical, Spigelia anthelmia, chemo-microscopy, pharmacognostic

Introduction

Medicinal plants are quite safe, less expensive and have no or minimal ill effects, unlike conventional drugs. They are used effectively in the treatment of diverse medical complications, but the absence of proper authentication and standardization reduces its acceptability. Due to this, they are susceptible to adulteration and species can be substituted which makes people doubt their potency. Therefore, it is imperative to create a proper identification of herbal plants. The wrong usage of medicinal plants proceeds wrong identification. The most common error is one common vernacular name is given to two or more entirely different species (Dineshkumar 2007). The importance of leaf epidermal and anatomical profile of a plant is usually done to create a taxonomic database that has been exclusively reported by several authors (Ayodele & Olowokudejo 2006); (Kadiri & Ayodele 2003); (Metcalfe & Chalk 1979). Many epidermal cells, trichomes, cell types, stomata, the arrangement of stomata, cell size, type, and distribution are very important botanical characters that can be used in identifying species boundary (Shokefun *et al.* 2014b; Stace 1984). Also, the taxonomic importance is the pollen features which have been utilized over the years in resolving phylogenetic relationship problems. Also, the pharmacognostic data has given valuable and detailed information of the species, generic, subfamily, tribe for different groups of herbal plants (Stuessy 2009). *Spigelia anthemia* is an annual weed, also known as pinkroot, that belongs to the family of Loganiaceae (Nathaniel

& Addison 2015). It is about 60 cm high with a scarcely branched stem and short-stalked, feather-like lobed leaves sets in whorls of four. The spikes with small purple or bright red flowers come out of the whorl. The fruit is a two-lobed capsule with warty seeds (Valkenburg 2003). The leaves are popularly used in the treatment of asthma and helminthiasis in several parts of Africa (Akah et al. 2003). Anthelmintic (Ademola et al. 2007) and cardioprotective activities have been reported. It has been revealed that the leaves contain anti-inflammatory and broncho-spasmolytic properties (Ezike et al. 2008). The plant is effective in the treatment of pericarditis and any other cardiovascular diseases of the heart. They are widely used for chronic catarrh, difficult breathing and deworming. It is given to angina pectoris patients accompanied by constricting pains that spread into the chest, to one or both the arms and up to the throat. *S. anthelmia* is an important remedy in the treatment of toothache, headache, common cold, heart disease, migraine, antibacterial, chronic mouth odor and pain, chest pain and fever. They fight against tapeworm and roundworm in the body (Okwu 2001). Pharmacognostic parameters like qualitative microscopy, quantitative leaf microscopy, microscopy, chemo-microscopy, and phytochemical studies are few of the basic parameters for standardization of herbal plants. Hence there is a need to provide a botanical and taxonomic standard which can guarantee the quality and prepare a monograph which would help in the proper identification of the plant.

Materials and Methods

Plant collection

The leaf was harvested within the premises of the Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria and was identified by a taxonomist at the taxonomy section of the institute. After collection, the left sample was air dried, powdered and stored in an airtight jar until required for use (Fig. 1).

Standardization parameters

Microscopic evaluation

1) Epidermal section (ES) preparations: The leaf sample was cut into sizeable portions and soaked in Nitric acid (HNO_3) in well covered Petri dishes for some hours depending on the plants. The lower and upper epidermis was soaked separately in the Petri-dishes. This is to macerate the mesophyll. Tissue disintegration was indicated by bubbles and the epidermal layer removed with forceps were transferred into a clean Petri dishes containing distilled water, it was then transferred again into another Petri dish containing 1ml of ethanol for some minutes, this enables hardening of the tissue cell. Afterward, safranin o was used in staining the tissues and distilled water was used to rinse again to remove the red stain. The epidermal layer was then placed on a microscopic slide, thereafter, a drop of glycerol was added on the epidermal layer that was on the slide, it was covered with coverslips and a sealant was used to ring the edges to prevent dehydration. Two slides were prepared for each epidermis (Abaxial and Adaxial) of the leaf sample. Methods followed those of (Radford et al. 1974; Khatijah & Zaharina 1998; Adedeji 2004; Metcalfe & Chalk 2004; Evans et al. 2005; Brain & Turner 1975).

Figure 1. *Leea guineensis* leaves.

The stomata index (SI) using the formula described by (Salisbury 1927).

$I=S/E+S \times 100$

Where I-Stomata Index

S-No of Stomata per unit area

E-No of epidermal cells in the same unit area

2) Transverse section (TS) preparations: Anatomical sections of the leaf were prepared by using a sledge micrometer on the specimen. The leaf blade was then put inside a container and safranin was used in staining the epidermis for some minutes. The outer cell layer was then cleaned in distilled water and later ethanol. Thereafter, it was stained again and rinsed with absolute ethanol. It was then placed into a container containing 1ml of Ethanol/Xylene until the epidermis is very clear. The sections were cleared with chloral hydrate solution and mounted on a slide with dilute glycerin (Evans et al. 2005; Brain & Turner 1975).

3) Light microscopy: The leaf epidermal preparations for both epidermal and transverse sections were properly labeled and examined using a light microscope with (x40, x10, x4) magnification. Photo-micrographic images of each sample were viewed with a digital camera mounted on Olympus photomicroscope. The micrometer eyepiece was viewed to make the necessary measurements and observations. For each micro-morphological character, measurements were randomly recorded for each of the microscopic slides. The mean and standard error values reported for each of the microscopic and quantitative parameters on the basis of their occurrence were calculated. Salisbury 1927 method was used to calculate the stomata index, and the formula used was written below.

$I=S/E+S \times 100$

Where I-Stomata Index

S-No of Stomata per unit area

E-No of epidermal cells in the same unit area

Macroscopic and organoleptic characters

The macroscopic and organoleptic characters of the leaf can be evaluated by using the sense organs. They provide a quick and easy way to establish a plants identity and in order to ensure proper standardization of a particular medicinal plant.

Organoleptic features such as shape, odor, size, taste, color, and fracture of stem bark, leaf structure like margin, apex, base surface etc. All these were described according to standard methods by (Evans et al. 2005; Brain & Turner, 1975). Images were captured using a digital camera.

Phytochemical estimation

Phytochemical screening was performed on the powdered leaf sample using standard biochemical procedures as described by (Harbone 1998). The preliminary evaluation was done to detect the secondary metabolites present such as flavonoids, phenols, alkaloids, saponin, phenols, anthraquinone, tannin, and cardiac glycoside.

Chemo microscopic evaluation

The powdered leave sample was placed on the microscopic slides and observed under a compound microscope for the detection of chemical substances like; cellulose, tannins, fat and oils, starch, lignin, calcium oxalate and calcium carbonate (Evans et al. 2005; Trease & Evans 1996).

Lignin test: The powdered plant was mounted in phloroglucinol followed by concentrated hydrochloric acid; a red coloration shows that lignin is present.

Cellulose test: The powdered whole plant was mounted in N/50 iodine solution followed by 66% sulphuric acid. A blue coloration indicates the presence of cellulose.

Starch test: The powdered plant was mounted in N/50 iodine. Bluish coloration indicates the presence of starch.

Calcium oxalate crystals test: Chloral hydrate solution was used in clearing the powdered plant sample. The crystals of calcium oxalate have definite sizes and shapes and they are very bright. On addition of 80% hydrochloric acid and viewing under a microscope, the disappearance of calcium oxalate crystals confirms their presence.

Calcium carbonate test: 2ml of the chlorate hydrate solution containing the powdered plant sample was taken and placed on the microscopic slide. 1-2 drops of the acetic acid solution were added on the sample. Evolution of gas shows that calcium carbonate is present.

Test for oils: The powdered plant was mounted in Sudan IV reagent. Pinkish coloration is an indication of the presence of oils.

Mucilage test: The powdered leaf sample was placed on the slide and Ruthenium red (a drop) was added, a pink coloration shows that mucilage is present.

Statistical analysis: The statistical analyses were carried out by analyses of variance ANOVA using SPSS version 20. The significant differences among mean values were calculated by Duncan's multiple range tests at $p<0.05$ and results were presented as mean ± standard deviation (SD).

Results and Discussion

Micro-morphology

The result indicated in Tab. 1. shows that the qualitative evaluation of the epidermal section of *Spigelia anthelmia* leaves on the microscopic slide using a microscope shows that a very clear and large cell, straight and rectangular to polygonal cell shape on both abaxial and adaxial epidermis is present. Presence of Anomocytic stomata, the epidermal walls have straight anticlinal walls. The abaxial epidermis as a non-glandular trichome, while trichome is absent on the adaxial epidermis. There is the presence of crystals on the abaxial epidermis and absent on the adaxial.

The result presented in Tab. 2., is of the quantitative leaf micromorphology of *Spigelia anthelmia*. The result indicates that cell length for abaxial is (84.55 µm), while on the adaxial it is (81.92 µm). The cell width for both the epidermis is comparably the same. The result of the cell density reflects that the epidermal cells on the abaxial (43.0 µm) are more than the adaxial (32.0 µm). The mean number of stomata is 5, while on the adaxial is 2. The stomata index for abaxial and adaxial are (10.42% and 5.88%) respectively. The palisade ratio is 28.5.

Macroscopic description

The macroscopic properties which cover organoleptic features via botanical description have provided a simple and quick means for proper identification of the plant. The macroscopic features and organoleptic characters show the features and characters shown in Tab. 3.

Phytochemical screening

The result of the qualitative preliminary assessment for phytochemicals of the crude powder of *Spigelia anthelmia* is expressed in Tab. 4. The result shows that phytochemicals are present in the leaves of *S. anthelmia.* The phytochemicals present include alkaloids, saponins, flavonoids, phenolics, and tannin, while anthraquinone and cardiac glycosides are absent.

Chemo-microscopic evaluation

Chemo microscopic evaluation of the plant species using the powdered samples, as described in Tab. 5., reveals that starch, cellulose, Starch, Crystals, and calcium carbonate are present, while fats, lignin, calcium oxalate, and mucilage are absent.

Table 1. Qualitative leaf micro-morphological characteristics of *Spigelia anthelmia*.

Characters	*Spigelia anthelmia*	
	Abaxial	Adaxial
Cell Shape Size	Rectangular to polygonal Large cells	Rectangular to polygonal Large cells
Anticlinal Walls	Straight	Straight
Stomata type Frequency	Anomocytic Rare	Anomocytic Rare
Trichome	Nonglandular	Absent
Micro-Crystal	Present	Absent

Table 2. Quantitative micro-morphological characteristics of *Spigelia anthelmia* leaves.

Characters	*Spigelia anthelmia*	
	Abaxial	Adaxial
Stomata index	10.42%	5.88%
Cell length (µm)	84.55	81.92
Cell Width (µm)	58.15	57.85
Cell density (µm)	43.0	32.0
Stomata length (µm)	-	-
Stomata Width (µm)	-	-
No of Stomata	5	2
Palisade Ratio	28.5	

Table 3. Macroscopic features of *Spigelia anthelmia* leaves.

Character	Observation
Colour	Greyish green
Odor	Faint
Surface	Smooth with a spot of brown nodules
Trunk	Simple without thorns but hairy
Fracture	Transverse
Shape	Cylindrical
Internodes	Short
Bark	Coarse
Shrinkage	Channeled and branched
Texture	Horny
Fracture Surface	Granular
Leaves	Opposite simple and entire
Taste	Bitter

Table 4. Phytochemical screening of *Spigelia anthelmia* leaves.

Phytochemicals	*Spigelia anthelmia*
Alkaloids	+
Saponin	+
Flavonoid	+
Tannin	+
Phenolics	-
Anthraquinone	-
Cardiac glycosides	-

Table 5. Result of Chemo microscopic evaluation.

Parameter	U1
Lignin	-
Starch	+
Fats	-
Calcium Oxalate Crystals	-
Calcium Carbonate	+
Mucilage	-
Crystals	+
Cellulose	+

These micro-chemicals have shown various pharmacological actions and thus, may be responsible for the activities associated with the plant.

Discussion

Medicinal plants are good sources of drug discovery. Standardization and correct authentication of the plant is very important to sustain purity and quality. The standardization parameters explored in this study include; microscopic, macroscopic, phytochemicals and chemo-microscopic of the plant. Evaluating all these parameters will ensure and help in maintaining quality and standard plant. It will prevent the plant from adulteration and wrong identification (Chanda 2014). The microscopic description of the qualitative features and quantitative parameters measured of the epidermal cells of *S. anthelmia* are summarized in Tab. 1. and 2. The transverse section (Fig. 2A) of the plant's leaf blade shows protoxylem, parenchyma, and metaxylem. The epidermal cells are very large and straight, having rectangular to polygonal on both surfaces of the plant species (Fig. 2C & 2D). It has a straight anticlinal wall and the stomata shape are both Anomocytic both on the lower (abaxial) and upper (adaxial) epidermis (Fig. 2C & 2D). *S. anthelmia* is amphistomatic because stomata are present on the lower and upper epidermis of the plant. Considering the abaxial side, the stomata present is higher than the adaxial side of the leaves, taking into cognizance the result obtained for stomata index which is (10.42% and 5.88%) for abaxial and adaxial respectively. The stomata present are a closed one. The non-glandular trichomes observed on the abaxial epidermis of the plant were long, hooked and has a short basal cell and a larger bent terminal cell (Fig. 2B). Meanwhile, they are absent on the adaxial epidermis. This is in consonance with the result reported by (Metcalfe & Chalk 2004). The quantitative epidermal studies show that the mean epidermal cells in *Spigelia anthelmia* are very large (Fig. 2C).

The average number of cells ranged from 32 to 43 on lower to upper epidermis. The stomata length and width cannot be calculated because they have a closed stoma. The cell length for abaxial and adaxial epidermis are (84.55 µm and 81.92 µm) respectively. While the cell width for both abaxial and adaxial are (58.15 µm and 57.85 µm). The number of available stomata on the lower epidermis is 5; while on the upper epidermis are 2. The palisade ratio is 28.5. The palisade ratio is also calculated for the number of cells inside each epidermis in ten places divided by ten. The characteristics diagnostic feature of *S. anthelmia* leaves showed to be greyish green in color. The leave was opposite, simple and entire with a faint characteristic odor and a bitter taste. The internodes are short and the trunk is simple without thorns but hairy. The shape is cylindrical. The fracture surface is granular and transverse. The surface is smooth with a spot of brown nodules. In shrinkage, they are always channeled and branched. Chemo-microscopically, the powdered leaves gave calcium carbonate crystals and starch grains, also the starch granules are of different sizes. The pharmacognostic parameters reported in this study shows that starch, calcium carbonate crystals, and cellulose are present, while lignin, fats, and mucilage are found absent. This study is comparably similar to that reported by Elufioye & Olaifa, 2015, but on the contrary to this research work, he reports that calcium oxalate crystal is present, instead of calcium carbonate detected in this study. In his report, all the

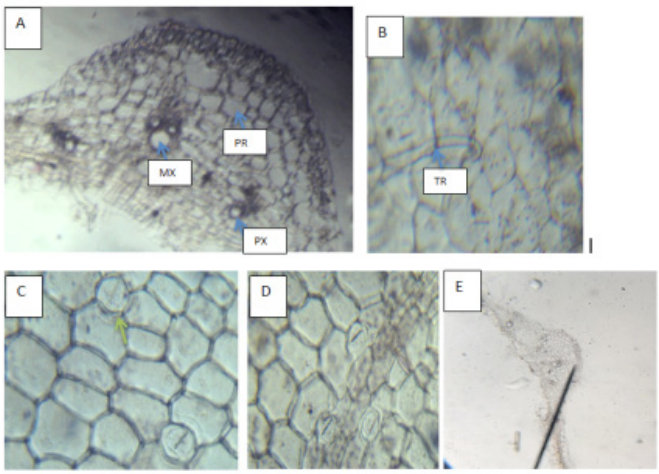

Figure 2. Leaf micrograph (LM) of Spigelia anthelmia leaves (A): Anatomical features of the leaf blade showing Protoxylem (px), Parenchyma (Pr) and Metaxylem (max) x40 mag; (B): Leaf clearing showing epidermal cells and non-glandular trichome (tr) on the abaxial epidermis; (C): Leaf clearing showing epidermal cells and Anomocytic type of stomata on the adaxial epidermis; (D): Leaf clearing showing the epidermal cells and secretory cavities (Anomocytic stomata) of the abaxial surface; (E): Anatomical features of the leaf blade x10 mag.

parameters are present. The qualitative evaluation of the crude powdered sample shows that some secondary metabolites in *S. anthelmia* leaves are present. The phytochemicals present include alkaloids, saponins, flavonoids, phenolics, and tannin, while anthraquinone and cardiac glycosides are absent. Tannins serve as an antidote and have a wound healing property; it is a good antioxidant (Norton 2000). Alkaloids possess antimicrobial, anti-inflammatory and anti-fungal property (Ghosal et al. 1996). Flavonoid also has an antioxidant property which may serve as protection against free radicals in the body (Kumar & Pandey 2013). Phenolics possess anti-aging properties (Brohem et al. 2011). Saponins are reported for hypoglycemic activity and antioxidant activity (Han et al. 2008; Chan et al. 2014). This preliminary phytochemical result gave valid information regarding the use of the plant for a particular biological activity to ascertain the environmental influence and plant's physiological performances.

Conclusion

The present research study shows that the examined plant species are very similar in their leaf epidermal features. Nonetheless, the presence of non-glandular trichomes on the abaxial of *S. anthelmia* can serve as a diagnostic character in describing its taxonomic relevance. While this work supports the earlier studies and reports of amphistomatic leaf epidermis, it has also added to the existing botanical information and may be used in showing the originality of each species in different groups, genus, and kingdoms, as well as in their taxonomic description. Also, the phytochemical screening result will form a basis for carryinging out standardization of crude drugs and suggest its use in the treatment of diverse diseases.

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