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Pharmacognostic Studies of the Leaves and Stem of *Diodia scandens* Sw in Nigeria

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ABSTRACT

Pharmacognostic studies of Diodia scandens Sw (Rubiaceae) were carried out. Diodia scandens Sw is a straggling herb with a taproot, slender stem and compound leaves used for treatment of various diseases such as dysentery, diarrhea, asthma, convulsion, epilepsy, oedema, gout, swelling and it is said to be anti-abortifacient, antidotes, antimicrobial, anti-inflammatory in Nigeria and other countries. The phytochemical screening of the leaf extract revealed the presence of saponins, tannins, cardiac glycosides and absence of flavonoids, phlobatannins, alkaloids and anthraquinones. Anatomical examination has revealed the occurrence of paracytic, anisocytic, diacytic and brachyparacytic on the abaxial surface ad only few paracytic on the adaxial surface. The stem showed druses of crystals. There are four different types of abnormal stomata which are vertical contiguous stomata, two stomata sharing one subsidiary cell, a stoma with unopened stomatal pore and a stomata with one guard cell. The surface of the epidermal anticlinical cell walls, guard cell area, stomata index, presence or absence of trichomes and venation characters can be used as a great diagnostic tool with a view of preparing a monograph.

Key words: Pharmacognostic Studies, *Diodia scandens* Sw, Rubiaceae, Anatomy, Nigeria.

INTRODUCTION

Plants have been used by man to cure diseases and heal injuries since time immemorial. The universal role of plants in the treatment of disease is exemplified by their employment in all major systems of medicine irrespective of the underlying philosophical premise (Trease and Evans, 2002).

Plants have been used throughout the world in folk medicine and as local cures for common ailments. Folk medicine gave rise to traditional systems of medicine in various diseases. World Health Organisation reported that of 119 plants derived pharmaceutical medicine, about 75% are

used in modern medicine in ways that correlated directly with the traditional uses as plant medicine by native culture (Herbal Medicine, 2005).

The basic needs of man such as food, shelter and fibres are obtained from plants and plant derivatives, wood and timber obtain from plants still form major raw materials for construction of doors, windows and the cotton that is still the most preferred is obtained from plants (Ashok and Pande, 2007).

Diodia scandens Sw has enormous usefulness and importance. In Nigeria, the leaves are used for curing eczema (antifungal property) as fodder to poultry, its juice is used to stop bleeding, its extract is used to treat bruises and minor cuts and drunk as metric during treatment of ear problems, it is also used as antiabortifacient (Etukudo, 2003).

In Western Africa, it is used as antidotes (venomous stings, bites and pain killers, venereal disease), the leaves are used as uricant (Burkill, 1985).

This project aims at establishing some diagnostic parameters such as macroscopic, microscopic and quantitative parameters of the crude drug (leaves) and also the constituents of the leaves which would be useful in preparing a monograph on the plant for its identification.

Significance of the Study

This to contribute useful information to the identification of *Diodia scandens* Sw, thus providing a useful tool for collection and preservation of the species.

Diodia scandens Sw is a straggling herb which has been in use in the Western African system of medicine. Whole parts of the plants are useful in curing of ailments (Ethnobotanic Survey, 2002). Carlquist (1961) stated that leaves of plant provide a variety of anatomical features. Garg and Srivastava (2005) defined leaves as the main photosynthetic organ, transpiratory organ and organ for gaseous exchange in most plants which is a lateral outgrowth from stem and comprises of leaf base, a petiole and lamina. Fahn (1967) stated that the leaf is the most variable organ both morphological and anatomically. Several authors have worked on the structure and development of stomata in a number of plants (Karatela and Gill, 1984, Guyot, 1971, Patel and Inamdar, 1971). Guyot (1971) found out that the first type of stomata to develop on the cotyloden was anomocytic and that anomocytic or anisocytic stomata appear on the first foliage leaf. Several authors have discussed the taxonomic significance of stomata (Esau, 1965, Essiett *et al*., 2006, Inyang, 1993). Essiett and Akpabio (2005) investigated the distribution of stomata in Asteraceae family using *Vernonia amygdalina*, *Emilia sonchifolia*, *Tridax procumbens* and *Chromoleana odorata* and reported that *Tridax procumbens* and *Chromoleana odorata* are amphistomatic, and both have matured stomata type such as anomocytic anisocytic, diacytic and paracytic. Kridemann *et al*. (2000) pointed out that abscisic acid (ABA) is a regulatory function hormone of the stomata. However, various facts pointed stomata as the main factor responsible for the physiological activities of the plant, abnormal stomata is the behaviour and hormonal imbalance in plant. Several authors have discussed the taxonomic significance of hairs (Stace, 1980, Gbile, 1986, Essiett and Akpabio, 2005). Stace (1980) reported that the trichomes morphology provides the most important epidermal feature in the taxonomy of the combretaceae family in which he asserted the combretaceous hair and recorded that trichomes is important from the specific to the family level in many plant groups.

Several workers who have used anatomical characters include Bakare (1991) on *Sida* (L.) and Solademi (1989) on *Solanum* species and many others have used the leaf as a taxonomic tool.

Watson and Dallwitz (1992) on the family Rubiaceae recorded septate hairs in Rubioideae and non-septate in Cinchonoideae and paracytic stomata as the most abundant. The importance of crystals in taxonomy as a diagnostic tool was emphasized by Trewelle (1976), Gibb (1974), Inyang (1993), Illoh and Inyang (1998) suggested that their mode of distribution is equally important in taxonomy. Basic phytochemical screening consists of performing simple chemical test to detect the presence of alkaloids, tannins, saponins, anthraquinones, flavonoids, cardenolides in plants extract through some preliminary test using appropriate chemical reagent (Sofowora, 1993). Essiett *et al*. (2006) discussed the phytochemical studies of *Hippocratea Africana*. Wild (Celastraceae) and recorded the presence of alkaloids, saponins, cardiac glycosides, flavonoids, tannins and anthraquinones in the plant extract.

Watson and Dallwitz (1992) reported on absence of ellagic acid in Rubiaceae family but presence of Arbutin, Ursolic acid and Anthraquinones detected only on 21 genera. Several authors have discussed the taxonomical significance of plant component using phytochemical screening. Barroso (2005) and Bacilupo and Cabral (1990) on the presence saponins on *Diodia scandens*, Gibbs (1974) reported on the presence of inulin in Rubiaceae. Akah *et al*. (1993) and Olive (1998) on the action of antioedema and analgesic actions of *Diodia scandens* extract on rats and mice, Onuaguluch (1998) reported on *Diodia scandens* activity against *E. carinatus* venom and Okoli *et al*. (2002) on endogenous antinutritional constituents of *Diodia scandens* for modern intensive animal production. Burkill (1985) reported that *Diodia scandens* extract has laxative and oxytoxic agent and the extract increased the threshold of pain stimulus in mice.

MATERIALS AND METHODS

The fresh leaves and stems of *Diodia scandens* Sw were collected from a bush in Nduetong Village in Uyo Local Government Area of Akwa Ibom State.

Macroscopic Examination

The macroscopic features of *Diodia scandens* Sw were examined using naked eyes and magnifying lens. Measurements of parameters such as leaf, size and petiole length were recorded. The shape of the leaf, leaf margin, apex, base, texture, colour, odour, taste and type of inflorescence were noted.

For the observation of the leaf morphology small sizeable portions of the leaves of each species were obtained from the standard median portion of the leaves. These portion were decolourised by boiling in 90% alcohol for about 30 minutes. The cleared leaves were then washed thoroughly in water before storing in 5% ethanol as described by Olatunji (1983).

Epidermal peels of abaxial and adaxial surfaces were made. The leaf material was placed on a glass tile irrigated with water and tissues above the epidermis were scrapped off carefully with a razor blade until the epidermis underneath was reached.

Peels from the leaf portions were stained in 1% solution of safranin O for 4 – 8 minutes, washed in water to remove excess stain before mounting on clean slides in 10% glycerol. Measurements of stomatal length and epidermal length and breath were made with a calibrated eye piece micrometer and final figures got with ocular constant. Guard cell area was estimated for the leaf surface using the following formula as described by Franco (1939). Guard cell area (GCA) = length x breadth x K.

Where K = Franco's constant = 0.7854

The stomatal index (SI) was estimated using the formula:

$$\frac{S}{E + S} \times 100$$

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

For the purpose of studying the venation patterns and crystal distribution, portions of the leaves taken from the standard median portion were decolourised by boiling of 90% ethyl alcohol at 20^{o}C for about 10 – 15 minutes, then washed in three to four changes of water to remove traces of alcohol. The portions were then transferred to 5% sodium hydroxide and boiled for 30 minutes for further decolourization. They were later washed thoroughly to remove alkaline solution. The partially cleared leaves were further cleared in 5% domestic bleach (Parazone) for about 20 n- 30 minutes under the sunlight which made clearing quicker. The portions were again washed in three to four changes of water, rinsed thoroughly with water and temporary mounts made in 25% glycerol.

All processed materials were preserved in 50% ethyl alcohol until when recorded. Illustrations were made with the aid of ocular micrometer and figures gotten with ocular constant.

Cold extraction was carried out on the materials, which was later concentrated to dryness in *vacuo* at 40^{o}C. The dry extract was subjected to phytochemical screening according to the methods of Sofowora (1993), Trease & Evans (2002) and Culliel (1982). Thin Layer Chromatography (TLC) was carried out on the dry extract to confirm the compounds detected in phytochemical screening.

Microscopical Examination
The powdered and transverse sections of the leaf were employed for this study; to carry out quantitative and qualitative studies, using the methods employed (Evans, 1996). Chemo-microscopical examination was carried out to detect the presence or absence of various chemical compounds such as starch, protein, lignin, mucilage and calcium oxalate crystals.

Quantitative Microscopy
The moisture content of the powdered leaves was determined by loss on drying method (African Pharmacopoeia, 1986). The ash value, acid insoluble ash and water-soluble ash were determined as described (British Pharmacopoeia, 1988). The water and alcohol extractive values were obtained using the method outlined (Brain and Turner, 1975).

RESULTS

Macroscopic Features/Observation
Macroscopic features of leaves observed are described as follows:

Diodia scandens Sw is an evergreen perennial herb, which has an alternate leaf arrangement, petiole is present. It has compound leaves, ovate to lanceolate in shape, reticulate venation, entire in margin, its apex is acute, its base is cuneate, it has glabrous surface and its texture is charteceous.

Diodia scandens Sw has a dark green colouration, tasteless, odourless and has solitary inflorescence.

Epidermal Cells (Shape and Size)
Epidermal cells show wide variations in shape and sizes. The cells of upper epidermis are slightly sinous while those of the lower epidermis are sinous.

Stomata
Stomata, although more numerous on the abaxial surface, than the adaxial surface (hypoamphistomatic). Stomata are usually absent in the coastal regions. Four stomata types are found in *Diodia scandens*. They are Diacytic, Anisocytic, Paracytic and Brachyparacytic. Diacytic and paracytic being more frequent. The Anisocytic and Brachyparacytic are rather infrequent. They occur individually or the different types are placed side by side even on the same surface of the leaf.

Abnormal Stomata
Various anomalous structures were observed in the material investigated. These include vertical contiguous stomata, two stomata sharing one subsidiary cell, a stoma with one guard cell and a stoma with unopened stomatal spore.

Hairs
The morphology of trichomes include their size, shape and frequency is of characteristics interest. They are scantily presence on the adaxial surface and densely present on the abaxial surface.

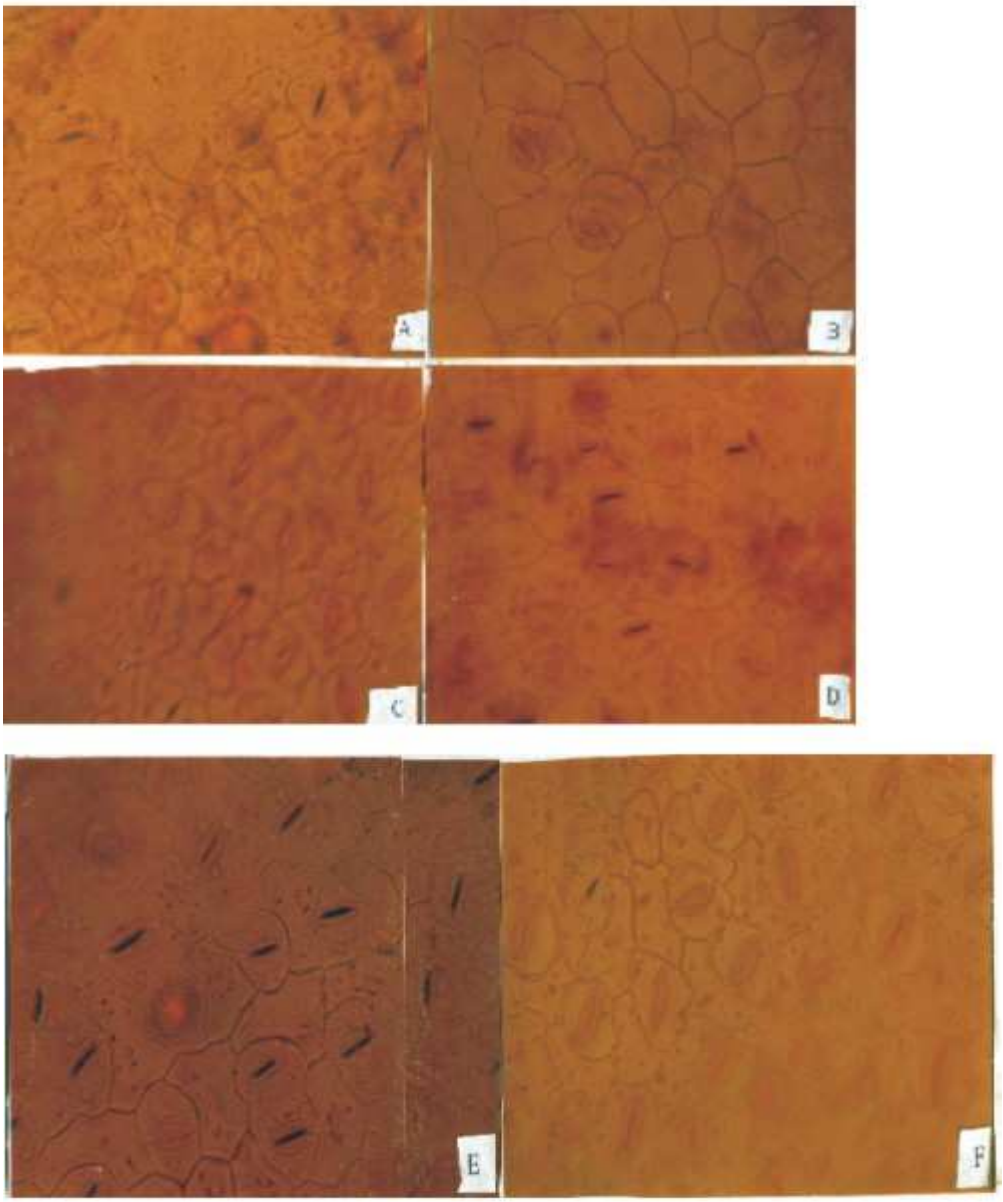

Plate 1: Surface views of the lamina of *Diodia scandens* showing the occurrence of the trichomes and types of Stomata (x400)

A. Abaxial surface showing paracytic and diacytic types of stomata
B. Adaxial surface showing epidermal cells walls and few paracytic stomata
C. Abaxial surface showing vertical contiguous stomata and two stomata sharing one subsidiary cell
D. Abaxial surface showing Anisocytic stomata with unopened stomatal pore (x100)
E. Abaxial surface showing a stoma with one Guard and an Aborted Guard Cell
F. Abaxial surface showing types of stomata

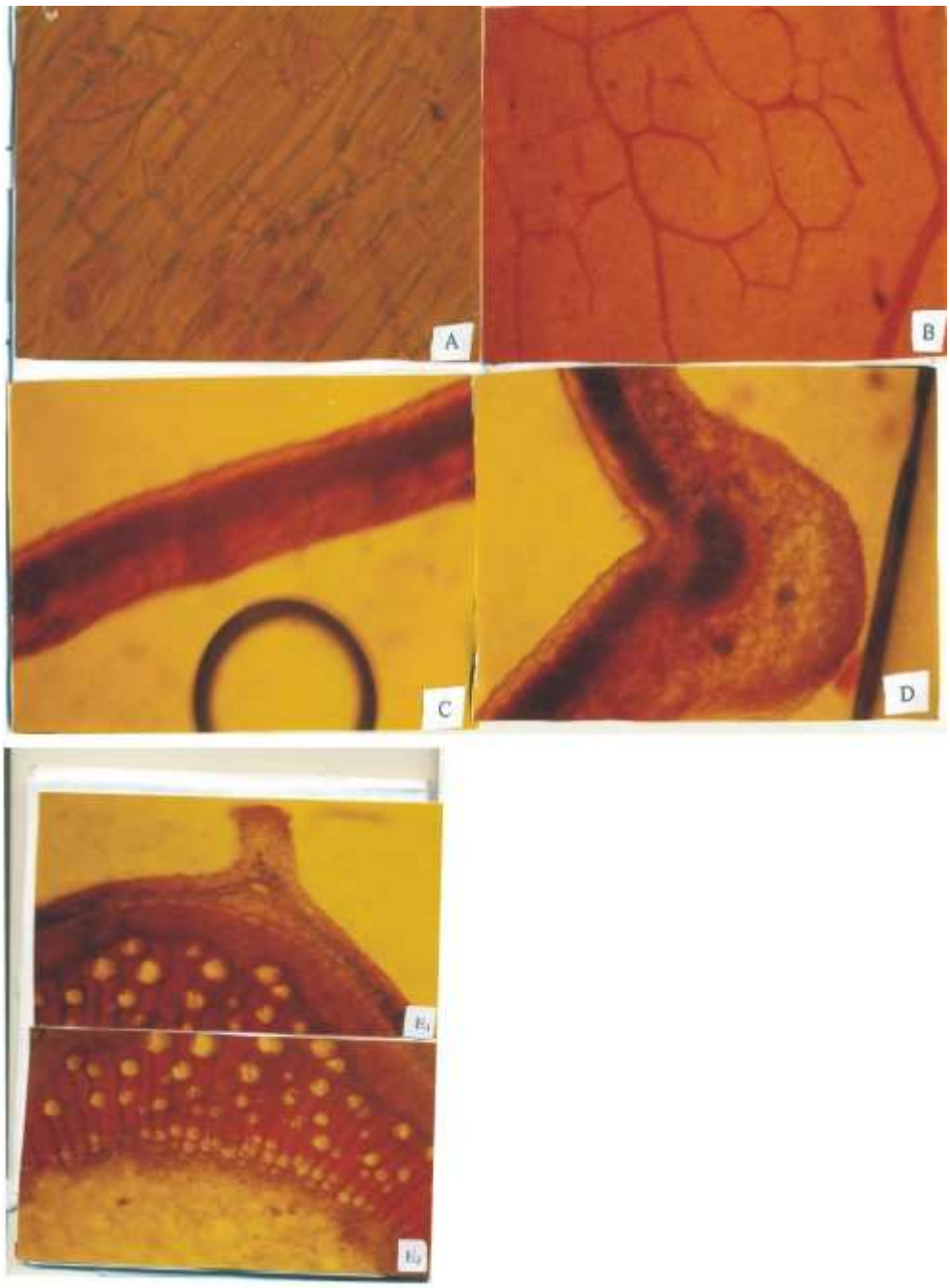

Plate 2:

A. Abaxial surface showing a stoma with one Guard Cell and an Aborted Guard Cell (x400)
B. Venation pattern of *Diodia scandens* (x100)
C. Transverse section of lamina of *Diodia scandens* with trichome on upper and lower surfaces (x100)
D. Transverse section of midrib of *Diodia scandens* (x100)
E_1E_2 Transverse section of the stem of *Diodia scandens* showing the general organization and distribution of tissues (x100).

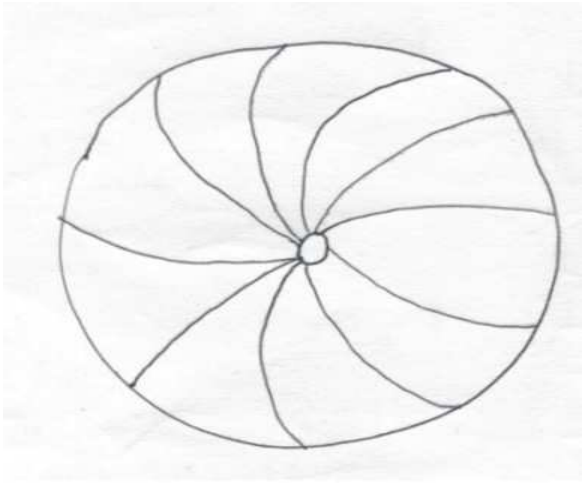

Fig. 1: Abaxial surface showing peltate trichome (x400)

Transverse Section of Lamina

Trichomes are scantily present on the abaxial surface, they are unicellular types. The epidermal calls are mostly rectangular to square in shape of different sizes. Cells walls are moderately thickened. The outer tangential wall was curved to straight covered by thin cuticle. Hypodermis were absent. Mesophyll was dorsiventral. The palisade layer was made up of single layer, cylindrical, variable in size with abundant chloroplasts. The spongy tissue has cells of irregular shape and sizes, and the cells are arranged loosely.

Transverse Section of Midrib

The outline of the transverse section of the midrib appeared convex on the abaxial and concave (hollow) at the adaxial surface. The epidermal cells are unisseriate, cells are rectangular to square in shape of various sizes. Cell walls are moderately thickened. Cortex consists of an outer part of angular collenchyma cells and inner part of polygonal paeenchyma cells on both adaxial and surfaces. It has only non-glandular and unicellular trichomes at the abaxial surface. The vascular bundle is bicollateral (two patches of ploem, one towards outer and another towards inner side, xylem present on the same radius). The vascular bundle is arc shaped. Crystals are present on parenchyma region of the cortex and xylem region of vascular bundle.

Transverse Section of the Stem

Trichomes are present, uniseriate and spaced. Epidermis is uniseriate and covered with thin cuticle. The epidermal cell is rectangular to square. Cell wall is moderately thickened. Periderm consists of many layers, its cells are rectangular to square. The cortex consists of *Lamellar collenchyma* and polygonal parenchyma. Endodermis is made up of one layer. Medullary ray is present (gap between vascular bundle and pith). Pericycle comprises of polygonal cells and small cells of parenchyma. Vascular system is bicollateral. The phloem cells are arranged in groups and present in form of strands. Xylem appears in form of continuous cylinder. Intra-xylary ploem is present in form of strand and at the periphery of the pith. The pith is unlignified and composed of polygonal parenchyma. Druses of calcium oxalate are abundant in pith and parenchyma of the cortex region.

Table 1: Leaf Characters of *Diodia scandens* Sw

Species	Leaf Length (cm)	Length Width (cm)	Petiole Length (cm)	Petiole Width (cm)	Leaf Shape
Diodia scandens	4.2	3.8	0.05	0.025	Ovate-Lanceolate

Table 2: Epidermal Features of *Diodia scandens* Sw

Stomatal Size (µm)				Epidermal Cell Size (µm)				Non-glandular Trichomes (µm)				Coastal cell size (µm)		Guard cell area (µm)^2		Length of Aeroles	Width of Aeroles	Number of Veinlet ending	Shape of Aeroles	Veinlet Endings	Stomata Distri-bution	Stomata Index		Epidermal Cell Wall	
Abaxial		Adaxial		Abaxial		Adaxial		Abaxial		Adaxial		Abaxial	Adaxial	Abaxial	Adaxial							Ab	Ad	Ab	Ad
L	B	L	B	L	B	L	B	L	B	L	B														
31.2	10.4	26.0	7.8	36.4	20.8	41.6	23.4	9.8	6.2	9.1	5.4	0.55X0.23	0.85X034	53.0	54.1	21.3	11.7	0-9	Varies from linear to curved single to branched	Spiral trac-heids, not swollen, surround-ded by bundle sheath cells which are of different size and shape	Hypo-amphi stomatic	59.6	20.0	Si-nous	Sligh-tly Sinous

Venation

The venation is pinnate, brochidodromous. The aeroles are of different shapes and sizes, areas of the veinlet endings per aerole ranges from 0 to 9, linear to curved, simple to branched. The veinlet ending usually consists of spiral tracheids, are not swollen. Areoles are pentagonal.

Phytochemical Screening

The result of preliminary phytochemical screening of the leaves of *Diodia scandens* Sw are summarized in Table 4. Result shows the presence of tannins, saponins, cardiac glycosides, absence of alkaloids, flavonoids, anthraquinones and phlobatanins.

Table 3: Chemo-Microscopical Result of Powdered Leaves

Test Reagents	Observation	Inference
5% iodine	Blue black colouration observed on a few starch grains in parenchyma cells of transverse section.	Starch (+++)
Phloroglucinol +conc. Hcl	Red colouration was not observed on the fibres of xylem tissue and phloem tissue were not lignified.	Lignin (-)
Sudan III	Light red colouration was observed around the vascular bundle.	Mucilage (++)
1% Picric acid solution	Yellow colour was observed.	Protein (++)
80% tetra-oxosulphate VI acid	The bright crystals disappeared on addition of this reagent.	Calcium oxalate crystal (+++)

-=Absence, +=Trace, ++=Moderately present, +++ =Abundantly present

Table 4: Result of the Phytochemical Screening Metabolites

Test	Observation	Inference
Saponin	Frothing persisted for 10 minutes	+++
Tannins	A blue black colouration	+++
Flavonoids	Absence of orange colouration	-
Alkaloids	Absence of turbidity and pink ppt	-
Phlobatannins	No reddish ppt formed	-
Anthroquinones	Absence of a rose pink colouration	-
Lieberman's test	A pink colour at interphase	-
Keller Killani's test	A brown ring obtained at interphase	++
Salkowski's test	Reddish brown colouration obtained	++

-=Absence, +=Trace, ++=Moderately present, +++ =Abundantly present

Table 5: Quantitative Evaluation of the Powdered Leaves

Evaluation parameters	Values (% w/w)
Moisture content	12.63
Ash content	20.53
Alcohol extractive value	4.20
Water extractive value	21.53

DISCUSSION

The morphological (macroscopic) observation made in this study are not different from Watson and Dallwitz (1992), Burkill (1985) and Ibrahim (2005). Anatomical features are widely used in systematics for identification, for placing anomalous groups in satisfactory position in classification and for indicating patterns of relationship that may have been observed by superficial convergence in morphological features (Essiett, 2004).

Variations were found in shapes and numbers of vascular bundles, the presence of calcium oxalate crystals and the presence or absence of trichomes on both abaxial surfaces. These variations could be used to distinguish the species.

Non-glandular unicellular trichomes on both adaxial and abaxial surfaces are considered interesting and the density of hairs was more abundant on the abaxial surface. The high density of thick and coated hairs probably serve to reduce the rate of transpiration in plants and this buttresses the importance of trichomes in taxonomy as a diagnostic tool as emphasized by Stace (1980) in the family of combretaceae and corroborates with Ibrahim (2005).

The epidermal cell size varies significantly. The adaxial surface has the largest epidermal cell size. The presence of abnormal with vertical contiguous stomata, two stomata sharing one subsidiary cell, a stoma with one guard cell and a stoma with unopened stomata pore is an interesting character in solanaceae and rubiaceae as highlighted by Metcalfe and Chalk (1979) and Ahmad (1963b). There is no evidence to suggest that such abnormalities are as the result of environmental or disease factor. Dehnel (1960) has induced stomata degradation by wounding in *Begonia aridicaulis*, but plants of *Brunfelsia Americana* and *Withania samnifera* extensively showing ‘single guard cell’ and degenerated ‘stomatal cell’ in their leaves, have been found to be quite normal and healthy. Thus, the cause of the occurrence of these abnormalities in nature as in many Solanaceae and Rubiaceae is yet to be determined. Although Jones and Mansfield (2000) and Krideman *et al*. (2000) pointed out that abscisic acid (ABA’s) imbalance in plant is responsible for abnormal stomata since it is a regulatory hormone found in the stomata. The findings in the *Diodia scandens* Sw studied exhibited this. This corroborates with Metcalfe and Chalk (1979) and Ahmad (1963b). Some attempts have been made to use stomatal character as an aid to classification. Ayensu (1972) had recorded anomocytic type of stomata in dioscoreales, Illoh and Inyang (1998) on the foliar epidermis and petiole anatomy in some Nigerian Solanum Linn. Species, Essiett and Akpabio (2005) on seven species and two varieties of Dioscorea. The presence of various type of stomata in the taxa is of taxonomidc interest in this study because it can be used to distinguish Diodia scandebs Sw which is hypoamphistomatic and this agrees with Watson and Dallwitz (1992) who reported that paracytic stomata is the most abundant in Rubuaceae family and more frequent on the abaxial surface as reported by Esua (1965) and Van Cothem (1970).

The transverse section of the lamina showed that the epidermal cells were of different shapes and sizes. The palisade cells were mainly one layer. Trichomes were present on the both adaxial and abaxial surfaces.

The transverse section of the midrib showed that the epidermal cells are uniseriate, rectangular to square in shape of various sizes. Transverse section appeared convex at the abaxial and concave at the adaxial surface. The vascular bundle is bicollateral and cortex consist of an outer part of collenchyma and inner part of polygonal parenchyma cells

The transverse section of the stem showed that the epidermal cell is uniseriate, covered with thin cuticle, rectangular to square in shape. Periderm consist of many layers. Cortex consists of lamellar collenchyma and polygonal parenchyma. Druses of calcium oxalate are abundant in the pith and parenchyma of cortex.

Most of the anatomical features of the stem in transverse section correlate with that of Metcalfe and Chalk (1979), Ibrahim (2005) and Watson and Dallwitz (1992), except the presence of crystals on the pith and parenchyma of the cortex region.
The possession of scale like trichomes by *Diodia scandens* supports the view of the Metcalfe and Chalk (1979) that peltate trichomes are more frequent in Rubiaceae and Solanaceae families.

Phytochemical Screening
Alkaloids which have some toxic effect were absent. Saponins which have anti-inflammatory, anti-yeast, antifungal, anti-parastic, anti-tumor, anti-viral and anti-abortifacient activities were present which corroborates with Barroso (2005) and Bacilupo and Carbral (1990) and confirms its usefulness to traditional medicine practitioners. Tannins which have astringent and detergent properties were also present and can be used against diarrhea (Trease and Evnas, 2002; Bruneton, 1999). Flavonoids, phlobatannins and anthraquinones were absent.

There has been an assertion by Trease and Evans (2002) that naturally cardiac glycosides are used for treatment of various diseases associated with the heart such as in controlling supraventicular (atrial) cardiac arrythmias, it also exert a slowing and strengthening effect on failing heart. The presence of this compound in *Diodia scandens* Sw could be useful in the treatment of diseases associated with the heart (Treases and Evans, 2002).

The quantitative evaluation is an important parameter in setting standard of crude drugs and the physical constant parameters could be useful in detecting any adulterant in the drug. Musa et al. (2002) recorded that the result of the moisture content that is not high indicated less chances of microbial degradation of the drug during storage and in this study, the moisture content value was 12.63%. The general requirement of moisture content in crude drug is that, it should not be more than 14% (British pharmacopoeia, 1980) and the value obtained in the research work (12.63%) is within the accepted range.

Edward et al. (1971) also emphasized that excessive moisture is considered an adulterant because of its added weight as well as the fact that excess moisture is conducive to the promotion of mold and bacterial growth, up to 5% is usually not considered excessive.

CONCLUSION

The chemical constituent obtained in this plant also lends credence to its use as fodder in poultry and ethnomedicine. Based on the phytochemical properties, several types of drugs could be produced from this plant, such as astringents, antifungal, anti-inflammatory, anti-venom, antidote, anti-oedema, anti-rheumatic and anti-inflammatory, anti-venon, antidote, ant-oedma, anti-rheumatic and anti-abortifacient drugs. The bioactive agents contained in the leaf shows a great medicinal value of the plant to man and edible as fodder to poultry.

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