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Phytochemical And Micro Anatomical Studies on Leaf, Stem and Petiole of Two Species of *Dioscorea* (*Dioscorea alata* L. and *Dioscorea bulbifera* L.)

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ABSTRACT: This study investigated the presence of bioactive compounds and micro anatomy on leaf, stem and petiole of *Dioscorea* species (*Dioscorea alata* and *Dioscorea bulbifera*). The phytochemical analysis revealed the presence of saponins, tannins, glycosides and absent of alkaloids, anthraquinone and phlobatanin in *Dioscorea alata.* Alkaloids, saponins, tannins, glycosides and flavonoids were present in *Dioscorea bulbifera* while anthraquinone and phlobatanin were absent. Microanatomical section of *Dioscorea alata* leaf showed amphistomatous epidermal cell with thickened cell wall and distinct middle lamella, bounded with intercellular air space, adaxial epidermal cells with eccentric nuclei, large central vacuole with thin peripheral layer of cytoplasm. The foliar epidermis of *Dioscorea bulbifera* leaf revealed anamocytic and anisocyticstomatas as well as dense hairy trichomes with cuticular striated cell wall on the adaxial and abaxial epidermis. Histological sections of *Dioscorea alata* stem revealed vascular bundles arranged in two concentric circles. The vascular bundle of the outer circle was smaller than the inner, with two metaxylem vessels together with one phloem unit present at both ends, however, the sections of *Dioscorea bulbifera* stem, showed bundles of inner circle with unpaired metaxylem with phloem unit at one end. Sections of *Dioscorea alata'*s petiole showed basically collateral vascular bundles arranged in a ring with the presence of two to three phloem units in each bundle with the presence of crystal granules. The sections of *Dioscorea bulbifera'*s petiole on the other hand showed longitudinal cell walls with the presence of sieve plates. These histological features and active compounds show that these plant parts are likely to have some medicinal compositions values that should be explored.

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Yam is the common name for some plant species in the genus Dioscorea family Dioscoreaceae that forms edible tubers. These are perennial herbaceous vines cultivated for the consumption of their starchy tubers in Africa, Asia, Latin America, the Caribbean and Oceania. There are many cultivars of yam. Yam tubers can grow up to 1.5 meters (4.9 feet) in length and weigh up to 70 kilograms and 3 to 6 inches high. Yams are monocots related to lilies and grasses (Dumont and Vernier, 2000) which belongs to the order Liliioflorae, family Dioscoreaceae and genus Dioscorea. It is considered to be among the most primitive of the angiosperms and contains over 600 species, of which only about ten are considered edible (Purseglove, 1988). Cultivated species include *Dioscorea alata, Dioscorea cayenesis, Dioscorea rotunata, Dioscorea esculenta, Dioscorea bulbifera, Dioscorea nummularia, Dioscorea pentaphyalla, Dioscorea hispida, Dioscorea trifida and Dioscorea dumentorum.* The range of *Dioscorea alata* cultivars was estimated to be not less than 85 in New Caledonia (Coursey, 1967). However, the diversity of cultivars has been reduced due to the progressive urbanization (Coursey, 1967). *Dioscorea alata* is a vigorously twining herbaceous vine, from massive underground tuber. Stems to 10m (30ft) or more in length, freely branching above; internodes square in cross sections, with corners compressed into "wings," these often red-purple tinged. Aerial tubers (bulbils) formed in leaf axils (not as freely as in *Dioscorea bulbifera*), elongates, to 10cm (4 in) x 3 cm (1.2 in), with rough bumpy surfaces. Leaves longed petiole, opposite (often with only 1 leaf persistent); blades to 20cm (8 in) or more long, narrowly heart shaped, with basal

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lobes often angular. The Flowers are small, occasional, male and female arising from leaf axils on separate plants (i.e., a dioecious species), male flowers in panicles to 30cm (1ft) long, female flowers in smaller spikes. Fruit a 3-parted capsule; seeds winged. *Dioscorea alata* tubers have variable shapes the majority being cylindrical. Its tubers are varying in number from one to five. The flesh of the tuber ranges in colour from white to purplish. The tubers used for the treatment of different diseases such as laxative and vermifuge, and as a treatment for fever, gonorrhea, leprosy, tumors, and inflamed hemorrhoids. The tubers are also believed to possess activities such as antimicrobial, antioxidant, anticough, anti-diabetic, anti-diarrhea, and anti-cancer activity (Shui and Leong, 2002). Furthermore traditionally *Dioscorea alata* is used prophylatically for chronic liver pain diseases. Several studies indicated that some Indian wild medicinal plants possess more potent antioxidant activity than common tubers and fruits and phenolics compounds were a major contributor to the antioxidant activity of these plants (Maga, 1978). *Dioscorea alata* tuber contains diosgenin, which is widely used as a precursor in the synthesis of steroid hormones such as progesterone, corticosteroids, and anabolic steroids. The most important sapogenins are diosgenin and yamogenin. *Dioscorea bulbifera* is a perennial vine with broad, alternate leaves, and two types of storage organs. The plant forms bulbils in the leaf axils of the twining stems, and tubers beneath the ground. These tubers are like small, oblong potatoes. Some varieties are edible and cultivated as a food crop, especially in West Africa. The tubers of edible varieties often have a bitter taste, which can be removed by boiling. They can then be prepared in the same way as other yams, potatoes and sweet potatoes. *Dioscorea bulbifera* is one of the most widely consumed yam species. It can grow up to 150ft tall. It can grow extremely quickly, roughly 8 inches per day, and eventually reach over 60ft long (Coursey, 1967). It typically climbs to the tops of trees and has a tendency to take over native plants. New plants develop from bulbils that form on the plant, and these bulbils serve as a means of dispersal. The aerial stems of air potato die back in winter, but resprouting occurs from bulbils and underground tubers. The therapeutic potentials of *Dioscorea bulbifera* has been documented in many parts of the world include; the treatment of sore throat, gastric cancer and carcinoma of rectum, and goiter in China (Jian, 1978; Gao and Kuroyanagi, 2001); treatment of tumor and leprosy in Bangladesh (Zarapata, 2005). Furthermore, various extracts of this plant have been reported to be usable as; anorexiant (Jindal *et al*., 1969), antitumor (Gao *et al.*, 2002), antihyperlipidemic (Mckoy *et al.*, 2003), antioxidant (Bhandari and Kawabata, 2004), plasmid curing agent (Shririam *et al*., 2008), antihyperglycemic (Ahmed *et al*., 2009), analgesic and anti-inflammatory (Nguelefack *et al*., 2011). The primary means of spread and reproduction are by the bulbils. The smallest bulbils make control of air potato difficult due to their ability to sprout at a very small stage. The vine produces small white flowers; however, these are rarely seen when it grows in Florida. The fruits are capsules. This means that yam will continue to have a high market potential in Nigeria. Yam also has ritual, medicinal and socio-cultural significance. It is the choice during ceremonies and festivities (Coursey, 1967). In some parts of Southeastern Nigeria, the meals offered to gods and ancestors consist principally of mashed yam. Some traditional ceremonies are celebrated with yam as the major food item such as the New Yam Festival in parts of West Africa. In parts of Igbo land in Southeastern Nigeria, it is customary for the parents of a bride to offer her seed yams for planting as a resource to assist her in raising family. Little work has been done on the phytochemical and micro anatomical studies on the species of *Dioscorea alata* and *Dioscorea bulbifera* hence this study aimed to add to the existing information the phytochemical constituents and microanatomy of the said species of *Dioscorea.*

MATERIALS AND METHODS

Collection of Plant Material and identification: The plant materials leaf, stem and petiole of *Dioscorea alata* and *Dioscorea bulbifera* used for this study were collected from a local cultivar in Abak Local Government Area of Akwa Ibom State and plant species were identified by a taxonomist in the Department of Botany, Akwa Ibom State University.

Phytochemical Studies: The leaves of each species studied were sun dried for 72 hours (3 days) and weighed. Fifty grams (50g) of the leaves were macerated in 96% ethanol using a pestle and a mortar. The extract was thereafter filtered and evaporated to dryness using a rotary evaporator set at 450C to constant weight and later, an exhort extraction machine. Residue yields were noted and a portion was used for the phytochemical screening following the method of Trease and Evans (1989) and Harborne (1977).

Preparation of plant parts for anatomical Studies: The fresh leaf, stem and petiole specimens of *Dioscorea alata* and *Dioscorea bulbifera* were cut into tiny pieces and the fresh samples were fixed in neutral buffered saline fixative for 72 hours. The plant parts were processed for paraffin blocks by passing them through graded concentrations of alcohol (70%, 90% and 100%) for one hour at two changes to completely

dehydrate and remove the fixatives from the plants tissues. After this step, the plants samples were passed through xylene which was used as the clearing agent for thirty minutes and two changes to remove the alcohol from the plant samples. Samples were then embedded in molten paraffin wax in preparation for thin sectioning at 10 to 15 microns using a rotary microtome. Thin ribbon of neatly sectioned stem, petiole and leaves were carefully spread on a water bath to obtain distinct sections of the embedded plant parts. They were then picked with the aid of well labeled frosted ended slides with glycerin as adhesive. The slides with the affixed sectioned plants tissues were maintained at 52 ^{o} C in an incubator to remove the excess paraffin wax. The whole plant parts were further routinely processed for histological study with methylene blue and safranine O / Fast Green stains, to study the cellular integrity and comparative anatomical differences in both species of the *Dioscorea* in this present study. Sections were viewed under the light microscope and photomicrographs were obtained using the microscope camera linked to a computer.

Sass's Safranin and Fast Green Technique: Sections on slides were deparaffinized and hydrated to distilled water. Sections were stained 1-12hours in aqueous safranin; the stained sections were washed in distilled water until no more dye was removed from the sections. Dehydrated in 30, 50 70 and 95% of ethyl alcohol, 2-5 minutes respectively ,thereafter sections were dipped 5-30 seconds in 95% ethyl alcohol and fast green , Sections were washed twice two minutes each in 100% ethyl alcohol , and dipped 5 seconds in carbol-xylene or a mixtrure of methyl salicylate and xylene to remove the last traces of water. Thereafter, the sections were cleared in 100% xylem for two changes. Coverslips were mounted.

Methylene blue Technique: Sections on slides were stained with a drop of methylene blue solution and a cover slip placed on top for 20-30 minutes. Excess solution of methylene blue was removed by allowing a paper towel to touch one side of the cover slip. The stained sections on slides were placed on the microscope, with 4x or 10x objective in position and viewed at higher magnification.

RESULTS AND DISCUSSION

The results of the phytochemical screening revealed the presence of saponins, tannins, glycosides and absent of alkaloids, anthraquinone and phlobatanin in *Dioscorea alata.* Alkaloids, saponins, tannins, glycosides and flavonoids were present in *Dioscorea bulbifera* while anthraquinone and phlobatanin were absent (Table 1).

Table 1: Qualitative analysis of *Dioscorea alata* and *Dioscorea bulbifera* leaf

Test	*Dioscorea alata*	*Dioscorea bulbifera*
Alkaloids		
i) Dragendroff's reagent	-	+
ii) Mayer's reagent	-	+
Saponins		
i) Frothing test	+	+
ii) Fehling test	+	+
Tannins		
i) Ferric chloride test	+ +	+
ii) bromine water test	+	+ +
Flavonids		
i) Shinoda reduction test	-	+
Cardiac glycoside		
i) Salkowski test	+	+
ii)Keller-kiliani test	+	+
iii) Lieberman's test	-	-
Anthraquinones		
i) Borntrager's test (free)	-	-
ii) Combined antraquinone	-	-
Phlobatannins		
Hydrochloric acid test	-	-

Key: +++ = High concentration; ++ = Moderate concentration; + = Trace concentration; - = Absent

Leaf Anatomy of *Dioscorea alata* and *Dioscorea bulbifera:* The upper (adaxial) and lower (abaxial) epidermis has one layer of cells and each is covered by a cuticle. The outer epidermis is also characterized by the presence of hairs in *Dioscorea bulbifera* species. Stomata are restricted only to the lower surface (hypostomatic).

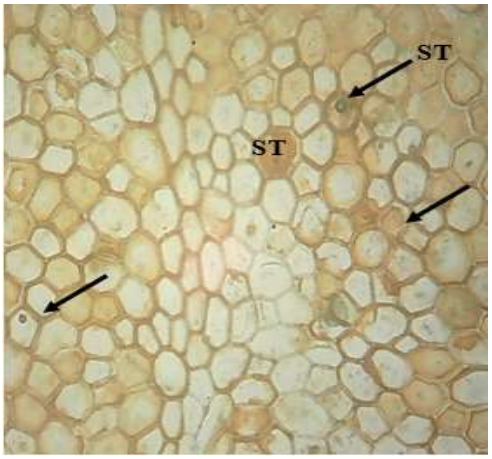

Plate 1: Anatomical section of *dios corea alata* leaf

Two stomata complex were identified namely amphistomatic or anisocytic and anamocytic stomata; *Dioscorea alata* possessed amphistomatous leaf showing epidermal cell with thickened cell wall of cells with distinct middle lamella, bounded with intercellular air space(arrows). At higher magnification, the anatomical sections of the leaf of *Dioscorea alata* is mostly adaxial epidermal cells with

eccentric nuclei, large central vacuole with thin peripheral layer of cytoplasm as well as anamocyticstomatas. The foliar epidermises of *Dioscorea bulbifera* leaf revealed anamocytic and anisocyticstomatas as well as dense hairy trichomes with cuticular striated cell wall on the adaxial and abaxial epidermises (Plates 1,2,3,and 4).

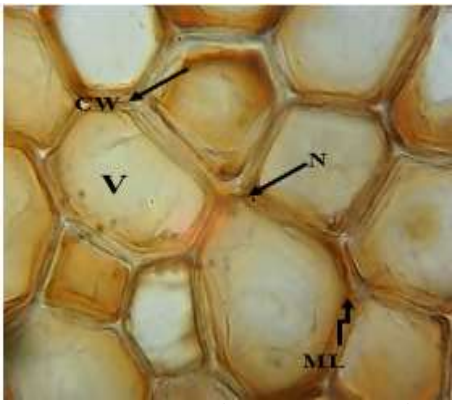

Plate 2: Anatomical section of *dios-corea alata* leaf at higher magnification

Plates 1 and 2: Anatomical section of *Dioscorea alata's* amphistomatous leaf showing epidermal cell with thickened cell wall of cells with distinct middle lamella, bounded with intercellular air space (arrows). At higher magnification (**Plate 2)**, the anatomical sections of the leaf of *Dioscorea alata* show mostly adaxial epidermal cells with eccentric nuclei, large central vacuole with thin peripheral layer of cytoplasm as well as anomocytic stomatas. ×100 and x400

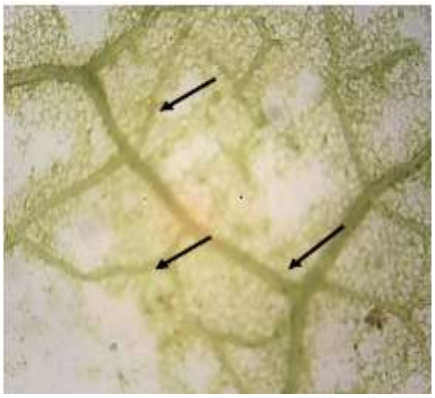

Plate 3: Anatomical sections Light green coloured foliar epidermis of *bulbifera* leaf of *dioscorea bulbifera*

Plates 3 and 4: Anatomical sections of light green coloured foliar epidermises of *Dioscorea bulbifera* leaf revealing both anomocytic and anisocyticstomatas as well as dense hairy trichomes with cuticular striated cell wall on the adaxial and abaxial epidermises (arrows) x100 and x400. (CW-cell wall; V-vacoule; N-nucleus; ML- middle lamella; ST-starch).

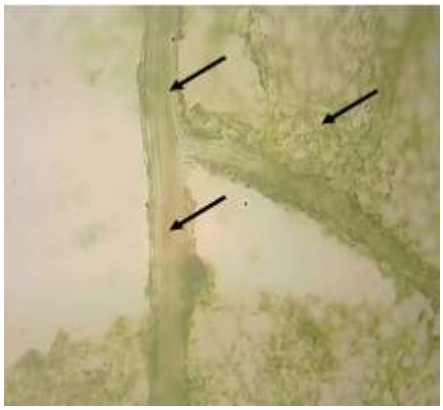

Plate 4: Anatomical section of leaf of *dio corea*

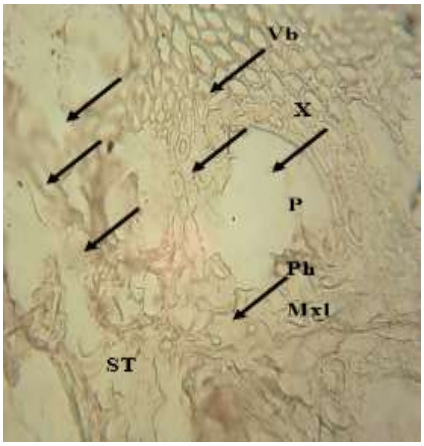

Plate 5: Anatomical section of *dios-corea alata* stem: Safranine O and Fast Green

Stem Anatomy of *Dioscorea alata* and *Dioscorea bulbifera:* Sections of stems of both species are with longitudinal ridges or wings, the epidermis consist of thin- walled rectangular, cuboidal or rounded cells. The epidermal cells are 1-cell layer. The cuticle is generally thin. The pith occupies the central position and composed of thin-walled hexagonal parenchyma cells. The vascular bundles of the stem are arranged in two concentric circles. The vascular bundle of the outer circle are smaller than the inner with 2 metaxylem vessels together with 1 phloem unit present at both ends in the species except but in *Dioscorea bulbifera*, where the bundles of inner circle have unpaired metaxylem with phloem unit at one end. The number of vascular bundles in transverse section varies within species (Plates 5 and 6). **Plate 5):** Anatomical section of *Dioscorea alata* stem where vascular bundles are arranged in two concentric circles. The vascular bundle of the outer circle is smaller than the inner with 2 metaxylem vessels

together with 1 phloem unit present at both ends (arrows).

(Plate 6): Anatomical section of *Dioscorea bulbifera* stem, where the bundles of inner circle have unpaired metaxylem with phloem unit at one end (arrows).
(Vb- vascular bundle; X-xylem; P-pith; Ph- phloem; mxl-metaxylem; ST-starch; N-nucleus).

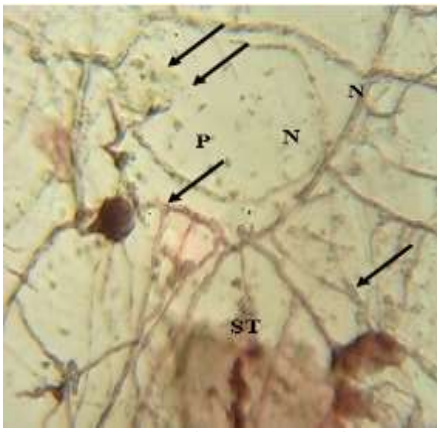

Plate 6: Anatomical section of *dioscorea bulbifera* stem: safranine O and fast Green

Petiole Anatomy of Dioscorea alata and Dioscorea bulbifera: The cortex is composed of collenchymatous tissues. The vascular bundles are arranged in a ring and are basically collateral with the presence of 2-3 phloem units in each bundle. The number of vascular bundles in each petiole is also variable both constant in both species. *Dioscorea bulbifera* section obtained was a longitudinal section which shows longitudinal cell walls with presence of sieve plates. Both species possess phloem and xylem. There is presence of crystal granules in one of the species (Plates 7 and 8).

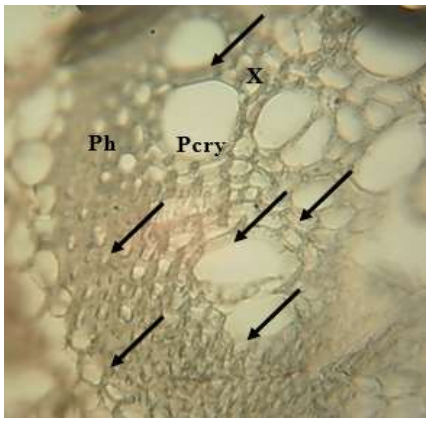

Plate 7: Anatomical section of petiole of *Dioscorea alata*: Safranine O and Fast Green

(Plate 7): Anatomical section of *Dioscorea alata*'s petiole showing vascular bundles arranged in a ring and is basically collateral with the presence of 2-3 phloem units in each bundle and presence of crystal granules. **(Plate 8):** Anatomical section of *Dioscorea bulbifera*'s petiole showing longitudinally cell walls with presence of sieve plates.

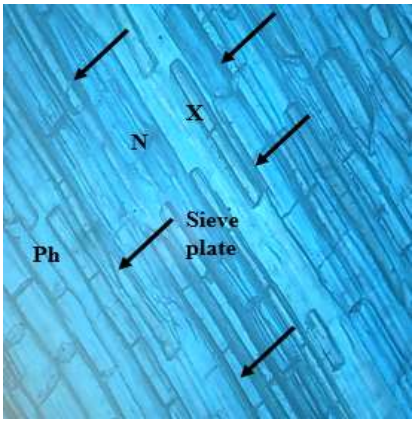

Plate 8: Anatomical section of *Dioscorea bulbifera*: Safranine O and Fast Green

There has been a tremendous interest in the assessment of phytochemical composition of staple foods such as yam, because they are considered to be economically, socially and culturally important in many tropic and subtropic regions of the world (Oko and Famurewa, 2015). It is traditionally known that yam tuber of which *D.* alata and *D. bulbifera* is not an exception, have potential ability to provide one of the cheapest sources of dietary energy in the form of carbohydrate (Ugwu, 2009). The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites like alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. (Odebiyi and Sofowora, 1978). Phenolic compounds like tannins found in plant cells are potent inhibitors of hydrolytic enzymes used by plant pathogens. Active constituents of plants usually interfere with growth and metabolism of microorganisms in a negative manner (Aboaba *et al.*, 2006). Plants synthesize these compounds to protect themselves from pathogens or disease or environment (Tyagi and Bohra, 2002). Other compounds like saponins also have antifungal properties. Saponins were detected in the leaves of *D. alata* and *D. bulbifera* in this present study. According to (Esenwah and Ikenebomeh, 2008), presence of saponin in *D. alata* and *D. bulbifera* as observed in this study, suggest that the leaves may have hypocholesterolenic effect, in that, saponins reduces the uptake of certain nutrient including glucose and cholesterol at the gut through intralumeral physicochemical interactions. This action tends to lessen the metabolic burden that would have been placed on the liver- hypocholesterolemic effect. Saponins have also been reported to possess the properties of precipitating and coagulating red blood

cells (Okwu, 2004). Therefore, in medicine, *D. alata* and *D. bulbifera* can be applied as antibleeding agent to arrest loss of blood in case of injuries. Vitamins and some vital minerals such as zinc and iron are reported to form insoluble complexes with saponin (Igile *et al*., 2013). Glycosides were also present in these studies in the form of cardiac glycosides. Glycosides, as reported in available literature, are the product of sugar condensation with the host of different varieties of organic hydroxyl or thiol compounds, of which the hemiacetal moiety of the carbohydrate plays an ignoble role in the condensation (Omojate *et al*., 2014). Therefore, the presence of this bioactive compound could be attributed to the presence of carbohydrate, which will serve as noble source of the sugar for condensation that will consequently yield the compound. These compounds have been reported, although to be chemically unrelated but possess common properties of intense bitter taste (Omojate *et al*., 2014). The bitters act on gustatory nerves, which results in increase flow of saliva and gastric juices (Omojate *et al*., 2014). The review of (Omojate *et al*., 2014) also reported that the tannic acid present in bitter principle of these cardiac glycosides either serves as an antiprotozoal, or to reduce thyroxine metabolisms. Presence of tannins in the leaves of *D. alata* and *D. bulbifera* is of health benefit, following the report of (Cowan, 1999) that advocated that the consumption of tannin containing beverages especially green teas and red wine, can prevent or cure a variety of illness. (Karou *et al.*, 2007) also stated that many physiological activities such as stimulation of phygocytic cells and wide range of anti-infective actions in plants have been attributed to tannins because of its molecular actions of forming complexes with proteins (Prakash and Hosetti, 2010). Tannins were able to inhibit the growth of insect and disrupt the digestive activities in ruminant animals (Karou *et al.*, 2007). Although, alkaloids which are one of the most efficient therapeutic phytochemicals in plants were not detected in the leaf of *D. alata* but was detected in *D. bulbifera*. The presence of flavonoids in *D. bulbifera* is of alternative advantage due to their anti-inflammatory and antimicrobial properties reported by Okwu (2004). Flavonoids are important class of polyphenols, structurally made of more than one benzene ring, which are found in plants (James, 2012). It has long been reported by (Cordell *et al*., 2001) that alkaloids and flavonoids are responsible for the antimicrobial activities in higher plants. Therefore, the presence of flavonoids detected in *D. bulbifera* indicated the pharmacological properties embedded in the leaf of *D. bulbifera*. The micro anatomical studies of the leaf, stem and petiole reveals an anomocytic character trait which contributes much towards variability. Presence of starch grain in stem and petiole is also an important character trait for differentiating among the species and high rate of variability (Onwuene and Charles, 1994). The results provides some useful features for future phylogenetic and taxonomic studies and these useful features included amphistomatic leaves, anomocytic and anisocytic stomata and smooth epidermal wall. Closed stomata pore, cuticularization of epidermal walls was observed in the adaxial and abaxial surfaces of the *Dioscorea bulbifera*. They had thicker lignified walls and mass of mucilage were evenly spread in the epidermal cell of the *Dioscorea bulbifera* leaf. The presence of mass of mucilage within the epidermal cell and the presence of glandular unicellular trichome with multicellular head is an indicative of the medicinal lodgment of bioactive principles in the plant and in a way may justify its use in traditional medicine. A combination of these features had been found useful for taxonomic distinction and recognition in angiosperm families. Anomocytic stomata type reported in this work agrees with a previous report of anomocytic stomata in the order Dioscoreales as in the case of *Dioscorea hispida* (Salmah *et al.*, 2013). The use of anatomical characters or traits for taxonomic studies has proved useful for identification of fragmented plant and herbarium specimens (Metcalfe and Chalk, 1957). Anatomy can provide useful information for establishing interrelations between taxa at the species and supra species levels. Sometimes it can also help in individual identifications. The internal structure of leaf is more affected by environmental factors and thus is of little value for delimiting taxonomic groups. Other characters of leaf, such as the epidermis and stomata have proved to be much more reliable for taxonomic consideration in many genera (Dickison, 2000; Yang and Lin, 2005; Strgulc-Krajsek *et al*., 2006). The petiole structure is of considerable taxonomic importance in many genera, since it is less affected by environmental changes (Metcalfe and Chalk, 1957). The result of the present study allows the selection of some diagnostic anatomical characters for the identification of Dioscorea species. Ayensu (1972) reported that all stomata in Dioscorea species were anomocytic which were found to be in consistent with the present study and with the reports of Abdulrahaman *et al.* (2009). Results of the present study shows presence of different types of stomata including, anisocytic, anomocytic and amphistomata etc were found congruent with those of Abdulrahaman *et al*., (2009).

Conclusion: It is concluded that *Dioscorea alata and Dioscorea bulbifera* is a plant with rich food and medicinal values. Presence of bioactive compounds such as tannin, saponin, glycosides, flavonoids and alkaloids in plant parts has shown the potential of

biological and pharmacological properties of this tuber crop. The micro anatomical studies of *Dioscorea alata and Dioscorea bulbifera* have shown that these lines of evidence are of taxonomic importance in Dioscoreaceae and this research work has provided information on certain aspects of anatomy/histology and phytochemistry of *Dioscorea alata and Dioscorea bulbifera*, tending support to similar conclusion by other investigators of this tuber crop.

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