



Micromorphological Characters in Wild Medicinal Species of *Dioscorea* (Dioscoreaceae)

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ABSTRACT

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Adulteration, misidentification, and substitution of wild *Dioscorea* species are common in Nigeria's herbal market and among traditional herbal practitioners. The present investigation reports a comparative micromorphological study of three wild species of *Dioscorea* L. used in ethnomedicine in South-western Nigeria to elucidate taxonomically significant characters, which would aid species identification. Physical and chemical methods were used in obtaining the epidermal leaf surfaces of the *Dioscorea* species. Leaves of *D. hirtiflora* Benth. and *D. bulbifera* L. were amphistomatic, whereas *D. dumetorum* (Kunth) Pax were hypostomatic. Diagnostic foliar epidermal characters include striated cell wall; presence of raphides in *D. hirtiflora* and *D. dumetorum* and secretory glands in *D. dumetorum* (wild) and *D. hirtiflora*. Glandular trichomes were observed in all species in addition to simple, elongated, unicellular trichomes in *D. dumetorum* and stellate trichomes in *D. hirtiflora*. Epidermal cells mainly were polygonal, straight to slightly wavy but deeply undulating in *D. dumetorum*. The largest and smallest mean epidermal cell sizes were obtained on the adaxial surfaces of *D. bulbifera* (mauve) and *D. dumetorum* (wild), respectively. Micromorphological characters in the *Dioscorea* species studied are taxonomically significant for species identification and could serve as diagnostic taxonomic tools for their standardization. A key for the identification of species is provided.

Keywords: Adulteration, *Dioscorea*, Micromorphology, Identification, Taxonomic tools

Introduction

Cases of misidentification and substitution of closely related herbal drugs are rampant among indigenes where these ethnomedicinal plants are found naturally. The documentation of cytomicroscopical diagnostic characters is imperative for proper recognition. The study of micromorphological characters is a veritable tool for identification purposes. Medicinal plant authentication is paramount, and histology is an inexpensive tool for herbal drug evaluations.¹ Studying divergent cellular structures, tissues arrangement and microscopic techniques including linear measurements, determination of leaf constants, and quantitative microscopy are important in plant identification for drug evaluation in Pharmacognosy.² Trichome type, stomata arrangement, stomata number, stomata index, palisade ratio from leaf epidermal studies are diagnostic tools for resolving taxonomic conflicts.^{3,4} In a comparative study of foliar microscopy of 25 *Ficus* species in Nigeria by Sonibare *et al.*,⁵ several epidermis, two and more layered hypodermis, 1-3 layers of palisade parenchyma as well as the pattern of vascular system contributed to further delimitation of the genus. Similarly, Ardhamy *et al.*⁶ reported the use of microscopic characters such as trichome, stomata, epidermis and crystals of calcium oxalate as identification tools for *Eleutherine bulbous*.

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Dioscorea L. species are perennial trailing rhizome plants which belong to the family Dioscoreaceae. Apart from being a major source of steroidal saponin used as precursors for sex hormones, they also serve as an essential source of energy giving food globally.⁷ The major edible species of yams occur in three isolated centres: West Africa, Southeast Asia and Tropical America, which are also considered areas for independent yam domestication and represent much diversity.⁸ Some yam species are edible, while the wild species are considered as famine food, and other species are sources of phytopharmaceuticals.⁹ Some valuable secondary metabolites from *Dioscorea* species include steroidal saponins, diterpenoids and alkaloids which are useful sources of pharmaceutical products.¹⁰⁻¹¹ The origin of *Dioscorea dumetorum* (Kunth) Pax is traced to the tropics in Africa.¹² Similarly, *D. bulbifera* L. is a vigorous climber plant native to West Africa¹³ with a wild variety existing as weeds in forests and virgin lands, while wild *D. hirtiflora* Benth. tubers are elongated, slim-like rod shape with reddish-brown colour.¹⁴⁻¹⁵ Traditionally, tubers of *D. dumetorum* are used in lowering blood sugar level. This has been proved scientifically by Iwu *et al.*¹⁶ in which regulated amount of alkaloid extract (disocoretine) isolated from the tubers serve as the active principle in the management of diabetes, thus, justifying their traditional uses. Similarly, Adeleye and Ikotun¹⁴ showed that 0.1% of purified dihydrodioscorine (an alkaloid) extracted from the bulbils and tubers of wild variety of *D. bulbifera* were fungitoxic. The bulbils are also widely used in traditional India and Chinese medicine for sore throat, gastric cancer and carcinoma of rectum and goitre.¹⁷ Ashidi *et al.*¹⁸ reported the leaf of *D. hirtiflora* for cancer treatment in Nigeria, while Sonibare and Abegunde¹⁹ reported medicinal application of the crude extract of tubers of *D. dumetorum* and *D. hirtiflora*. Species misidentification of botanical drugs due to diverse indigenous names given to the same species or several species with the same indigenous name is rampant in herbal market.²⁰ Therefore, leaf anatomy description of crude drugs may complement macroscopic description

of medicinal plants, which could be helpful in preparing monographs for plant identification. Before now, our research group has documented exploitation of the petiole, nodal segment and tuber/bulbil for identifying cultivars of *D. bulbifera* and *D. dumetorum*.²¹ Interestingly, no report from literature search has documented foliar micromorphological characters of the cultivars of *D. bulbifera* and *D. dumetorum*. The present study, therefore, investigates micromorphological characters of *D. bulbifera* cultivars (mauve and yellow); *D. dumetorum* (wild and edible); and *D. hirtiflora* (wild) using epidermal peel and mid-rib section of the leaves.

Materials and Methods

Plant collection and authentication

Fresh specimen of *D. hirtiflora* collected from Ode-Ekiti, Ekiti State in June, 2015 and cultivars of *D. dumetorum* and *D. bulbifera* collected from Baare village, Apata and Alabata village, Moniya in Ibadan (June, 2015) were identified and authenticated by Mr A. Adeyemo at the Forest Herbarium Ibadan (FHI) of the Forestry Research Institute of Nigeria. Voucher specimens were deposited in FHI as *Dioscorea hirtiflora* - FHI 108911, *Dioscorea dumetorum* (wild) FHI 110357, *Dioscorea dumetorum* (edible) - FHI 110356, *Dioscorea bulbifera* (mauve) - FHI 110358, *Dioscorea bulbifera* (yellow) - FHI 110355.

Leaf epidermal preparation

For epidermal preparation, fresh leaves 3-5 cm² were excised from the standard median part of the leaf lamina near the mid-rib. The scraping technique of Metcalfe and Chalk²² was used for the physical method of epidermis preparation while nitric acid treatment reported by Mohajer *et al.*²³ with slight modifications was used for chemical method. Briefly, for the preparation of epidermal peels by chemical method, fresh leaves were irrigated in concentrated trioxonitrate (V) acid (HNO₃) in a covered petri dish and warmed in a water bath (60°C) for 3-5 min. Bubbles indicated tissue disintegration and the epidermis was carefully transferred into petri dishes containing distilled water for cleansing (X4). Tissue debris was cleared off the epidermis with fine-hair, and epidermal peels of adaxial and abaxial were carefully obtained using a pair of forceps and a mounting needle. Epidermal peels were further cleared in 40% NaOCl solution (2.5 % w/v) for 10-15 min. The decolorized epidermal peels were then rinsed thoroughly

in distilled water (X5). The cleared surfaces were dehydrated, stained with Safranin O, and mounted as described above.

Palisade ratio determination

From the cleared epidermal peel, the number of palisade cells present in four adjacent epidermal cells was taken and divided by four to obtain the palisade ratio.²³

Stomatal index determination

Several epidermal cells and stomata per square mm were observed from the cleared leaf. From the data, stomatal index (St In) was calculated using the standard formula²⁴, $St\ In = [St \times 100 / (Ep + St)]$; Where St is the number of stomata per unit area, Ep is the number of epidermal cell in the same unit area. Other features like epidermal cell size, stomata, and trichome were measured quantitatively using a calibrated light microscope with eyepiece and stage micrometer.²⁵⁻²⁶ Similarly, stomatal number and trichome number were recorded per mm square. Qualitative and quantitative measurements were examined in twenty fields of view from five different slides.

Statistical analysis

Microsoft excel package (2019) was used to calculate the mean for each examined feature in a total of twenty fields of view from five different slides.

Results and Discussion

Morphological description

The leaf shape of the investigated *Dioscorea* species is cordate except in cultivars of *D. dumetorum* where it is ovate, leaf apex is acuminate in all and the base cordate except in *D. dumetorum* where it is decurrent (Figure 1). The leaves of *D. bulbifera* (yellow) and *D. dumetorum* (edible) are larger and wider than leaves of other cultivars. Bulbils obtained from wild *D. bulbifera* (mauve) were smaller, rough, brownish and more angular, whereas *D. bulbifera* (yellow) produced bigger bulbils, greyish, smooth and more rounded. In wild *D. dumetorum*, the tubers appear greyish with many scars and adventitious roots attached, while the edible type is brownish with smooth surfaces. Leaves of *D. dumetorum* are trifoliate and the tubers occur in cluster form, while the tubers of *D. hirtiflora* are slim, elongated and brownish.

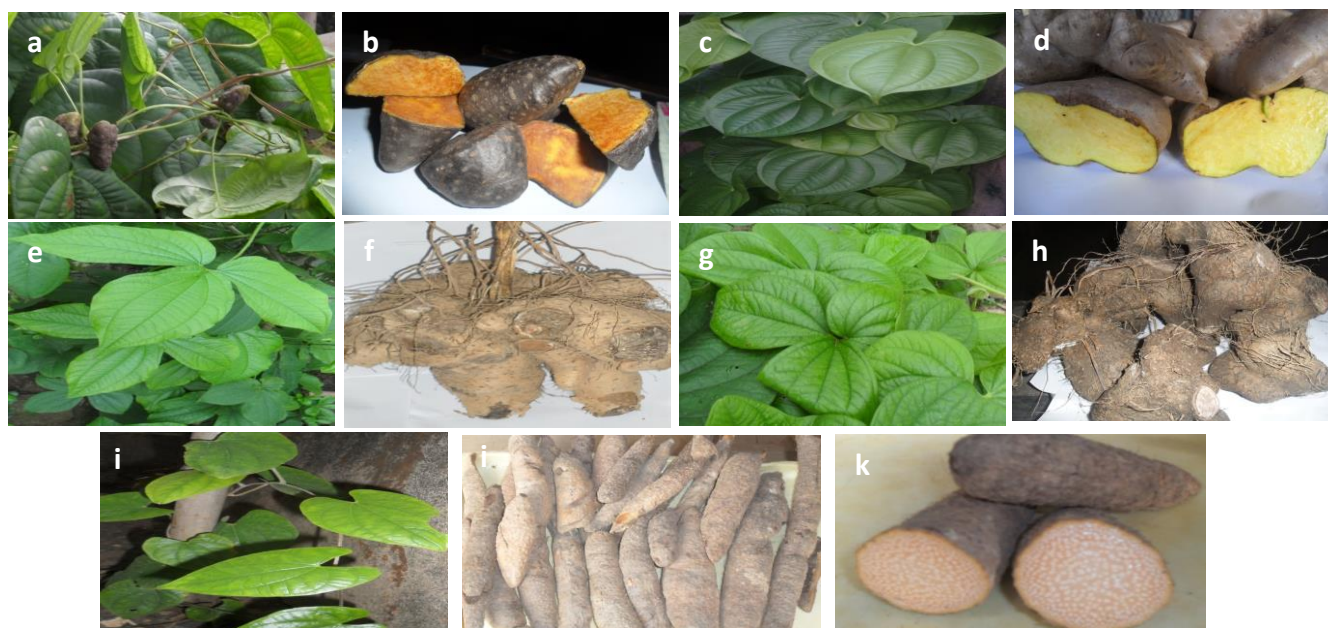


Figure 1: Morphological description of *Dioscorea* species. a. Leaf shape of *D. bulbifera* (mauve) b. Bulbils of *D. bulbifera* showing mauve colour when cut c. Leaf shape of *D. bulbifera* (yellow) d. Bulbils of *D. bulbifera* showing yellow colour when cut e. Leaf shape of *D. dumetorum* (wild) f. Tubers of *D. dumetorum* g. Leaf shape of *D. dumetorum* (edible) h. Tubers of *D. dumetorum* i. j. Leaf shape of *D. hirtiflora* j. Long slender tubers of *D. hirtiflora* k. Cut section of *D. hirtiflora*.

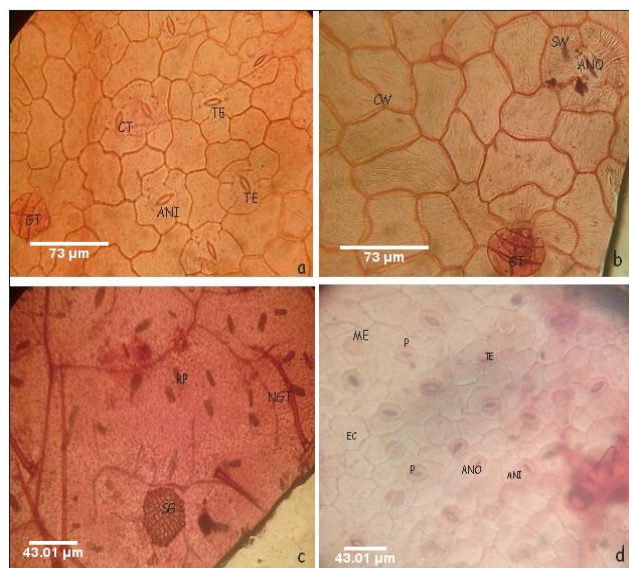


Figure 2: Epidermal cells, stomata and cuticular striations. a, b. Abaxial and adaxial surfaces of *D. bulbifera* (mauve) c. Adaxial surface of *D. dumetorum* (Edible) d. Abaxial surface of *D. hirtiflora*. Stomata types: ANI-Animocytic; ANO-Anomocytic; CT-Contiguous stomata; P-Paracytic; TE-Tetracytic, CW- Cell wall, EC- Epidermal cell, GT- glandular Trichome, NGT- Non-glandular trichome, Me-Meristemoid, RP-Raphides, SG- Secretoty glands, SW-Striated wall

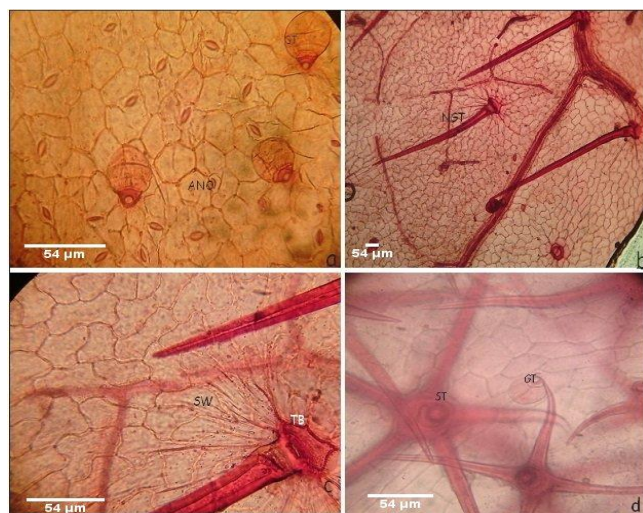


Figure 3: Trichome types. a. Abaxial surface of *D. bulbifera* (yellow) b, c. Adaxial and abaxial surfaces of *D. dumetorum* (edible) d. Abaxial surface of *D. hirtiflora*. GT-Glandular trichome, NGT- Non-glandular trichome, St- Stellate trichome, Tb- Trichome base, SW- striated wall

Qualitative description of epidermal features

Epidermal cells are rectangular, polygonal and irregular (Figure 2). The anticlinal walls are straight to slightly wavy in all species except in the abaxial surfaces of *D. dumetorum* cultivars where they are deeply undulating to sinuous (Table 1). Leaves of *D. hirtiflora* and cultivars of *D. bulbifera* are amphistomatic, while *D. dumetorum* leaves are hypostomatic. In cultivars of *D. bulbifera* (mauve and yellow) anisocytic, anomocytic, paracytic, tetracytic and contiguous stomata were present. In *D. hirtiflora*, stomatal type present include anisocytic, anomocytic, diacytic and paracytic while in *D. dumetorum*, stomatal type present include anisocytic, anomocytic, paracytic and tetracytic (Figure 2). Diacytic stomata featured prominently in *D. hirtiflora* (Figure 2d). Cuticular striations were on the abaxial surface of *D. hirtiflora*, adaxial

surface of cultivars of *D. bulbifera* and on both surfaces of *D. dumetorum* cultivars.

Epidermal cell shape being rectangular, polygonal and irregular with straight, slightly wavy, undulating and sinuous anticlinal wall pattern supports earlier report by Shah and Gopal²⁷ in stomata and trichome description of six species of *Dioscorea* (*D. bulbifera*, *D. oppositifolia*, *D. hispida*, *D. wallichii*, *D. belophylla* and *D. alata*). Similarly, Abdulrahman *et al.*²⁸ had also reported paracytic and anisocytic stomata in *D. bulbifera* while paracytic and tetracytic were also documented in *D. dumetorum* by the same authors. Stomata arrangement and types are known as the best taxonomic hierarchy criteria and offer efficient basis for exploring phylogenetic relationship in taxonomic hierarchy.²⁹ As many as four stomata types-anomocytic, anisocytic, paracytic and diacytic were found in the *Dioscorea* species.

Stomatal clusters occur where two stomata are side by side without any common subsidiary cell. They are unique leaf epidermal features reported only in a very limited number of genera of vascular plants.³⁰ Interestingly, stomatal cluster was observed in wild *D. bulbifera* (mauve) but absent in the yellow cultivar. This is in agreement with cluster stomata-contiguous stomata found in *D. oppositifolia* and *D. bulbifera* by Shah and Gopal.²⁷ They have also been reported in other monocotyledons in the families Gramineae³¹ and Amaryllidaceae.³² Changqi *et al.*³³ also reported similar cluster stomata pattern in *Camellia hemiana* and *C. tsingpiensis*. Being a very rare feature, it could serve as a diagnostic tool for distinguishing between the cultivars of *D. bulbifera*. Diacytic stomata featured only in *D. hirtiflora* but was absent in other species. Meristemoid was found by the side of a fully developed stoma in *D. hirtiflora*. Shah and Gopal²⁷ described such meristemoid as occurring occasionally and were found as a deeply stained cell by the side of the stoma. It may develop into a stoma, thus forming contiguous stomata or enlarging as a subsidiary cell so that the stoma has one subsidiary cell.

Cuticular striations have featured prominently as diagnostic features in plant taxonomy. For example, the cuticle on the epidermal leaf surface of *Baccharis* species have been reported to usually possess striations and they have been shown to be in a perpendicular and concentric ring in the direction of trichome base and stomata, respectively³⁴⁻³⁵. Cuticular striations on the abaxial surfaces of *D. hirtiflora*, cultivars of *D. bulbifera* and on both surfaces of *D. dumetorum* cultivars are possibly diagnostic features common to the family Dioscoreaceae. The striations radiate from the guard cells of the stomata and trichome bases. We had also reported cuticular striations in the wild and micropropagated leaves of *D. bulbifera*.²⁵

Calcium oxalate crystals in the form of raphides, which occurred in bundles of four to five (acicular in shape), were scattered on the surfaces of *D. hirtiflora* and cultivars of *D. dumetorum* but were more prominent on the abaxial surfaces (Figure 2c). Secretory glands were on the abaxial surfaces of wild *D. dumetorum* and *D. hirtiflora* (Figure 2c). Glandular trichomes with unicellular stalk and multicellular heads were found in all the investigated *Dioscorea* species (Figure 3a). Simple elongated unicellular trichomes were present in *D. dumetorum* (Figure 3b), while stellate trichomes were abundant on the abaxial surfaces of *D. hirtiflora* (Figure 3d). In addition, trichomes with unicellular stalk and unicellular head were found on the surfaces of *D. hirtiflora* and *D. dumetorum* (wild). Scars left behind due to fallen trichomes were described as trichome bases (Figure 3c).

Raphides are bundles of narrow, elongated needle-shaped crystals, usually of similar orientation, with pointed ends at maturity. Of the three types of calcium oxalate crystals in monocotyledons (raphides, styloids, and druses), raphides are the most common.³⁶ According to Ayensu³⁷, raphides are generally present in Dioscoreaceae. Epidermal peel of *D. hirtiflora* and cultivars of *D. dumetorum* showed needle-like bundles of four to five raphides scattered on the both surfaces, but more abundant on the abaxial surfaces. The present observation of raphides in *D. hirtiflora* and *D. dumetorum* is in conformity with reports of earlier workers on the presence of raphides in Dioscoreaceae. However, its absence in the cultivars of *D. bulbifera* could be characteristic and be employed to separate *D. bulbifera* from *D. dumetorum* and *D. hirtiflora*. Another diagnostic feature present on the abaxial surfaces of *D. hirtiflora* and wild *D. dumetorum* was secretory gland, which was absent in others.

Table 1: Micromorphological features of epidermal cells and their dimensions.

Taxa	Leaf surface	Epidermal cell shape, pattern of anticlinal wall, striation	Epidermal cell length (μm)	Epidermal cell breadth (μm)	Epidermal cell number (mm^2)
<i>D. hirtiflora</i>	adaxial	polygonal, straight-slightly wavy, not striated	64.9	44.0	59.2
	abaxial	polygonal, straight-slightly wavy, striated	46.8	26.5	111.6
<i>D. bulbifera</i> (mauve)	adaxial	polygonal, straight-slightly wavy, not striated	82.1	43.4	31.1
	abaxial	Irregular, slightly wavy, not striated	67.9	28.5	41.6
<i>D. bulbifera</i> (yellow)	adaxial	polygonal, straight, striated	70.4	42.5	30.1
	abaxial	polygonal, straight-slightly wavy, not striated	52.9	30.6	56.6
<i>D. dumetorum</i> (edible)	adaxial	rectangular, straight-slightly wavy, striated	54.3	31.1	45.8
	abaxial	Irregular, deeply undulating-sinuuous, striated	48.3	17.8	96.5
<i>D. dumetorum</i> (wild)	adaxial	polygonal, straight-slightly wavy, striated	50.1	26.0	70.3
	abaxial	Irregular, deeply undulating-sinuuous, striated	45.3	15.8	107.1

Table 2: Micromorphological features of stomata and palisade ratio.

Taxa	Leaf surface	Stomatal type	Stomatal frequency (mm^2)	Stomata length (μm)	Stomata breadth (μm)	Stomatal index (%)	Palisade ratio
<i>D. hirtiflora</i>	adaxial	anisocytic, anomocytic, diacytic, paracytic	9.7	16.8	8.0	22.0	10.1
	abaxial	anisocytic, anomocytic, diacytic, paracytic tetracytic	15.0	16.5	6.8	16.5	6.8
<i>D. bulbifera</i> (mauve)	adaxial	anisocytic, anomocytic,	-	22.3	5.4	-	9.4
	abaxial	anisocytic, anomocytic, paracytic, tetracytic, contagious	7.6	23.1	7.5	16.6	4.3
<i>D. bulbifera</i> (yellow)	adaxial	anisocytic, anomocytic	-	19.1	5.8	-	10.9
	abaxial	anisocytic, anomocytic, paracytic, tetracytic	11.8	19.9	7.1	20.5	5.1
<i>D. dumetorum</i> (edible)	adaxial	absent	absent	absent	absent	absent	4.8
	abaxial	anisocytic, anomocytic, paracytic, tetracytic,	20.4	15.3	6.0	18.9	4.4
<i>D. dumetorum</i> (wild)	adaxial	absent	absent	absent	absent	absent	4.8
	abaxial	anisocytic, anomocytic, paracytic, tetracytic,	15.2	14.3	5.5	14.1	2.8

∴ not determined.

The presence of trichomes in plants are protective in nature due to secretion of secondary metabolites, especially to buds of some plants offering them first line of defence against intruding organisms.³⁸⁻³⁹ Glandular secretory trichomes can influence plant functions by their physical properties (size, density) and can affect host disease and pest resistance based on the phytochemicals they secrete.³⁸ Several workers have found studies on trichomes taxonomically significant.⁴⁰ Metcalfe and Chalk²² had often used presence of a particular trichome type to delimit species, genera or even whole families. Trichome was found more useful taxonomically in the study of the genus *Baccharis* and was successfully adopted than any of the other epidermal characters in distinguishing the species into sections³⁴.

In the present study, glandular trichomes (capitate unicellular stalk with multicellular head) were found in all the *Dioscorea* species. Diagnostic characters like secretory oil glands, stellate and glandular trichomes were in consonant with observations from the epidermal peel of regenerated and wild *D. hirtiflora* reported by Adeniran *et al.*⁴¹ in the assessment of genetic fidelity of *D. bulbifera* and *D. hirtiflora* and medicinal bioactivity produced from their tuberous roots. However, the uniqueness of stellate trichomes in *D. hirtiflora* and

simple unicellular trichome in cultivars of *D. dumetorum* are noteworthy. Also, of significant interest is the presence of glandular trichomes with capitate unicellular stalk and unicellular head on the abaxial surfaces of *D. hirtiflora* and *D. dumetorum* (wild). The cultivars of *D. dumetorum* are distinguishable by this diagnostic character.

Quantitative description of epidermal features

Mean frequency of epidermal cell and dimensions are summarized in Table 1 while stomatal frequency, stomatal index, stomatal dimensions and palisade ratio for the studied *Dioscorea* species are presented in Table 2. Different types of trichomes, their frequencies and dimensions are summarized in Table 3. Generally, mean palisade ratio, glandular trichome size, and epidermal cell size were higher on the adaxial surfaces than the abaxial surfaces of the studied species.

Different diagnostic epidermal features like stomatal index and palisade ratio have been known to have constant range for particular species and are not affected by geographical variation or age⁴² and are useful for characterization, standardization and identification of specific species. To distinguish the two cultivars, on the abaxial

surface, stomatal index was higher in yellow *D. bulbifera* (20.5) and edible *D. dumetorum* (18.9) compared with their varieties- mauve *D. bulbifera* (16.6) and wild *D. dumetorum* (14.1). In contrast, stomatal size in abaxial was higher in mauve *D. bulbifera* (23.1 by 7.5 μm) and edible *D. dumetorum* (15.3 by 6.0 μm) compared with their varieties- yellow *D. bulbifera* (19.9 by 7.1 μm) and wild *D. dumetorum* (14.3 by 5.5 μm). Stomatal size was inversely proportional to the stomatal number in the present investigation and therefore conforms to earlier report by Metcalfe and Chalk.²² Palisade ratio of *D. bulbifera* (yellow) and *D. dumetorum* (edible) were higher than their varieties on the abaxial and adaxial surfaces except on the adaxial surfaces of varieties of *D. dumetorum* where there was no difference. Epidermal cell number/ mm^2 was highest in *D. hirtiflora* (111.6) and lowest in mauve *D. bulbifera* (41.6) on the abaxial surface but highest in wild *D. dumetorum* (70.3) and lowest in yellow *D. bulbifera* (30.1) on the adaxial surface.

Qualitative and quantitative microscopic characters have been reported to be vital in plant identification and drug evaluation in Pharmacognosy, especially when they are in fragmented form.² Specifically, Gul *et al*⁴³ reported the use of quantitative parameters like dimension of trichomes, epidermal cells and stomatal index which were successfully utilized in delimiting 22 Lamiaceae species from 15 genera. The present investigation conforms to Gul *et al*⁴³ assertion that quantitative measurements like epidermal cell size, glandular trichome size, and non-glandular trichome size served as an important tool in distinguishing the three wild *Dioscorea* species and indeed can also aid recognition of cultivars of *D. bulbifera* and *D. dumetorum*.

Transverse section of leaf showing the mid-rib region

Transverse section of the leaf showing the mid rib (Figure 4) revealed a single compact epidermal layer, a single upper palisade cell and a compact polygonal but angular collenchyma cells in all species. Xylem and phloem vessels occurred centrally and surrounded by sclerenchyma cells. In the outer epidermal layer of the transverse section of *D. hirtiflora* and cultivars of *D. dumetorum* were many stellate trichome and simple unicellular trichomes with fewer glandular trichomes, while in *D. bulbifera* trichomes were absent.

The arrangement of cells and tissues in the mid rib section followed the same pattern except for presence or absence of trichomes in the outer epidermal cell. This is useful in identifying the crude drug when fragmented.

The micromorphological characters obtained from the three wild *Dioscorea* species in South-western Nigeria may be useful and serve as complementing tools for correctly identifying these botanical drugs, particularly when accompanied by other taxonomic or systematic information, including morphology and DNA sequences. Interestingly, our research group has distinguished between the

cultivars of *D. Dumetorum* (the wild and edible cultivars) using DNA barcoding.¹⁵

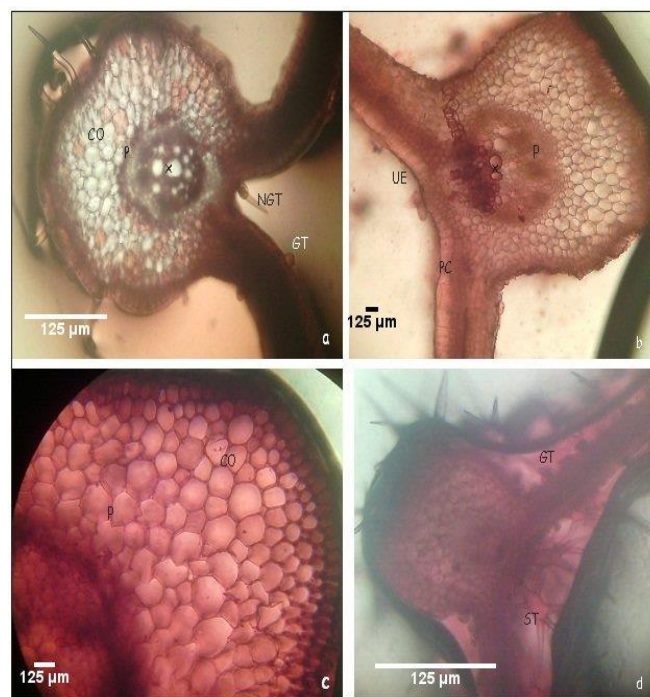


Figure 4: Transverse section of mid-rib region of *Dioscorea* species. a. *D. dumetorum* (edible) b. *D. bulbifera* (mauve) c. *D. bulbifera* (yellow) d. *D. hirtiflora*. CO-Collenchyma, P- parenchyma, X- Xylem, UE- Upper epidermis, PC- Palisade cells, GT- Glandular trichome, NGT- Non-glandular trichome, ST- stellate trichome

Conclusion

The diagnostic features provided in this study, such as stomatal type, trichome, secretory glands, epidermal shapecalcium oxalate crystals (raphides) and anticlinal walls, along with their quantitative measurements, may be useful in preparing sets of standards for the identification of *Dioscorea* species, providing a valuable tool for collecting and preserving these species. In the appendix, an indented dichotomous key is provided for species delimitation based on quantitative and qualitative variance in foliar micromorphological features.

Table 3: Micromorphological features of trichome, calcium oxalate and secretory glands.

Taxa	Leaf surface	*Trichome type	Trichome frequency (μm)	Trichome Length (μm)	Trichome breadth (μm)	Calcium Oxalate	Secretory glands
<i>D. hirtiflora</i>	adaxial	G, St	23.8	48.5	37.3	raphide	absent
	abaxial	G, St	4.5	41.0	30.3	raphide	present
<i>D. bulbifera</i> (mauve)	adaxial	G	-	57.3	41.8	absent	absent
	abaxial	G	2.1	49.0	41.3	absent	absent
<i>D. bulbifera</i> (yellow)	adaxial	G	-	77.0	42.0	absent	absent
	abaxial	G	2.6	50.9	41.4	absent	absent
<i>D. dumetorum</i> (edible)	adaxial	SU, G	-	57.8	31.5	raphide	absent
	abaxial	SU, G	5.8	45.8	28.5	raphide	absent
<i>D. dumetorum</i> (wild)	adaxial	SU, G	-	55.5	27.0	raphide	absent
	abaxial	SU, G	7.5	39.3	26.5	raphide	present

*main trichome types (G-glandular, St-stellate, SU-simple unicellular), -: not determined.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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