RESEARCH ARTICLE

ISSN: 0331 - 670X

https://doi.org/10.51412/psnnjp.2022.27

The Nigerian Journal of PHARMACY

AS MEN OF HONOUR WE JOIN HANDS

THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

Vegetative anatomical characteristics and trichome morphology of the monotypic *Martynia annua* L. (Martyniaceae) for use in identification and crude drug search

Ogundipe Oluwatoyin Temitayo[1] and Kadiri Akeem Babalola[1]

[1]Department of Botany, Faculty of Science, University of Lagos, Akoka, Lagos Nigeria

ARTICLE INFO

Article history:

Received 23 July 2022
Revised 14 August 2022
Accepted 25 August 2022
Online Published 30 October 2022

Keywords:

Eudicot,
light microscopy,
pharmacognosy,
taxonomy,
vegetative and reproductive parts

* *Corresponding Author:*
akadiri@unilag.edu.ng
https://orcid.org/0000-0003-4002-9652
+234 802 067 3756

ABSTRACT

Background: Leaf epidermis and indumenta structure of *M. annua* were studied with the aid of microscopy for easy identification of the plant materials when in fragments and for crude drug search. Stomata and trichome morphology appear significant in identifying the species. Paracytic and anomocytic stomatal types are basic but polocytic is additional on the abaxial surface of the epidermis.The overwhelming reports on the folkloric uses of *Martynia annua* L. (Martyniaceae) and the sole reliance on exo-morphology for its identification which gives room to error form the basis for this study. Moreso, the need to utilize as many data as possible for accurate identification of the plant is another important reason for its choice for investigation.

Methods: A total of 20 plant samples were examined. 100 leaves and 40 stems, petioles and midribs obtained from both herbarium samples of the plant were examined. The epidermis was recovered after treatment with concentrated trioxonitrate (v) acid (HNO_3) in capped specimen bottles for about 8–24hrs to macerate the mesophyll. Samples were stained with acidified phloroglucinol and then one drop of toluidine blue. They were mounted on glass slides and examined under the microscope (XSZ-170BN Olympus) and photomicrographs were taken with microscope eyepiece camera (Toupview 3.7). Line drawings were made with camera lucida drawing instrument.

Results: Interesting folia epidermal features were encountered. These include 3 stomatal types, two main types of trichomes namely: - Type 1: Long to short multicellular-glandular trichomes with globular heads and Type 2: Unicellular non-glandular globular-headed trichomes with stalk or no stalk.

Conclusion: The report of leaf teeth in *M. annua* which is the begonioid type is novel and cell shape of the perivascular structures is oval to polyhedral. A suite of these characters will aid identification of *M. annua* when a whole fresh plant specimen is absent.

1. Introduction

Martynia annua L. (Martyniaceae) whose common name is Tiger's or Devil's claw is the only species of the genus *Martynia* L. whose synonyms included *M. perennis* L. and *M. foliis-dentatis* L[1]. It belongs to the family Martyniaceae[2] and its habitat preference is the moist waste dump sites. It is generally used as food and medicine[3] in its native South America and throughout the tropical regions of the world including Nigeria[4,5], where it has naturalized. It is characterized by sticky, hairy leaves, orchid-like flowers and woody, beak shaped pods[(4,3)]. The plant can be identified with the exo-morphological characters but there is paucity of data on its anatomical characteristics. Folkloric accounts reported *M. annua* has having a wide spectrum of medicinal uses[3,7,8,9]. It is used in the treatment of epilepsy, sore throat and inflammatory disorders, anthelmintic, analgesic, anti-pyretic, anti-bacterial, anti-convulsant, antifertility, anti-nociceptive, anti-oxidant, CNS depressant, anti-diabetic and it has wound healing activity.

Previous accounts have only documented limited information on the leaf epidermis and stomata while a few other data from pharmacognostic research has been reported[3,5]. Given that enormous data is required for adequate description of any plant taxon for its systematic comprehension[6] and *M. annua* with very limited taxonomic data, this study was undertaken. The anatomical features of the leaf epidermis and trichome morphology of the species were investigated with a view to add to the existing criteria for easy identification of the plant samples even when they are available as fragments or adulterated with other plant materials, the common state of herbal materials in the African medicinal plants markets.

2. Materials and methods

A total of 100 leaves and 40 stems, petioles and midribs obtained from herbarium specimens were examined. The herbarium samples were obtained from University of Lagos (LUH) and Forestry Research Institute of Nigeria (FHI) while the dried samples were preserved as herbarium specimens (Table 1). The anatomical methods and terminologies[10,11,12], with some modifications. For leaf epidermal study, 5 leaves from each of the 20 plant samples were investigated. The epidermis was examined after acid treatment which involved cutting 3–7 cm^2 from the standard median part of the leaf lamina, near the mid-rib following the approaches of [13, 14] with some modifications. Dried herbarium leaf samples were boiled in water for 30 min and subsequently soaked in concentrated trioxonitrate (v) acid (HNO_3) in capped specimen bottles for about 8–24 hrs to macerate the mesophyll. Tissue disintegration was indicated by bubbles and the epidermal layers were separated and transferred into Petri dishes containing water for cleansing. Tissue debris was cleared off the epidermis with fine-hair brush and washed in several changes of water. Drops of different grades of ethanol 50% – 100%, were added in turn to dehydrate the cells. The preparations were later stained with Safranin O in 50% alcohol for about 5 min before mounting in glycerine on glass slides. The epidermal layers were mounted on glass slides with the uppermost surfaces facing up, covered with cover-slips and ringed with nail varnish to prevent dehydration. For anatomical study of the petiole, midrib and stem, 2 specimens were investigated per plant sample. They were freely sectioned using razor blade and thin slices obtained were kept in water before transferring onto glass slide where a few drops of absolute ethyl alcohol were added for tissue hardening. Samples were counter-stained using 2-3 drops of acidified phloroglucinol and then one drop of toluidine blue[15,16]. Excess stain was washed off with 50% ethyl alcohol before a drop of glycerine was added. The prepared specimens were protected with cover slips and then ringed with nail lacquer to prevent dehydration. All preparations were observed with XSZ-170BN Olympus microscope (Tokyo, Japan) and photomicrographs were taken with Toupview 3.7 microscope eyepiece camera, version UCMOS08000KPA-U-NA-N-M-CY-NA attached to a Pentium IV computer and digital images were captured, and analysed. Line drawings were made with camera lucida drawing apparatus.

Table 1: Herbarium data of the specimens of *M. annua* used for the study

(1)- Magbagbeola & others, 26/6/81, FHI 10094743; (2)- Daramola & Ihe, 13/9/1978, FHI 86445; (3)- Keay, R.W.J. 16/6/62, FHI 46308; (4)- Emwiogbon, J. A., 22/7/65, FHI 56802; (5)- Onochie, C. F. A., 26/5/60 FHI 34001; (6)- Umana O. A., 28/7/59, FHI 34507; (7)- Gbile Z. O., 26/9/78,FHI 84316; (8)- Obaseki J. K. O., 27/6/49, FHI 23827; (9)- Onochie C. F. A., 14/7/48, FHI 10019117; (10)- Sofoluwe F. O., 19/4/58, FHI 38160; (11)- Olorunfemi & others, 16/7/80, FHI 93511; (12)- Odewo T.K., 26/4/89, FHI 103698; (13)- Abrah L. H., September, 1950, FHI 36176; (14)- Okeke & Adebusuyi, 12/5/59, FHI 18248; (15)- Emwiogbon J. A., 29/7/66, FHI 60351; (16)- Olorunfemi & others, 15/6/79, FHI 92195; (17)- Onyeachusim O., 24/4/63, FHI 47506; (18)- Odewo & others , 6/8/79, FHI 91236; (19)- Kadiri and Ogundipe, 7/8/2017, LUH 9517; (20)- Kadiri and Ogundipe, 8/8/2017 LUH 9518.
Each record follows collector(s) name, date of collection and herbarium number respectively.

The abbreviations of the herbaria[36]

3. Results

All the twenty (20) herbarium samples of the species collected between 1948 and 2017 by different workers and deposited in two herbaria in Nigeria were investigated (Table 1). The micro-features of the leaf and stem of *M. annua* as seen under the microscope are shown in Figures 1-5 and Tables 2 and 3. The leaf is amphistomatic (stomata are present on both surfaces of the epidermis). On the adaxial surface, the anticlinal wall is sinuous with anomocytic stomatal type and gland ducts within the guard cells (Fig. 1A). On the lower epidermis, gland ducts were recorded on the straight-curved anticlinal walls and 3 different stomatal types were found namely, anomocytic, paracytic and polocytic (Table 1B). 1-2 nerve endings were recorded within the leaf areole whose shape is either rectangular or triangular (Fig. 1C). The proximal, median and distal sections of the petioles investigated appear uniform with centrally-located collateral vascular bundles and copious perivascular tissue (Fig. 2A). On the adaxial surface, the petiole is widely furrowed whereas on the abaxial surface, it is boat-shaped (Fig. 2A) The midrib is dome-shaped and U-shaped adaxially and abaxially respectively (Fig. 2B) while the vascular bundle is U-V-shaped. The vascular bundle is collateral and circular as well. The perivascular bundle comprises collenchyma, sclerenchyma and parenchyma whose cell shapes varies from oval to polyhedral (Fig. 3C, E, F).

3.1 Trichome and leaf teeth morphology

Trichomes were recorded on the leaf lamina, margin, petiole, midrib and stem, pedicel and petals of the plant. On the epidermal surfaces- glandular, multicellular, long to short trichomes with globular heads were recorded (Fig 1D). The petiole is entirely pubescent on the surface (Fig. 2A) while the midrib surface is covered with multicellular glandular trichomes (Fig. 2B). The stem is covered with trichomes of different types namely (i) multicellular globular headed, (ii) unicellular globular headed and (iii) unicellular non-glandular which may be stalked or non-stalked (Fig. 3A, B, D). Simple multicellular conical trichomes were found on the leaf teeth as marginal projection (Figs. 4A, 4B, 4G).

The simple multicellular glandular trichomes found has four sub- types namely; cylindrical-headed, found on the lamina (Fig. 4D, 4E), petiole (Fig. 5A), pedicel (Fig. 5B) and stem (Figs. 3A, B, D, F, 5F) and the nodular type found on the leaf margin (Fig. 4F). The globular trichomes are non-stalked and short-statured as recorded on the leaf lamina (Fig. 4G, F) and petals (Fig. 5D), stalked-multicellular and long as recorded on the petals (Fig 5C, D_2) and stem (Figs. 3A, F) while knob-headed simple multicellular glandular trichomes were also found on the petals (Fig. 5E).

The leaf margin is dentate having continuous and generally outward pointing teeth. The tooth type is begonioid; it is dome-shaped comprising two longitudinal strands of vascular bundles with one of them supplying the terminal tooth. It is surrounded by abundant parenchymatous cells and two layers of rectangular collenchyma cells (Fig. 4A). The tip of the teeth usually has simple multicellular conical trichomes (Fig. 4B) and there is a furrow between any two teeth which usually bears a multicellular trichome (Fig. 4C).

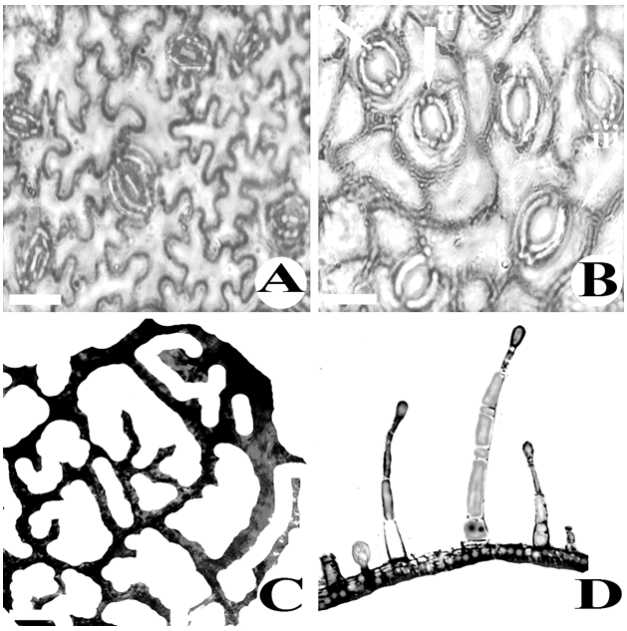

Figure 1: Leaf micro-morphology of *Martynia annua*. A: Upper surface leaf epidermis showing sinuous walls paracytic stomata and gland ducts on the guard cells. B: Lower surface leaf epidermis showing gland ducts on the straight-curved anticlinal walls and stomatal types, (i) polocytic (ii) paracytic (iii) anomocytic. C: Leaf areole with 1-2 nerve endings with rectangular to triangular shape. D: Unicellular and multicellular globular head glandular trichomes recorded on the leaf. Scale bar is 50 µm except D which is 150 µm.

Table 2: Quantitative and qualitatative pharmacognostic characteristics of leaf epidermis of *Martynia annua*

Surface	Stomata					Epidermal cells			
	Mean number per mm²	Length Min(M±S.E) Max (µm)	Width Min(M±S.E) Max (µm)	Type	Stomatal Index (%)	Mean number per mm²	Length Min(M±S.E)Max (µm)	Width Min(M±S.E) Max (µm)	Wall thickness (µm) / pattern
Adaxial	47	7.5(8.6±1.0) 9.8	8.75(9.8±0.7)12.25	Anomocytic, paracytic	29.3	54	32.5 (34.525±1.7)45 .8	12.3 (14.5±0.7)15. 8	3.5 / sinuous
Abaxial	36	19.2(21.3±0. 3)23.1	13(11.3±0.2) 15.8	Anomocytic, paracytic, polocytic	32.4	48	27.4(28.3±0.5) 30.8	17(18.3±0.4)2 0.9	5.3 / straight to curved

Min(Mean±S.E)Max (µm) = Minimum value (Mean value ± Standard error) Maximum value .

Table 3: Quantitative trichome characteristics of *Martynia annua*.

Trichomes					
Surface		Number per mm²	Length Min(M±S.E)Max (µm)	Width Min(M±S.E)Max (µm)	Trichome Index (%)
Adaxial		23	140(211.8±8.2)280	10.5(19.3±2.6)26.3	29.9
Abaxial		13	183.8(222.3±9.8)280.3	17.5(20.7±1.0)22.8	21.3
	Stem	49	120(280±14.3)294.8	21(28±2.4)31.5	
	Petiole	54 (68)	183.8(206.5±10.9)238.5	24(34.3±8.4)39.6	
	Midrib	66 (88)	105(220.5±11.2)302.7	10.5(21.9±4.1)35	

Min(Mean ±S.E)Max (µm) = Minimum value (Mean value ± Standard error) Maximum value. Values in parenthesis are number of trichomes found on the undersurface.

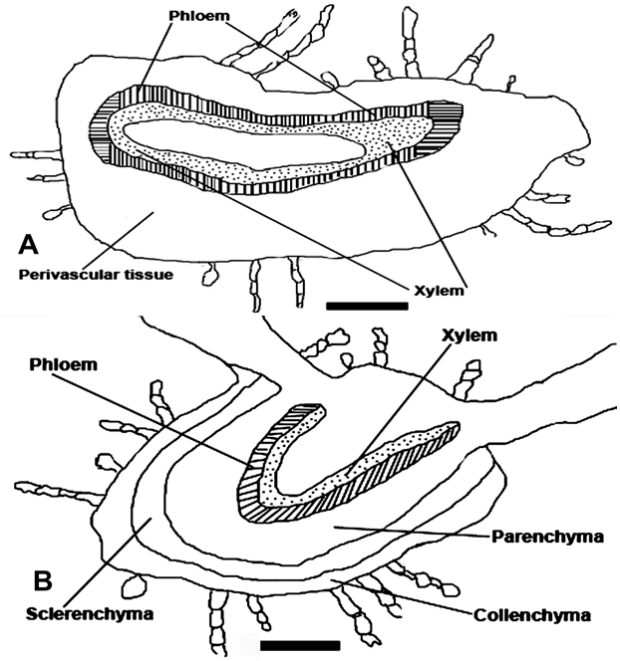

Figure 2: Anatomy of petiole and midrib of *Martynia annua.* A: Petiole. B: Midrib.
Scale bar is 250 µm.

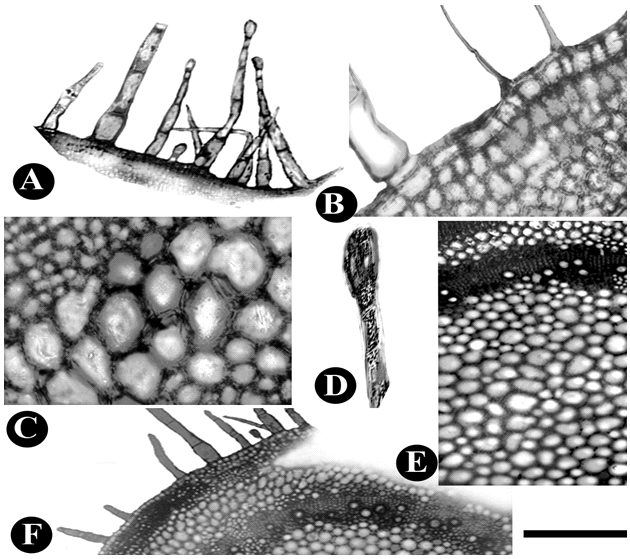

Figure 3: Stem anatomical characteristics of *Martynia annua*. A: Trichome types: i- multicellular globular head glandular trichomes, ii- Unicellular globular head glandular trichome iii-Unicellular non-glandular, trichomes. B: Unicellular non glandular and multicellular globular head glandular trichomes with broken parts. C, E, F: Cortical portion of the stem showing oval shaped and differently thickened collenchyma cells, oval-circular lignified sclerenchyma cells and polyhedral-shaped parenchyma cells. D. Globular head of the glandular trichomes. Scale bar is 25 µm except A and D which are 200 µm

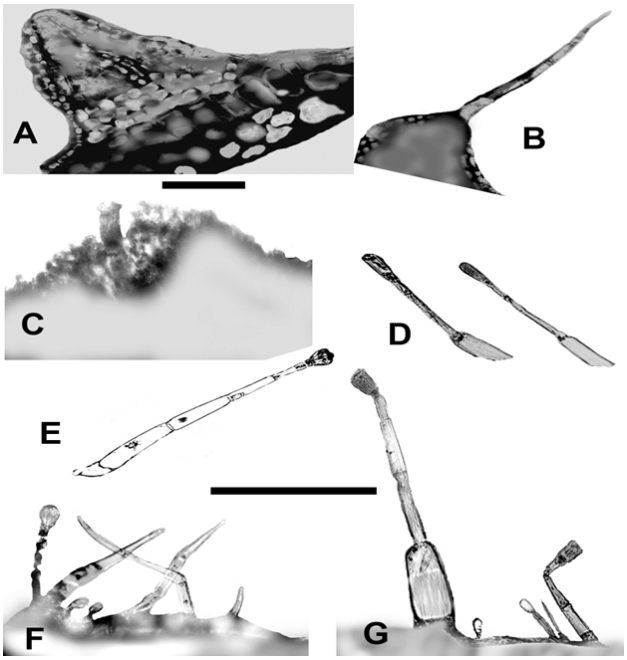

Figure 4: Trichome types found on leaf teeth, leaf margin and lamina of *Martynia annua*. A-C: leaf teeth, D, E, G: Hair types on the leaf lamina. F: Hair types on the leaf margin. Scale bars: A= 50 µm. B-G= 250 µm.

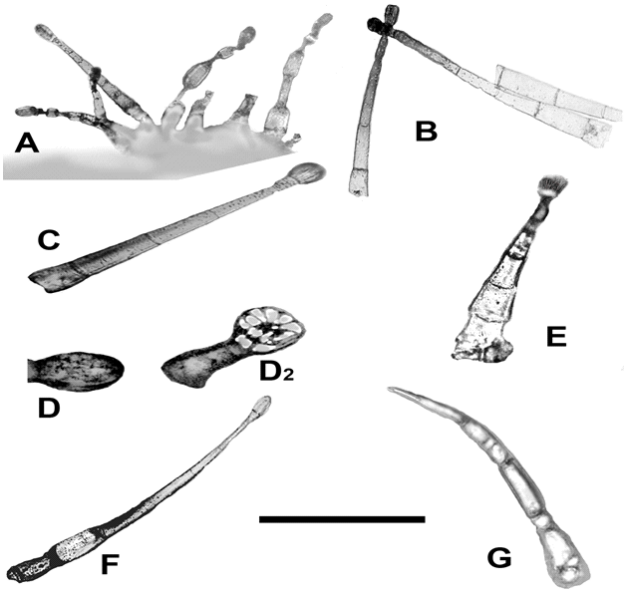

Figure 5: Trichome types found on leaf lamina, stem, pedicel and petals of *Martynia annua*. A= petiole, B= pedicel, C-E= petal, F= stem, G= leaf lamina. Scale bar: 250 µm.

4. Discussion

An updated account of the existing anatomical features of *M. annua* is presented. This is to sufficiently define the species for purposeful use in crude drug research and identification in case there is a mix-up with samples of another related species. The studied plant parts namely:- leaf, stem, petiole and midrib and petals of the species were selected because of their taxonomic relevance in the family Martyniaceae and angiosperms in general[5,17,18,19,20.21,22]. The leaf is amphistomatic, a condition whereby stomata are present on both surfaces of the leaf. with the abaxial surface having greater number and diversity namely, polocytic paracytic and anomocytic. Paracytic stomatal type has been indicated as the major type for the species[17]. However, multiple stomata types have been reported in many plant taxa and they are very useful for clear distinction of species, especially in the species-rich genera [13,14,22]. Trichome is another important character that can be used to describe *M. annua* as its parts are covered by trichomes. They were recorded in the epidermis, petiole, midrib, stem, pedicel, petals and leaf teeth. There are two main types which are long to short multicellular glandular trichomes with globular heads and unicellular non-glandular globular headed which are either stalked or non-stalked. The simple multicellular glandular trichomes can be further sub-divided into 4 forms which are cylindrical-headed as found on the lamina, petiole, pedicel and stem and the nodular type on the leaf margin; the globular trichomes which may be non-stalked and short-statured as recorded on the leaf lamina and petals, or stalked-multicellular and long as found on the petals and stem while knob-headed simple multicellular glandular trichomes were found on the petals.

Trichome index as used to define *Grewia lasiocarpa* E. Mey. ex Harv[23] varied from 21.3-29.9% on the surfaces of the epidermis in *M. annua*[23]. Trichomes of *M. annua* are generally greater in number on the upper part of all the structures studied except the leaf epidermis while the midrib and petiole had longer trichomes than other plant parts studied. However, trichomes have been employed to define the edible and medicinal *G. lasiocarpa* where three different types peltate, capitate and non-glandular (simple, stellate, multiangulate-stellate) trichomes were found on the leaves and stem bark[23]. Similarly, seed trichomes have been used for subspecific differentiation between the Kenya and Tanzanian populations of *Hibiscus altissimus* Horny[24]. Trichomes are taxonomically useful[25.] Their other uses include serving as a chemical store; useful for monitoring pollution, helpful in minimizing transpiration as well as in the control of the plant body temperature, and prevention of herbivory[6,26].

Other anatomical features of the species which are taxonomically useful are 1-2 nerve endings recorded within the rectangular - triangular leaf areoles, furrowed petiole on the adaxial surface as well as dome-shaped midrib on the adaxial surface and its centrally located vascular bundle. Nerves ramification in the areole has been employed amongst other leaf epidermal features for plant classifications and species distinction while petiole and midrib anatomical features have been used in resolving taxonomic confusion in many plant taxa[22,27,28,29].

Leaf teeth are projections on the leaf blade margin and they are structurally variable with characters useful for taxonomy and phylogeny[30]. The tooth type in *Martynia annua* is begonioid with associated colleters which are sticky in nature because of the mucilage they contain[30]. The begonioid tooth is characteristic of margins and the floral parts in eudicots[31]. Colleters as a component of the leaf teeth, especially young leaves is one of the mucilage-secreting hairs that clothe many plant surfaces and they have been used to distinguish two different groups in the genus *Ilex*[30] and have been described on leaves[32,33], stems, flowers and other organs of more than 60 dicotyledonous families[34].

A suite of these anatomical characters can be used for distinguishing *M. annua* especially when in dried fragments or mixed up with other medicinally useful plant samples; the usual state the medicinal herbs are offered for sale in the trado-medical plant markets in Africa. These characters can be helpful in pharmacognostic research work on the species[6,8,9] and as already enunciated and documented for other taxa in angiosperms[10,35]. We therefore suggest further work particularly on the chemical constituents of the trichomes of *M. annua* because they are copious on the plant body appearing as glandular structures which microscopy has revealed to serve as the repository of the plant chemicals.

5. Conclusion

Anatomical characteristics obtained from some vegetative and reproductive parts of *Martynia annua* have been documented for use in the identification of the species. When the plant samples are fragmentary or mixed up with another plant sample, the documented characteristics are sufficient for distinguishing the species.

Acknowledgement

This is another contribution to the project on taxonomic understanding of the medicinally useful monotypic plant

taxa by the Department of Botany, University of Lagos, Nigeria towards drug search. The encouragement and contribution of the senior author to crude drug research have made this work successful. He has passed on the torch of knowledge from the botanical perspectives to assist the pharmocognosists and other crude drug researchers in Nigeria. The authors are grateful to the herbarium curators at the Forest Herbarium, Ibadan and University of Lagos for granting access to laboratory infrastructure and the plant samples investigated.

References

1. Richard H 1969. Nomenclatural History and Typification of *Martynia* and *Proboscidea* (Martyniaceae). *Taxon*, 18 (5): 527-534.
2. Willis JC 1973. *A Dictionary of the flowering plants and ferns.* Cambridge University Press, Cambridge. 1213pp.
3. Kenwat R, Prasad P, Satapathy T, Roy A 2013. *Martynia annua*: An Overview. *UK Journal of Pharmaceutical and Biosciences*, 1(1): 7-10.
4. Hutchinson J, Dalziel JM 1958. *Flora of West Tropical Africa*. White Friars Press Ltd, London. 295pp.
5. Inamdhar JA, Gangadhara M 1977. Studies on the trichomes of some Euphorbiaceae. *Feddes Repertorium,* 88: 1033–111.
6. Davis PH, Heywood VH 1963. *Principles of Angiosperm Taxonomy*. Oliver and Boyd. Ltd., Edinburgh. 556 pp.
7. Santram L, Singhai AK 2011. Preliminary pharmacological evaluation of *Martyni annua* Linn leaves for wound healing. *Asian Pacific Journal of Tropical Biomedicine*, 1(6): 421–427.
8. Malik JK, Katare V 2020. Pharmacological activities of *Martynia* (*Martynia annua* Linn.): A Brief Review. *Asian Plant Research Journal*, 4(2): 46-51.
9. Akin-Obasola BJ, Obe BW, Adewumi AE 2021. Effect of *Martynia nnua* (Devil's Claw) Leaf meal on the reproductive and growth performance of O*reochromis niloticus* (*Nile tilapia*). *Pakistan Journal of Nutrition,* 20: 18-24.
10. Ajayi GO, Kadiri AB, Ibiloye AE 2015. Pharmacognostic characteristics of the West African *Jateorhiza macrantha* (Hook.F.) Exell & Mendonça (Menispermaceae) *International Journal of Pharmacognosy and Phytochemical Research*, 7(5): 1001-1006.
11. Kadiri AB, Ayodele AE 2010. Anatomical characteristics of some commercial timbers from Nigeria. 1. Structures of wood elements. *Nigerian Journal of Botany,* 23(1): 143-150.
12. Wilkinson HP 1989. Leaf Anatomy of Menispermaceae tribe Tilicoreae Miers. *Botanical Journal of the Linnean Society,* 125-174.
13. Kadiri AB, Olowokudejo JD 2016. Systematic significance of foliar epidermis and tendril morphology in three West African genera of Cucurbitaceae: *Momordica* L., *Luffa* Mill. and *Trichosanthes* L, *Webbia,* 71(1): 91-105.
14. Ogundipe OT, Kadiri AB 2012. Comparative foliar epidermal morphology of the West African species of Amaranthaceae Juss. *Feddes Repertorium*, 123: 97–116.
15, Carson FL 1997. *Histotechnology. A Self-Instructional Text.* American Society of Clinical Pathologists, Chicago. 160pp.
16. Cutler DF 1978. *Applied Plant Anatomy*. Longman Group Limited. New York. 103pp
17. Metcalfe CR, Chalk L 1950. *Anatomy of Dicotyledons*. Clarendon Press, Oxford. 724pp.
18. Metcalfe CR, Chalk L 1979. *Systematic Anatomy of the leaf and stem. In: Anatomy of Dicotyledons.* Oxford Science Publication. Great Britain. 276pp
19. Stace CA 1965. Cuticular studies as an aid to plant taxonomy. *Bulletin of the British Museum* (*Natural History*) *Botany* 4: 1–71.
20. Radford AE, Dickson WC, Massey JR, Bell CR 1976. *Vascular Plant Systematics.* Harper and Row, USA. 886pp.
21. Kadiri AB, Zeng G, Liu B, Ferguson DK, Yang Y 2021. Leaf epidermal micromorphology of *Beilschmiedia* Nees (Lauraceae) from Africa. *Taiwania,* 66(3): 378–38. DOI: 10.6165/tai.2021.66.378.
22. Kadiri AB, Muellner-Riehl AN 2021. Comparative leaf micromorphology of *Drypetes* and *Putranjiva* (Putranjivaceae) and its taxonomic significance. *Botanical Journal of the Linnean Society,* 195(2): 139-160.
23. Akwu NA, Naidoo Y, Singh M 2020. The anatomy and histochemistry of *Grewia lasiocarpa* E. Mey. ex Harv. (Malvaceae). *SuidAfrikaanse Tydskrif vir Natuurwetenskap en Tegnologie*, 39(1): 1-17.
24. Geoffrey M 1995. Seed trichome variation in *Hibiscus* section *Furcaria* in East Africa *Journal*

of East African Natural History, 84 (1): 65-75.

25. Ančev M, Goranova V 2006. Trichome morphology of eleven genera of the Tribe Alysseae (Brassicaceae) occurring in Bulgaria. *Willdenowia* 36: 193–204.

26. Zhang X, Lyu J, Zeng Y, Sun N, Liu C, Yin S 2021. Individual effects of trichomes and leaf morphology on $PM_{2.5}$ dry deposition velocity: A variable-control approach using species from the same family or genus. *Environmental Pollution*, 272: 1-8.

27. de Almeida-Jr. EB, Araújo JS, Santos-Filho FS, Zickel CS. 2012. Leaf morphology and anatomy of *Manilkara* Adans. (Sapotaceae) from northeastern Brazil. *Plant Systematics and Evolution,* 299: 1–9.

28. Okeke CU, Iroka CF, Izundu AI, Okeke NC, Onwuasoeje CI, Nyananyo BL 2015. Comparative systematic leaf and petiole anatomical studies of the genus *Stachytarpheta* found in Awka, Nigeria. *Journal of Medicinal Plant Studies,* 3(4): 82-84.

29. Anorue GU, Olowokudejo JD, Kadiri AB 2020. Petiole anatomy of some selected species of the family Verbenaceae (Lamiales) in Nigeria. *Indian Journal of Plant Sciences*, 9: 1-14.

30. Riosi ABM, Menino GCO, Dalvi VC 202. Leaf teeth in eudicots: what can anatomy elucidate? *Botanical Journal of the Linnean Society*, 193: 504–522.

31 Gonzalez AM, Tarragó JR 2009. Anatomical structure and secretion compounds of colleters in nine *Ilex* species (Aquifoliaceae) from southern South America. *Botanical Journal of the Linnean Society*, 160: 197–210.

32. Thomas V, Dave Y 1989. Structure, origin, development and senescence of colleters in *Nerium indicum* Mill. (*N. odorum* Soland., Apocynaceae). *Korean Journal of Botany*, 32: 163–172.

33. Thomas V, Dave Y 1990. Structure and necrosis of stipular colleters in *Mitragyna parvifolia* (Rubiaceae). *Belgian Journal of Botany,* 123 67-72.

34. Thomas V 1991. Structural, functional and phylogenetic aspects of the colleter. *Annals of Botany,* 68: 287–305.

35. Kadiri AB, Agboola OM, Fashina FO 2013. Ethnobotanical survey and phyto-anatomical studies of some common plants used for the treatment of epilepsy in some rural areas of South west Nigeria. *Journal of Pharmacognosy and Phytochemistry,* 2(4):175-182.

36. Holmgren PK, Keuken W, Schofield EK 1990. Index Herbariorum, Part 1: The herbaria of the World. *Regnum Vegetabilis*, 106: 1–456.