

# Reliable identification of five taxa of *Euphorbia* (*Euphorbiaceae*) of ethnobotanical value by morphological characters of the leaves

Onyebuchi E. Nwankwo<sup>1</sup>, Eucharia C. Ezigbo<sup>2</sup>, Samuel A. Odewo<sup>3</sup>,  
Sunday E. Nwachukwu<sup>1</sup>, Babatunde A. Ajani<sup>3</sup> & Luke T. Soyewo<sup>3</sup>

<sup>1</sup> Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria;  
e-mail: ephrainnwankwo8@gmail.com (corresponding author)

<sup>2</sup> Department of Biotechnology and Environmental Biology, Veritas University,  
Abuja, Nigeria

<sup>3</sup> Herbarium Unit, Forest Research Institute of Nigeria, Ibadan, Nigeria

Received: March 13, 2021 ▷ Accepted: July 31, 2021

**Abstract.** This study is aimed at using macro and micro morphological features of the leaves for reliable identification of five taxa of *Euphorbia*. Standard methods are used in the study. The obtained results have shown that the leaf surface of *E. hirta* is pubescent, while in the other four taxa is glabrous. As their diagnostic features, *E. hirta* has an oblique leaf base and tetracytic stomata on the abaxial surface and *E. heterophylla* shows variable leaf shapes, with incised margins and actinocytic stomata on the abaxial surface. *E. hyssopifolia* has oblong leaves with rounded base and anisocytic stomata, while *E. milii* has obovate leaves with cuneate base and hypo-paracytic stomata. Cordate leaf base and staurocytic stomata on the abaxial surface are diagnostic of *E. prostrata*. Foliar epidermis of *E. hyssopifolia* and *E. prostrata* is reported here for the first time.

**Key words:** *Euphorbia*, *Euphorbiaceae*, identification, leaves, morphological characters, stomata

---

## Introduction

The genus *Euphorbia* L. is cosmopolitan and contains over 2000 species (Kumar & al. 2010; Okanume & al. 2017). According to Hutchinson & Dalziel. (1954), 30 species of the genus were found in West Africa and about 21 of them are well represented in Nigeria. The *Euphorbia* species are mostly herbs, occasionally shrubs but seldom trees (Davis & al. 1994; Bolaji & al. 2015). The five *Euphorbia* taxa, namely *E. hirta* L., *E. heterophylla* L., *E. hyssopifolia* L., *E. milii* Des Moul., and *E. prostrata* Aiton, are used for treatment of various diseases, such as chronic cough, asthma, rheumatism, dengue, toothache, constipation, bacterial and inflammatory diseases (Kumar & al. 2010; Okanume & al. 2017; Ekpo & Pretorius 2007; Perera & al. 2018).

Proper identification of the plant specimens by studying their various parts for true diagnostic features is necessary for their application in drug development (Odewo & al. 2020). Brinckmann, (2011) and Howard & al. (2012) have stated that problems of misidentification of many medicinal plants have caused much havoc in traditional medicine and cost lives. Although there have been earlier publications on these five taxa of *Euphorbia*, there has been no detailed study of the characters of their leaves. Hence, the present study aims at using macro morphological and micro morphological features of the leaves for reliable identification of the five taxa of *Euphorbia* of ethnobotanical value.

## Material and methods

Fresh specimens of *E. hirta*, *E. heterophylla*, *E. hyssopifolia*, *E. milii*, and *E. prostrata* have been collected from Abakaliki metropolis and identified at Ebonyi State University and National Herbaria in Nigeria. The voucher numbers are EBS-H-0230, EBS-H-0231, EBS-H-0232, EBS-H-0233, and EBS-H-0234, respectively.

**Macromorphological study.** The qualitative and quantitative characters of the taxa were studied following the method of Nwankwo & Ayodele (2017). Leaf length and width were measured by a metre ruler. From each specimen, leaves were randomly selected and measured also by a metre ruler.

**Foliar epidermal study.** The epidermal preparation methods followed Ayodele & Olowokudejo (1997). Standard median portions of the mature leaves of the five *Euphorbia* taxa obtained by cutting with razor blade were soaked in concentrated trioxonitrate (v) acid for about three to seven minutes, depending on the nature of leaves. Appearance of air bubbles on the surface of the leaves indicated their readiness for separation. The leaves were transferred into water in a Petri dish by a pair of forceps. Adaxial and abaxial surfaces were carefully separated by teasing them apart and pulling the epidermis back with camel hair brush and dissecting needle. A camel hair brush was also used to remove the adhering tissue debris. The separated surfaces were rinsed in distilled water and then transferred into 50% ethanol for about two to

three minutes to harden. They were rinsed again in distilled water and stained with methyl blue for about five minutes; the excess staining was washed off in water. The samples were mounted in 25% glycerol on slides, with the edge of the cover slips sealed with nail varnish to prevent dehydration. The slides were labelled appropriately and examined under a light microscope, and photomicrographs of each slide were taken at a magnification of  $\times 400$ , using a Canon digital camera fixed on a light microscope and connected to a personal computer.

## Results

The results have shown that the five *Euphorbia* taxa possessed simple leaves and were amphistomatic and trichomic, except for *E. milii*, which had paracytic stomata only on the abaxial surface and no trichomes. The epidermal cell shapes were mostly isodiametric on the abaxial surfaces of the five taxa. The results are summarized in Tables 1 to 3, with images in Figs 1, 2.

## Discussion

The five species have simple leaves and capsuled fruits with three chambers. The leaf surfaces of the species are all glabrous, except for in *E. hirta*, which has pubescent leaves. The foliar epidermal features

**Table 1.** Qualitative characters of the five studied *Euphorbia* taxa.

| Characters \ Species   | Lt     | Ls                | Hb    | La        | Lb      | If                | Lsf       | Lm      | Lar       |
|------------------------|--------|-------------------|-------|-----------|---------|-------------------|-----------|---------|-----------|
| <i>E. hirta</i>        | Simple | Elliptic          | Herb  | Acute     | Oblique | Axillary cymose   | Pubescent | Serrate | Opposite  |
| <i>E. heterophylla</i> | Simple | Lyrate            | Herb  | Acute     | Acute   | Terminal cymose   | Glabrous  | Incised | Alternate |
| <i>E. hyssopifolia</i> | Simple | Oblong/lanceolate | Herb  | Acute     | Rounded | Terminal racemose | Glabrous  | Serrate | Opposite  |
| <i>E. milii</i>        | Simple | Obovate           | Shrub | Mucronate | Cuneate | Terminal cymose   | Glabrous  | Entire  | Whorled   |
| <i>E. prostrata</i>    | Simple | Elliptic          | Herb  | Mucronate | Cordate | Axillary cymose   | Glabrous  | Entire  | Opposite  |

**Legend:** Lt – leaf type, Ls – leaf shape, Hb – habit, La – leaf apex, Lb – leaf base, If – inflorescence, Lsf – leaf surface, Lm – leaf margin, Lar – leaf arrangement.

**Table 2.** Quantitative characters of the five studied *Euphorbia* taxa.

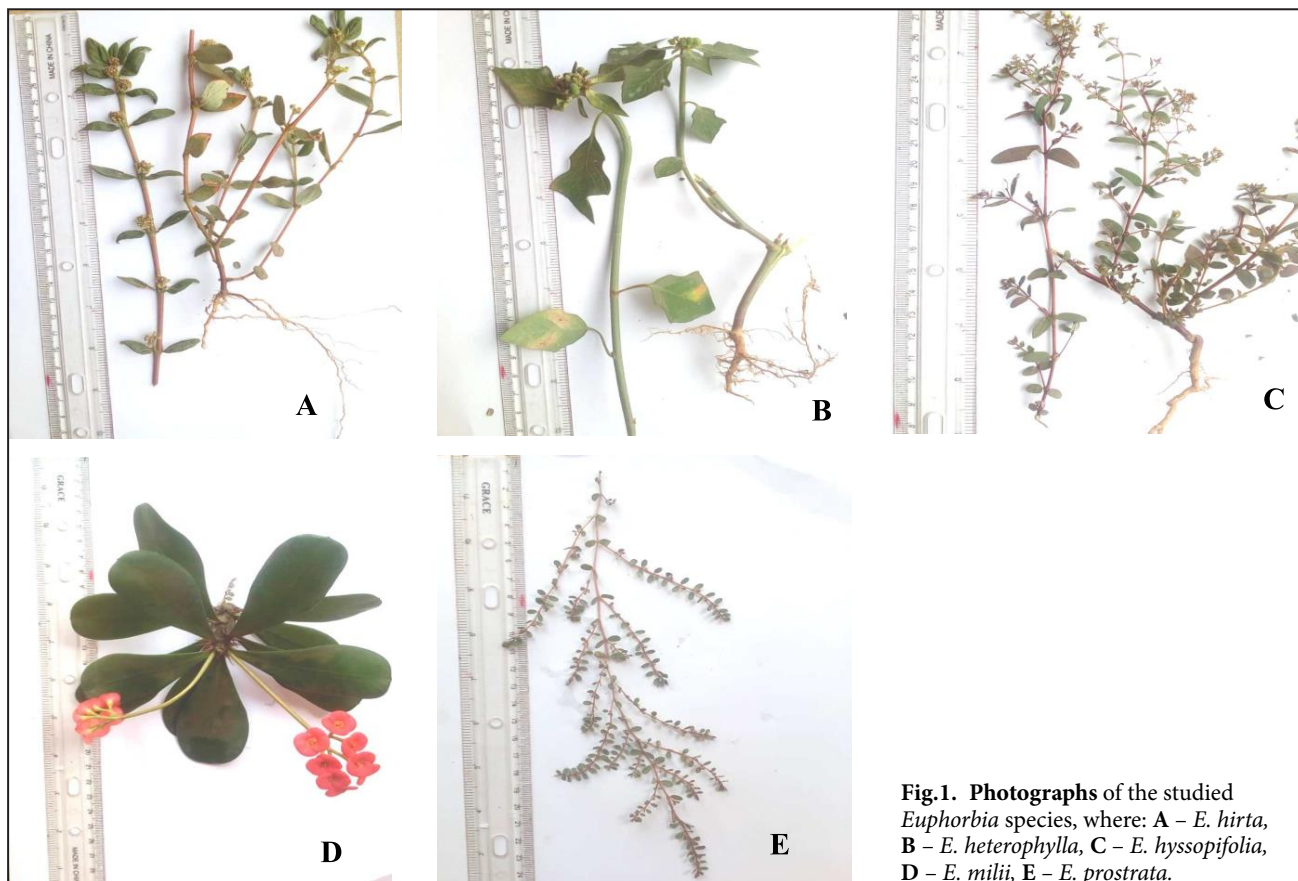
| Characters \ Species | <i>E. hirta</i>       | <i>E. heterophylla</i> | <i>E. hyssopifolia</i> | <i>E. milii</i>       | <i>E. prostrata</i>   |
|----------------------|-----------------------|------------------------|------------------------|-----------------------|-----------------------|
| Leaf length          | 0.5(2.4 $\pm$ 0.2)3.5 | 2.8(4.4 $\pm$ 0.2)6.2  | 0.6(2.2 $\pm$ 0.2)2.8  | 6.5(7.9 $\pm$ 0.2)9.1 | 0.2(0.5 $\pm$ 0.1)0.6 |
| Leaf width           | 0.4(1.1 $\pm$ 0.1)1.8 | 1.9(2.6 $\pm$ 0.1)3.5  | 0.2(0.7 $\pm$ 0.1)0.8  | 2.1(3.1 $\pm$ 0.1)3.7 | 0.1(0.2 $\pm$ 0.1)0.4 |
| Petiole length       | 0.0(0.1 $\pm$ 0)0.3   | 0.1(0.8 $\pm$ 0.1)1.8  | 0.1(0.1 $\pm$ 0.0)0.3  | 0.0(0.0 $\pm$ 0.0)0.0 | 0.0(0.0 $\pm$ 0.0)0.0 |

**Notes:** All measurement are in centimetres.

Table 3. Qualitative characters of the foliar epidermis of the five studied *Euphorbia* taxa.

| Species                | Stomatal type |        | Cell shape |              | Anticlinal wall pattern |          | Trichome |        |
|------------------------|---------------|--------|------------|--------------|-------------------------|----------|----------|--------|
|                        | Ad.           | Ab.    | Ad.        | Ab           | Ad.                     | Ab       | Ad.      | Ab.    |
| <i>E. hirta</i>        | Ans.          | Tr/Ans | Pentagonal | Isodiametric | Undulate                | Sinuous  | Satr.    | Satr.  |
| <i>E. heterophylla</i> | Ans.          | Ats    | Pentagonal | Hexagonal    | Straight                | Straight | Satr.    | Satr.  |
| <i>E. hyssopifolia</i> | Ans.          | Ans.   | Pentagonal | Isodiametric | Undulate                | Sinuous  | Satr.    | Absent |
| <i>E. milii</i>        | Absent        | Pr.    | Irregular  | Isodiametric | Undulate/sinuous        | Sinuous  | Absent   | Absent |
| <i>E. prostrata</i>    | Ans.          | Str.   | Irregular  | Isodiametric | Straight                | Sinuous  | Absent   | Absent |

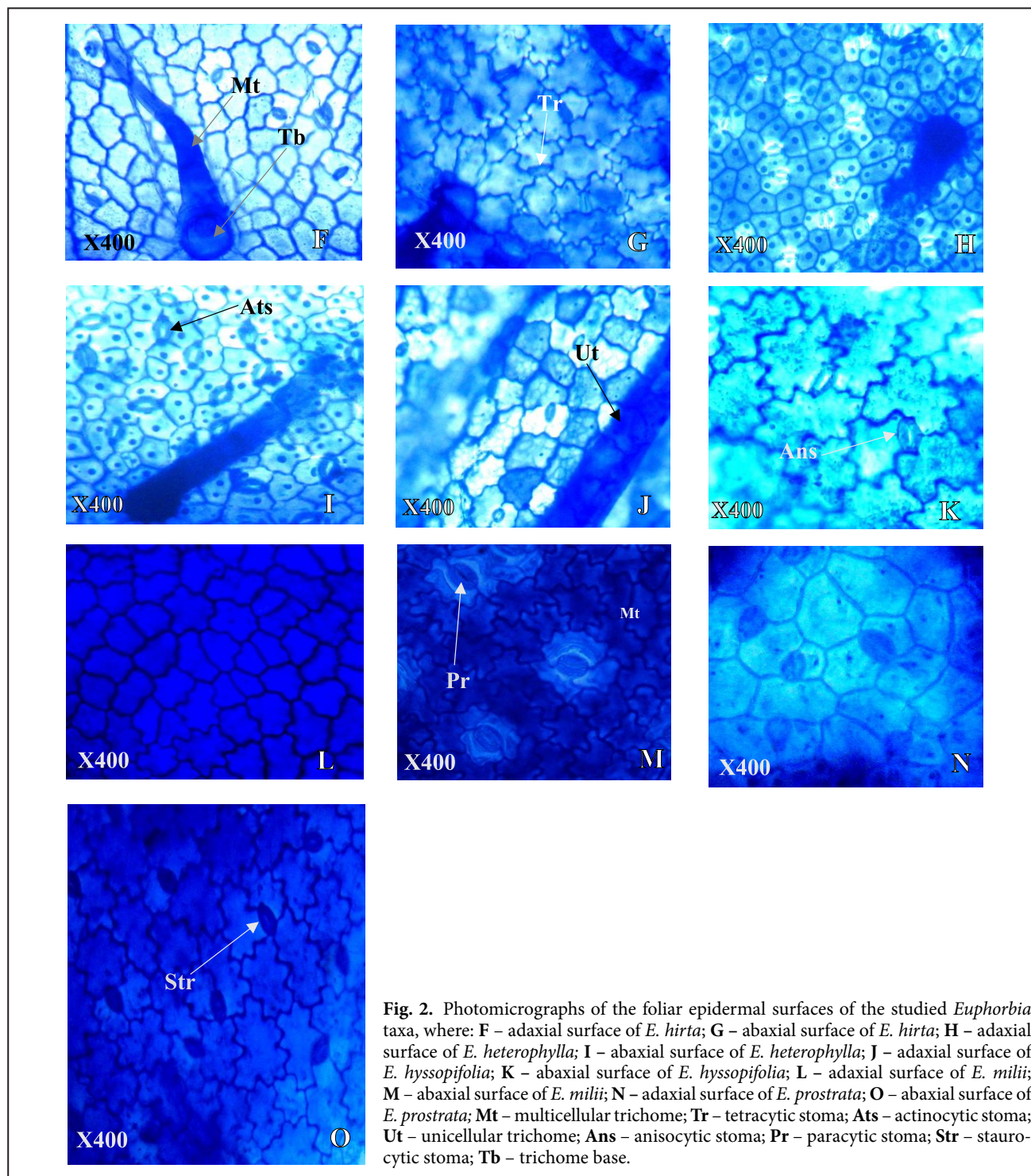
**Legend:** Ad. – adaxial; Ab. – abaxial; Ans. – anisocytic stomata; Pr – paracytic stomata; Str – staurocytic stomata; Tr. – tetracytic stomata; Ats – actinocytic stomata; Satr. – single-arm trichome.



**Fig.1. Photographs** of the studied *Euphorbia* species, where: **A** – *E. hirta*, **B** – *E. heterophylla*, **C** – *E. hyssopifolia*, **D** – *E. milii*, **E** – *E. prostrata*.

of *E. hirta* and *E. milii* are almost in agreement with the results of Okanume & al. (2017), except that the number of arms (single or double) of the trichomes in *E. hirta* have not been specified. Besides, the cell shape of *E. hirta* and *E. milii* which is isodiametric on the abaxial surface, has been inadvertently reported as undulate. The five studied taxa were amphistomatic and amphitrichomic, except for *E. milii*, which was hypostomatic. The reported amphistomatic feature of *E. hirta* and *E. heterophylla* and the hypostomata of *E. milii* are in agreement with Essiett & al. (2012). Talebi & al. (2017) have reported anisocytic stomata only on the adaxial and abaxial surfaces of eighteen different *Euphorbia* taxa studied by them, but our studies have

recognized different types of stomata ranging from paracytic to staurocytic on the adaxial and abaxial surfaces of the five studied taxa, except for *E. milii* (Table 3, Fig. 2). Disagreement in the reports of Talebi & al. (2017) and this one may be due to different environmental influences on the taxa, considering Stace's (1965) statement that environment affects the epidermal features of plant species. *Euphorbia heterophylla* has shown sunken anisocytic stomata on the adaxial surface, which may be due to the plant's efforts to reduce the rate of transpiration, as the study was carried out during the dry season. Undulate and sinuous anticlinal walls have been common among the studied taxa. The trichomes observed in the five



*Euphorbia* taxa have been single-arm unicellular and multicellular trichomes. Inferring from Margarita's (1992) assumption that paracytic stomata are more primitive than the other stomata, *E. milii*, which possessed paracytic stomata, is primitive, while the species with tetracytic/staurocytic stomata are advanced. *Euphorbia hirta* can be easily recognized

by the pubescent leaves and stems, with head inflorescence and tetracytic stomata on the abaxial surface. *Euphorbia heterophylla* possesses cymose inflorescence, hollow stems with leaves of variable shape, and actinocytic stomata on the abaxial surface. *E. hyssopifolia* can be recognized by its red pigmented stem, terminal racemose inflorescence and anisocytic

stomata on the abaxial surface. *Euphorbia milii* is a shrub with thorns and paracytic stomata on the abaxial surface only, while *E. prostrata* is a decumbent herb with small leaves, axillary cymose inflorescence and staurocytic stomata on the abaxial surface.

## Conclusion

The leaf shape, surface, size, position of inflorescence, and type of stomata on the abaxial surfaces of the five studied taxa are very significant for identification of these *Euphorbia* taxa. Part of the present studies is novel by contributing the fact of the foliar epidermis of *E. hyssopifolia* and *E. prostrata*, which is lacking in the works other authors, and is now recorded. Single-arm trichomes have been reported for the first time here.

**Acknowledgements.** The first author is grateful to his project colleagues, Samuel Akinniyi Odewo and Babatunde Adeyemi Ajani, who helped in collection and authentication of the taxa. The efforts of Eucharia Chizoba Ezigbo, who proofread the article and analyzed the data, can never be forgotten, as well as of Sunday Egbe Nwachukwu and Luke Temitope Soyewo, who helped in the laboratory work. Thank you all.

**Conflict of interest:** There is no actual or potential conflict of interest in relation to this article.

## References

- Ayodele, A.E. & Olowokudejo, J.D.** 1997. Systematic importance of leaf epidermal characters in West African species of family *Myrtaceae*. – Boletim da Sociedade Broteriana (Portugal), **68**: 35-728.
- Bolaji, A.O., Olojede, C.B., Famurewa, A.A. & Faluyi, J.O.** 2015. Morphological and cytological studies of *Euphorbia hyssopifolia* L. and *Euphorbia heterophylla* L. from Nigeria. – Nigerian Journal of Genetics, **28**: 15-18.
- Brinckmann, J.** 2011. Reproducible efficacy and safety depend on reproducible quality: matching the various quality standards that have been established for botanical ingredients with their intended uses in cosmetics, dietary supplements, foods, and medicines. – Herbal Gram, **91**: 40-55.
- Davis, S.D., Heywood, V.H. & Hamilton, A.C.** 1994. Centres of plant diversity. A guide strategy for their conservation. WWF IUCN, pp. 503-527.
- Ekpo, O.E. & Pretorius, E.** 2007. Asthma, *Euphorbia* species and its anti-inflammatory properties. – South African Journal of Science, **103**: 201-203.
- Essiett, U.A., Illoh, H.C. & Udoh, U.E.** 2012. Leaf epidermal studies of three species of *Euphorbia* in Akwa Ibom State. – Pelagia Research Library: Advances in Applied Sciences Research, **3** (4): 2481-2491.
- Howard, C., Socratous, E., Williams, S., Graham E., Fowler M.R. & Scott N.W.** 2012. Plant ID-DNA-based identification of multiple medicinal plants in complex mixtures. – Chinese Medicine, **2**: 7-18.
- Hutchinson, J. & Dalziel, J.M.** 1954. Flora of West Tropical Africa. 2<sup>nd</sup> ed., vol. **1**. Crown Agent of Overseas Governments and Administrations, Mill Bank, London.
- Kumar, S., Malhotra, R. & Kumar, D.** 2010. Antidiabetic and free radicals scavenging potential of *Euphorbia hirta* flower extract. – Indian Journal of Pharmaceutical Science, **72**(4): 533-537.
- Margarita, B.** 1992. Principles of comparative stomatographic of flowering plants. – Botanical Review, **58**(1): 49-99.
- Nwankwo, O.E. & Ayodele, A.E.** 2017. Taxonomic studies of the genus *Indigofera* Linn., in Nigeria. – International Digital Organisation Journal for Scientific Research, **2**(3): 10-26.
- Odewo, S.A., Nwankwo, O.E., Adeniyi, I.M. & Odozie, E.C.** 2020. Comparative studies of two medicinal plants: *Petiveria alliacea* L. and *Hillieria latifolia* (Lam.) H. Walter (*Petiveriaceae*) based on foliar anatomy. – Plants and Environment, **2**(2):54-58.
- Okanume, O.E., Dalang, B., Agaba, O.A. & Ezeabara, C.A.** 2017. Taxonomic significance of foliar epidermis in four *Euphorbia* species in Jos, Plateau State, Nigeria. – Analele Universitatii din Oradea, Fascicula Biologie, **24**(1): 19-24.
- Perera, S.D., Jayawardena, U.A. & Jayasinghe, C.D.** 2018. Potential use of *Euphorbia hirta* for dengue: A systematic review of scientific evidence. – Journal of Tropical Medicine, **8**: 1-7.
- Stace, C.A.** 1965. The taxonomic importance of leaf surface current concept of plant taxonomy. – Bulletin of British Museum (Natural History) Botany, **4**: 3-7.
- Talebi, S.M., Noori, M. & Naniz, H.A.** 2017. A study of epidermal leaf anatomy of 18 *Euphorbia* taxa from Kerman Province, Iran. – Biologija, **63**: 126-133.

