Anatomical and Palynological Studies of Some Bougainvillea Glabra Cultivars

Chnar Najmaddin^{1*}, Mosleh M. Saeed²

¹Biology department, College of Sciences, Salahaddin University, Erbil, Iraq ²Science Research Center, College of Sciences, Duhok University, Duhok, Iraq ^{1*}Correspond Author: <u>chnar.fathulla@su.edu.krd</u>

ABSTRACT

The present study is undertaken to find the anatomical and palynological differences between Bougainvillea cultivars (Bougainvillea glabra cv. Miss Manila, Bougainvillea glabra cv. Barbara Karst, Bougainvillea glabra cv. Killie Campbell, Bougainvillea glabra cv. California Gold, Bougainvillea glabra cv. Jamaica White, Bougainvillea glabra cv. Terracota, and Bougainvillea glabra cv. Mini-tige) that have been taken from plantation of the Ministry of Municipal/Erbil/Iraq. The results showed that cultivars differ in the shape of the transverse sections of the stem, petiole, midrib, lamina, and margin. Raphides crystals, and fibers are present in the stem and petioles. Anomalous secondary structure present in the stem and petioles of some cultivars. Starch grains and tannins are found in all cultivar stems. Accessory vascular bundles are present in some petioles of the cultivars. Trichomes are presented in all parts of the plant sections which are multicellular or unicellular and glandular or non-glandullar. The shapes of leaf epidermal cells are usually straight and polygonal. The stomatal apparatus is presented on the adaxial and abaxial side of the leaf; the type of stomatal is anisocytic, anomocytic, parasitic, hexacytic and hemiparacytic. Pollen grains are prolate and spheroidal to oblong. The exine ornamentation is reticulate, while Bougainvilleg alabra cy. Mini-tige is spinules. Cluster analysis using MVSP version 3.2 showed that the anatomical evidence is of taxonomic significance to distinguish the cultivars and find a close relationship between the cultivars.

INTRODUCTION

Nyctaginaceae consists of 400 species belonging to 30 genera and many of them have anomalous secondary thickening characterized by the development of successive rings of collateral vascular bundles, it is mainly distributed in the tropical and subtropical areas of the world and some genera are extending into the temperate regions as southern Africa. *Bougainvillea* is evergreen shrubby vine and popular for its long-lasting and colourful flower bracts which appear periodically throughout most of the year but are especially plentiful in winter and spring. Although flower bracts of purple or red are commonly distributed, cultivars are now available in apricot, white, yellow, and orange (Gilman, 1999).

Anatomical studies of Nyctaginaceae, described by Metcalfe and Chalk (1950); Bittrich and Kuhn (1993). *Boerhavia* L., *Commicarpus* Standl., *Mirabilis* L., *Phaeoptilum* Radlk and *Pisonia* L. are located in southern Africa, south of the Zambezi River, Botswana, Lesotho, southern Mozambique, Namibia, South Africa, Swaziland and Zimbabwe (<u>Lu and Gilbert</u> 2003). Because of the presence of medullary bundles and supernumerary cambium in *Boerhaavia* species stem these behaviours attract the attention that it is the anastomists plants (Rajput and Rao, 1998).

The environmental factors effects on the variation of anatomy and morphology of Nyctaginaceae, for example *Allionia* species, are changed in size and number rather than distinctive features in the vegetative and reproductive organs. Thus, they are strongly affected by growing conditions of the plants especially intensity of light and duration exposure, availability of moisture, nutrient supply and temperature. The differences present in the amount of secondary tissue of the stems and roots **Keywords:** Nyctaginaceae family, *Bougainvillea*, anatomy of Nyctaginaceae, pollen grains of Nyctaginaceae, anatomy of *Bougainvillea*, morphology of Nyctaginaceae family.

Correspondence: Chnar Naimaddin

Biology department, College of Sciences, Salahaddin University, Erbil, Iraq Email: <u>chnar.fathulla@su.edu.krd</u>

while the plants are at the same stage of development (Phillips, 1976).

Nyctaginaceae pollen grains are 17–200 µm in diameter, they are spheroidal, prolate or oblate and 3 or 4 colpate, pantocolpate or pantoporate. The pores are covered by a pore plate which might be either roughened or became spinulose. The exine is very thick and varies in sculpturing (Struwig et al., 2013). Based on pollen morphology Heimerl (1934) divided the Nyctagineae into four subtribes, such as Nyctagininae subtribe. The environment effect on the pollen competitive performance, as in Mirabilis jalapa, used the number of pollen grains and the number of pollen tubes per pistil as the measures of pollen competition. Pollen germination and pollen tube penetration are set into the style and pollen tube growth rates (Niesenbaum and Schueller, 1997).

Cain and Harrison (1960) proposed the term phenetic relationship to elucidate the relationship among the species based on the overall similarity. Phenetics was widely applied in elucidating relationships among different palm genera like *Arenga*, *Bactris*, *Phoenix*, *Reinhardia* and *Drymopholeus*. The preliminary studies of Malayan species show that the genus *Calamus* is paraphyletic (Sreekumar, 2004).

MATERIAL AND METHODS

Plant samples

The plant samples were collected from Plantation of the Ministry of Municipal/Erbil/Iraq in 2016, and fixed in a mixture formalin, alcohol and acetic acid Formalinalcohol-acetic acid (FAA).

Plastic Method (Arildite Method)

The glutaraldehyde (2.5%) fixed samples were post-fixed in 1% osmium tetroxide, dehydrated, cleared in acetone

and embedded in araldite mixture. Half of micrometer thick sections were stained by 1% toludine blue in 1% borax (Ruzin, 1999).

Paraffin Method

Pieces of samples have been put in FAA and have been dehydrated using a series concentration of alcohol after which the samples were cleared by xylene for 3-4 hrs. Later, they were embedded in a mixture of xylene and paraffin for 30 min., then transferred into pure paraffin wax and left in it at 60° C for overnight. After that preparation of paraffin blocks were made and sections were prepared with the thickness of 8 µm using the rotary microtome. Then the sections were stained using safranin and fast green or light green. Finally, the sections were mounted by DPX (Najmaddin and Mahmood, 2016). **Stomata**

The leaf samples were obtained from the fresh materials while the leaf epidermis was prepared by mechanical scraping. The samples were observed and viewed under a light microscope with camera attached, using Analysis Image Analysis Software.

Pollen grains preparation

Flowers were obtained from fresh materials. Pollens were gathered from the open flowers or mature flower buds and pollen grains were prepared for the light microscope (LM) from methods described by Erdtman (1952).

Phenetic study

Cluster analysis is a term used to describe a set of numerical techniques in which the main purpose is to divide the objective studies into discrete groups. Cluster analysis by UPGMA (unweighted pair-group method using arithmetic averages) suggests the existence of geographic and ecologic groups with higher genetic similarities (Aras *et al.*, 2005).

Cluster analysis was carried out using the Multi Variate Statistical Package (MVSP) for Windows, version 3.2. The character states were extracted from the obtained results, 46 characters or variable, from the anatomical data that had been used previously. Each data set from the anatomy results was analyzed individually but at the end all the data from the individual sets were merged to perform an overall analysis which involved 46-character states and 7 cultivars of *Bougainvillea glabra*.

RESULTS AND DISCUSSION

General Anatomical Description (TS)

The results of the present investigation showed that *Bougainvillea glabra* possesses the different shapes of stem outline and the presence of raphides, secretory cells with tannins and fibers contain simple pits, as has been reported by Metcalfe and Chalk (1950). It also reported that collenchyma (lacunar) and starch grains are present in all cultivars. In the internode region of the complete stems, cells become packed with or large compound number of grains of starch. Occasionally, bundles of raphides may fill some of the cells (Phillips, 1976).

This study shows the process of anomalous growth in the stem; the secondary anomalous growth in the stem appears to be initiated in a cambium that strands as a continuation of the outer vascular bundle's cambium (Figure 1, 2, 3). Discrete vascular strands are produced and imbedded in the fibers and relatively thin cell walled of parenchyma cells are collectively called as conjunctive tissue (Phillips, 1976; Rajput and Rao, 1998).

This research shows the presence of tannins only in *Bougainvillea glabra* cv. Mini-tige petiole, and it is absence in another cultivars (Figure 4, 5). The tannins are

absent in other parts of all cultivars (Figure 6, 7, 8). Tannin idioblasts and raphides are recorded for the family by various authors. The tannin idioblasts are absent in *Commicarpus* leaves, and that they are not always present in the epidermal cells of *Boerhavia* leaves as well. As a result, this character cannot reliably be used to distinguish between the two genera. The distribution of the raphides is not in a specific pattern and it has no taxonomic value (Struwig *et al.*, 2011).

In this investigated, it appears that the trichomes are multicellular glandular, unicellular non-glandular, multicellular non-glandular and unicellular glandular have no stalk in the stems, petioles, lamina and margins in all cultivars (Figure 1-8). The trichomes are present in all the organs of both *Boerhavia* and *Commicarpus*, however they differ in the size, length, distribution and abundance. They are uniserial and multicellular. They are terminating in a head which is either globose or clavate. The walls of the trichomes are impregnated with numerous crystalline granules. The trichomes secrete a substance which makes the organs extremely sticky (Struwig *et al.*, 2011).

General Description of Epidermis

This research showed that the stomata in some cultivars is present in both, adaxial and abaxial surface, as well as anisocytic, anomocytic, parasitic, hexacytic and hemiparacytic. The epidermis cells are polygonally shaped and have straight anticlinal walls (Figure 9, 10, 11). Xuan et al. (2011) reported in Bougainvillea spectabilis the stomata were observed on the abaxial epidermis of the leaf. The form of epidermal cells was polygonal, and the patterns of anticlinal walls were straight: the stomata were anisocytic. While Chew (2010) mentioned that the stomata of genus Bougainvillea were absent on the adaxial surface and that there were differences in the shapes of the pavement cells that found on the abaxial against the adaxial side of the leaf. The epidermal studies of fifteen Indian cultivars of Bougainvillea shows that, the epidermal cells are polygonal isodiametric, or elongated with thick straight arched or have slightly sinuous walls. The mature stomata are anomocytic, paracytic and with a single subsidiary cell. Fifteen cultivars of Bougainvillea are separated on the basis of bract colour, stomatal frequency and index per unit area as reported by Inamdar et al. (1980).

Pollen grains

The pollen morphology could easily serve **as** a diagnostic character. The pollen of the constituent genera or species of each of these larger taxa is alike in aperture structure, sexine pattern and within a general size range (Nowicke and Luikart, 1971). This work revealed that the shape of *Bougainvillea glabra* cultivars is in equatorial view, which is spherical, prolate or oblong. The polar view is spherical (Figure 12) and (Table 1). The exine ornamentation is reticulate, while Bougainvillea glabra cv. Mini-tige is spinules. According to Erdtman (1952); Pramanick et al. (2015), the pollen grains of family Nyctaginaceae were either 3-4 colpate, or 6 polyrugate or polyforate. Bougainvillea glabra var. typical has pollen grains in tricolpate, oblate, and reticulate (muri provided with scattered spinuloid excrescences). Perveen and Qaiser (2001) reported that the pollen grains are generally spheroidal and pantopo-rate with tubuliferous-spinulose tectum. Chaves et al. (1998) mentioned that the pollen grains of the Salpianthus species studies have the range of size from 25 to 47 μ m (polar view) and 22 to 47 μ m

(equatorial view), and their shapes vary from oblate spheroidal to subprolate. The exine ornamentation is always spinulose with a perforated tectum.

Phonetics description

Phenetics is the method for establishing relationships between the organisms based on overall similarity characters relationships. Phenetics or taximetrics is the application of multivariate numerical techniques in a systematical process (Sreekumar, 2004). Dendrogram which is constructed from anatomical characters and resulting from cluster analysis. The absolute similarity is marked by 1.00, (Figure 13) shows at GGSc= 0.82 the *Bougainvillea glabra* cv. Barbara Karst to occupy a single branch sister to the cluster consisting of *Bougainvillea glabra* cv. Jamaica White and *Bougainvillea glabra* cv. California Gold. While at GGSc= 0.74 consisting of two similar groups, *Bougainvillea glabra* cv. Mini-tige and *Bougainvillea glabra* cv. Terracota that is similar to *Bougainvillea glabra* cv. Killie and *Bougainvillea glabra* cv. Miss Manila. According to Swanepoel and Villiers (1987), numerical analysis based on ampelographical and morphological characteristics showed distinct differences between American Vitis pp. and hybrids and European cultivars of Vitis vinifera. With respect to the ampelographic characteristics, the hybrids tended to cluster with at least one of their parents. Regarding the morphological characteristics, this direction was not pronounced, and this could be mainly ascribed to the differences in indumenta.



Figure 1. T.S section of stems: A. *Bougainvillea glabra* cv. Miss Manila, B. *Bougainvillea glabra* cv. Barbara Karst, C. *Bougainvillea glabra* cv. Killie Campbell, D. *Bougainvillea glabra* cv. California Gold. E: epidermis, C: collenchyma, V: vascular bundles, St: stone cell, AN: anomalous vascular bundles, T: tannins, trichomes (small black arrow). A, D (plastic method). B, C (paraffin method). A, B, C, D=4X



Figure 2. T.S section of stems: A. *Bougainvillea glabra* cv. Jamaica White, B. *Bougainvillea glabra* cv. Terracota, C. *Bougainvillea glabra* cv. Mini-tige. E: epidermis, C: collenchyma, V: vascular bundles, St: stone cell, AN: anomalous vascular bundles, T: tannins, trichomes (small black arrow), PE: periderm. B, C (plastic method). A (paraffin method). A, B, C=4X.





Figure 3. T.S section of stems: A. *Bougainvillea glabra* cv. Miss Manila, B. *Bougainvillea glabra* cv. Barbara Karst, C. *Bougainvillea glabra* cv. Killie Campbell, D. *Bougainvillea glabra* cv. California Gold, E. *Bougainvillea glabra* cv. Jamaica White, F. *Bougainvillea glabra* cv. Terracota. trichomes (small black arrow), G, H. TS section of the stem. E: epidermis, C: collenchyma, V: vascular bundles, R: raphides, Ch: starch grains, T: tannins, S: secretory canal, F: fiber. A, B (plastic method). CDE (paraffin method). B, D =10X, A, C, E, F, G= 40X.





Figure. 4. T.S section of stems: A. Bougainvillea glabra cv. Miss Manila, B. Bougainvillea glabra cv. Barbara Karst, C. Bougainvillea glabra cv. Killie Campbell, D. Bougainvillea glabra cv. California Gold, E. Bougainvillea glabra cv. Jamaica White, F. Bougainvillea glabra cv. Terracota, G. Bougainvillea glabra cv. Mini-tige. E: epidermis, C: collenchyma, V: vascular bundles, R: raphides, T: tannins, S: secretory canal, trichomes (small black arrow), accessory vascular bundle (large black arrow). E, G (plastic method). A, B, C, D, E (paraffin method). A, B, C, D, E, F, G= 4X.



A

Systematic Reviews in Pharmacy



Figure 5. T.S section of stems: A. *Bougainvillea glabra* cv. Miss Manila, B. *Bougainvillea glabra* cv. Barbara Karst, C. *Bougainvillea glabra* cv. California Gold, D. *Bougainvillea glabra* cv. Jamaica White, E. *Bougainvillea glabra* cv. Terracota, F. TS section of petiole. E: epidermis, C: cortex, V: vascular bundles, R: raphides, S: secretory canal, F: fiber, Ch: starch grains, trichomes (small black arrow). E (plastic method). A, B, C, D (paraffin method). A, C, D=10X, B, E= 40X.





Figure 6. T.S section of stems: A. *Bougainvillea glabra* cv. Miss Manila, B. *Bougainvillea glabra* cv. Barbara Karst, C. *Bougainvillea glabra* cv. Killie Campbell, D. *Bougainvillea glabra* cv. California Gold, E. *Bougainvillea glabra* cv. Jamaica White, F. *Bougainvillea glabra* cv. Terracota, G. *Bougainvillea glabra* cv. Mini-tige. C: cortex, V: vascular bundles, R: raphides, S: secretory canal, trichomes (small black arrow), accessory vascular bundle (large black arrow). A, B, C, D, E, F, G (paraffin method). A, B, C, D, E, G =10X, F=4X.





Figure 7. T.S section of stems: A. *Bougainvillea glabra* cv. Miss Manila, B. *Bougainvillea glabra* cv. Barbara Karst, C. *Bougainvillea glabra* cv. Killie Campbell, D. *Bougainvillea glabra* cv. California Gold, E. *Bougainvillea glabra* cv. Jamaica White, F. *Bougainvillea glabra* cv. Terracota, G. *Bougainvillea glabra* cv. Mini-tige. up: upper epidermis, lo: lower epidermis, V: vascular bundles, R: raphides, PA: palisade layer, SP: spongy layer, Sch: schizogenous cavity, trichomes (small black arrow). A, B, C, D, E, F, G (paraffin method). A, B, C, D, E, F, G= 40X.





Figure 8. T.S section of stems: A. *Bougainvillea glabra* cv. Miss Manila, B. *Bougainvillea glabra* cv. Barbara Karst, C. *Bougainvillea glabra* cv. Killie Campbell, D. *Bougainvillea glabra* cv. California Gold, E. *Bougainvillea glabra* cv. Jamaica White, F. *Bougainvillea glabra* cv. Terracota, G. *Bougainvillea glabra* cv. Mini-tige. V: vascular bundles, R: raphides, trichomes (small black arrow). A, B, D, F (plastic method). C, E, G (paraffin method). A, B, C, D, E, F, G= 40X.



Figure 9. Epidermis: A, B, C. adaxial surface of *Bougainvillea glabra* cv. Miss Manila, D. abaxial surface of *Bougainvillea glabra* cv. Miss Manila. stomata (curve arrow). A, B, C, D=40x



Figure 10. Epidermis: A. adaxial surface of *Bougainvillea glabra* cv. Barbara Karst, B. abaxial surface of *Bougainvillea glabra* cv. Barbara Karst, C. adaxial surface of *Bougainvillea glabra* cv. Killie Campbell, D. abaxial surface of *Bougainvillea glabra* cv. Killie Campbell. E. adaxial surface of *Bougainvillea glabra* cv. California Gold, F. abaxial surface of *Bougainvillea glabra* cv. California Gold. Stomata (curve arrow). A, B, C, D, E, F=40X.



Figure 11. Epidermis: A. adaxial surface of *Bougainvillea glabra* cv. Jamaica White, B. abaxial surface of *Bougainvillea glabra* cv. Jamaica White, C. adaxial surface of *Bougainvillea glabra* cv. Terracota, D. abaxial surface of *Bougainvillea glabra* cv. Terracota, E. adaxial surface of *Bougainvillea glabra* cv. Mini-tige. F. E. adaxial surface of *Bougainvillea glabra* cv. Mini-tige. Stomata (curve arrow). A, B, C, D, E, F=40X.



Figure 12. T.S section of stems: A. *Bougainvillea glabra* cv. Miss Manila, B. *Bougainvillea glabra* cv. Barbara Karst, C. *Bougainvillea glabra* cv. Killie Campbell, D. *Bougainvillea glabra* cv. California Gold, E. equatorial view of *Bougainvillea glabra* cv. Terracota F. polar view of *Bougainvillea glabra* cv. Terracota, G. *Bougainvillea glabra* cv. Jamaica White, H. *Bougainvillea glabra* cv. Mini-tige. A, B, C, D, E, F, G, H=100X.

Table 1: Pollen grains feature

Cultivars	Polar view	Equatorial view	Size P/E	Sculpture ornamtation	Colpus	Porus	spinules
<i>B. glabra</i> cv. Miss Manila	spherical	circular	0.872	reticulate	3	absent	absent

<i>B. glabra</i> cv. Barbara	Sub-prolate	circular	1.044	reticulate	3	absent	absent
Karst							
B. glabra cv. Killie	spherical	circular	0.987	reticulate	3	absent	absent
Campbell							
B. glabra cv.	spherical	circular	0.882	reticulate	3	absent	absent
California Gold							
B. glabra cv. Jamaica	Sub-prolate	circular	1.004	reticulate	3	absent	absent
White							
B. glabra cv.	Sub-prolate	oblong	1.863	reticulate	3	absent	absent
Terracota							
B. glabra cv. Mini-	spherical	circular	0.952	reticulate	3	absent	present
tige							



Figure 13. Dendogram of anatomical characters of seven cultivars of *Bougainvillea glabra*.

CONCLUSION

In this investigate concluded the cultivars differ in the outline shape of the stem, petiole, midrib, lamina, and margin. Raphides crystals, and fibers are present in the stem and petioles. Anomalous secondary structure present in the stem and petioles. Starch grains and tannins are found in all cultivar stems. Accessory vascular bundles are present in some petioles of the cultivars. Trichomes are presented which are multicellular or unicellular and glandular or non-glandular. The type of stomatal is anisocytic, anomocytic, parasitic, hexacytic and hemiparacytic. Pollen grains are prolate and spheroidal to oblong. The Cluster analysis using MVSP showed that the anatomical evidence is of taxonomic significance to distinguish the cultivars.

ACKNOWLEDGEMENT

This work was doing in Biology department, College of Sciences, Salahaddin University, Iraq. Thanks, the Plantation of the Ministry of Municipal, we thank any one help us to doing this work.

REFERENCES

1. Aras, S., Polat, J. B., Cansaran, D. and Soylemezoglu, G. 2005. RAPD analysis of genetic relations between *Buzgulu* grape cultivars (*Vitis vinifera*) grown in different parts of Turkey. Act. Bio. Crac. Ser. Bot. 47: 77-82.

- Bittrich, V. and Kühn, U. 1993. Nyctaginaceae. *In*: Kubitzki, K., Rohwer, J. G. and Bittrich, V. (Eds), Flowering Plants, Dicotyledons, Magnoliid, Hamamelid and Caryophyllid families. The Families and Genera of Vascular Plants. 2nd ed. Berlin, Springer, Heidelberg, pp.473-486.
- 3. Cain, A. and Harrison, G. A. 1960. Phyletic weighting. J. Zoo. 135 (1): 1 -31.
- 4. Chavez, R. P., Nava, R. F., Grafström, E., Pfaler, M. V. and Nilsson, S. 1998. On the pollen of *Salpianthus* (Nyctaginaceae). Gra. 37: 352-357.
- 5. Chew, S. 2010. Anatomical features of Bougainvillea (Nyctaginaceae). Gue. J. 4 (1): 72-78.
- 6. Erdtman, G. 1952. Pollen morphology and plant taxonomy-angiosperms. Stockholm, Almqvist and Wiksell.
- 7. Gilman, E. F. 1999. *Bougainvillea* spp. Cooperative Extension Service Institute of Food and Agricultural Sciences, University of Florida.
- Heimerl, A. 1934. Nyctaginaceae. *In*: Engler A, Prantl K (eds) *Die Natürlichen Pflanzenfamilien*, 2nd ed. Leipzig, Wilhelm Engelmann, pp. 1887-1909.
- Inamdar, J. A., Gangadhara, M., Avita, S. and Rao, N. V. 1980. Epidermal studies in some Indian cultivars of *Bougainvilleas*. Fed. Rep. 91: 259–266.
- 10. Lu D, Gilbert MG (2003) *Bougainvillea*. Flo Chi 5: 430-434.
- 11. Metcalfe, C. R. and Chalk, L. 1950. Anatomy of the dicotyledons Vol. II. London, Oxford University Press.

- 12. Najmaddin, C. and Mahmood, A. J. 2016. Anatomically and palynologically studies of some *Carthamus Tinctorius* genotypes. Int. J. Bio. Sci. 3: 1-13.
- Niesenbaum, R. A. and Schueller, S. K. 1997. Effects of pollen competitive environment on pollen performance in *Mirabilis jalapa* (Nyctaginaceae). Se. Pla. Repr. 10: 101–106.
- 14. Nowicke, J. W. and Luikart, T. J. 1971. Pollen morphology of the Nyctaginaceae. Gra. 11: 145-150.
- Perveen, A. and Qaiser, M. 2001. Pollen Flora of Pakistan -XXVII Nyctaginaceae. Tur. J. Bot. 25: 385-388.
- 16. Phillips, B. G. 1976. Anatomy and developmental morphology of *Allionia* L. (Nyctaginaceae), University of Arizona.
- Pramanick, D. D., Mondal, M. and Maiti, G. G. 2015. Pollen morphological studies on some members of the family Nyctaginaceae in India. Asi. J. Pla. Sci. Res. 5: 72-76.
- Rajput, K. S. and Rao, K. S. 1998. Cambial anatomy and absence of rayes in the stem of *Boerhaavia* species (Nyctaginaceae). Ann. Bota. Fenn. 35: 131-135.
- 19. Ruzin, S. E. 1999. Plant Microtechnique and microscopy. New York, Oxford University Press.
- 20. Sreekumar, V. B. 2004. Systematics and phylogeny of the genus *Calamus* linn. (arecaceae) in the western ghats. Dissertation, University of Calicut.
- Struwig, M., Jordaan, A. and Siebert, S. J. 2011. Anatomy of the southern African *Boerhavia* and *Commicarpus* species (Nyctaginaceae). Bang. J. Pla. Tax. 18: 105-115.
- Struwig, M., Siebert, S. J. and Jordaan, A. 2013. Pollen morphology of members of southern African *Boerhavia* and *Commicarpus* (Nyctaginaceae). Both. 43: 15–22.
- Swanepoel, J. J. and Villiers, C. E. D. 1987. A numerical - taxonomic classification of *Vitis* spp. and cultivars based on leaf characteristics. Sou. Afr. J. Eno. Vit. 8: 31-35.
- 24. Xuan, X., Wang, Y., Ma, S. and Ye, X. 2011. Comparisons of stomatal parameters between normal and abnormal leaf of *Bougainvillea spectabilis* Willd. Afr. J. Bio. 10: 6973-6978.