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RESEARCH ARTICLE

FOLIAR MICROMORPHOLOGY OF *EUPHORBIA GOLONDRINA* L.C. WHEELER (EUPHORBIACEAE) FROM CAMEROON

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ABSTRACT

Euphorbia golondrina L.C. Wheeler (Euphorbiaceae) is an important plant used in traditional medicine in Cameroon. There exists a dearth of scientific literature on the anatomical and morphological attributes of this plant. Hence, the ultrastructure and crystal deposits of *Euphorbia golondrina* L.C. Wheeler (Euphorbiaceae) were assessed and illustrated for the first time using light microscopy (LM), scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDXS) with a view to explore the taxonomic significance of foliar epidermal features and their role in biosynthesis of plant secondary metabolites. Fresh leaves of *E. golondrina* were prepared for analysis using standard methods. The leaves are amphistomatic and characterized by paracytic stomata which were more on the abaxial surface than the adaxial surface. Anticlinal walls were undulate in pattern while the epidermal cell shape was irregular. Stomata indices range from 30.20 % *to* 60.20 % in the abaxial and adaxial surfaces respectively. The leaves have eglandular trichomes which were sparsely distributed over the entire surface. The EDXS analysis revealed Mg, Al, Si, Fe, S and Ca ions as predominant mineral components. The absence of glandular trichomes on the leaf of this plant suggests that some other tissues on the leaf are responsible for the secretion of secondary metabolites.

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INTRODUCTION

The family Euphorbiaceae habours about 300 genera and over 7500 species with the genus Euphorbia L. being a major contributor to the pantropical species and most species rich in the Neotropics (Ali *et al*., 2008; Ali *et al*., 2009). The genus *Euphorbia* and family Euphorbiaceae were named in honour of a Greek physician to King Juba II of Mauritania called Euphorbus believed to have used *Euphorbia resinifera* latex to cure the King swollen belly ailment (Lovell, 1998; Van Damme, 2001). Cameroon with a great diversity of habitats is one of the botanically rich countries in Africa with over 9.000 species of plants and 160 endemics (Sunderland *et al*., 2003; Onana, 2011).

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Most of the endemic taxa are found around mount Cameroon and other highlands that constitute part of western Cameroon which is less than 1 % of the geographical area of west Africa and encompasses 50 % of the flora of Tropical West Africa (Cable and Cheek, 1998). There are about 174 species of *Euphorbiaceae* in the Cameroon Guineo–Congolian rainforest, of which 146 species occur in the southwest region. Onana (2011) reported 28 species of Euphorbia in the checklist of Vascular plants of Cameroon of which 9 species corroborate the checklist of the mount Cameroon region of the southwest region published by Cable and Check (1998).

Many of the herbaceous, leafy species of *Euphorbia* are commonly called "spurges." This word derives from the Old French word espurgier (Latin expurgare), which means "to purge" refereeing to the medicinal property of some of the species. The sap of many herbaceous *Euphorbia* species have traditionally been used as a purgative, or laxative. Many

Euphorbia species have not annihilate their economic importance as weeds, medicinal plants, ornamentals and wild vegetables. Since time immemorial, *Euphorbia* species have found wide application as medicinal plants including the use for the treatment of skin diseases, gonorrhoea, migraine, cough, dysentery, intestinal parasites and wart cures (Kinghom and Evans, 1975; Singia and Pathak, 1990; Usher, 1974). Even today, many Euphorbia plant concoctions, fresh latex and teas are used in alternative medicine. *E. tirucalli* is known for its curative features against diseases like warts, cancer, gonorrhea, arthritis, asthma, cough, earache, neuralgia, rheumatism, toothache, excrescences, tumours and others (Duke, 2000; Van Damme, 1989). *Euphorbia thymifolia* is used as an anti-viral against simplex virus-2 (Van Damme, 1989) whereas *E. maculata* is said to cure cholera, diarrhoea and dysentery (www.botanical.com.). *E. Pulcherrima* is grown in parks, lawn gardens and homes as an ornamental plant (Khalil *et al*., 2014).

Euphorbia golondrina L.C. Wheeler which naturally grows in Central America, is a medicinal plant used by the people of Mexico (Wheeler, 1940). It is a herbaceous annual weedy plant of the Chaesmyce subgenus of the genus Euphorbia that grows to about 50cm in height on alluvial and marginal soils at road verges, cultivated fields and is geographically restricted to USA, Mexico and Cameroon (Ndam *et al*., 2015).The leaves of this invasive plant are glabrous, opposite, oblong, petioles with entire margines and rounded base on a slender stem. The staminate flowers are creamy-white with a cluster incase in a cyathium. The plant is of free blooming nature, flowering from June to November. An infusion of the roots is used traditionally in the treatment of diabetes while a decoction of the leaves mixed with *Senna alata* is used against conjunctivitis, gastritis, enterocolitis, tonsils, vaginitis, hermorrhoids, prostatism and snake poison (Aleksandroff, 2011; Rudriguez, 2013). Ethnobotanical information revealed that the white latex from the stem is applied topically in the treatment of warts and painful swellings by the Mundani people of the mount Bambouto Caldera, Western Cameroon.

Sufficient interest seems to have been revived during the past two decades on the role of internal organization of the individual organs of plants. Plant secondary metabolites are produced by plants and stored in various organs including the leaves, stem and roots (Asrafi *et al*., 2008). Leaves occupy a prominent position in this regard and their various features such as venation, stomata and trichomes were found useful in solving taxonomic and phylogenetic issues (Badmus and Afolayan, 2010). Plants communicate with their external environment, protect and maintain essential internal physiological and biochemical processes through specialized epidermal structures (Otang *et al*., 2014). Information on their morphology, therefore, has bearing on a wide variety of issues. Eglandular and glandular trichomes which originate from epidermal cells are functionally termed as hairs that cover the aerial surfaces of plants. Utrastructures of plants such as stomata, trichomes and lenticels which can be unicellular to multicellular are recognized for their varying roles in gaseous exchange, transpiration, protection against arthropods and herbivores, act as attractants to pollinators or for fruit dispersal, storage and secretion of bioactive compounds which include phytochemicals with biological activities of interest to many industries (Ascensão *et al*., 1998, Heinrich *et al.,* 2002; Wagner, 1991; Werker *et al*., 1994; Dyubeni and Buwa, 2012). Many trichomes are glandular and have a secretory funtion. Several macrocyclic diterpenoids with antibacterial, anticancer, Prostaglandin E2 inhibitory, anti multidrug resistant, prolyl endopeptidase inhibitory, antifeedant, anti Human Immunodeficiency Virus and analgesic activities have been recently reported from numbers of *Euphorbia* species. Members of *Euphorbia* are rich in phenolics, steroids, alkaloids, glucosinolates, tannins, aromatic esters, diterpenoids, tetracyclic triterpenoids, pentacyclic Triterpenoids, essential oils and several bioactive constituents (Ahmad *et al.,* 2002a, Ahmad *et al.,* 2002b; Jassbi *et al.,* 2004, Vasas *et al.*, 2004, Pervaiz *et al.,* 2004; Feizbakhsh *et al.,* 2004; Ravikanth *et al.,* 2002; Hohmann *et al.,* 2003; Corea *et al.*, 2005; Ahmad *et al.,* 2006). Sesquiterpenes and monoterpenes isolated from plants are of scientific interest due to their plant growth inhibitory properties and can be used as lead compounds in bioherbicide production (Marimoto and Komai, 2005; Seigler, 1994) and phytochemicals produced from the glandular trichomes have high concentration and activity as compared to those produced from other parts of the plant (Duke *et al*., 2000).

The taxonomic relevance of foliar epidermal characters of angiosperms has been well documented (Inamdar and Gangadhara 1977; Kotresha and Seetharam 1995, 2000; Olowokudejo and Pereira-Sheteolu 1988).The utility of foliar epidermal features in distinguishing the taxonomic group Euphorbia was equally clearly established (Stace, 1965, 1984; Dilcher, 1974; Raju, 1981; Rao and Raju, 1985, 1988; Manohari, 2004). Anatomical and micromorphological analyses of the leaf can also provide relevant evidence for the taxonomy and identification of medicinal plants species even when they are available as leaf fragments by the pharmaceutical industry (Afolayan and Adebola, 1992). The genus has been the subject of several chemotaxonomic, foliar morphological studies and phytochemical investigations. Despite the myriad of studies on the pharmacological profile of the plants of this genus, there still exists a dearth of scientific literature on the phytochemical and chemotaxonomic attributes of this important medicinal plant. In addition to the scanty reports on the medicinal uses of this plant, no information was found in literature on the micromorphology and ultrastructure of the leaf appendages of *E. golondrina.* Information on the foliar anatomy and micromorphology can further enlighten our established perceptions on the inherent interrelationships between structure and function as regards the synthesis and secretion of bioactive secondary metabolites by the leaves of plants. This study was therefore undertaken for the first time, using both light and scanning electron microscopy to obtain information on the micro-morphological features of *E. golondrina* which would help in its identification and authentication and to determine the elemental composition of the leaf sections by energy dispersive X-ray spectroscopy.

MATERIALS AND METHODS

Plant materials

The plant used in this study was obtained in 2014 from their natural habitat around the Afala hills in the western flank of the Mt Bambouto Caldera (latitudes 5º38 N and 5º43 N and

longitudes 9º58 E and 10º06 E), Wabane municipality of Western Cameroon. The plant was authenticated at the Department of Botany and Plant physiology, University of Buea, Cameroon and a voucher specimen (SCA/1687) was deposited in the Herbarium of the Limbe Botanic Garden (SCA).

Leaf Epidermal studies (Light microscopy)

Collected fresh leaves of *E. golondrina* were viewed on a light microscope (Lm) with an in-built digital camera for analysis according to the procedure of Coopoosamy and Naidoo (2011). Adaxial and Abaxial Leaf epidermal preparations involved cutting 1-3 cm^2 portions from the standard median portion of the leaf lamina near the mid-rib using a sharp razor and then swelled by boiling in water for thirty minutes. The leaf pieces were later soaked in concentrated nitric acid (HNO_3) in capped specimen bottles for about eight to twenty-four hours to macerate the mesophyll. Tissue disintegration was indicated by bubbles, and the epidermises were transferred into Petri dishes containing water for cleansing and then, epidermises were separated with forceps and mounting needles. Tissue debris was cleared off the epidermises with a fine-hair brush and washed in several changes of water. Drops of different grades of Ethanol: 50 %, 70 %, 75 % up to 100 % were added in turn to harden the cells. Preparations were later stained with Safranin O in 50 % alcohol for about five minutes before mounting in glycerine on the glass slide. The epidermises were mounted on the glass slide with upper surfaces facing up and then covered with cover-slips and ringed with nail varnish to prevent dehydration. Slides were examined with Motic light microscope at x10 and x40 magnifications. For stomatal measurements, observations were made on the epidermal preparations and stomatal guard cell length determined using an ocular micrometer. Stomatal and epidermal cell frequency was estimated by counting the number of stomata per field of view at varying magnifications. These values were then converted to stomata per mm^2. Stomatal index (SI) was calculated according to the formula of Salisbury (1927):

SI =[number of stomata/(number of stomata + number of epidermal cells)]*100

Scanning electron microscopy and Energy Dispersive X-ray Spectroscopy (SEM-EDXS)

The standard protocol used for SEM was adopted from Dyubeni and Buwa (2012). From freshly harvested leaves, 4-6 mm of the upper and lower parts were removed and fixed in 6 % glutaraldehyde in 0.05 M sodiumcacodylate for 24 hours. The samples obtained after washing in 0.05 M cacodylate buffer (pH 7.5) were rinsed in distilled water 2-3 times using a pasture pipette. This was then dehydrated in a graded series of ethanol 10-100 % for 20 min per rinse. The samples were then stored in 100 % ethanol in the refrigerator till use. This was followed by critical point drying with liquid CO_2 in Hitachi HCP-2 critical point dryer. Each dried sample was mounted onto an aluminium specimen stub with double-sided carbon coated sputter-coating with gold-palladium (Eiko IB. 3 Ion Coater). Both the adaxial and abaxial surfaces of the leaves were examined at varying magnifications using JEOL (JSM-6390LV) scanning electron microscope (SEM), operated at 10-15 kV accelerated voltage. All the representative features examined were captured digitally using Microsoft image programmed for windows. The energy dispersive X-ray spectroscopy-SEM, involved both fixing and dehydration procedure as in SEM, while a FEI QUANTA 200 oxford EDXanalyser was used for the analysis of the chemical elements present in the leaves and representative spectra presented. A focused beam of electrons was used to scan the leaf epidermis at the point where examination of its chemical composition was desired. Identification of elements with the EDXS is hinged on emission of characteristic X-rays by the epidermal cells under bombardment with electrons. The dispersed spectra produce a pattern of X-rays characteristic of the element excited. Only the most intense emissions, the so-called K′ and Kα lines, were analyzed with the spectrometers.

RESULTS AND DISCUSSION

Light and scanning electron microscopy

The foliar ultra structures of *E. golondrina* are presented in (Figs. 1–2 and Table 1). The lower and upper epidermis is singled layered and impregnated with epicuticular waxes on the leaf surface to form a quaternary sculpturing of flakes (Fig.1 A and 1B).

Table 1. Foliar micromorphological characteristics of *Euphorbia golondrina* L.C. Wheeler leaf from Cameroon

Stomatal characteristics	Abaxial	Adaxial
No of epidermal cells	111	69
Mean number of subsidiary cells/stoma	4	4
Mean guard cell length	60.2µm	56.2 µm
Mean guard cell width	2.39 µm	2.40 µm
Guard cell index	190 μm^2	95.94 μm^2
Number of stomata/ mm^2	8	2
Stomatal index	60.2±0.1%	30.2±0.2%
Pattern of anticlinal wall of epidermal cell	Undulating	Undulating

Plant epicuticular wax or bloom consisting mainly of straight-chain aliphatic hydrocarbons with a variety of substituted functional groups, function to decrease surface wetting and moisture loss. In several groups of plants, hydrophobic cuticles are very resistant and have a high fossilization potential and when habouring epicuticular wax can reflect UV light, such as the white, chalky, wax coating of *Dudleya brittonii*, which has the highest ultraviolet light (UV) reflectivity of any known naturally occurring biological substance (Mulroy,1979). The Pattern of anticlinal wall of epidermal cell is undulate while the epidermal cell wall shape is irregular on both adaxial and abaxial surfaces of the leaf of *E. golondrina* (Fig.1B and 1C). The frequency of epidermal cells in the abaxial and adaxial surfaces was 111 and 69 cells/mm^2 respectively. Stomata density was 8stomata/mm^2 in the abaxial surface and 2stomata/mm^2 in the adaxial (Table 1). The leaves are amphistomatic but the number of paracytic stomata on the adaxial surface is much lower than on abaxial surface (Fig 1E, IF and Table 1). This is a common phenomenon in most angiosperms (Ashafa *et al*., 2008; Eichert and Fernandez, 2011). The presence of paracytic stomata in the tribe Euphorbieae were reported by Metcalfe and Chalk (1950) and Raju and Rao (1977).

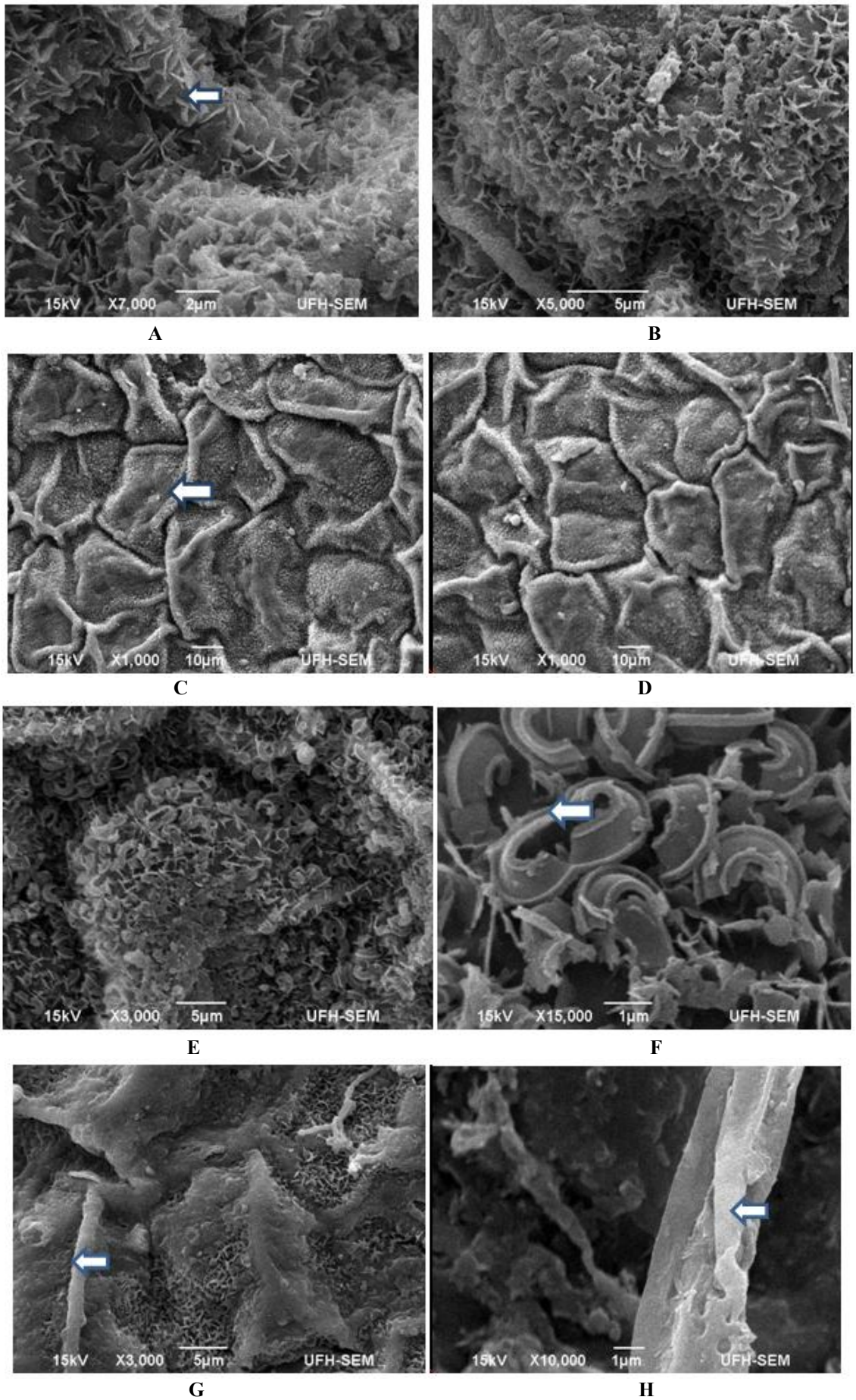

Fig.1. Scanning electron micrographs of Euphorbia *golondrina* L.C. Wheeler leaf from Cameroon. A) Epicuticular waxes in abaxial surface (B) Epicuticular waxes in adaxial surface. Note the wax platelet (arrowhead). C) Epidermal cell walls revealed by SEM in abaxial surface. D) Epidermal cell walls revealed by SEM in adaxial surface. Solid arrowheads = junction between epidermal outer periclinal and anticlinal cell walls. E) Paracytic stoma in abaxial surface; (F) higher magnification of paracytic stoma on abaxial surface (arrow). G) Eglandular lateral trichomes on adaxial surface. H) Higher magnification of flaccid glandular trichome

Same studies recorded anisocytic, anomocytic, and diacytic stomata in 50 species belonging to 17 different tribes of the Euphorbiaceae. In their opinions the paracytic type forms the basic stomatal type for the family Euphorbiaceae because of common occurrence in majority of tribes studied. Kakkar and Paliwal (1974) observed anomocytic, anisocytic, paracytic and cyclocytic type of stomata in various species of *Euphorbia.* There were equal number of guard cells surrounding each stoma both at the abaxial and adaxial surfaces with the mean guard cell length and width being 56.2-60.2 µm and 2.39-2.40 µm respectively (Table 1). Stomatal index has proved useful in aiding the recognition of plant species (Otang *et al*., 2014).

Energy dispersive X-ray spectroscopy (EDXS)

Results of qualitative X-ray microanalyses of the epidermis of *E. golondrina* show some crystal deposits were found on the leaves surfaces and some near the stomata (Fig.2). The chemical nature of the crystals showed that they were predominantly composed of Carbon (C), magnesium (Mg), oxygen (O), iron(Fe), calcium (Ca), sulfur (S) silicon (Si), and aluminium (Al) on the PET crystal detector (Fig. 2), while gold(AU) was assumed to be derived from the spur coater. The peak heights of the elements in the spectra show their comparative measures.

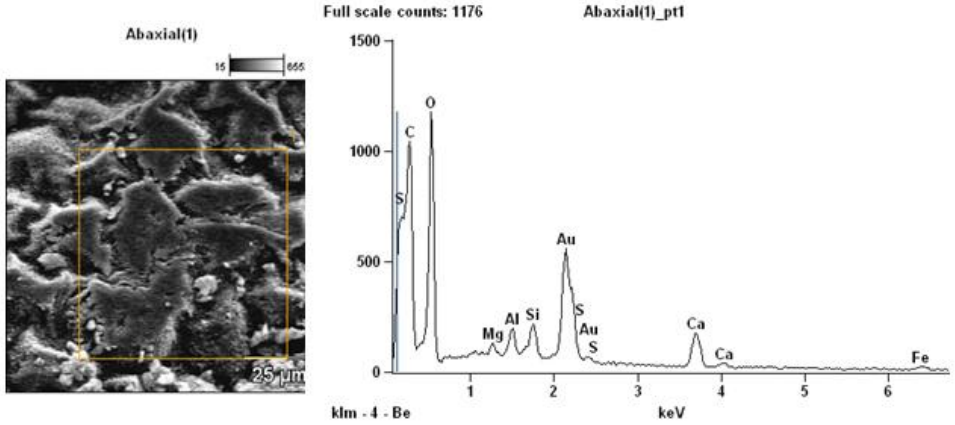

Fig.2. X-ray spectra of various elements detected in the leaf epidermis of *Euphorbia Golondrina* L.C. Wheeler from Cameroon, micrograph on the left show the point of focus of the electron beam

The stomata index was larger on the abaxial surface (60.2 %) than on the adaxial surface (30.2 %). SEM confirm the presence of eglandular trichome on the leaves of *E. golondrina*, The trichome is a simple, lateral, flat base atternuated type with equal representation on the abaxial and adaxial surfaces (Fig. 1G and 1H). The presence of trichome and stomata on the abaxial surface of the leave may be an adaptation to the often extreme environmental conditions in its restricted phytogeographical abode in the USA and Mexican deserts of Texas and Chihuahua respectively (Karabourniotis and Liakopoulos, 2005; Ndam *et al*., 2015). Metcalfe and Chalk (1950, 1979) documented basically three types of trichomes, viz., glandular, eglandular and stinging types. Rao and Raju (1985) opinionated trichome types and their distribution in 250 species of the family Euphorbiacea.

According to Gales and Toma (2006), trichomes found in *Euphorbia* were simple, unicellular or multicellular, and uniseriate. Kakkar and Paliwal (1974) presented the same observations. According to Werker (2000), trichomes are defined as unicellular or multicellular appendages which originate from epidermal cells only, and which develop outwards from the surface of various plant organs. Eglandular trichome abundance and distribution over the surface of the leaves are geared, possibly against foraging insects and airborne propagules of fungi as well as heat and moisture regulation. Positive correlation between trichome density and insect resistance has been demonstrated in various plant species (Afolayan and Adebola, 1992).

Based on the elemental X-ray microanalyses, the mineral crystals present at the level of the mesophyll of *E. golondrina* were probably mixtures of calcium oxalate, calcium sulphate and silica. Based on the relatively high carbon and oxygen peaks and the lower sulphur and obvious calcium peaks; calcium oxalate was considered as the major component and calcium sulfate as the minor component. Mineral formation in plants is common phenomenon (Franceschi and Nakata, 2005) and the most abundant minerals formed by plants are silica, calcium carbonate and calcium oxalate. Calcium is an essential plant nutrient for growth but when in excess, calcium is often precipitated in the form of calcium salts such as oxalate, carbonate, sulfate, phosphate, silicate, citrate, tartrate and malate (Prychid and Rudall, 1999). Crystal formation and their functions have been proposed, including roles in cellular ion balance, in plant defense against herbivore, in tissue rigidity and support, in detoxification of oxalic acid or aluminium (Franceschi and Nakata, 2005;Otang *et al*., 2014). Hence, the co-occurrence of aluminum suggests the potential role of the crystals in detoxification of aluminum and heavy metals, as reported in the past (Kuo-Huang *et al*., 2007). However, additional knowledge from cell and molecular biology is necessary to yield a more coherent, although certainly more complex, general theory of plant crystallization (Lersten and Horner, 2006). Franceschi and Horner (1980) opinionated that the presence of oxalate in crop plants caused a negative impact on human health acting as a toxin, and in CaO kidney stone formation. Therefore, considering the great deal of crystallization occurring at the mesophyll of *E. golondrina* leaf,

the traditional use of the plant should be very carefully monitored to ensure the safety of the recipients' spectrum. Their presence in the trichomes most probably increases the mechanical stability of the leaf appendages. It could be assumed that the anionic contents of these crystal deposits found on the leaves of this plant were produced in other foliar tissues since glandular trichomes believed to be site of secretory unit for plants were not present in *E. golondrina*

Conclusion

The present work summarizes current knowledge of some diagnostic microscopic characters of *E. golondrina* leaf surface. This study has identified the presence of eglandular tichomes, paracytic stoma, amphistomal epidermal surface, waxy quaternary sculpturing of flakes deposit near the stomata and mineral components of Mg, Al, Si, Al, Fe, S and Ca ions. Information on the foliar anatomy and micromorphology can further enlightened our perceptions on the inherent interrelationships between structure and function as regards the synthesis and secretion of bioactive secondary metabolites by plants. Therefore, the stomata, trichomes and epidermal cells can be effectively used to identify and distinguish different plant species and draw parallels or convergence with the molecular evidence. Since no glandular trichomes were present on the leaves of this herb, the bioactive components present in this plant may be produced in some other tissues in the leaf other than the trichomes.

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REFERENCES

Ahmad, V.U, Hussain, H., Hussain, J., Jassbi, A.R., Bukhari, I.A., Yasin, A., Choudhary, M.I and Dar, A. 2002a. New bioactive diterpenoid from Euphorbia decipiens. Z. Naturforsch 57: 1066-1071.

Ahmad, V.U., Hussain, J., Hussain, H., Farooq, U., Ullah, F, Lodhi, M.A and Choudhary, M.I. 2006. Two new diterpene polyesters from Euphorbia decipiens. *Nat Prod Res.,* 19: 267-274.

Ahmad, V.U., Zahid, M., Khan, T., Asim, M and Ahmad, A. 2002b. Chemical constituents of Euphorbia heteradenia. *Proceed Pak Acad Sci.,* 39: 201-206.

ALeksandroff, S. 2011. Plants in the Mayan Culture. Imago (1St ed.), Playal de Carmen, Mexico. pp. 283.

Ali, M.S, Ahmed, S., Saleem, M. 2008. Spirowallichiione: A Rearranged Multiflorane from Euphorbia wallichii Hook F. (Euphorbiaceae). Mol., 13: 405-411.

Ali, I., Naz, R., Khan, W.N., Gul, R. 2009. Biological screening of different root extracts of Euphorbia wallichii. *Pak J Bot.*, 4: 1737-1741.

Afolayan, A.J and Adebola, P.A. 1992. Leaf morphology and anatomy of Hyptis suaveolens (Linn.) *Poit. Boiling Res. Commun*., 4: 25-33.

Ascensão, L., Figueiredo, A.C, Barroso, J.G, Pedro, L.G, Schripsema, J, Deans, S.G and Scheffer, J.C. 1998. Plectranthus madagascariensis: morphology of the glandular trichomes, essential oil composition, and its biology activity. *Intern J Plant Sc.,* 159: 31–38.

Ashafa, A.O.T, Grierson, D.S and Afolayan, A.J. 2008. Foliar micromorphology of Felicia muricata Thunb., A South African medicinal plant. Pak J Biol Sc., 11: 1713–1717.

Ashrafi, Z.Y, Mashhadi, H.R, Sadeghi, S., Alizade, H.M. 2008. Allelopathic effects of sunflower (Helianthus annuus) on germination and growth of wild barley (Hordeum spontaneum). *J Agric Tech.,* 4: 219-229.

Badmus, A.A, Afolayan, A.J. 2010. The foliar micromorphology of Arctoitis arctotoides (L.f) O.Hoffm. *J Med Plant Res.,* 4(16): 1643–1646.

Cable, S and Cheek, M. 1998. The plants of mount Cameroon. A conservation checklist. Roy Botanic gard., Kew, pp. 198.

Coopoosamy, R.M, Naidoo, K.K. 2011. Anatomical features of the medicinal importance of Aloe excelsa (Berger). *Afr J Biotech.,* 10(39): 7622–7632.

Corea, GC, Fattorusso, E, Lanzotti, V. 2005. Amygdaloidins A-L, twelve new 13α-OH jatrophane diterpenes from Euphorbia amygdaloides L. Tetra., 61: 4485-4494.

Dilcher, D.L. 1974. Approaches to the identification of angiosperm leaf remains. *Bot Rev.,* 40: 1-157.

Dold, A.P, Cocks, M.L. 1999. Preliminary list of Xhosa plant names from Eastern Cape, South Africa. *Bothalia* 29, 267-292.

Duke, S.O., Dayan, F.E., Romagni, J.G and Rimando, A.M. 2000. Natural products as sources of herbicides: current status and future trends. Weed Res., 40: 99-111.

Dyubeni, L., Buwa, L.V. 2012. Foliar micromorphology of Salvia greggii A. Gray (Lamiaceae). *Afric J Plant Sc*., 6: 32-38.

Eichert, T., Fernández, V. 2011. Uptake and release of elements by leaves and other aerial plant parts. In Marschners' mineral nutrition of higher plants. P. Marschner, mineral nutrition of higher plants. P. Marschner, editor. *Academic Press, Oxford,* pp. 71-84.

Feizbakhsh, A., Bighdeli, M., Tehrani, M.S., Rustaiyan, A and Masoudi, S. 2004. Chemical constituents of essential oil of Euphorbia teheranica Boiss, a species endemic to Iran. *J Essent Oil Res*.,16: 40-41.

Franceschi, V.R, Horner, Jr H.T. 1980. Calcium oxalate crystals in plants. *Bot Rev.,* 46: 361–364.

Franceschi, V.R and Nakata, P.A. 2005. Calcium oxalate in plant formation and function. Ann Rev Plant Biol., 56: 41-71.

Gale, R.C, Toma, C. 2006. Comparative anatomy of the vegetative organs of some Euphorbia species (euphorbiaceae juss.) from the Romanian flora. *Rom J Biol-Plant Biol*., 51: 39-47.

Heinrich, G, Pfeifhofer, H.W., Stabentheiner, E., Sawidis, T. 2002. Glandular hairs of Sigesbeckia jorullensis Kunth (Asteraceae): morphology, histochemistry and composition of essential oil. *Ann Bot.*, 88: 459–469.

Hohmann, J., Rédei, D., Forgo, P., Molnár, J., Dombi, G, Zorig, T. 2003. Jatrophane diterpenoids from Euphorbia

mangolica as modulators of the multidrug resistance of L5128 mouse lymphoma cells. *J Nat Prod.,* 66: 976-979.

Inamdar, J.A, Gangadhara, M. 1977. Studies on the trichomes of some Euphorbiaceae. *Feddes Repert*., 88: 103-111.

Jassbi, A.R, Zamanizadehnajari, S., Tahara, S. 2004. Chemical constituents of Euphorbia marschalliana Boiss. Z. Naturforsch., 59: 15-18.

Kakkar, L., Paliwal, G.S. 1974. Studies on leaf anatomy of Euphorbia L. V. Epidermis. Proc Ind Nat Sci Acad., 40: 55-67.

Karabourniotis, G., Liakopoulos, G. 2005. Phenolic compounds in plant cuticles: Physiological and ecophysiological aspects. *Adv Plt Physiol.*, 8: 33-47.

Khalil, A.T, Shinwari, Z.K, Qaiser, M, Marwat, K.B. 2014. Phyto-therapeutic claims about euphorbeaceous plants belonging to Pakistan; an ethnomedicinal review. *Pak. J Bot.,* 46(3): 1137-1144.

Kotresha, K., Seetharam, Y.N. 2000. Epidermal micromorphology of some species of Cassia L. (Caesalpiniaceae). Phytomorp., 50(3-4): 229-237.

Kuo-Huang, L.L., Ku, M.S.B, Franceschi, V.R. 2007. Correlation between calcium oxalate Crystals and photosynthetic activity of palisade cells of shadeadapted Peperomia gilbella. Botanic Stud., 48: 155-164.

Lerstern, N.R, Horner, H.T. 2006. Crystal macropattern development in Prunus Serotina (Rosaceae, Prunoideae) leaves. *Ann Bot.,* 97: 723-729.

Lovell, C.R. 1998. Some biblical plants of dermatological importance. Clin Dermatol.,16: 33-40.

Manohari, A.L.S. 2004. Studies on the Foliar Epidermology, Architecture and Anatomy of some Apocynaceae. PhD Thesis, Andhra University, Visakhapatnam, India, pp. 204.

Marimoto, M., Komai, K. 2005. Plant growth inhibitors: patchoulane-type sesquiterpenes from Cyperus rotundus L. *Weed Biol Manage.,* 5: 203-209.

Metcalfe, C.R, Chalk, L. 1950. Anatomy of the Dicotyledons. Oxford University Press, Oxford, p. 724.

Metcalfe CR, Chalk L. 1979. Anatomy of the Dicotyledons (2nd edition). Oxford University Press, Oxford, p. 276.

Mulroy, T.W. 1979. "Spectral properties of heavily glaucous and non-glaucous leaves of a succulent rosette-plant". Oecologia 38(3): 349–357.

Ndam, L.M., Mih, A.M., Fongod, A.G.N., Tening, A.S., Temenu, N.A and Fujii Y. 2015. First record of Euphorbia golondrina L. C. Wheeler (Euphorbiaceae) in Cameroon. *J Biodiversity Environ Sc*., 6 (4): 428-434.

Ogunkunle, A.T.J, Oladele, F.A. 2008. Leaf epidermal studies in some Nigerian species of Ficus L. (Moraceae). *Plant system Evol.*, 274: 209-221.

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