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ULTRASTRUCTURAL MICROMORPHOLOGY OF *BULBINE ABYSSINICA* A. RICH. GROWING IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA

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Abstract

The genus *Bulbine* (Asphodelaceae) comprises about 40 species in South Africa. *Bulbine abyssinica* is a succulent member of the genus that occurs from the Eastern Cape, through Swaziland, Lesotho, and further north to Ethiopia. The species is often used in traditional medicine to treat rheumatism dysentery, bilharzia and diabetes. Inspite of its ethno medicinal value, not much data concerning the micro-morphological features is available in literature. The present study was undertaken to examine the ultra-morphological features of the leaf, stem and root of the plant using light and scanning electron microscopes and the elemental composition. The elemental compositions of the plant parts were done using energy dispersive x- ray spectroscopy. The mean length and width of the guard cells in the abaxial surface are 0.15 ± 0.002 mm and 0.14 ± 0.002 mm, respectively while those of the adaxial surface are 0.14 ± 0.001 mm and 0.12 ± 0.001 mm, respectively. The electron microscopy revealed the presence of crystals in the leaves, stems and roots. The EDXS microanalysis of the crystals revealed the presence of sodium, silicon, potassium and calcium as the major constituents. The leaf also showed the presence of iron and magnesium, while the stem had aluminium, phosphorous and magnesium. The X-ray analysis of the roots also revealed the presence of sulphur and aluminium. The presence of these elements, which are vital in maintaining good health status, suggests the potential role of *B. abyssinica* in the treatment of infections and some chronic diseases, especially diabetes mellitus.

Key words: *B. abyssinica*, Scanning electron microscopy, X-ray spectroscopy, Mineral elements.

Introduction

The use of plant materials for the treatment of various diseases is very common in African countries. In traditional African medicine, various *Bulbine* species are used to treat a number of conditions including sexually transmitted diseases, wound infections, dysentery and urinary tract infections (Wanjohi *et al.,* 2005; Van Staden & Drewes, 1994).

The genus *Bulbine* (Asphodelaceae) comprises about 40 species in South Africa. These plants are mostly herbs with leaves that are evergreen and succulent in appearance. They have thick fleshy tuberous roots and are easy to grow (Wanjohi *et al.,* 2005). *Bulbine* species are commonly used by traditional healers in South Africa in the treatment of wounds, burns, rashes, itches, ringworm, cracked lips and herpes (Hutchings *et al.,* 1996).

Bulbine abyssinica is a succulent, perennial herb with a rhizomatous base which grows in small clusters. The plant is a hardy, water-wise plant that offers a brilliant yellow display when in flower. Both flowers and fruit have an attractive bicolored (yellow and black) appearance (Pooley, 1998). The roots are many, slender or swollen. It has soft, dark green leaves which are grass-like and up to 350 mm long. Mature fruits are black, 4mm in diameter and often covered with the faded perianth persisting as a cap. *B. abyssinica* occurs from the Eastern Cape, through KwaZulu-Natal, Swaziland, Lesotho, Free State, North-West, Gauteng, Mpumalanga, Limpopo and further north to Ethiopia (Pooley, 1998).

B. abyssinica is often used in traditional medicine to treat rheumatism dysentery, bilharzia and cracked lips (Wanjohi *et al.,* 2005). In South Africa, the whole plant is used by traditional healers in the management of diabetes mellitus (Oyedemi *et al.,* 2009).

The stem and root of *Bulbine* species are known to contain anthraquinones such as chrysophanol and knipholone which have anti-bacterial properties (Van Staden & Drewes, 1994; Van Wyk, 1995). Some anthraquinones have been isolated from the roots of *B. abyssinica* (Dagne &Yenesew 1994; Bezabih *et al.,* 1997). From the fruits of *B. abyssinica*, three new dimeric anthracene derivatives namely; abyquinone A, abyquinone B and abyquinone C have also been isolated (Wanjohi *et al.,* 2005). Though advances have been made to scientifically validate the use of the plant in traditional medicine, their medicinal value remains obscure.

Production of such compounds with therapeutic properties has been reported in many plant species. In most cases, the source of these bioactive compounds has been attributed to the trichomes (Afolayan & Meyer, 1995). In addition, among the various cell contents, crystals such as calcium oxalate of different types are found in different organs of the plant. Such crystals occur in almost every part of the plant, including both the vegetative and reproductive organs (Prychid & Rudall, 1999).

Crystals such as calcium oxalate play key roles in plant's defense. They may also be waste products as a result of crassulacean acid metabolism, an adaptive feature of plants in arid habitants (Franceschi & Nakata, 2005; Badmus & Afolayan, 2005). Some crystals mediate in reducing the transpiration rate of the leaf and wilting, hence preventing excessive water loss during dry spell (Wintola & Afolayan, 2013). Crystals have been reported to have great therapeutic value including curing cuts, wounds and also in fractures. Presence or absence of crystals, and their dimensions are useful in correct identification of crude drugs. This helps in detection of adulterants (Masram & Harisha, 2012). Therefore, developmental and structural studies of these trichomes and crystalline bodies, according

to Afolayan & Meyer (1995) and Masram & Harisha (2012) can shed light on the nature of these secreted materials and their functional significance.

Despite the pharmacological and therapeutic uses of *B. abyssinica*, no information on its ultrastructural morphology is available in literature. Therefore, the objective of this study was to examine the ultrastructural morphology and elemental compositions of the leaves, stems and roots using scanning electron microscope (SEM) and to relate our findings to their possible functional role in the production of therapeutic compounds.

Materials and Methods

Plant material: The leaves, stems and roots of *B. abyssinica* were collected from lower Ncera location in Nkonkobe Municipality of the Eastern Cape Province, South Africa. This area lies at the latitude 30° 00 to 34°15'S and longitudes 22° 45' to 30° 15'E (Afolayan & Wintola, 2014). It is bounded by the sea in the East and the drier Karoo (semi-desert vegetation) in the West. The elevation ranges from sea-level to approximately 2,200 m in the north and the vegetation is veld type, known as the Eastern Cape thorn veld (Masika & Afolayan, 2003). The voucher specimen (voucher no. KibMed 2014/01) was deposited in the Giffen's herbarium, University of Fort Hare, South Africa for authentication.

Light microscopy: Microscopic examinations of the epidermal parts of the leaf were carried out according to the procedure of Ogunkunle & Oladele (2008); Otang *et al.* (2014). Leaf samples of 1 to 3 cm were sectioned from the mid portion of the adaxial and abaxial surfaces of mature leaves using a razor blade. The sections were washed with distilled water for 2-3 minutes. These sections were placed on clean glass slides with 1-2 drops of distilled water, covered with a cover slip and observed under a Motic light microscope. The microphotographs were taken with a digital camera that was fitted to the light microscope (Akyol, 2014). The stomata density was estimated at 10X magnification. These values were converted to stomata per mm^2 (Wilkinson, 1979). The density of the epidermal cells, guard cell length and width, guard cell indices were estimated according to the method of Wintola & Afolayan (2013).

Scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM-EDXS): Fresh leaves, stems and roots were cut into segments (both cross sectional and longitudinal sections) of 4-6 mm in length and fixed in 6% glutaraldehyde with pH 7.3 for 12 h. The sections were rinsed with 0.05M sodium cacodylate buffer (pH 7.5). Each sample was later rinsed in distilled water and dehydrated in a graded series of ethanol 10-100% for 20 min per rinse. The sections were dried in a Hitachi HCP-2 critical point dryer and mounted on aluminium stubs with double-sided carbon coated sputter coating with gold palladium (Elko IB-3 Ion Coater). The samples were examined at varying magnifications using JEOL (JSM-6390LV) scanning electron microscope (SEM) that was operated at 10-15 kV accelerated voltage. The energy dispersive X- ray spectroscopy (EDXS) involved both fixing and dehydration procedure as in SEM, while the elemental analysis was done using energy dispersive x-ray analyser which was coupled to SEM, manufactured by Thermo Electron Corporation, 6733B-IUUSN, USA. The Noran system six software was used for imaging.

Results and Discussion

Different features were observed in the leaves of *B. abyssinic*a. The mean stomata densities on the abaxial and adaxial surfaces were 21.53 ± 1.68 and 9.81 ± 1.01 per mm^2 respectively. This amphistomatic feature shows the paracytic stomata surrounded by the guard cells. The stomata are embedded within the epidermal layer with two subsidiary cells surrounding each stoma (Fig. 1A & B).

The epidermal cells are symmetric rectangular in shape with undulating cell walls on both surfaces (Fig. 1C & D). The density of the epidermal cells was not statistically different in the lower and upper surfaces, that is, 21.74 ± 0.85 and 21.94 ± 1.36 per mm^2, respectively. The guard cells are dumbbell shaped outlined by a thick inner and thin outer walls which are horizontally embedded to the subsidiary cells (Fig. 2A & 2B). The mean length and width of the guard cells on the abaxial surface are 0.15 ± 0.002 mm and 0.14 ± 0.002 mm respectively while those of the adaxial surface are 0.14 ± 0.001 mm and 0.12 ± 0.001 mm respectively. The guard cell index is $1.67x10^{-8}$ μm^2 and $1.30x10^{-8}$ μm^2 in the abaxial and adaxial surfaces respectively. There is no statistical difference in the guard cell indices and mean length and width of the guard cells in both abaxial and adaxial surfaces ($p \leq 0.05$).

The micromorphology of the leaf and root section of *B. abyssinica* as seen under SEM is presented in Fig. 3 A-D, showing the distinctive stomata with the presence of mineral crystals positioned in the stomata pores, scattered in the intercellular spaces within the plant surface and inside the root cavities.

The micromorphology of the stem is presented in Fig. 4A and B showing a cross section of the vascular bundle of the xylem (arranged in ringed shapes) and phloem tissues. The xylem tissue serves as a vessel for transporting water, dissolved minerals and fibres which support the plant. The phloem transports organic substances through the stem. The structure of the stem shows the presence of droplets of mineral sediments on the phloem tissue which are translocated to all parts of the plant (Fig. 4B).

The X-ray microanalysis of the leaf, stem and root of *B. abyssinica* generated spectra of the following micro and macro mineral elements: carbon (C), oxygen (O), sodium (Na), silicon (Si), potassium (K) and calcium (Ca). The leaf spectra also indicated presence of iron (Fe) and magnesium (Mg). The stem spectra indicated presence of aluminium (Al), phosphorous (P) and magnesium (Mg), while the root had sulphur (S) and aluminium (Fig. 5, 6 and 7). Gold (Au) was probably from the spur coater. The mineral constituents of this plant are an indication of its ethno-pharmacological importance. For instance, the high peaks of carbon, oxygen, phosphorus and sodium shows the abundance of these elements while calcium, magnesium, potassium and iron were in moderate quantity (Table 1). Aluminum, silicon and sulphur were found in small quantity.

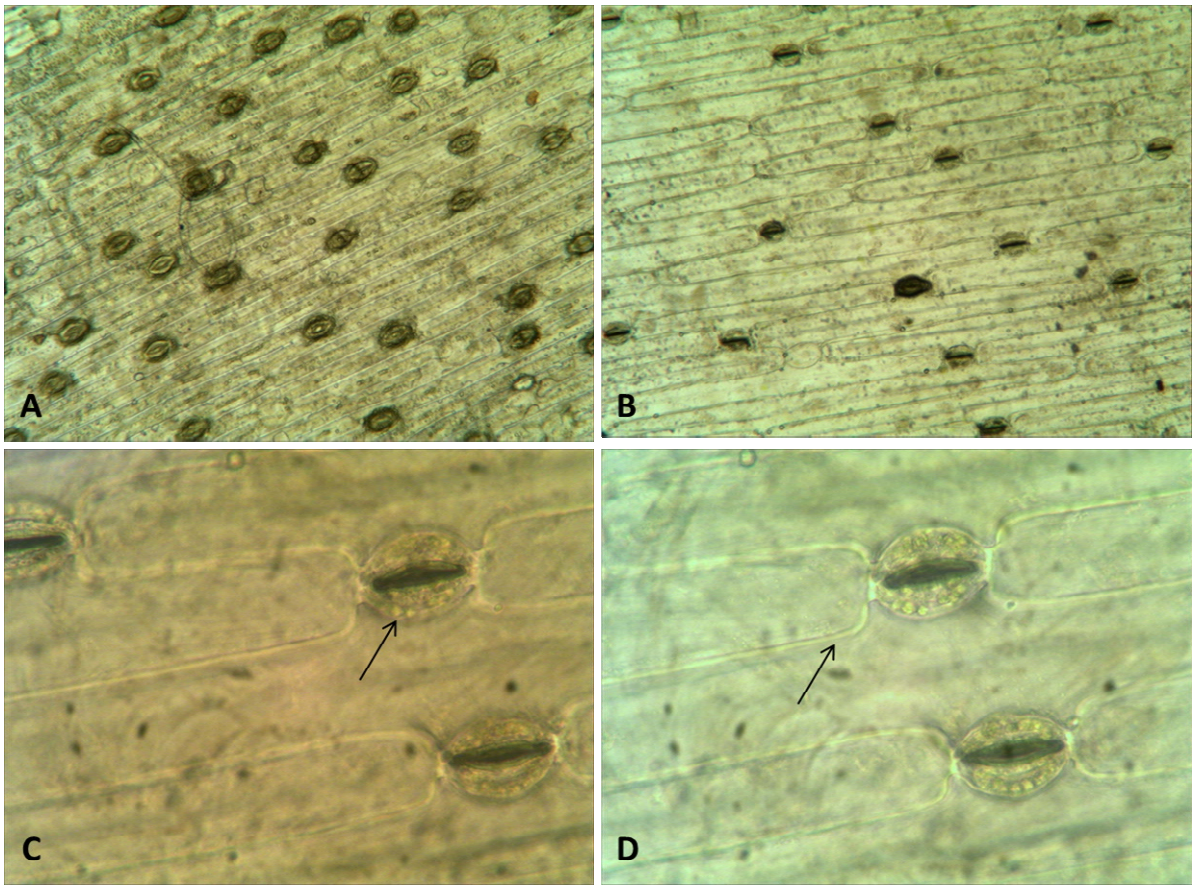

Fig. 1. Ultra morphological features of the leaf of *B. abyssinica* (A) stomata distribution on the abaxial leaf surface (10X); (B) epidermal cells and stomata distribution on the adaxial leaf surface (10X); (C) Guard cells and dumbbell stomata (arrow) in the adaxial leaf surface (40X); D) Rectangular epidermal cells (arrow) on abaxial leaf surface (40X).

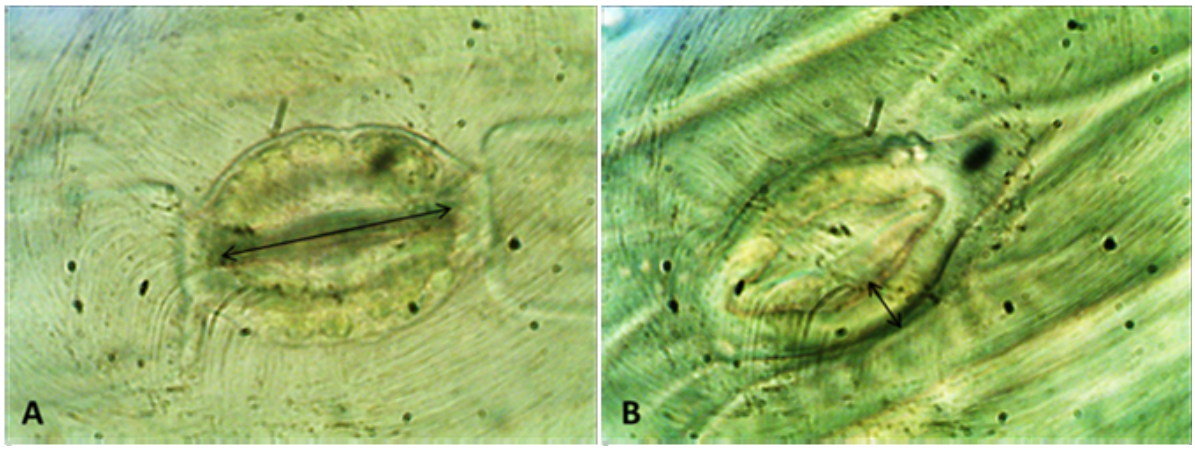

Fig. 2. Stoma, dumbbell shaped guard cell, (A) abaxial surface (100X); (B) Adaxial surface (100X).

These mineral elements play different metabolic roles in human. For example, calcium is a key element known in maintaining bones and teeth, regulation of nerve and muscle function. It plays a vital role in enzyme activation during blood clotting (Pravina *et al.*, 2013). Sulfur serves a structural function in cartilage, bone, tendons and blood vessel walls (Parcel, 2002). In addition, sulphur plays a significant role in protein synthesis, cell regeneration and blood cleansing (Afolayan & Otunola, 2014). Potassium is the main intracellular cation in the human body and is required for vital cellular processes. It is involved in regulating acid–base balance, blood pressure, cell membrane function and basic cellular enzymatic reaction (Greenlee *et al.*, 2009; Chatterjee *et al.*, 2011). Magnesium acts as a cofactor to several enzymes like kinase, which participate in energy and protein production processes

(Adhikari *et al.,* 2006). It's also vital in strengthening cell membrane structure (Jahnen-Dechent & Ketteler, 2012).

Iron is an important element, it is found in the portion of the cell involved in energy production, neurotransmitter synthesis and in maintaining a stable immune system. Iron functions as haemoglobin in the transport of oxygen (Lieu *et al.,* 2001). Phosphorus is located in every cell of the body and is vitally functions as a constituent of bones, teeth, phosphorylated metabolic intermediates and nucleic acids. It serves buffering action in the formation of high energy compounds (Soetan *et al.,* 2010). Sodium is the principal cation in extracellular fluids. It regulates plasma volume and acid-base balance, involved in the maintenance of osmotic pressure of the body fluids, preserves normal function of the nervous and muscle (Constantin *et al.,* 2011).

Silicon is an essential component of mucopolysaccharides, hyaluronic acid and chondroitin-4-sulfate, which are important constituents of connective tissue. This is a biological cross-linking agent contributing to the structure and resiliency of connective tissue and calcification of bones hence plays a role in wound healing (Price *et al.,* 2013). The presence of these elements accounts for the pharmacological use of *B. abyssinica* in management of diabetes mellitus and complications associated with the disease such as wound healing (Oyedemi *et al.,* 2009).

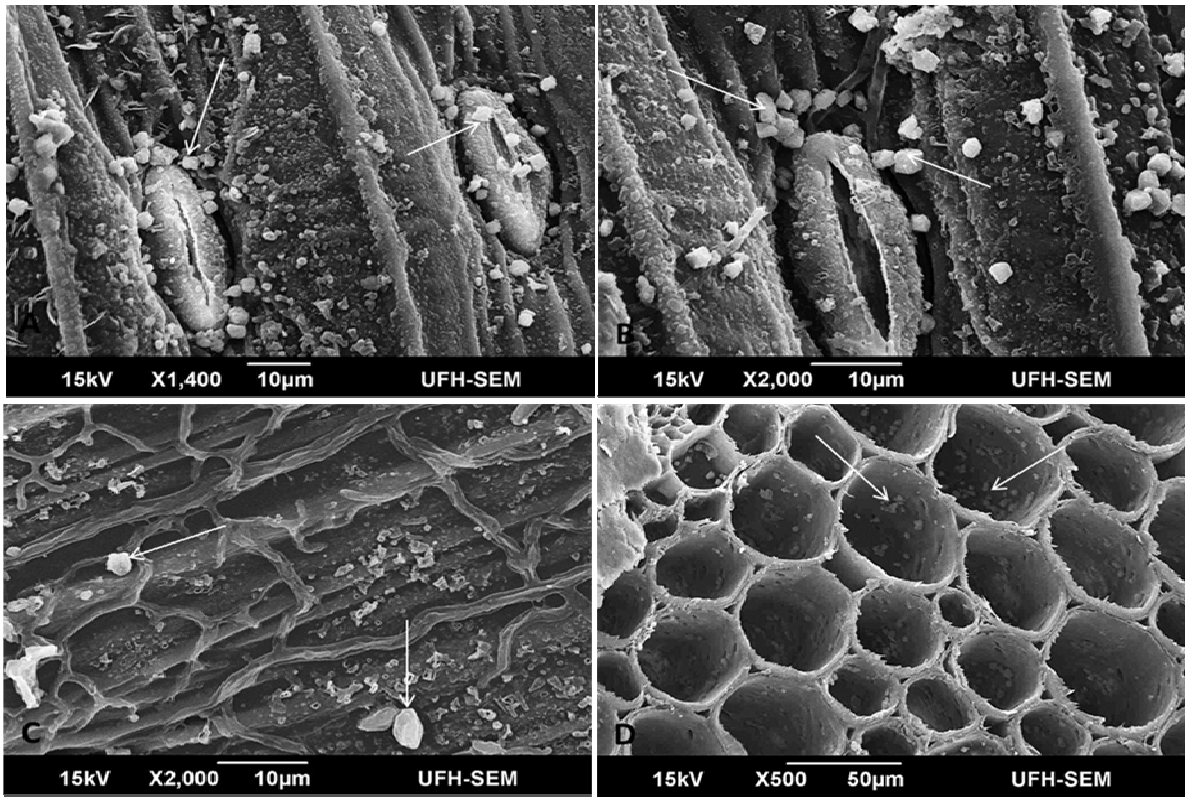

Fig. 3. Scanning Electron Micrograph of *B. abyssinica* leaf and root (A) Crystal deposit on the abaxial surface the leaves; (B) concentrated crystals within the stomata on the adaxial surface; (C) fragments of crystals scattered in abaxial surface; (D) crystal deposits in the root cross section.

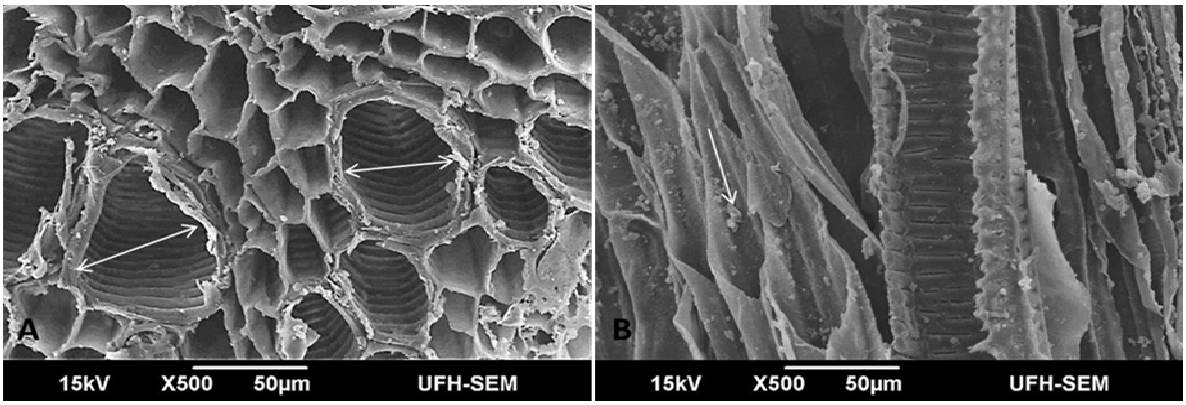

Fig. 4. Scanning Electron Micrograph of *B. abyssinica* stem (A) cross sections showing the inner rings of xylem tissues; (B) stem having mineral sediments on the phloem tissue.

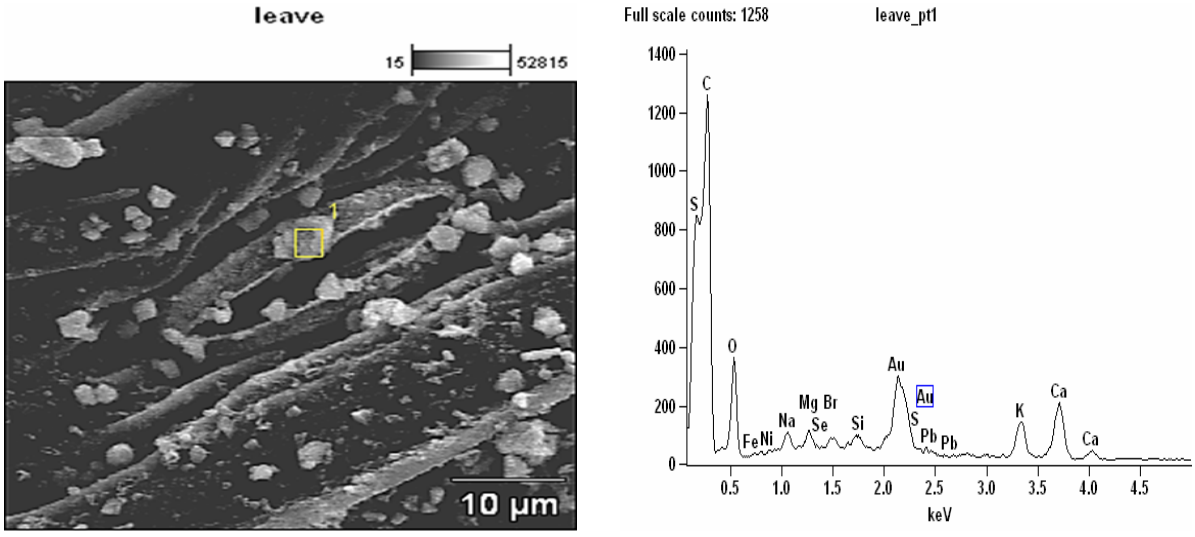

Fig. 5: Energy Dispersive X-ray analysis of crystal deposits in the stomata pore of *B. abyssinica*; micrograph showing the point of focus of the electron beam.

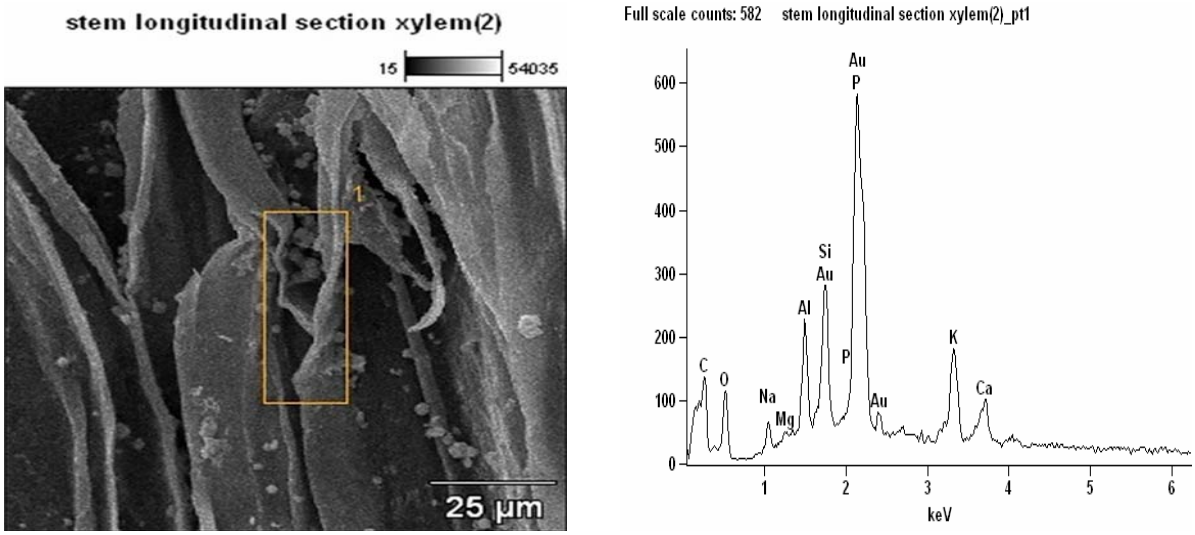

Fig. 6. Energy Dispersive X-ray analysis of crystal deposits in the vascular tissue of *B. abyssinica*; micrograph showing the point of focus of the electron beam.

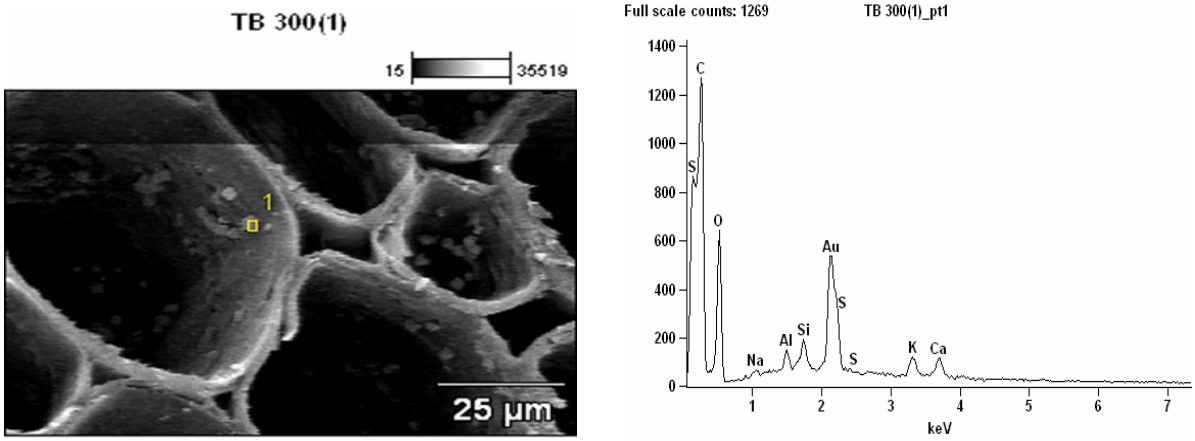

Fig. 7. Energy Dispersive X-ray analysis of crystal deposits in the extracellular space of *B. abyssinica* root; micrograph showing the point of focus of the electron beam.

Table 1. Percentage elemental composition of crystal deposits on *B. abyssinica* leaf, root and stem.

Element (%)	Leaf	Root	Stem
Carbon	32.68 ± 0.46	23.92 ± 0.37	2.22 ± 0.15
Oxygen	18.63 ± 0.61	20.34 ± 0.42	4.84 ± 0.29
Sodium	1.22 ± 0.09	0.31 ± 0.05	0.67 ± 0.10
Magnesium	0.92 ± 0.10	0	0.13 ± 0.07
Silicon	0.83 ± 0.07	1.16 ± 0.06	3.26 ± 0.17
Iron	0.03 ± 0.01	0	0
Potassium	1.80 ± 0.10	1.80 ± 0.10	4.79 ± 0.31
Calcium	2.01 ± 0.11	2.01 ± 0.11	2.55 ± 0.16
Phosphorus	0	0	0.09 ± 0.09
Sulphur	0	0.18 ± 0.13	0
Aluminium	0	0.73 ± 0.05	2.17 ± 0.14
Gold	49.56 ± 8.07	49.56 ± 8.07	79.27 ± 7.81

Conclusion

The present study reveals the micro-morphological characteristics of the leaf of *B. abyssinica* such as amphistomatic epidermal surfaces, rectangular epidermal cells, mean guard cell length and width, subsidiary cells surrounding the stomata and crystal deposits. The crystal deposits are present in the vascular system and extracellular spaces of the roots. It also shows the presence of crucial micro and macro mineral elements which may probably account for its ethno-pharmacological importance. This knowledge will help in partially validating the use of the plant in traditional medicine.

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References

Adhikari, S., J.A. Toretsky, L. Yuan and R. Roy. 2006. Magnesium, essential for base excision repair enzymes, inhibits substrate binding of N-methylpurine-DNA glycosylase. *J. Biol. Chem*., 281(40): 29525-29532.

Afolayan, A.J. and G.A. Otunola. 2014. Ultrastructure and elemental analysis of *Hypoxis hemerocallidea*: a multipurpose medicinal plant. *Afr. J. Tradit. Complement. Altern. Med*., 11(4): 39-43.

Afolayan, A.J. and J.M. Meyer. 1995. Morphology and Ultrastructure of secreting and nonsecreting foliar trichomes of *Helichrysum aureonitens* (Asteraceae). *Int. J. Plant. Sci*., 156 (4): 481-487.

Afolayan, A.J. and O.A. Wintola. 2014. A survey of medicinal plants used in the treatment of dysentery in Amathole district municipality, South Africa. *Pak. J. Bot*., 46(5): 1685-1692.

Akyol, Y. 2014. The morphological and anatomical studies on endemic C*rocus biflorus* Miller subsp. *Pulchricolor* (Herbert) Mathew (Iridaceae) in Turkey. *Pak. J. Bot*., 46(2): 573-578.

Badmus, A.A. and A.J. Afolayan. 2010. The foliar micromorphology of *Arctotis arctotoides* (L.f.) O. Hoffm. *J. Med. Plants. Res*., 4(16): 1643-1646.

Bezabih, M., S. Motlhagodi and B.M. Abegaz. 1997. Isofuranonaphthoquinones and phenolic and knipholone derivatives from the roots of *Bulbine capitata*. *Phytochemistry*, 46: 1063-1067.

Chatterjee, R., H. Yeh, D. Edelman and F. Brancati. 2011. Potassium and risk of Type 2 diabetes. *Expert. Rev. Endocrinol. Metab*., 6(5): 665-672.

Constantin, M. and I. Alexandru. 2011. The role of sodium in the body. *Balneo Res. J.*, 2(1): 70-74.

Dagne, E. and A. Yenesew. 1994. Anthraquinones and chemotaxonomy of the Asphodelaceae. *Pure. Appl. Chem*., 66: 2395-2398.

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

Greenlee, M., C.S. Wingo, A.A. McDonough, J. Youn and B.C. Kone. 2009. Narrative Review: Evolving concepts in potassium homeostasis and hypokalemia. *Ann. Intern. Med*., 150(9): 619-625.

Hutchings, A., A.H. Scott, G. Lewis and A. Cunningham. 1996. *Zulu medicinal plants: an inventory.* University of Natal Press, Pietermaritzburg, South Africa.

Jahnen-Dechent, W. and M. Ketteler. 2012. Magnesium basics. *Clin. Kidney. J*., 5(1): 3-14.

Lieu, P.T., M. Heiskala, P.A. Peterson and Y. Yang. 2001. The roles of iron in health and disease. *Mol. Aspects. Med*., 22(2):1-87.

Masika, P.J. and A.J. Afolayan. 2003. An ethno-botanical study of plants used for the treatment of livestock diseases in the Eastern Cape Province, South Africa. *Pharm. Biol*., 41: 16-21.

Masram, H.G. and C.R. Harisha. 2012. Importance of calcium oxalate crystals in family *Vitaceae*. *Int. J. Comprehensive Pharmacy*., 3(2): 1-3.

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

Otang, W.M., D.S. Grierson and R. Ndip. 2014. Foliar micro-morphology of *Gasteria bicolor* Haw. (Asphodelaceae) from South Africa. *Afr. J. Tradit. Complement. Altern. Med*., 11(4): 71-76.

Oyedemi, S.O., G. Bradley and A.J. Afolayan. 2009. Ethnobotanical survey of medicinal plants used for the management of diabetes mellitus in the Nkonkobe

municipality of South Africa. *J. Med. Plants. Res*., 3(12): 1040-1044.

Parcel, S. 2002. Sulfur in human and applications in medicine. *Altern. Med. Rev*., 7(1): 22-44.

Pooley, E. 1998. A field guide to wild flowers of KwaZulu-Natal and the Eastern Region. Natal Flora Trust Publications, Durban, South Africa.

Pravina, P., D. Sayaji and M. Avinash. 2013. Calcium and its role in human body. *Int. J. Pharm. Biomed. Res*., 4(2): 659-668.

Price, C.T., K.J. Koval and J.R. Langford. 2013. Silicon: A review of its potential role in the prevention and treatment of postmenopausal osteoporosis. *Int. J. Endocrinology*., 2013: 6. DOI:10.1155/2013/316783.

Prychid, C.J. and P.J. Rudall. 1999. Calcium oxalate crystals in monocotyledons: A review of their structure and systematics. *Ann. Bot*., 84: 725-739.

Soetan, K.O., C.O. Olaiya and O.E. Oyewole. 2010. The importance of mineral elements for humans, domestic animals and plants: A review. *Afr. J. Food. Sci*., 4(5): 200-222.

Van Staden, L.F. and S.E. Drewes. 1994. Knipholone from *Bulbine latifolia* and *Bulbine frutescens*. *Phytochem*., 35(3): 685-686.

Van Wyk, B.E., A. Yenesew and E. Dagne. 1995. Chemotaxonomic significance of anthraquinones in the roots of *Asphodeloideae* (Asphodelaceae). *Biochem. Syst. Ecol*., 23(3): 277-281.

Wanjohi, J.M., A. Yenesew, J.O. Midiwo, M. Heydenreich, M.G. Peter, M. Dreyer, M. Reichert and G. Bringmann. 2005. Three dimeric anthracene derivatives from the fruits of *Bulbine abyssinica*. *Tetrahedron*., 61: 2667-2674.

Wilkinson, H.P. 1979. The plant surface (mainly leaf), Part 1: Stomata. In: *Anatomy of dicotyledons*. (Eds.): C.F. Melcalfe and L. Chalk. Clarendon Press, Oxford Chap 10(1): 113.

Wintola, O.A. and A.J. Afolayan. 2013. The foliar anatomy and micromorphology of *Aloe ferox* Mill. (Asphodelaceae). *Afr. J. Tradit. Complement. Altern. Med*., 11(2): 350-357.

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