http://dx.doi.org/10.4314/ajtcam.v11i2.21

THE FOLIAR ANATOMY AND MICROMORPHOLOGY OF *ALOE FEROX* MILL. (ASPHODELACEAE)

Olubunmi Abosede Wintola[1] and Anthony Jide Afolayan[1]*

[1]Medicinal Plant and Economic Development Research Centre, Department of Botany, University of Fort Hare, South Africa.

***Email**: Aafolayan@ufh.ac.za

Abstract

Background: The genus *Aloe* has been the subject of several chemotaxonomic and phytochemical investigations. *Aloe ferox* is an important source of biologically active compounds like anthrones, chromones, pyrones, and flavonoids. It is a plant used extensively in traditional medicine. Despite the myriad of studies on the pharmacological profile of the plant, there still exists a dearth of scientific literature on the anatomical and morphological attributes of this important medicinal plant.
Materials and methods: Hence, the objective of this study was to examine the foliar anatomy and micromorphological characteristics of *A. ferox* using light microscope and scanning electron microscope and to determine the elemental composition of the leaf sections by energy dispersive x-ray spectroscopy.
Results: Epidermal cells varied from round, square to hexagonal, with distinctive anticlinal walls and sunken stomata, equally distributed on both the adaxial and abaxial surfaces were observed. There were vertically elongated palisade cells with numerous chloroplasts in the palisade mesophyll layer. The EDXS analysis revealed Ca, K, S, Al, Mg, and Si as the major constituents of crystals present in the mesophyll.
Conclusion: 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.

Keywords: *Aloe ferox*; foliar micromorphology; anatomy; light microscope; scanning electron microscope; x- ray spectroscopy.

List of abbreviations: SEM – Scanning electron microscope; EDXS – Energy dispersive X-ray spectrometer; EDS - Energy dispersive spectrometer; EMU – Electron microscope unit.

Introduction

Globally, the genus Aloe (Aspodelaceae) comprises of about 500 species (Viljoen, 2008). However, over 120 species of the genus are confined to South Africa known as the center of diversity of Aloes (Dagne et al., 2000). Aloineae, a well-defined tribe of monocotyledonous family Asphodelaceae, offers additional insight into the inheritance of leaf anatomical characters. Several chemotaxonomic and phytochemical investigations have focused on this genus best known for the variety of chemical compounds accumulated on their leaves surfaces. This genus has several pharmacological importances such as anti-bacterial, anti-fungal, anti-tumor and hepatoprotective effects (Kametani et al., 2007; Norikura et al., 2002). It has proved to be one of the most important sources of biologically active compounds like anthrones, chromones, pyrones, coumarins, alkaloids, glycoproteins, naphthalenes and flavonoids. It has also been used extensively in traditional medicine and has maintained its popularity over the cause of time (Dagne et al., 2000).

Aloe ferox Mill is a species of Aloe known as Cape aloe, native to South Africa and is widely distributed in Free State, Western and Eastern Cape Provinces of South Africa (Shackleton and Gambiza, 2007). It is usually an arborescent single-stemmed perennial shrub of 2 - 3 m in height. The plant has large thick greenish succulent leaves of about 35 - 45 cm long and 4 - 6 cm wide with brown spines on the margin. It has attractive bright red or orange flower in erect, candle-shaped clusters compactly arranged on 5 - 12 racemes (Van Wyk et al., 1996; Wintola and Afolayan, 2010). It thrives in varied climatic conditions and diverse ecological niches such as in bushveld, road sides, gardens and undisturbed places.

For centuries, it has been used therapeutically for the treatment of constipation (Watt and Breyer-Brandwijk, 1962; Jia, et al., 2008). Today, the plant has been accepted widely for its antiseptic, cleansing, laxative, mosturising and anti-inflammatory properties (Van Wyk et al., 2002; Loots et al., 2007; Wintola et al., 2010). The fresh and dried whole leaf, juice (gel) and leave pulp of this plant are used directly as infusion and decoction for the treatment of various diseases such as skin cancer, gastrointestinal disorder, inflammation, burns and wound healing, psoriasis, tooth abscesses, sexually transmitted infection, arthritis, rheumatism, conjunctivitis and eye ailments as well as insect repellant (Watt and Breyer- Brandwijk, 1962; Githens, 1979; Van Wyk et al., 1997; Grierson and Afolayan, 1999; Crouch et al., 2006; Kambizi et al., 2007; Loots et al., 2007; Jia et al., 2008; Wintola and Afolayan, 2010). Today, *A. ferox* is an important source of drugs because of its pharmacologically active phytochemicals such as 1, 8-dihydroxy-3-hydroxymethyl-9,10-anthracenedione (aloe-emodin), l, 8-dihydroxy-3-methyl-9, 10 anthracenedione (chrysophanol) and 10-*C*-*β*-*D*-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9-anthracenone (aloin A) which are effective against bacteria and fungi (Afolayan et al., 2002; Kambizi et al., 2004; Wintola and Afolayan, 2011).

Since some bioactive compounds of plants are found in the appendages of leaves (Afolayan and Adebola, 1992; Aliero et al., 2006; Koduru et al., 2006), differences in the leaf surface characteristics among some typical members of the genus Aloe have been reported by Grace et al. (2009) who indicated the ultrastructure of the maculate groups of Aloe. However, there is a dearth of scientific literature on the anatomical and morphological attributes of *Aloe ferox*. Hence, the objective of this study was to investigate the foliar anatomy and micromorphological characteristics of *A. ferox* by using light microscope and scanning electron microscope and the elemental composition of the leaf sections using energy dispersive x-

http://dx.doi.org/10.4314/ajtcam.v11i2.21
ray spectroscopy. 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.

Anatomical and micromorphological analyses of the leaf can also provide relevant evidence for the taxonomy and identification of medicinal plants by the pharmaceutical industry (Afolayan and Adebola, 1992).

Materials and Methods

Plant materials

The plant used in this study was obtained from their natural habitat around the East camp road of the University of Fort Hare Alice campus. The plant was authenticated at the Department of Botany, University of Fort Hare, and a voucher specimen WinMed 2009/01 was deposited in the Griffen's herbarium of the University.

Light microscopy

Collected leaves of *A. ferox* were sectioned using razor blade and mounted on a slide and viewed on a light microscope (Lm) with an in built digital camera for analysis according to the procedure of Coopoosamy and Naidoo (2011). Slides were viewed using a Motic photomicroscope and results were recorded on 210 motic image version 2.0 digital recordings. Sections of the upper and lower epidermis of the fresh leaf were cut with a sharp razor blade, transferred onto a slide and protected with a slide slip. Observations were made on the epidermal preparations and stomatal density was estimated by counting the number of stomata per field of view at 10X magnification. These values were then converted to stomata per mm^2. The stomata index (SI) was estimated using the following formula, Stomatal index I = [S/(E + S)] X 100, as defined by Franco, 1939; Wilkinson, 1979; Ogunkunle and Oladele, 2008.

Where S is the number of stomata per unit area, E is the number of ordinary epidermal cells in the same area.

Scanning electron microscopy (SEM)

From a freshly harvested plant, 4-6 mm of the upper and lower parts were removed and fixed in 6% glutaraldehyde in 0.05 M sodium cacodylate for 24 h. 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 EDX analyser was used for the analysis of the chemical elements present in the leaves.

Results

SEM-EDXS

The cross section of the fresh leaf of *A. ferox* is presented in Figure 1. The thick cuticle has a rough surface, impregnated with wax and the rind tissue is beneath. The rind consists of many layers of cells interspersed with chloroplast synthesised constituents such as secondary metabolites which ooze out as yellowish exudates when the leaf was cut or damaged (Figure 1A). Below the rind tissue is the watery parenchyma (Figure 1B) responsible for the storage of many plant sythesised constituents.

A **B**

Figure 1: (A) Morphology of leaf section of *Aloe ferox;* **(B)** Cut surface of *leaf* showing: cuticle, rind and parenchymatous tissue.

Light microscopy (Lm)

Based on the detailed microscopic examination a distinctive array of fine and smooth surface epidermal sculpturing was observed. The leaf sculpturing appears complex with more or less hexagonal epidermal cells (Figure 2). The stomata on the epidermal cells of the leaves were evenly distributed (amphistomatal) on the lower and upper epidermal surfaces (Figures 2A and 2B). The frequency of epidermal cells in the abaxial and adaxial surfaces was 368 and 336 cells/mm^2 respectively. Stomata density was 9 stomata/mm^2 in the abaxial surface and 8 stomata/mm^2 in the adaxial

http://dx.doi.org/10.4314/ajtcam.v11i2.21
surface. The stomatal indices were 2.39 and 2.30 in the abaxial and adaxial surfaces respectively. The stomata were very numerous, deeply sunken below the general surfaces of the leaf with cuticular membrane extending throughout the stomata complex, overarched by four well developed lobes one from each of the four surrounding epidermal cells, and composed of a small outer chamber above the guard cells (subsidiary cell) mostly common to xerophytes plants (Figures 2C and 2D).

From the cross section, the main regions observed were the epidermis with thicker curticle cover, several layers of undifferentiated chlorenchymatous and water storage parenchyma cells (Figure 3). Distinct organelles like the chloroplast were observed in the cross section of the leaf when viewed under higher magnification (100X) while the upper and lower epidermal scrapping under oil immersions showed the thickened cell walls distinctly (Figure 4A-4B).

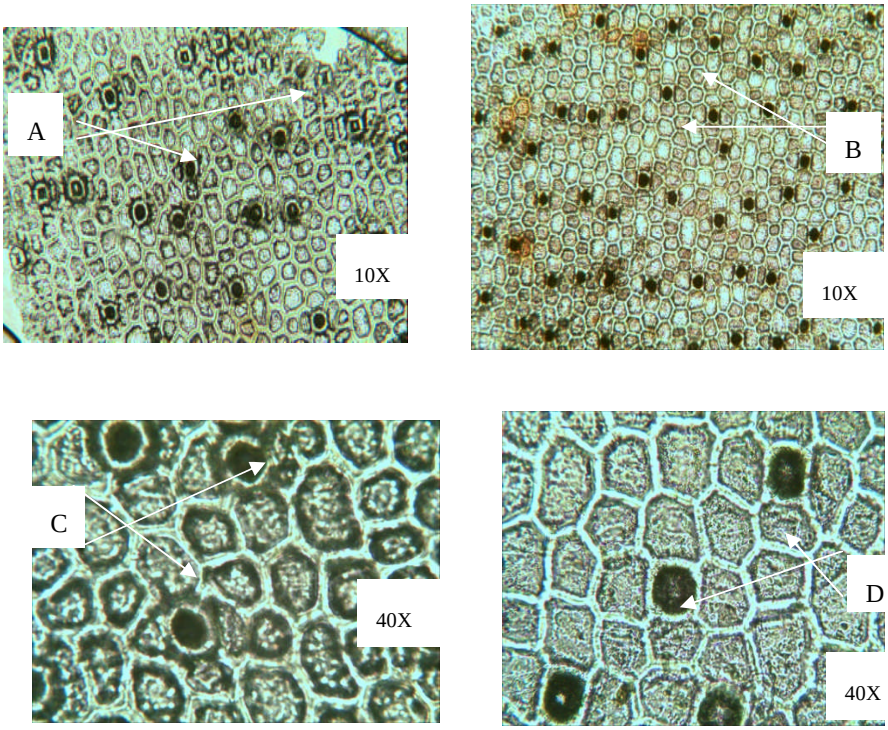


Figure 2: Epidermal surfaces of *A. ferox* leaf (LM); **(A)**: stomata distribution in the abaxial surface (10X); **(B)**: stomata distribution in the adaxial surface (10X); **(C)**: epidermal cells in abaxial surface (40X); **(D)** epidermal cells in adaxial surface (40X).

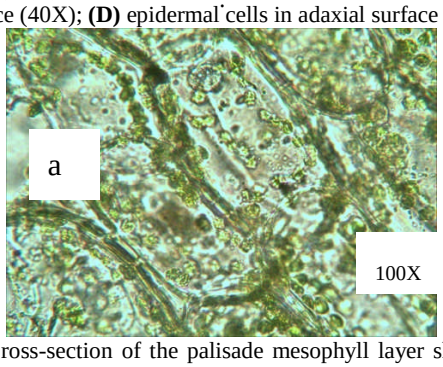
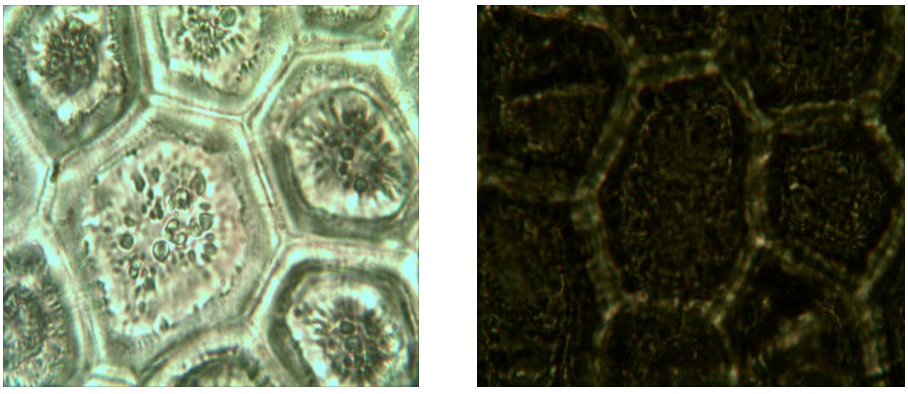


Figure 3: Cross sections of *A. ferox* leaf (LM); Cross-section of the palisade mesophyll layer showing palisade mesophyll cells with chloroplasts (100X)



A B

http://dx.doi.org/10.4314/ajtcam.v11i2.21
Figure 4: Epidermal cells in *A. ferox* leaf surfaces (LM-100X). **(A)** = Polygonal epidermal cells in lower surface with thick cell walls; **(B)** = Polygonal epidermal cells in upper surface with thick cell walls

EM-E

The micromorphology of the leaf of *A. ferox* as seen under SEM is presented in Figures 5-7. The epidermal surface portrays a smooth mosaic-like pattern in the abaxial surface (Figure 5A) and a rough pattern on the adaxial surface (Figure 5B). Cells of varying shapes ranging from square to round were observed and the stomata were present on both surfaces of the epidermis (Figures 5A and 5B). The leaf surface morphology showed the distinctive sunken stomata with aperture over arched on either side by a cuticular rim of guard cells (Figure 6A). The cuticular rim of dumb bell-shaped guard cells (subsidiary or accessory cells) overarching the stoma differ in structure from those of the other epidermal cell (Figure 6B). These dense rugulose tertiary sculpturing of the stomata were narrow in a closing state and square in the open state. The epidermal layer near the stomata was covered by thick curticle and a layer of wax deposited as flakes on the leaf surface to form a quaternary sculpturing of flakes (Figures 6B, C and D).

There are thin layers of thin walled cells believed to be the vascular bundle beside the palisade mesophyll cells (Figure 7A). The inner parenchymatous cells contained larger, solitary and needle-like styloid crystals, found inside the cells beneath the inner chlorenchymatous cells. The numerous needle-like crystals were found inside the intercostals cells just beneath the inner side of the chlorenchymatous cells (Figures 7B- D).

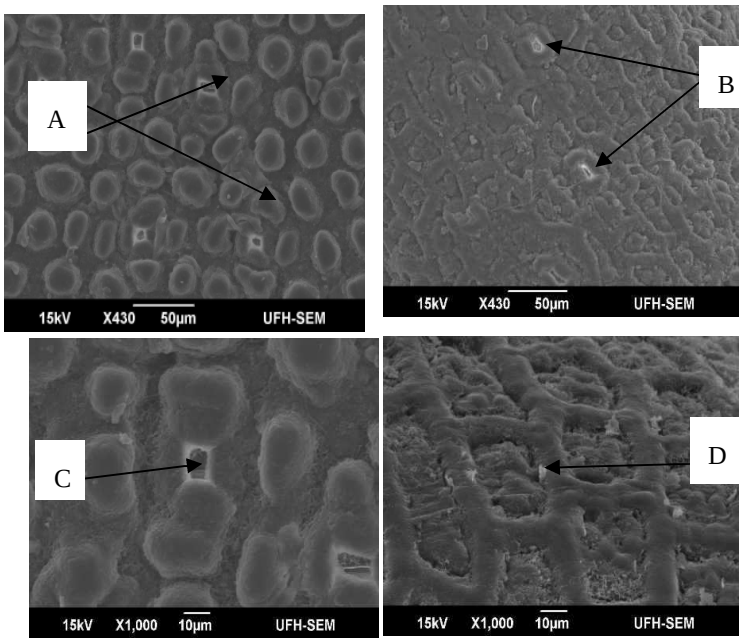
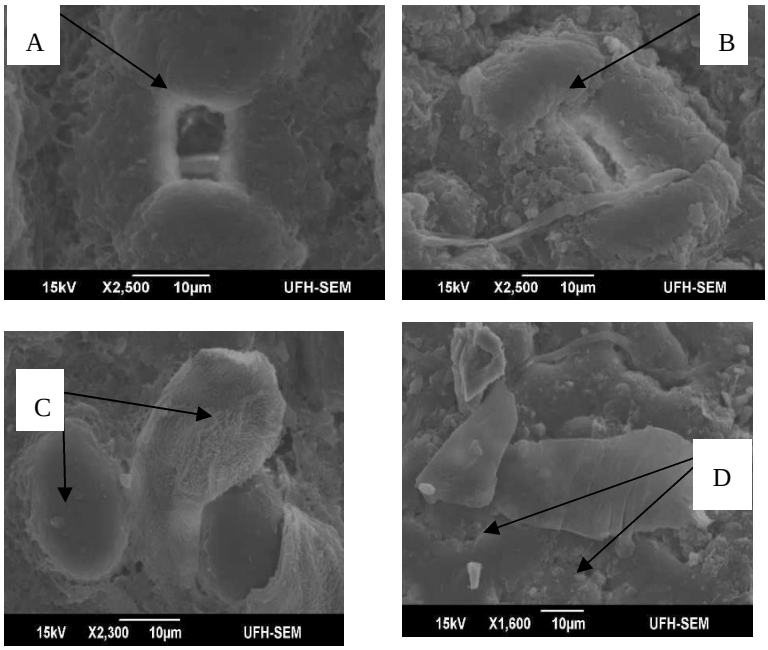


Figure 5: Epidermal surfaces of *A. ferox* leaf (SEM); **(A)** = stomata distribution in lower surface; **(B)** = stomata distribution in upper surface; **(C)** = sunken stoma and subsidiary cells in lower surface; **(D)** = rectangular epidermal cells with anticlinal walls.



http://dx.doi.org/10.4314/ajtcam.v11i2.21
Figure 6: Stomatal architecture of *A. ferox* leaf (SEM); **(A)** = sunken stoma with rectangular aperture and dumbbell-shaped guard cells; **(B)** = sunken stoma surrounded by waxy deposition of flakes; **(C)** = waxy deposition of flakes; **(D)** = waxy deposition of flakes and mineral crystal bodies overshadowing stomatal aperture.

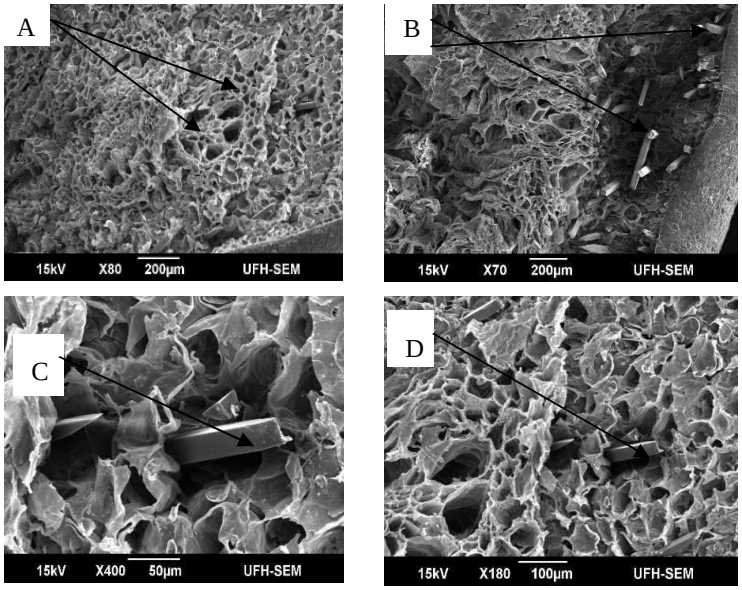
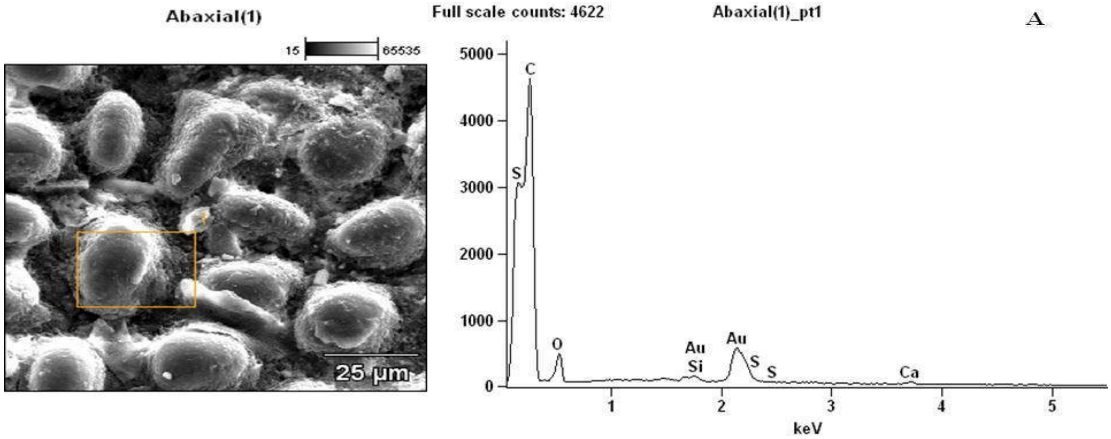


Figure 7: Cross section of mesophyll layer of *A. ferox* leaf (SEM); **(A)** vascular bundle; inner ring of xylem tissue surrounded by phloem; **(B - D)** solitary, needle-like styloid crystals in the mesophyll layer**.**

The X-ray microanalysis of the leaf structure of *A. ferox* showed the spectra of the following elements: calcium (Ca), potassium (K), sulphur (S), aluminium (Al), magnesium (Mg) and silicon (Si) on the PET crystal detector (Figures 8 and 9). The spectra of calcium (Ca) and sulphur (S) were prominent in all the tissues analysed while aluminium (Al) was only observed around the stomata in both the abaxial and adaxial epidermal surfaces (Figures 8B and C). Potassium (K) was only detected around a stoma cell on the abaxial epidermal leaf surface. Silicon (Si) was present in both abaxial and adaxial epidermal surfaces (Figures 8B and C) and magnesium (Mg) was observed in the spectrum of the abaxial epidermal surface (Figure 8B).



http://dx.doi.org/10.4314/ajtcam.v11i2.21

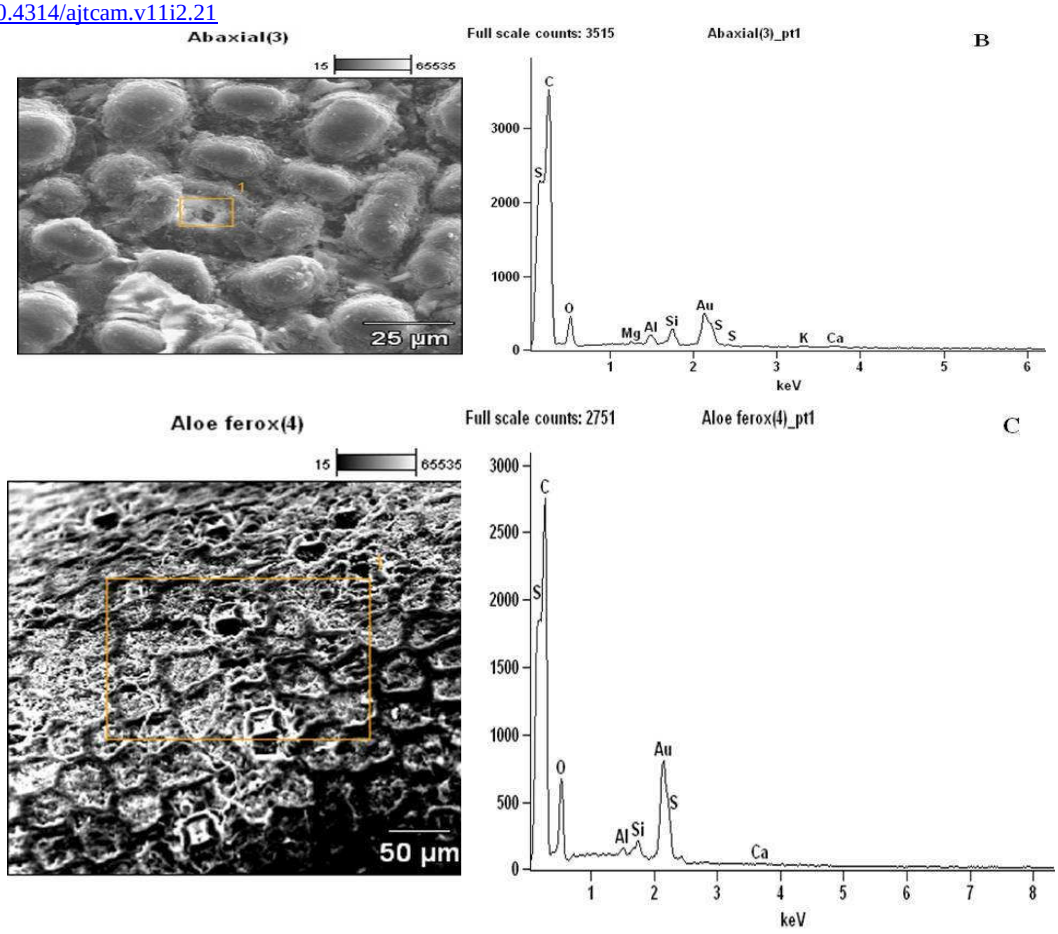


Figure 8: Characteristic X-ray spectra of *A. ferox* leaf; **(A)** spectrum of lower epidermal surface; **(B)** spectrum of stoma and guard cells; **(C)** spectrum of upper epidermal surface

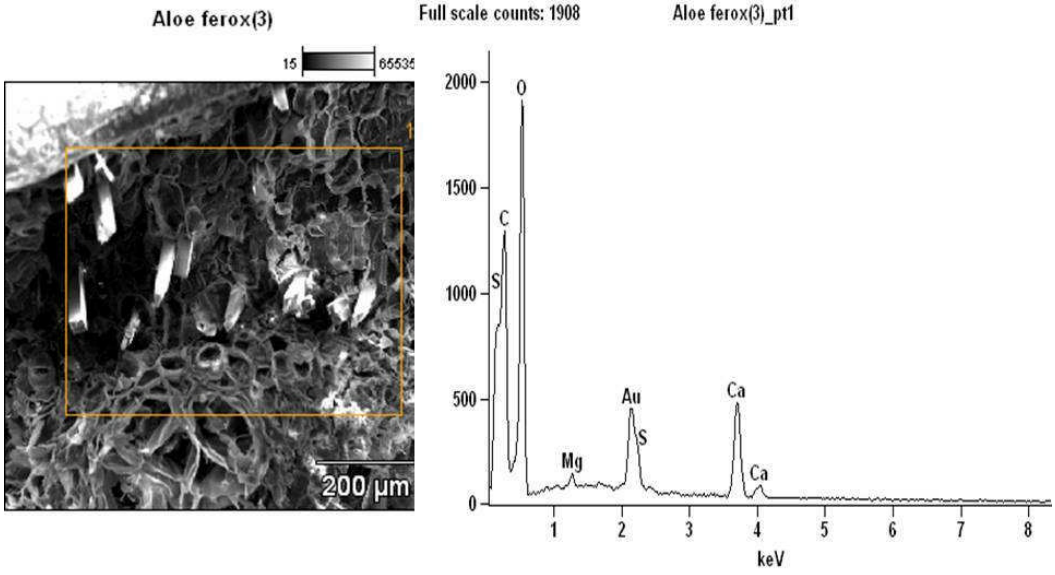


Figure 9: X-ray spectrum of mineral crystals deposits in the mesophyll tissue

The relatively higher sulphur peak detected from the EDXS analysis is indicative of the presence of calcium sulphate as the major components of the crystals present on both adaxial and abaxial epidermal surfaces. On the other hand, calcium oxalate was considered as the major components of crystals present in the mesophyll cells, based on the high oxygen peaks detected in the corresponding spectrum.

http://dx.doi.org/10.4314/ajtcam.v11i2.21

Discussion

Leaves can be classified based on shape, sizes, texture and colour. These variable features are reflections of the arrangement of the internal tissues. The present study provides useful information on the foliar anatomy and micromorphology of *A. ferox* like other members of the Asphodelaceae.

Investigations of the leaf surface of *A. ferox* under the light microscope revealed a rich source of diagnostic characteristics which may be difficult to see otherwise. For instance, the cuticular sculpturing of the leaf can be used as a diagnostic tool in the identification of species or groups of similar species (Cutler, 1985). The rough, thick interface and rind consisting of cells interspersed with chloroplast synthesised constituents were observed features in the cuticle of *A. ferox* which have also been implicated in other Aloe species Coopoosamy and Naidoo (2011). Although epidermal features such as stomata position, size and surface papillation in Aloe are species specific and varied from one species to another, these variations have been attributed to direct genetic control as well as environmental variables (Cutler, 1982).

Taxonomically, the observed dense leaf surface with indistinct cell walls, sunken epistomatal chamber surrounded by poorly developed lobes in *A. ferox,* are consistent with those of the maculates species of Aloe as reported by Grace et al. (2009). Stomata were deeply sunken in *A. ferox* with apertures over arched on either side by a cuticular rim. This is typical of species of plants, including *Aloe* species, growing in water-stressed conditions. Similar characters in Aloe with each stoma surrounded by four subsidiary cells were observed by Brandham and Cutler (1978).Contrary to the reports of Coopoosamy and Naidoo (2011). that indicated that the stomata distribution in *A. excels* was more on the abaxial than the adaxial epidermal surface, an amphistomatic nature of *A. ferox* leaf with stomata that appeared similar on both adaxial and abaxial surfaces was observed in this study. While Santos et al. (2008) indicated that stomatal density was related to the age of the plant and the direct influence of environmental conditions, stomatal apertures are regulated by changes in the solute content of the guard cells. (Talbott and Zeiger 1998). Talbott and Zeiger (1998) reported that the starch-sugar hypothesis has been replaced by the guard cell osmoregulation of K^+ hypothesis. The role of potassium as the major osmotically active solute in the guard cells of open stomata has been demonstrated quantitatively. Stomatal opening in light was associated with an increase of guard cell potassium concentration from 0.2 to 0.5M and stomatal aperture was linearly related to the potassium concentration (Fischer, 1971).

Crystallization is the most common way by which plants neutralise abundant calcium absorbed in ionic solution and remained when water vapour transpires. Crystal formation and its distribution in various organs are a common phenomenon in higher plants (Lerstern and Horner, 2006). The crystals are often classified as drusa, sand, prismatic, raphides or styloid (Maiti et al., 2002; Franceschi and Nakat, 2005; Lerstern and Horner, 2006; Badmus and Afolayan, 2010). They are always formed within cells, which are remarkably diverse among angiosperms (Metcalfe and Chalk 1950). In this study, the inner parenchymatous cells of *A. ferox* believed to be storing the exudates contained some larger, solitary and rod-like crystals. Even though, the significance of the crystals and its formation in plants is unknown, various functions have been attributed to their presence. Some of these functions included their involvement in photosynthesis, detoxification of heavy metals or oxides in plant and regulation of calcium level in plant tissues and organs (Kuo-Huang et al., 2007).

From the analysis of the spectra observed in *A. ferox* leaf, Ca, C, O, and S were predominantly found in the crystals. Eventhough, foliar crystals in some plants have been found to be mainly composed of Al, K, Na and Si (Ashafa et al., 2008). These elements were oriented in different ways depending on their locations in the cells. The peaks of carbon, sulphur and oxygen were preponderantly higher on both adaxial and abaxial epidermal surfaces indicating the presence of calcium sulphates crystals on these layers. In the cross section of the parenchymatous tissue, oxygen peaks was higher than that of sulphur showing that the crystals were made up of calcium oxalate and the possibility of calcium sulphates crystals was inevitable. These crystalline bodies are assumed to be waste as a result of crassulacean acid metabolism which is a typical adaptive feature of plants in arid habitats. Calcium oxalate crystals have been shown to occur in almost every part of plant, including both the vegetative and reproductive organs (Prychid and Rudall, 1999). They are often present near veins, possibly due to transport of calcium through the xylem and are sometimes associated with air space formation. This could assist in reducing the transpiration rate of the leaf and wilting, hence preventing excessive water loss during dry spell.

Chemical analysis with EDXS is based on the principle that each element on the periodic table has a well defined characteristic X-ray spectrum (Otang et al., 2011). X-rays have characteristic energies/wavelengths and can be detected using either a solid state energy dispersive spectrometer (EDS) detector, Some of the elements (C, O, S and N) detected on the leaf surface of *A. ferox* are natural minerals required by all living tissues for proper growth and development, while gold (Au) was believed to be derived from the spur coater. 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 *Aloe ferox*. The peak heights of these elements in the spectra showed their comparative measures in the leaf surface of *A. ferox.*

Conclusions

The present work summarises current knowledge of some diagnostic microscopic characters of *A. ferox* leaf surface. The morpho-anatomical features identified include: paracytic stomata, amphistomal epidermal surface, waxy quaternary sculpturing of flakes deposit near the stomata. Vertically elongated palisade cells with chloroplasts were observed in the inner mesophyll layer, mesophyll cells contained large amounts of styloid calcium oxalate crystals. These micro-taxonomical characters in addition to existing macro-characters would be useful in correct identification of *A. ferox*.

Acknowledgments

This research was supported by grants from Govan Mbeki Research and Development Centre, University of Fort Hare, South Africa. We also thank Ms N. Matyumza at the Electron Microscope Unit (EMU) for her assistance with the SEM work.

http://dx.doi.org/10.4314/ajtcam.v11i2.21

References

1. Afolayan, A.J., Grierson, D.S., Kambizi, L., Madamombe, I. and Masika, P.J. (2002). In-vitroantifungal activity of some medicinal plants. S. Afr. J. Bot., 68: 72-76.
2. Afolayan, A.J. and Adebola, P.A. (1992). Leaf morphology and anatomy of *Hyptis suaveolens* (Linn.) Poit. Boil. Res. Comm., 4: 25-33.
3. Aliero, A.A., Grierson, D.S. and Afolayan, A.J. (2006). Antifungal activity of *Solanum pseudocapiscum.* Res. J. Bot., 1: 129-133.
4. 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. Bio. Sci., 11:1713-1717.
5. Badmus, A.A. and Afolayan, A.J. (2010). The foliar micromorphology of *Arctoitis arctotoides* (L.f) O.Hoffm. J. Med Plt. Res., 4(16): 1643-1646.
6. Brandham, P.E. and Cutler, D.F. (1978). Influence of chromosomes variation on the organization of the leaf epidermis in a hybrid Aloe. Bot. J. Linnean soc., 77: 1-16.
7. Coopoosamy, R.M. and Naidoo, K.K. (2011). Anatomical features of the medicinal importanceof *Aloe excelsa* (Berger). Afr.J.Biotech., 10 (39): 7622-7632.
8. Crouch, N.R., Symmonds, R. Spring, A. and Diederichs. N. (2006). Fact sheet for growing popular medicinal plant species. In Commercializing Medicinal plants; Diederichs N., Ed.; A Southern African Guide. Sun Press; Stellenbosch, South Africa, p 100-102.
9. Cutler, D.F. (1985). Taxonomic and ecological implications of leaf surface features in Aloe and Haworthia (Liliaceae) as seen with the SEM. Proceedings of Royal Microscopial Society, 20, 23.
10. Cutler, D.F. (1982). Cuticular sculpturing and habitat in certain Aloe species (Liliaceae) from Southern Africa. In the plant curticle, Cutler, D.F., K.L. Alvin, C.E. Price, Ed;Academic Press, London, pp. 425-444.
11. Dagne, E., Bisrat, D., Viljoen, A., Van Wyk, B.E. (2000). Chemistry of Aloe species. Curr.Org. Chem., 4: 1055-1078.
12. Franceschi, V.R. and Nakat .P.A. (2005). Calcium oxalate in plants; formation and function. Ann. Rev. Plt. Biol. 56: 41-71.
13. Franco, C. (1939). Relation between chromosome number and stomata in Coffea. Bot.Gaz., 100: 817-829.
14. Fischer, R.A. (1971). Role of potassium in stomatal opening in the leaf of *Vicia faba*. Plant Physiol., 47: 555-558.
15. Githens, T.S. (1979). Drug plants of Africa. University of Pennsylvania Press,Philadelphia, Pennsylvania.
16. Grace, O.M., Simmonds, M.S.J., Smith, G.F. and Van Wyk, A.E. (2009). Documented utility and bio-cultural value of Aloe L. (Aspodelaceae): A review. Econ. Bot., 64: 167-178.
17. Grierson, D.S. and Afolayan. A.J. (1999). An ethnobotanical study of plants used for the treatment of wounds in the Eastern Cape, South Africa. J. Ethnopharmacol., 67: 327-332.
18. Jia S., Lang, M. and Guo. L.H. (2008). Photoelectrochemical detection of oxidative DNA damage induced by Fenton reaction with low concentration and DNA associated Fe++. J. Phy. Chem. Bull., 112: 4461-4464.
19. Kametani, S., Kojima, A., Kikuzaki, H., Kennedy, D.O., Honzawa, M. and Matsui-Yuasa. I. (2007). Chemical constituents of Cape Aloe and their synergistic growth-inhibition effect on Ehrlich ascites tumor cells. Biosci. Biotech. Biochem. 71: 1220-1229.
20. Kambizi, L., Sultana, N. and Afolayan. A.J. (2004). Bioactive compounds isolated from *Aloe ferox*: a plant traditionally used for the treatment of sexually transmitted infections in the Eastern Cape, South Africa. Pharm. Bio., 42: 636-639.
21. Kambizi, L., Goosen, B.M., Taylor, M.B. and Afolayan. A. J. (2007). Anti-viral effects of aqueous extracts of *Aloe ferox* and *Withania somnifera* on herpes simplex virus type 1 in cell culture. S. Afr. J. Sci., 103: 359-60.
22. Koduru, S., Grierson, D.S. and A.J. Afolayan. (2006). Antimicrobial activtity of *Solanum aculeastrum.* Pharmaceut. Biol., 44: 283-286.
23. Kuo-Huang, L.L., Ku, M.S.B. and Franceschi. V.R. (2007). Correlation between calcium oxalate Crystals and photosynthetic activity of palisade cells of shade-adapted Peperomia gilbella. Bot. Stud., 48: 155-164.
24. Lerstern, N.R. and Horner. H.T. (2006). Crystal macropattern development in *Prunus Serotina (Rosaceae, Prunoideae*) leaves. Ann. Bot., 97: 723-729.
25. Loots, D.T., Van Der Westhuizen, F.H., Botes, L. (2007). Aloe ferox leaf gel phytochemical content, antioxidant capacity and possible health benefit. J. Agric. Chem., 55: 6891-6896.
26. Maiti, R.K., Villareal, L.R., Trevino, A.V. and Valades-Cerda., M.C. (2002). Some aspects on pharmacology of ten species of the family *Solanaceae* utilization in traditional medicine. Cald. 24: 317-321.
27. Metcalfe, C.R. and Chalk, L. (1950). Anatomy of the dicotyledons (2 vol.). Clarendon Press, Oxford, pp 1500.
28. Norikura, T., Kennedy, D.O. Nyarko, A.K., Kojima, A. and Matsui-Yuasa.I. (2002). Protective effect of Aloe extract against the cytotoxicity of 1,4-napththoquinone in isolated rat hepatocytes involves modulations in cellular Thiol levels. Pharmacol. Toxicity., 90: 278-284.
29. Ogunkunle, A.T.J. and Oladele. F.A. (2008). Leaf epidermal studies in some Nigerian species of Ficus L. (Moraceae). Plt. Syst. Evol., 274: 209-221.
30. Otang, W.M., Grierson, D.S. and Ndip. R.N. (2011). Effect of the acetone extracts of *Arctotis arctotoides (Asteraceae*) on the growth and ultra structure of some opportunistic fungi associated with HIV/AIDS. Int. J. Mol. Sci., 12: 9226-9235.
31. Prychid, C.J. and Rudall. P.J. (1999). Calcium oxalate crystals in monocotyledons: a review of their structure and systematics. Ann. Bot., 84: 725-739.
32. Santos, L.D.T., Thadeo, M. L., Iarema, R.M., Meira, S.A. and Ferreira. F.A. (2008). Foliar anatomy and histochemistry in seven species of Eucalyptus. Rec Arvore (Online). 32 (4): 769-779.
33. Shackleton, C.N. and Gambiza. J. (2007). Growth of *Aloe ferox* Mill. at selected site in the Makana region of the Eastern Cape. S. Afr. J. of Bot., 73: 266-269.
34. Talbott, L.D. and Zeiger. E. (1998). The role of sucrose in guard cell osmo regulation. J. Exptal.Bot.49: 329-337.
35. Van Wyk, B.E., Van Oudtshoorn, B. and Gericke, N. (1997). Medicinal plants of South Africa. Briza publication, Pretoria, South Africa.
36. Van Wyk, B.E., Van Oudtshoorn B. and N. Gericke. (2002). Medicinal Plant of South Africa, 2nd Ed.; Briza publications: Pretoria, South Africa, pp 156-157.
37. Van Wyk, B.E., and G.F. Smith. (1996). Guide to Aloes of South Africa. Briza publications, Pretoria, South Africa, pp 303.
38. Viljoen, A. (2008). Indigenous South African medicinal plants part 7: *Aloe ferox* (Cape Aloe). S. Afr. Pharm. J., 47.
39. Watt, J., and Breyer-Brandwijk, M.G. (1962). The Medicinal and poisonous plants of Southern and Eastern Africa. An account of their medicinal and other uses, chemical composition, pharmacological effects and toxicology in man and animal. E and S Livingstone (Ltd) Edinburg, pp. 996-1000.
40. Wilkinson, H.P. (1979). The plant surface (mainly leaf), Part 1: Stomata. In: Melcalfe, C.F., L.Chalk (eds) Anatomy of dicotyledons: Clarendon Press, Oxford Chap 10, vol 1. P 113.
41. Wintola, O.A. and Afolayan A.J. (2011). Phytochemical constituents and antioxidant activities of the whole leaf extract of *Aloe ferox* Mill. Pharmacog. Mag., 7: 325-333.
42. Wintola, O.A. and Afolayan. A.J. (2010). Ethnobotanical survey of plants used for the treatment of constipation within Nkonkobe Municipality of South Africa. Afr. J. Bio. 9: 7767- 7770.
43. Wintola, O.A., Sunmonu, T.O, and Afolayan, A.J. (2010). The effect of *Aloe ferox* Mill. in the treatment of loperamide- induced constipation in Wistar rats. BMC Gastroenterol. 10: 1-5.