

ULTRASTRUCTURE AND ELEMENTAL ANALYSIS OF *HYPOXIS HEMEROCALLIDEA*: A MULTIPURPOSE MEDICINAL PLANTAnthony J. Afolayan^{1*} and Gloria A. Otunola^{1,2}¹ Centre for Phytomedicine Research, Department of Botany, University of Fort Hare, Alice 5700, South Africa.² Department of Home Economics and Food Science, University of Ilorin, Nigeria.*Corresponding author E-mail: aafolayan@ufh.ac.za; adeglo2004@yahoo.com**Abstract**

Background: Herbal medicine is a popular means of medical management in some parts of the world especially in Africa. *Hypoxis hemerocallidea* Fisch.C.A.Mey. & Avé-Lall, also known as African potato of the Hypoxidaceae family, is one of the medicinal plants that have enjoyed long usage as an herbal medicine in South Africa. In this study, the morphology and elemental constituents of *H. hemerocallidea* leaf was investigated to correlate the functional role of the ultrastructure in the production of therapeutic compounds.

Materials and Methods: Fresh leaves of *H. hemerocallidea* were prepared for analysis using standard methods. The ultrastructure and crystal deposits of the plant were assessed using scanning electron microscopy (SEM), and energy dispersive x-ray (EDX).

Results: It was observed that the leaves were characterised by multicellular glandular and non glandular trichomes which are sparsely distributed over the entire surfaces. The glandular trichomes (GTs) in *H. hemerocallidea* leaf have boubous heads which are probably filled with secretions, while the non glandular trichomes were long, fibrous and sparse. EDX-SEM of *Hypoxis hemerocallidea* leaf revealed that carbon, oxygen, nitrogen and silicon are the major components of the deposits, while other elements such as iron, sulphur, sodium, calcium, magnesium, potassium, manganese, iodine, chromium and iodine were present in small but variable amounts.

Conclusion: The presence of these elements which are crucial to maintaining good health, in addition to other bioactive constituents might be accountable for the multipurpose therapeutic uses of *Hypoxis hemerocallidea* in the treatment of cancers, HIV/AIDS related diseases, urinary tract infections, cardiovascular disorders, diabetes and other chronic ailments of humans.

Key words: mineral elements, *Hypoxis hemerocallidea*, multipurpose medicinal plant, scanning electron microscope, trichomes, ultrastructure, EDX-MS analysis.

Introduction

Hypoxis hemerocallidea Fisch. C.A.Mey. & Avé-Lall, also known as African potato is a medicinal plant that is widely distributed in South Africa. It has enjoyed long usage as a herbal medicine in this area. The genus *Hypoxis* belongs to the Hypoxidaceae family which are easily recognizable because of their bright yellow star-shaped flowers and strap-like leaves. *Hypoxis* species have a long history of medicinal use in Africa, with eleven different species reportedly used in South Africa (Sathekege et al., 2010; Bourkes and van de Venter, 2011; Appleton et al., 2012).

H. hemerocallidea is characterized by strap-like leaves, bright yellow star-shaped flowers, tuberous stock or corm. It is commonly, though erroneously, referred to as African potato because of its potato-like shape (Van Wyk et al., 2002; Owira and Ojewole, 2009). Different preparations of the plant have been reported in the treatment of a wide variety of ailments such as cancers, nervous disorders, immune-related diseases, urinary tract infections, diabetes, anti inflammation, anti microbial, and cardiovascular diseases (Singh 1999; Ojewole, 2006; Steenkamp et al., 2006; Larporta et al., 2007; Nair et al., 2007).

Production of epicuticular compounds with therapeutic properties have been reported in many plant species. In most cases, the source of these bioactive compounds has been attributed to the trichomes. Developmental and structural studies of trichomes, according to Afolayan and Meyer (1995), can shed light on the nature of the secreted materials and the functional significance of the glands.

Despite the wide pharmacological and therapeutic uses of this plant, little or no information on its ultra-morphology is available in literature and the scanning electron microscopy technique is an invaluable tool for analyzing complex structures and elemental detection. The objective of this study therefore, was to examine the ultra-structural morphology and elemental composition of the leaf of *H. hemerocallidea* using scanning electron microscope (SEM) and to relate our findings to their possible functional role in the production of therapeutic compounds.

Materials and Methods

The leaves of *H. hemerocallidea* (voucher no. Otun.2013/04) were collected in April 2013, from Alice in the Eastern Cape Province of South Africa. The plant was identified by Prof D.S Grierson of the Department of Botany, University of Fort Hare.

Fresh leaves were cut into segments of about 4 to 6 mm in length and fixed for 24 hrs in 6 % glutaraldehyde in 0.05 M sodium cacodylate buffer, rinsed with the buffer solution, and dehydrated in a graded series of ethanol (10 to 100 % 3 xs) at 20 min per rinse. This was followed by critical point drying with liquid CO₂ in a Hitachi HCP-2 critical point dryer. Each dried sample was mounted onto aluminium specimen stubs with double-sided carbon coated adhesive discs and sputter-coated with gold-palladium (Eiko IB-3 Ion Coater). Both the adaxial and abaxial surfaces of the leaves were examined at varying magnifications using a JEOL (JSM-6390LV) scanning electron microscope, operated at 10–15 kV acceleration voltage.

The energy dispersive X-ray spectroscopy-SEM, involved both the fixing and dehydration procedures described for SEM, while an EDX Analyzer (FEI QUANTA 200 Oxford) was used for the analysis.

Results and Discussion

The ultrastructure of both abaxial and adaxial surfaces of *H. hemerocallidea* was observed as being characterized by multicellular trichomes (glandular and non-glandular) that are sparsely distributed all over the leaves (Fig 1). The non glandular trichomes (NGTs) were long and sparse (Fig 1A & C). In some species the fibrous trichomes help in protecting the aerial shoot of the plant against water loss due to

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evaporation, attack by pests such as insects and airborne fungal particles (Aliero et al., 2006; Pendota et al., 2008; Ashafa et al., 2008). Fibrous trichomes also form protective layers on leaf surfaces.

Glandular trichomes (GTs) in *H. hemerocallidea* leaf have boubous heads which are probably filled with secretions. They have long stalks and gland cells (1B). Afolayan and Meyer (1995) observed that both GTs and NGTs originated from a single epidermal cell in *Helichrysum aureonitens*. According to the authors, it may be difficult to distinguish between the two types in the early stages of development, but as development continues, the GT could be identified by the presence of two basal cells, while the NGT retains a one-celled base. Also, the apical cell of the NGT is very long, dead and fibrous (Fig 1C).

Formation of a subcuticular space in which secretory substances accumulate (Fig 1D) is common in many types of glands. The secretory products are usually released through pores in the cuticle or breaks in the cuticle caused by external pressure. Investigation of developmental and structural trichomes can shed light on the nature of secreted material and their functional significance (Afolayan and Meyer, 1995; Pendota et al., 2008). Therefore, it is possible that the therapeutic compounds produced by *H. hemerocallidea* are among the exudates produced on its leaves.

Glandular trichomes have been reported to secrete chemicals potentially useful in the pesticide, flavour and pharmaceutical industries (Peter and Shanower, 1998).

Trichomes in *Mentha pipenta* (pepper mint), *Artemisia annua* and *Helichrysum aureonites* secrete menthone, artemisinin and flavonoids respectfully, which are used in several pharmaceutical drugs such as cough syrups, asthma inhalers, antimalarial and antioxidants (Afolayan and Meyer, 1995; Peter and Shanower, 1998). The glandular trichomes in *H. hemerocallidea* may contain the bioactive compounds that are responsible for the plant's pharmaceutical properties.

A total of 12 elements were detected on the leaves of *H. hemerocallidea* with carbon, oxygen, nitrogen and silicon being the most abundant (Fig 2), while iron, sulphur, sodium, calcium, magnesium, ptassium, manganese, iodine, chromium and iodene were also found in small but variable amounts (Table 1). The type of mineral element present in plants could determine their dietary and medicinal funnctions of the plant. Trace elements, also referred to as essential elements, act as co-factors to many enzymes in promoting many metabolic processes, general well being and cure of diseases (Prasad, 1993). Recent research has added chromium, nickel, vanadium, selenium, silicon, molybdenum, iron and arsenic to the list of essential elements (Narendhirakannan et al., 2004).

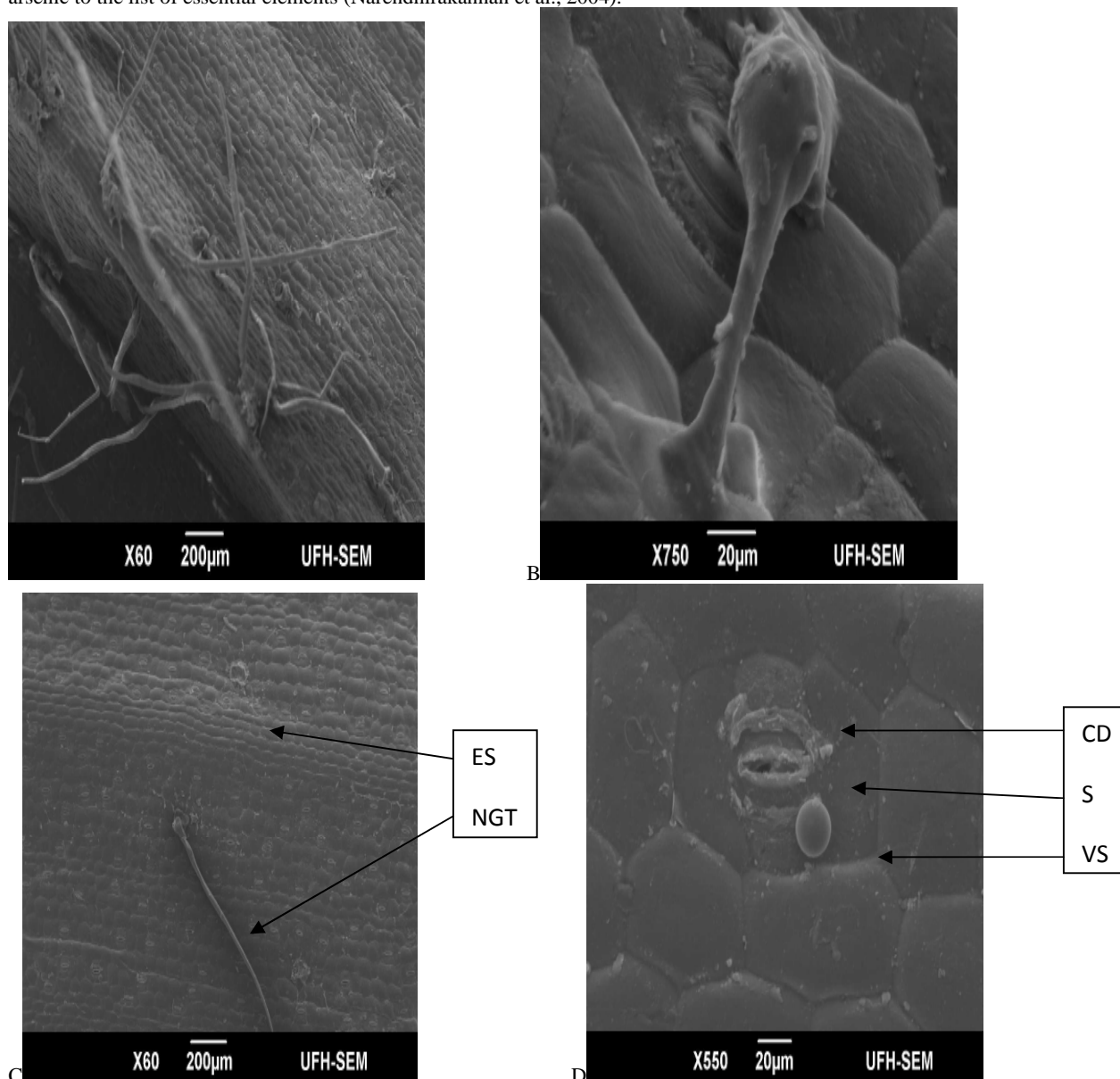


Figure 1: Scanning Electron Micrograph of *H. hemerocallidea* leaf. A- Abaxial surface showing glandular (GTs) and non-glandular (NGTs) trichomes. B- Glandular trichome with boubous head (BH), long stalk (LS) and base (B). C- Adaxial surface of the leaf showing epidermal surface (ES) and mature, dead, non glandular trichome (NGT). D- Crystal deposit (CD), stoma (S) and vessel probably filled with secretion (VS).

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In the present study, EDX observations showed that *H. hemerocallidea* possess a wide range of trace elements which have been reported to be crucial in maintaining good health. This could as well account for the many therapeutic uses of the plant. For example, sodium and potassium are responsible for maintaining the electrolyte balance of the cells, water balance and distribution, blood pressure, acid-base balance, muscle and cell nerve function, heart function, kidney and adrenal functions (Agunbiade et al., 2012). These elements (sodium and potassium) were present in significant amounts in the leaves of *H. hemerocallidea*.



Image Name: Hhem leaf(2)

Accelerating Voltage: 15.0 kV

Magnification: 1100

Full scale counts: 2412

Hhem leaf(2)_pt1

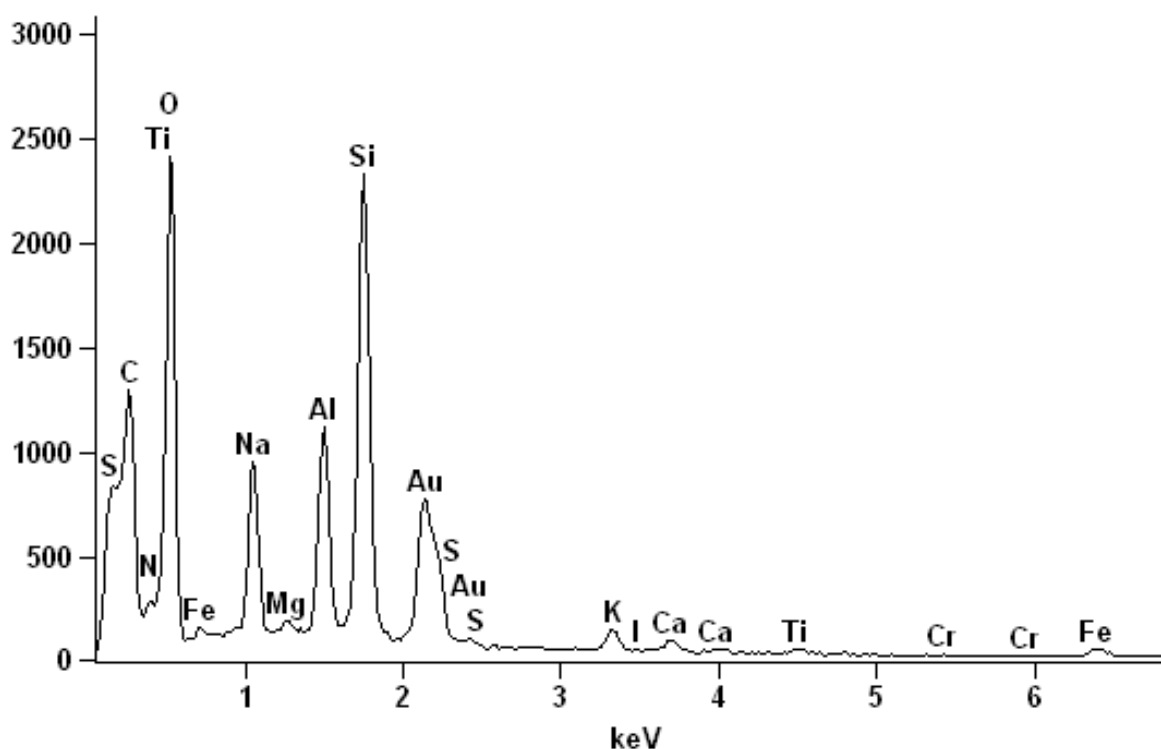


Figure 2: Energy Dispersive X-ray analysis of crystal deposit of *H. hemerocallidea* leaf (the rectangle indicates the area analysed) and the various elements in them.

Calcium is essential for healthy bones, teeth, blood and regulation of skeletal, heart and tissue muscles, while magnesium has been reported to be helpful in fighting heart disease, stroke and in cell repair. Iron may increase packed cell volume, boost the immune system and prevent anaemia in humans (Larson and Wolk, 2007; Agunbiade et al., 2012). These could account for the therapeutic uses of *H. hemerocallidea* in cardiovascular diseases, immune deficiency diseases, as a tonic and treatment of nervous disorders (Maduna, 2006; Drewes et al., 2008; Owira and Ojewole, 2009).

Chromium is an essential mineral that is necessary for normal glucose and lipid homeostasis especially in the proper function of insulin (Balk et al., 2007). The element has been reported to play crucial roles in normoglycaemia and management of diabetes mellitus especially

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through the activation of the beta-cells in the pancreas (O'Connell, 2001; Narendhirakannan et al., 2004; Balk et al., 2007; Larsson and Wolk, 2007). Its presence in *H. hemerocallidea* could account for the use of the plant in the treatment of diabetes.

In addition to its use for goitre treatment, iodine serves as an important antiseptic, it also possess anti-bacterial, anti-viral and anti-mycotic properties as well as anti-fungal, anti-acidosis, cystocidal and viricidal effects (Alekseev and Tatyana, 1967). This supports the age-long use of *H. hemerocallidea* in the treatment of microbial and viral infections as well as HIV/AIDS (Albrecht, 1995; Mills et al., 2005; Owira and Ojewole, 2009).

Sulphur is used in the treatment of skin disorders and irritations, such as eczema, psoriasis, acne and antimicrobial agents (www.who.int/info/8680883_medical-uses-sulfur.html). Because sulphur aids in the synthesis of essential proteins, cleanses the blood and is necessary for cell regeneration, administering the mineral to HIV and AIDS patients may slow the progression of the disease by inhibiting the wasting process.

Table 1: Percentage (%) elemental composition of crystal deposits on *H. hemerocallidea* leaf as shown by Energy Dispersive X-ray analysis

Element	Amount (%)
Carbon	15.13±0.29
Oxygen	31.51±0.32
Nitrogen	6.33±0.58
Silicon	9.39±0.10
Sodium	4.26±0.08
Potassium	0.75±0.08
Calcium	0.62±0.04
Magnesium	0.26±0.05
Manganese	0.07±0.13
Iron	1.50±0.13
Chromium	0.07±0.07
Iodine	0.32±0.11

Conclusion

The results from this study documents the ultra-structure and energy dispersive x-ray analysis of *H. hemerocallidea*. It also reveals the presence of elements such as iron, sulphur, chromium and iodine which in combination with other bioactive compounds could be responsible for the therapeutic uses of *H. hemerocallidea* in the treatment of various skin diseases, microbial infections, immunostimulant for HIV/AIDS, cancer, diabetes, cardiovascular diseases and a plethora of other ailments as variously reported in the literature.

Declaration of interest

The authors declare that they have no competing interest.

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