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FOLIAR MICRO-MORPHOLOGY OF *GASTERIA BICOLOR* HAW. (ASPHODELACEAE) FROM SOUTH AFRICA

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Abstract

Background: The succulent genus, *Gasteria*, which comprises 16 species, is endemic to South Africa and has its main centre of distribution in the Savanna Region of the Eastern Cape. Whereas *G. bicolor* has been investigated phyto-chemically and pharmacologically, not much data concerning the anatomical and micro-morphological features can be found in literature. **Materials and Methods:** This study was undertaken, using light and scanning electron microscopy to obtain information on the micro-morphological features of this important medicinal plant to facilitate its identification and authentication. The elemental composition of the leaf was determined by energy dispersive X-ray spectroscopy (EDXS).
Results: The epidermal cells are either hexagonal or pentagonal in form, and are compactly arranged with undulate anti-clinal cell walls. The epidermal cell width was approximately 50 µm. Stomata apertures are elliptical and the upper epidermis of the leaf has paracytic stomata which are slightly raised above the epidermal surface with 4 to 5 subsidiary cells surrounding each stoma. Based on the EDXS microanalysis, the mineral crystals present at the level of the mesophyll of *G. bicolor* were probably mixtures of calcium oxalate, calcium sulphate and silica.
Conclusion: The co-occurrence of aluminum suggests the potential role of the crystals in detoxification of aluminum and heavy metals, as reported previously.

Key words: Foliar micro-morphology, *Gasteria bicolor*, light microscopy, scanning electron microscopy

Introduction

Medicinal plants are of great value in the cure and treatment of diseases. Over the years, scientific research has expanded our knowledge of medicinal plants and new drugs. As people are becoming aware of the potency and side effects of synthetic drugs, there is an increasing interest in plant-based medications (Sultana *et al.*, 2011). Herbal drug technology includes all the necessary steps for the conversion of botanical materials into medicines (Serrano *et al*. 2008). The first step in quality control of medicinal plants is to ensure the authenticity of the desired species for the intended use because mis-identification can cause serious health problems to consumers, as well as publicity and legal sanctions for the pharmaceutical industry (da Silva *et al*., 2009). Describing medicinal plants in a systematic manner is based on multiple approaches of pharmacognostic, taxonomy and chemical analysis, including documentation of their biological and geographical source, cultivation, collection, processing, morphological, microscopic and chemical characters (Serrano *et al*., 2008).

Microscopy is an essential analytical tool in ensuring that the proper identification of a medicinal plant is achieved. Advances in light and scanning electron microscopes accuracy have increased and re-enforced the capability of microscopy as a veritable means of botanical identification. Micro-anatomy sometimes provides additional information that gross anatomy does not posit (da Silva *et al*., 2009). Microscope descriptions can include the characterization of the histological structures, cells and cell contents visible only via light microscopy (LM) and scanning electron microscopy (SEM) (Serrano *et al*., 2008).

The succulent genus, *Gasteria*, compring 16 species, is endemic to South Africa and has its main centre of distribution within the Savanna Region of the Eastern Cape (Dagne *et al*., 1996). Previously, this genus was classified in the large heterogeneous Liliaceae family (Dagne *et al*., 1996), but is now classified under the family Asphodelaceae. Three new dihydroanthracenones namely 3,4-dihydro-2,6,9-trihydroxy-8-methyl-1(2*H*)-anthracenone (gasteriacenone A), 3,4-dihydro-2,4,9-trihydroxy-6-methoxy-8-methyl-1(2*H*)-anthracenone (gasteriacenone B) and 3,4-dihydro-4,6,9-trihydroxy-7-carbomethoxy-8-methyl-1(2*H*)-anthracenone (gasteriacenone C) were isolated from the stems and leaves of *Gasteria bicolor*. Their structures were elucidated using spectroscopic methods including 2D NMR techniques. The *in vitro* antifungal activity of *G. bicolor* was investigated against a panel of opportunistic fungi in HIV/AIDS in our previous study (Otang *et* al., 2012). Whereas *G. bicolor* has been investigated phytochemically and pharmacologically, not much data concerning the anatomical and micro-morphological features can be found in literature. This study was therefore undertaken, using both light and scanning electron microscopy to obtain information on the micro-morphological features of this important medicinal plant which would help in its identification and authentication. The elemental composition of *G. bicolor* leaves was determined by energy dispersive X-ray spectroscopy (EDXS).

Plants communicate with their external environment, protect and maintain essential internal physiological and biochemical processes through specialized epidermal structures. Information on their morphology, therefore, has bearing on a wide variety of issues. Apart from their proven value in systematic taxonomy, specialized epidermal structures represent adaptations to a wide range of ecologies and their role in host–parasite interactions (Carpenter, 2006). Foliar micro-morphological features are also useful in the identification and authentication of many plants, hence finding relevance in the standardization of herbal products obtainable from various medicinal plants indigenous to most communities **(**Sonibare & Osiyemi, 2012).

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Materials and methods

Plant material

G. bicolor was supplied by an herbalist and its identity was authenticated at the Griffin Herbarium of the Department of Botany, University of Fort Hare, where a voucher specimen was deposited (Voucher No. W31).

Leaf epidermal studies (Light microscopy)

Microscopic examinations of the epidermal sections of the leaf were carried out according to the procedure of Ogunkunle & Oladele (2008). Leaf samples of 1 to 3 cm were sectioned from the mid portion of the adaxial and abaxial surfaces of a mature leaf using a razor blade. The sections of the leaf were washed with distilled water for 2-3 minutes. Then the sections were placed on clean glass slides with 1-2 drops of sterile distilled water, covered with a cover slip. Prepared slides were observed under a Motic light microscope and the microphotographs were taken with a digital camera, fitted into the light microscope and the images were captured digitally using Microsoft image programme for Windows.

SEM and Energy Dispersive X-ray Spectroscopy (SEM-EDXS)

The procedure used for SEM was adopted from Dyubeni and Buwa (2012). Fresh leaves and stems were cut into segments of approximately 4-6 mm in length and fixed for 24 hrs in 6% glutaraldehyde in 0.05M sodium cacodylate buffer (pH 7.5). Each sample was rinsed in distilled water and dehydrated in a graded series of ethanol (20-100%) at 20 minutes per rinse. Critical point drying was done in Hitachi HCP-2 Critical Point Dryer with liquid carbondioxide. Each dried sample was mounted unto aluminium specimen stubs with double-sided carbon coated adhesive discs and sputter-coated in Eiko IB · 3 Ion Coater with gold-palladium. Morphology and anatomy of both the adaxial and abaxial surfaces of the leaves were examined at varying magnifications using JEOL (JSM-6390LV) SEM, operated at 10 – 15 kV acceleration voltage. SEM was coupled with elementary analysis by FEI QUANTA 200 Oxford electron/energy dispersive spectroscopy (EDS Analyzer). Features and images examined were captured digitally using Microsoft image programme for Windows.

EDXS analysis of the leaf epidermal sections was adopted from (Otang *et al.*, 2012). Different regions of the epidermal sections of the leaf were micro-analyzed and the representative spectra are presented (see results). A focused beam of electrons was used to scan the leaf epidermis at the point where examination of its chemical composition was desired. The identification of elements with the EDXS was based on the 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

Both the lower and upper epidermis is single layered with thin cuticle and many stomata which are densely distributed on both surfaces (amphistomatic leaf), of the epidermis (Figs 1a and b). The epidermal cells are either hexagonal or pentagonal in form, are compactly arranged with undulate anti-clinal cell walls (Figs 1c-1f, 3a and 3b). Epidermal cell width is approximately 50 µm. Stomatal apertures are elliptical (Figs 2a and 2b) and the upper epidermis of the leaf has paracytic stomata which are slightly raised above the epidermal surface with 4 to 5 subsidiary cells surrounding each stoma (Figures 2a, 2b and 3c).

The guard cells are thick walled with expanded ends (Figs 2a and 2b) and their neighboring cells have no prominent wax. LM examinations of the transverse sections of *G. bicolor* leaf blade (Figs 2e and 2f), reveal vertically elongated palisade cells with chloroplasts towards the lower epidermis, while the SEM micrographs shows the presence of mineral crystals present at the level of the mesophyll (Figures 3e and 3f).

The main difficulty in fixing the botanical identity of medicinal plants in traditional systems arises due to the local name(s) of these plants, with local name(s) applied to more than one plant species (Sultana *et al.*, 2011). For instance, *G. bicolor* has the vernacular and trade name of "Intelezi" within the Eastern Cape Province of South Africa. However, this same vernacular name is also being used for other plants such as: *Aloe boylei*, *A. ecklonis*, *A. maculate* and *A. tenuior* (Dold & Cocks, 1999) by many herbalists, local communities and herb sellers, due to the morphological similarities among the plants. This problem of nomenclatural controversy may lead to misuse of this plant for specific diseases; Girach *et al.* (1998) therefore stressed the need for authentic botanical identification of herbal plants used in traditional medicine, in order to maintain herbal drug efficacy. In the present study, diagnostic anatomical and morphological features presented for *G. bicolor* are very useful for authentication at a microscopic level to distinguish it from closely related plants.

Probably, more work has been carried out on stomatal structure and development, and on their use in assessing taxonomic relationships and evolutionary pathways, than any other leaf character (Tahir & Rajput, 2009). The shape, size, distribution and orientation of the stomata and the various thickenings and ornamentation of the guard cells are all characters, which are frequently used in taxonomic work (Tahir & Rajput, 2009). By using the concept of Silva *et al.* (2008) the stomata of the *G. bicolor* are classified as paracytic type. It is also called regular celled type of stomata. In the paracytic type, guard cells are accompanied by two subsidiary cells, the longitudinal axis of which is parallel to that of the guard cells and aperture (Perveen et al., 2007).

Energy dispersive X-ray spectroscopy (EDXS)

Results of qualitative X-ray microanalyses of the epidermis of *G. bicolor* show the specific spectra of the following elements: Carbon (C), hydrogen (H), oxygen (O), nitrogen (N), calcium (Ca), sulfur (S) silicon (Si) and aluminium (Al) on the PET crystal detector (Fig. 4), 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.

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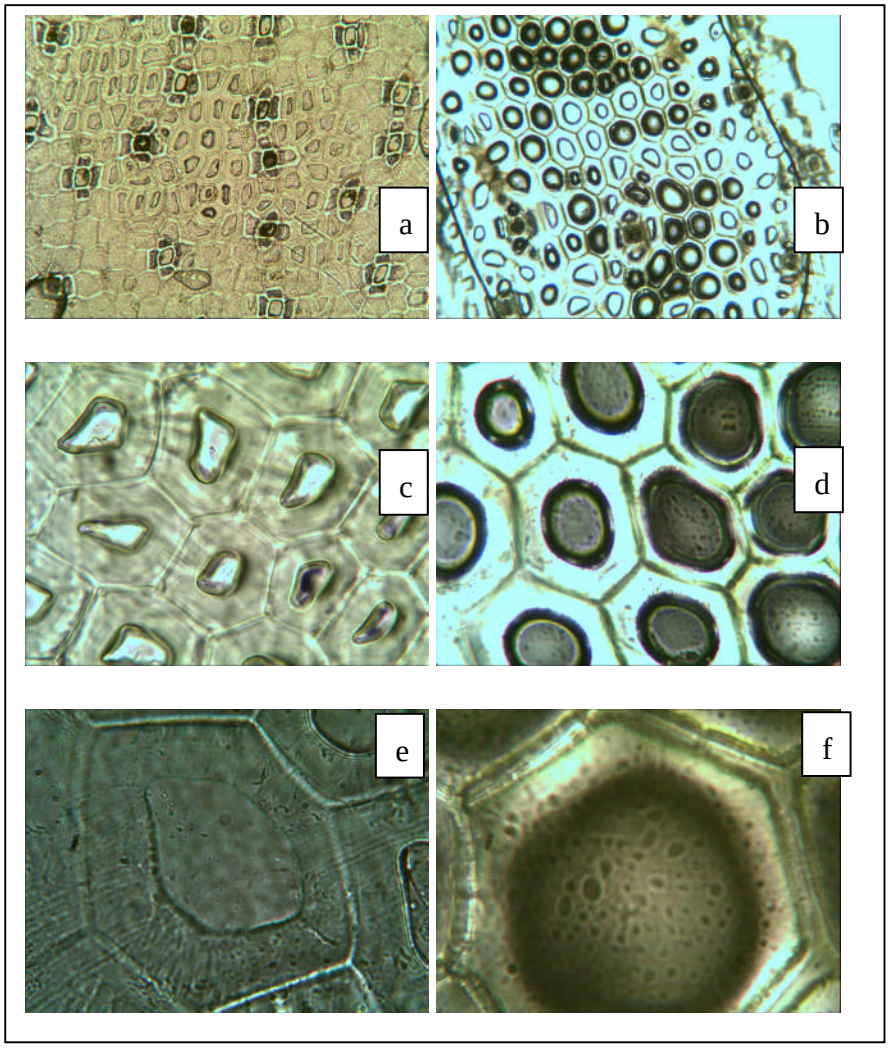

Figure 1: Epidermal surfaces of *G. bicolor leaf* (LM); **(a)** distribution of stomata in adaxial surface (10X); **(b)** distribution of stomata in abaxial surface (10X); **(c)** hexagonal epidermal cells in abaxial surface (40X); **(d)** hexagonal epidermal cells in adaxial surface (40X); **(e)** pentagonal epidermal cells in abaxial surface (100X); **(f)** pentagonal epidermal cells in adaxial surface (100X).

Based on the elemental X-ray microanalyses, the mineral crystals present at the level of the mesophyll of *G. bicolor* 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 (Franceschi & Nakata, 2005) and the most abundant minerals formed by plants are silica, calcium carbonate and calcium oxalate. Although calcium is an essential plant nutrient, excess calcium is often precipitated in the form of calcium salts such as oxalate, carbonate, sulfate, phosphate, silicate, citrate, tartrate and malate (Weiner & Dove, 2003). A number of roles for crystal formation have been proposed, i.e. roles in cellular ion balance, in plant defense against herbivore, in tissue rigidity and support, in detoxification of oxalic acid or aluminium (Franceschi & Nakata, 2005). Hence, the co-occurrence of aluminum suggests the potential role of the crystals in detoxification of aluminum and heavy metals, as reported previously (Mazen, 2004). 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 & Horner, 2006). Franceschi & Horner (1980) reported that oxalate in crop plants caused a negative impact on human health acting as a toxin, and in CaOx kidney stone formation. Therefore, considering the great deal of crystallization occurring at the mesophyll of *G. bicolor* leaf, the traditional use of the plant should be very carefully monitored to ensure the safety of the recipients.

Conclusion

Reliable identification of medicinal plants is necessary to guarantee the safety of the users. The present study has shown that microscopy could increase the accuracy of medicinal plant identification. Knowledge of some important botanical microscopic characters such as the shape,

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elevation and sunken state of the stomata, presence and absence of wax, on the guard and subsidiary cells could serve as important taxonomic parameters for the authentication of *G. bicolor*.

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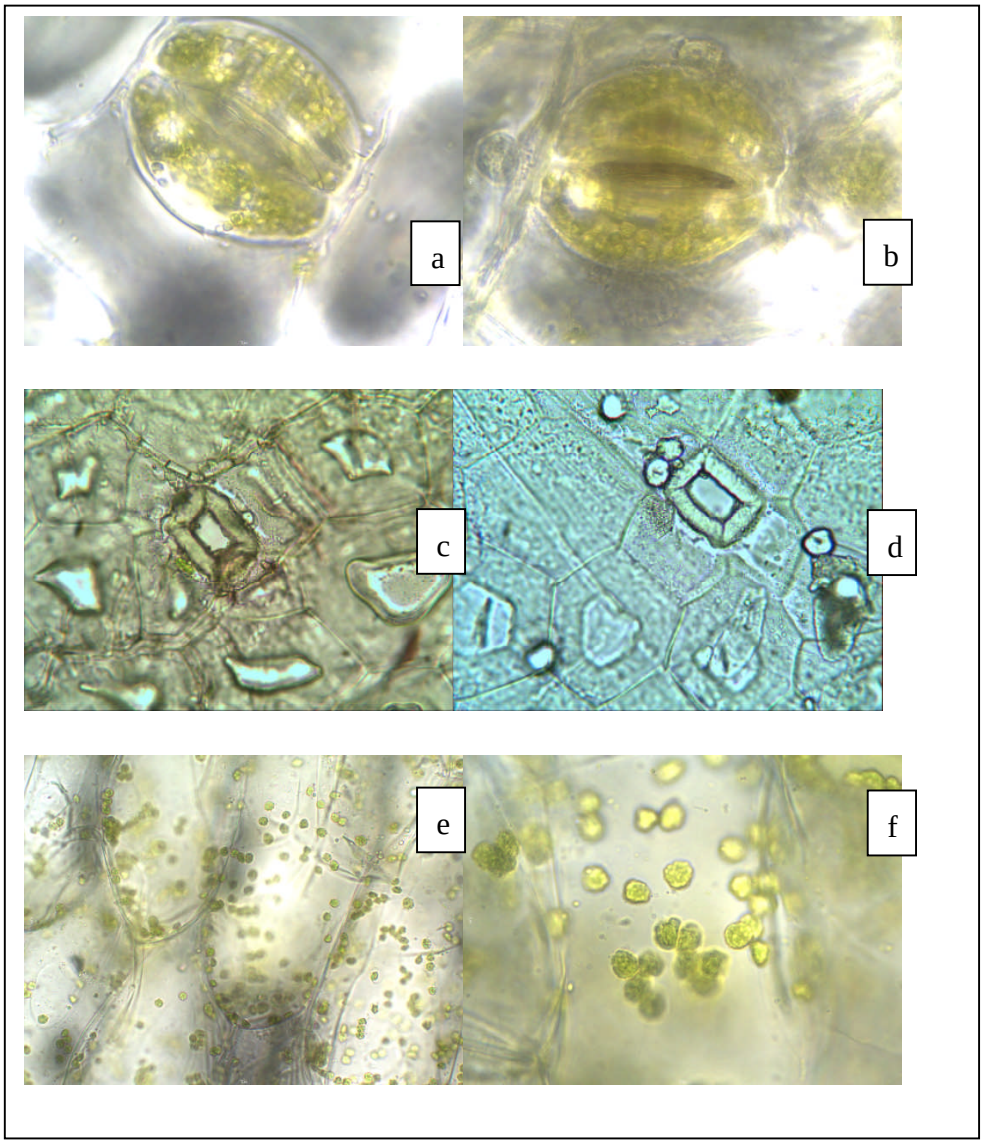

Figure 2: Epidermal surface and cross section of *G. bicolor* leaf (LM); **(a)** stoma, bean-shaped guard cell and subsidiary cell in abaxial surface (100X); **(b)** stoma, bean-shaped guard cell and subsidiary cell in abaxial surface (100X); **(c)** vertically elongated palisade cells with chloroplasts in cross section (40X); **(d)** hexagonal epidermal cells (100X); **(e)** vertically elongated palisade cells with chloroplasts in cross section (100X); **(f)** chloroplasts in palisade cells of *G. bicolor* leaf blade.

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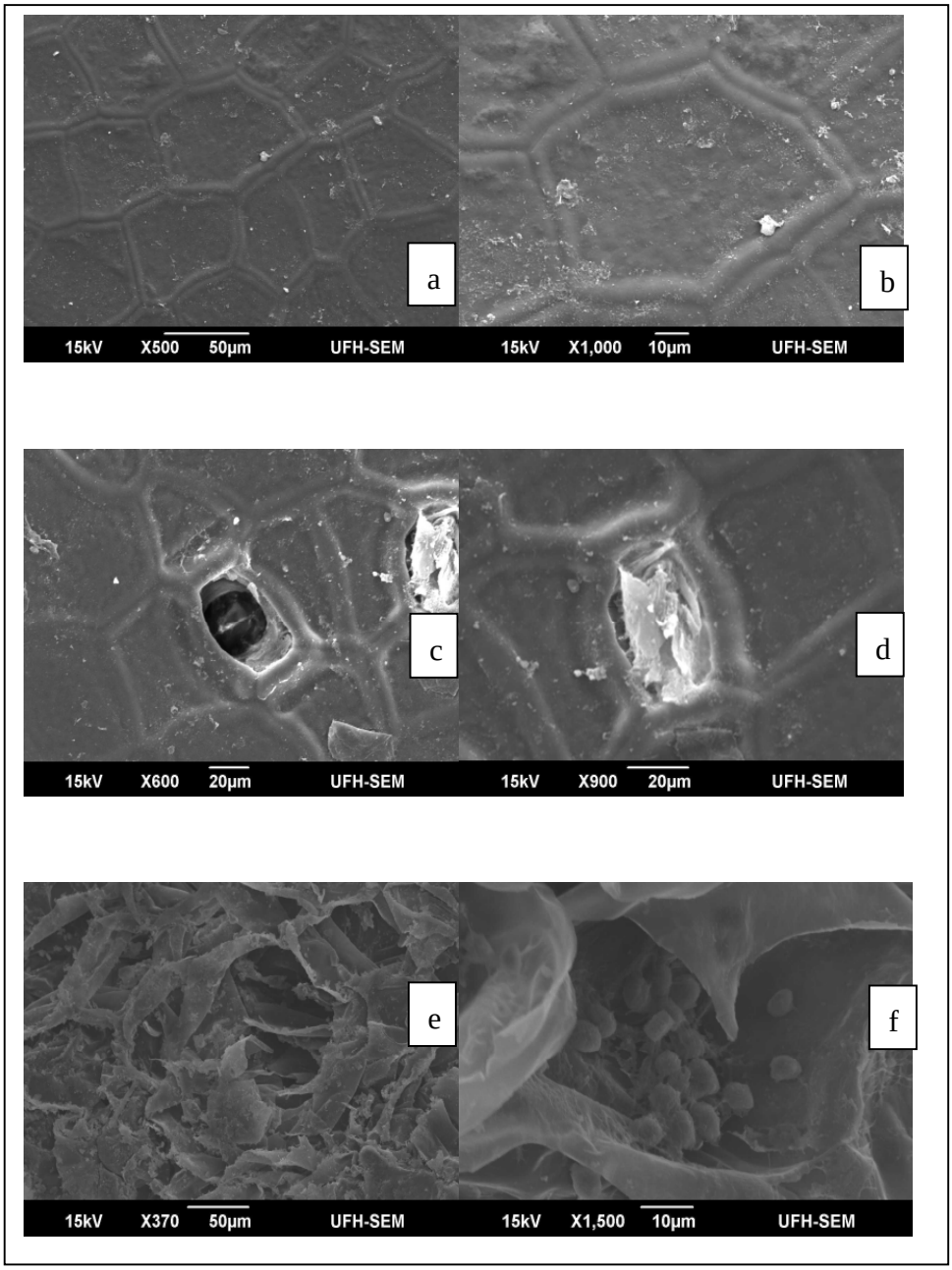

Figure 3: Epidermal surface of *G. bicolor leaf* (SEM); **(a)** pentagonal epidermal cells in abaxial surface; **(b)** pentagonal epidermal cells with anti-clinal walls in abaxial surface; **(c)** paracytic stoma in abaxial surface; **(d)** higher magnification of paracytic stoma on abaxial surface; **(e)** deposition of mineral crystals in mesophyll **(f)** deposition of mineral crystals in mesophyll (higher magnification).

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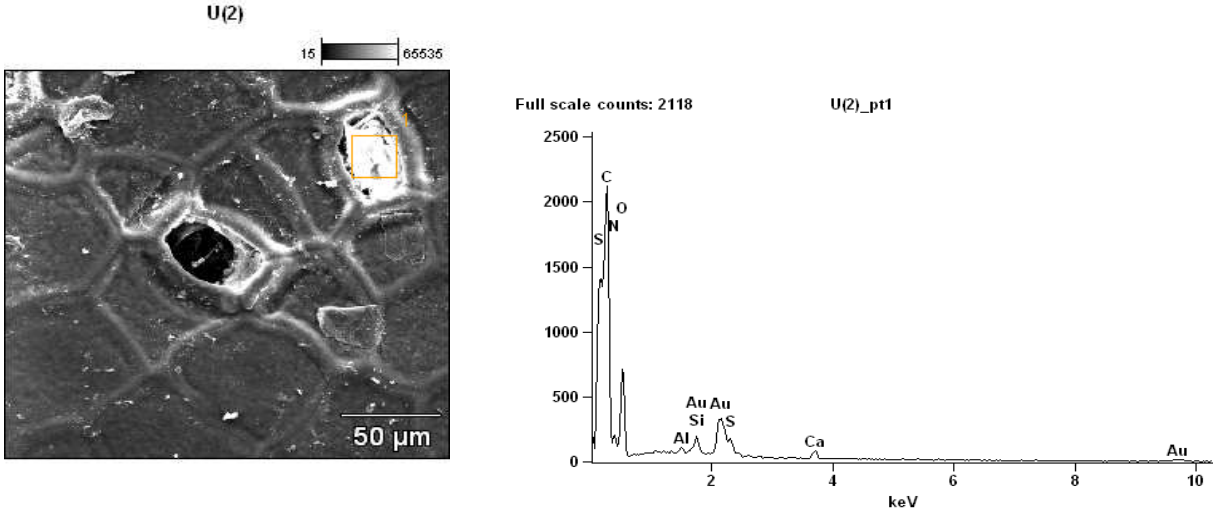

Figure 4: X-ray spectra of various elements detected in the leaf epidermis, micrograph on the left show the point of focus of the electron beam (anticathode).

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