Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2021.15.23

Research Article
Micro-morphological Information of *Carpobrotus edulis* (L.) Bolus: A Southern African Herbal Plant

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Abstract

Background and Objective: *Carpobrotus edulis* has become one of the most recognized herbal plants in the Savanna Regions of Eastern Cape and Western Cape in South Africa. This study analyzed the foliar morphology and elemental composition of the plant *Carpobrotus edulis* (L.) bolus. **Materials and Methods:** Fresh leaves of the plant were investigated using the JEOL (JSM-6390LV) Scanning Electron Microscope (SEM). The elemental composition of the leaf was determined by Energy Dispersive X-ray Spectroscopy (EDXS). **Results:** The epidermal surface of *C. edulis* was cuticularised with numerous stomata density which was more on the adaxial surface 392.7±0.2 mm^2 than the abaxial surface 360.6±0.6 mm^2. Both large and small bulbous glandular trichomes were stalked above the epidermis. Mineral elements present were druses, raphides, C (37.53±0.54 wt.%), O (45.94±0.72 wt.%), Si (6.23±0.06 wt.%), Ca (24.34±0.35 wt.%) and Au (80.32±8.64 wt.%). **Conclusion:** The study provides for the first time the micro-morphological information on the leaf of *C. edulis* L. bolus.

Key words: *Carpobrotus edulis*, micro-anatomy, cuticularised stomata, glandular trichomes, epidermis and abaxial surfaces

Citation: Omoruyi, B.E., D.I. Ighodaro, A.J. Afolayan and G. Bradley, 2021. Micro-morphological information of *Carpobrotus edulis* (L.) bolus: A Southern African herbal plant. Asian J. Plant Sci., 20: 15-23.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Carpobrotus edulis belongs to the Aizoaceae family, a dicotyledonous midday flowering plant consisting of 20 species[1]. The plant was previously classified as *Mesembryanthemum edulis* carpet vygies with glistening globular bladder cells covering the succulent horizontal stems, fruit and leaves[2,3]. Seven of the *C. edulis* species are indigenous and widespread in Southern Africa, especially in the Eastern Cape Province, Northern Cape, KwaZulu-Natal, Free State, Western Cape, Swaziland and Lesotho[4,5]. Rapid growth occurs year round along highways, beaches and in other public and private landscapes. Flowers are extremely flamboyant with several stamens that are bordered by stigma appearing like star fish during late winter-spring (August-October), which are pollinated by solitary bees, honey bees, carpenter bees and many beetle species[6]. Due to its rapid growth, the plant has been widely promoted as an evergreen ornamental plant capable of holding great amount of water in its leaves for a very long period of time[7]. This helps it to Beverly resistant to harsh coastal climatic conditions, such as drought, wind and fire[3].

The plant is widely used in South Africa because it possesses antimicrobial, antifungal, antitumor and antioxidant properties[8-11]. In previous studies, several chemical compounds present in *C. edulis* leave extract were analyzed, using GC-MS and LC-ESI-TOF/MS techniques and found out that the plant contains flavonoids, phenolic acids, lipid derivatives, purine derivatives, pyrimidine derivatives, vitamin B and quinolinone derivatives. Combinations of these compounds were found to significantly inhibit HIV-1 protease activity[12]. The most important features of traditional medicine in South Africa are based on diversity, flexibility, easy accessibility, relative low cost and low side effects. However, one of the major setbacks is the species misidentification due to broad similar morphology of its members. SEM micro-anatomy has been the best analytical tool for the accurate identification and quality assessment of plant organelles[13]. Among Aizoaceae families, such as Ruschioideae, anisocytic and anomocytic or paracytic stomata have been observed[14]. Variations in stomata type, size, abundance, length and density are taxonomically important features in angiosperm plant studies[15-17]. Trichomes in the form of bulbous, capitate-sessile and capitate-stalked exist among Asteraceae, Lamiaceae and Verbenaceae plants[18]. They are characterized organelles with cell factories that store different classes of secondary metabolites[19]. Their morphological diversity contributes to the plant fitness and sustainability in any environment[20]. Calcium crystals are bio-minerals occurring in many organs or tissues within plants. They appear naturally in various shapes and are classified into five major forms: druses crystals, prism crystals, styloids crystals, raphides crystals and sand crystals[21]. Their primary role may vary depending on the plant, organ and tissue in which they occur[22]. Some acts as tissue calcium regulator, while others act as a defence against herbivory, tissue support and as an internal reservoir for calcium, ion balance[23]. Based on these characters, the micro-characters of the leaf of *C. edulis* were analyzed using SEM and EDXS, with a view to contribute to the knowledge of the genus and generating data to enhance the proper identification and classification of the specie.

MATERIALS AND METHODS

Plant collection: Fresh green leaves of *C. edulis* were collected from Ntselamanzi location, Alice, Eastern Cape Province, South Africa, in August, 2013. The plant was identified by Dr. O.J. Sharaibi and Professor D.S. Grierson of the Department of Botany, University of Fort Hare, South Africa, in line with the South African National Biodiversity Institute (http://pza.sanbi.org/carpobrotus-edulis). A voucher sample (Omo 2013/1-Omo 2013/20) was deposited in Giffen's herbarium of the Department of Botany, University of Fort Hare.

Cross section of *C. edulis* leaves: The thick fresh green leaves have a smooth surface, succulent, sharply 3-angled, with a triangular cross section, 4-10 cm long, 5-12 mm wide with tiny serrations along the outermost part (Fig. 1a). When leafs were cut, the external surface contains chlorenchyma layers which were seen all round the periphery and colourless water-storage tissue in the core (Fig. 1b).

Scanning Electron Microscopy (SEM): The triangular cross section of green fresh leaves was prepared for SEM according to Otang *et al.*[24]. Briefly, sections of 4-6 mm thick were cut using table knife and were fixed for 12 h in 8% Glutaraldehyde in H_2O, pH 7.3 (Cat no 111-30-8, Merck, South Africa) and then rinsed with 0.05 M sodium cacodylate buffer, pH 7.5 (Cat no E11650, Science service, Unterhachinger, Munchen, Germany). Samples were further rinsed three times with distilled water and then subjected to dehydration in 100% ethanol (Cat no 64-17-5, Sigma-Aldrich, South Africa) at various concentrations ranging from 10-100% with 20 min per rinse. This was followed by critically drying the samples with $\geq$99.8% liquid CO_2 (Cat no 124-38-9, Merck, South Africa) in an auto sampler 810 critical point drier (Cat no 91090, Electron microscopy sciences, Hartfield, US) and then mounted on aluminium stubs using two-sided adhesive carbon tape

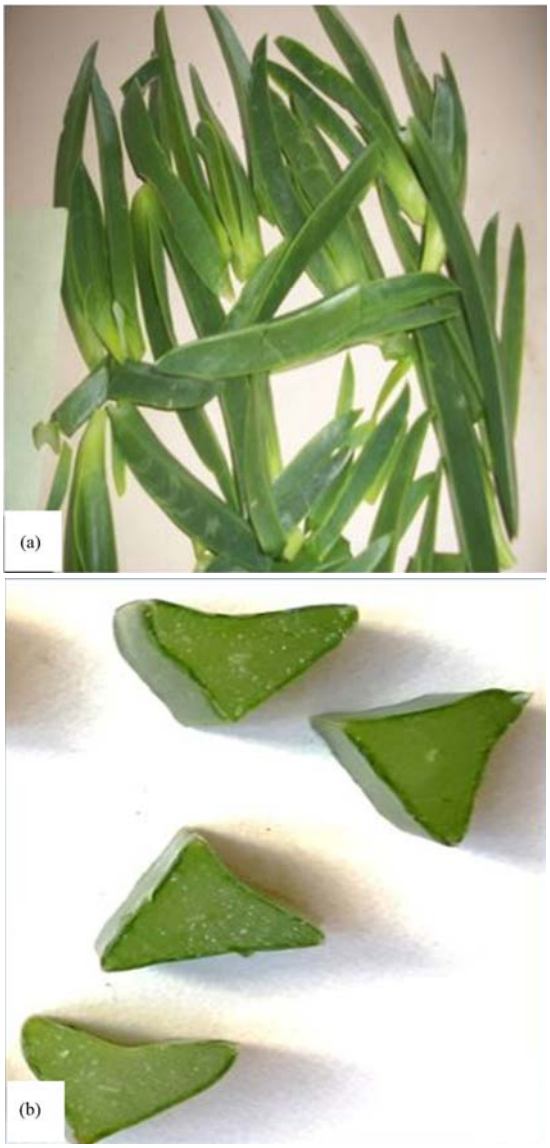

Fig. 1(a-b): Leaves morphology

(a) Morphology of leaves section of *C. edulis* and (b) Cut surface of leaves showing 3-angled and triangular in cross-section, chlorenchyma and colourless water-storage tissues

coating with gold-palladium (Elko IB-3 Ion coater, Hartfield, US). Samples were examined at varying magnification using JEOL scanning electron microscopy (SEM) (Cat no 9193088, JSM-6390LV, Congress Ave, USA Int.) that was operated at 10-15 kV accelerated voltage.

Energy Dispersive X-ray Spectroscopy (EDXS): The elemental analysis of the samples was carried out using the energy dispersive X-ray analyser (Cat no 51733, Thermo Electron Corporation, 6733B-IUUSN, USA) connected to the scanning electron microscope. Electron images were captured using Noran system six imaging software (Cat no 1221, Thermo Electron Corporation, 6733B-IUUSN, USA). All epidermal features examined were stomata type, location, distribution, pore length, density, trichomes shape, size and distribution on both the external and internal epidermis, crystals, shape, size and distribution as well as mineral nutrients were evaluated.

RESULTS AND DISCUSSION

Scanning electron microscopy (SEM)

Stomata observation: The SEM analysis of the triangular cross-sections of the leaves showed cuticularised epidermal cells with numerous paracytic stomata extending throughout the stomata complex (Fig. 2). The paracytic stomata were fully opened on both the external and internal epidermal surface (Fig. 2a-c). The mean stomata pore length on the adaxial surface was 12.50±0.6 µm and the mean adaxial stomata pore width was 12.49±0.3 µm while the mean abaxial stoma pore length was 13.05±0.3 µm with mean stomata pore width of 11.1±0.4 µm. The mean stomata density on the abaxial surface was 392.7±0.2 mm^2 while the mean stomata density on the abaxial surface was 360.6±0.6 mm^2. The stomata pores were surrounded by guard cells on both surfaces. Calcium Oxalate (CaOX) crystals were observed at the pit areas close to the guard cells. The guard cells length on both surfaces was 23.2±1.2 µm; the guard cells density on the adaxial surface was 325.2±0.7 mm^2. Stomata density and distribution is a natural phenomenon in most angiosperm families. They are the main channels through which plants regulate water and gas exchange with the environment[25]. The stomata morphology has long been regarded as a useful taxonomic tool in differentiating among Aizoaceae families as some others have been observed as anisocytic and anomocytic or paracytic; the last type seems to be more frequent in c than in any of the other subfamilies[14]. The unique features of the paracytic stomata is the occurrence of bladder idioblasts on the leaf and stem surfaces of many Aizoaceae[14], furthermore, thick outer epidermis walls parallel to guard cells and sunken stomata and the incrustation of the outer wall with calcium oxalate crystals (Fig. 2c).

Distribution of trichomes: A random distribution of trichomes revealed by SEM was grouped into large (approx. 60 µm in diameter) and small (aprox. 20 µm in diameter) bulbous glandular trichomes. These structures occurred as heads of stalked above the epidermis on both adaxial and abaxial leaf

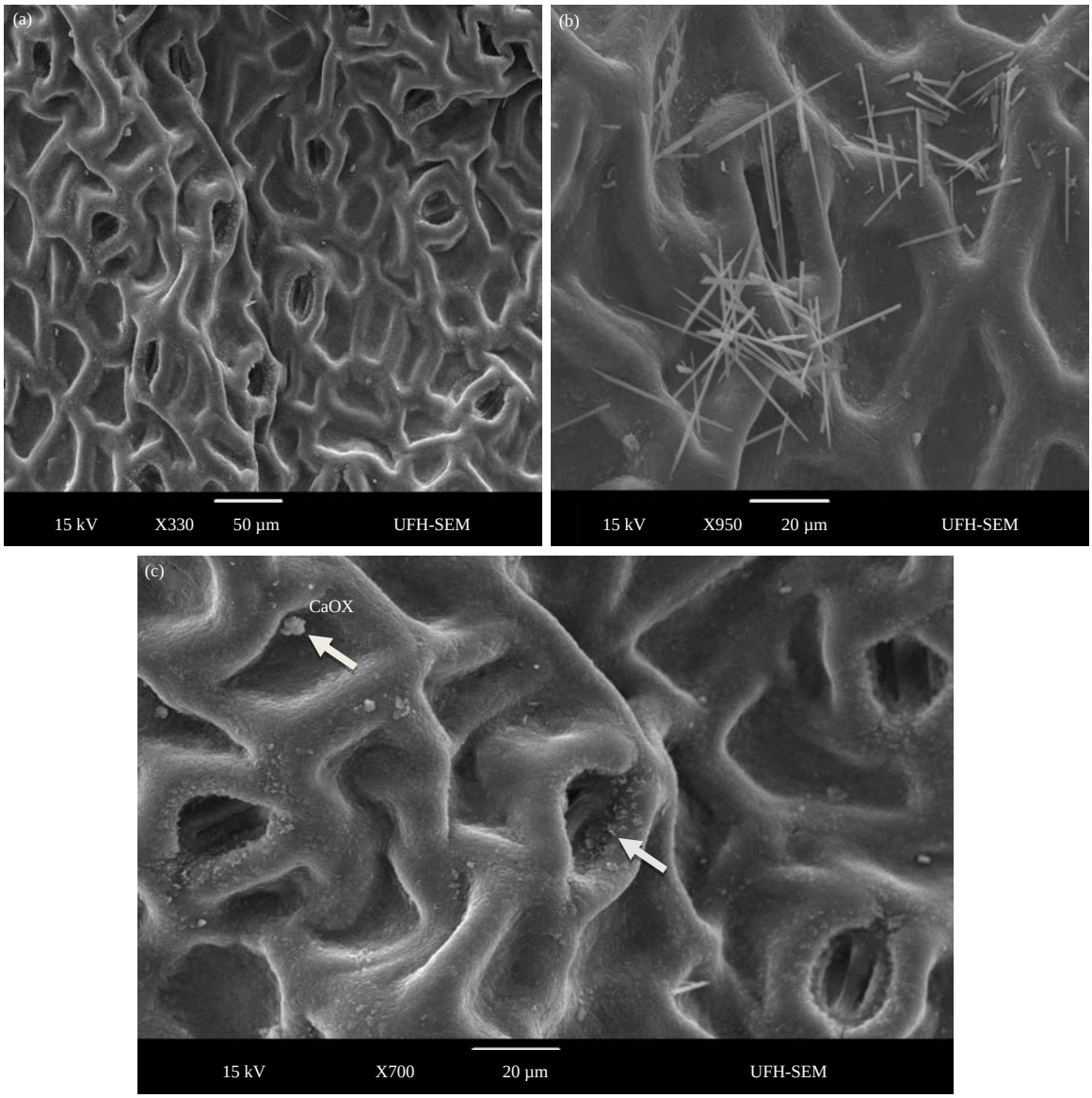

Fig. 2(a-c): Cuticularised epidermal surfaces of *C. edulis* leaf (SEM)

a: Paracytic stomata distribution in adaxial surface, b: Paracytic stomata distribution in abaxial surface and c: Higher magnification showing stomata pore guard cells and calcium oxalate (CaOX)

surfaces. Distribution ratio of 1:1 between large and small bulbous glands in the adaxial and abaxial surfaces per square millimeter was 29 and small 48, respectively (Fig. 3a, c and d). Bulbous glands have also been observed in *Lippiascaberrima*, the distribution ratio counted per square millimeter on both adaxial and abaxial sides were 24 large and 26 small[18]. The X-ray analysis of the bulbous Glandular Trichomes (GT) mineral elements showed that C, S, O, P, Au, Cl, Ca and Na were most predominant on the adaxial epidermal surface (Fig. 3b). The large bulbous glands may secrete duct cells with diameter of 20±2.0 µm head above (Fig. 3d), which may contain bioactive compounds and essential oils[26]. The development of trichomes from the epidermis usually results from differential enlargement and subsequent division of the epidermal cells and their derivatives[15]. Trichomes serve as photo-protective against light stress[16] as significant defence mechanism against abiotic and biotic stresses and assist in decreasing water loss and facilitate acclimation to xeric environments[26].

Appearance of crystals: Two types of crystals were identified in this study. The druses type was found in the adaxial pore region of the stomata (Fig. 4a and c). The druse crystals were

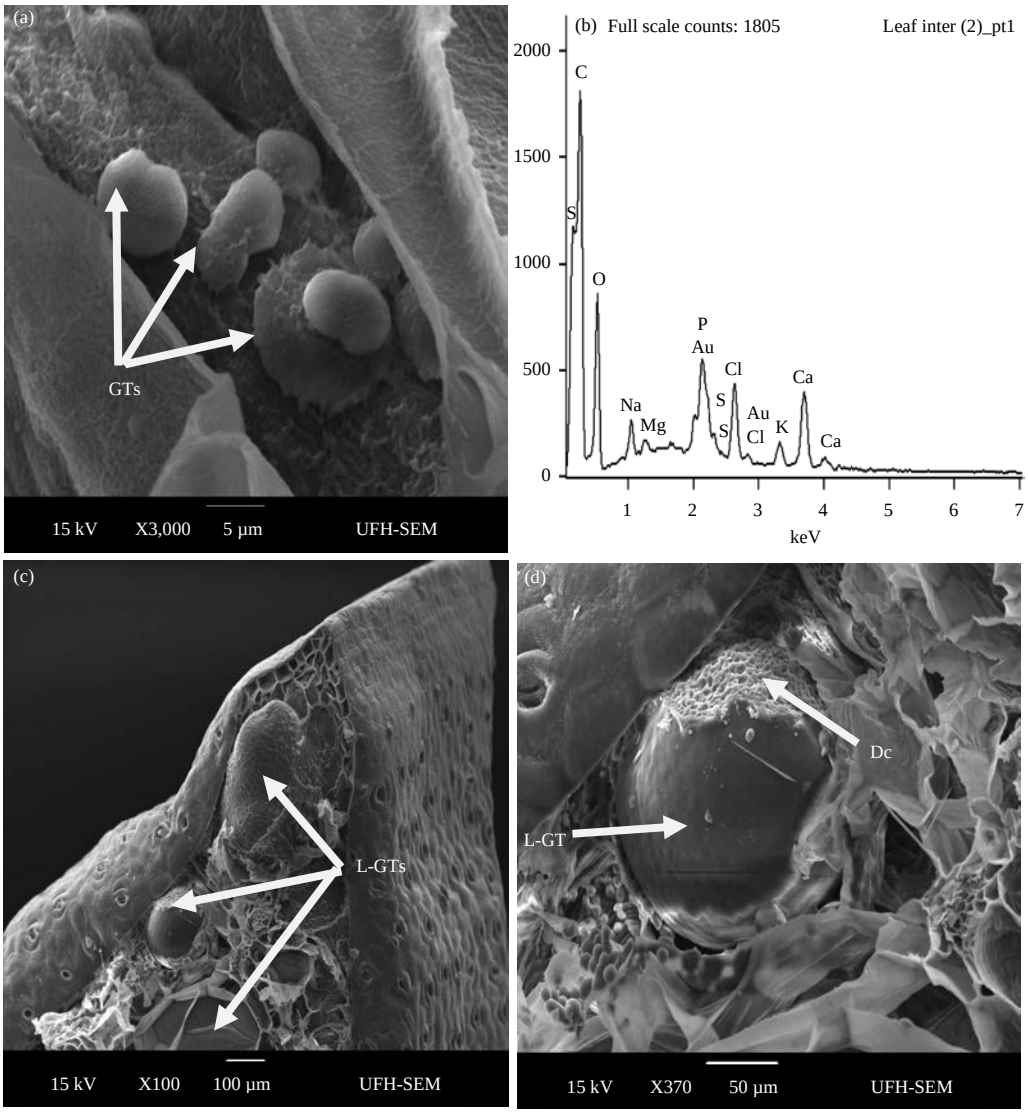

Fig. 3(a-d): Micromorphology of trichomes of *C. edulis* (SEM)

a: Stalked head of bulbous glandular trichomes (GTs) in the adaxial surface, b: X-ray analysis of the bulbous GT mineral elements showing C, S, O, P, Au, Cl, Ca and Na on the adaxial epidermal surface, c: Distribution of large (L-GTs) and small (S-GTs) in the abaxial surface higher magnification of L-GT, comprising of duct cells (Dc) are visible

rectangular aggregate in shape and they are known strictly for calcium ionic balance in plant tissues, photosynthesis and mechanical support[27]. The X-ray micrograph of the point focus of druses crystal show that C, S, Si, O, Au, Ti, Cl, Ca and K were the major mineral elements present on the adaxial stomata pores (Fig. 4b and d).

The raphides types were stored internally as bundles around the adaxial and abaxial regions of stomata and GTs area (Fig. 5a-d). They appear in the form of dual-pointed needles, arranged and packed in bundles of about 2-3 mm long[28]. They are described as monohydrate crystalline bundles that act as defence mechanism against plant predator. The X-ray micrograph of the mineral elements found in both adaxial and abaxial surfaces of raphides crystals were C, Ca, Cl, O, S, Au, Na and K (Fig. 5e and f). Crystallization is the most common way by which plants neutralise abundant calcium absorbed in ionic solution and remained when water vapour transpires[27]. Crystal formation and its distribution in various organs is a common phenomenon in higher plants[29]. Their functional role is to protect the plant from any predator that tries to feed on their leaves[30]. Crystals are found more in leaves than in stems and they are remarkably diverse among angiosperms.

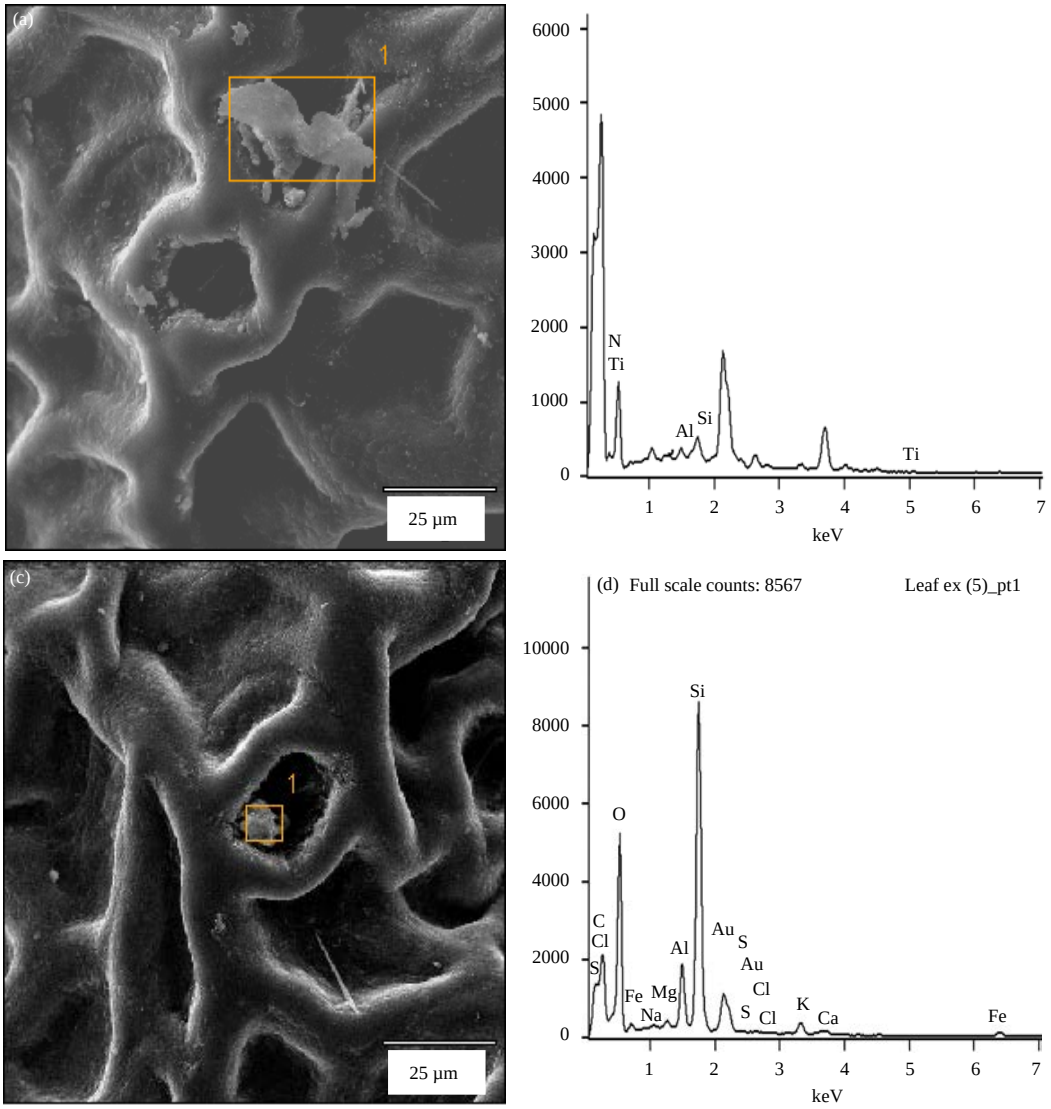

Fig. 4(a-d): Deposits of crystals in the leaf surface of *C. edulis*

a and c: deposits of druses crystals located in the adaxial stomata pores, b and d: X-ray micrograph of the point focus of druses crystal showing C, S, Si, O, Au, Ti, Cl, Ca and K as the mineral elements present on the adaxial stomata pores

Table 1: Total composition of the mineral elements found in the druses and raphides crystals of *C. edulis* epidermal surfaces by EDX spectroscopy

Elements		External surface	Internal surface	Total (wt. %)
Carbon	C	21.97±0.29	15.56±0.25	37.53±0.54
Nitrogen	N	0.56±0.56	0	0.56±0.56
Oxygen	O	19.28±0.34	26.66±0.38	45.94±0.72
Sodium	Na	0.52±0.05	0.85±0.05	1.37±0.10
Magnesium	Mg	0.20±0.03	0.38±0.05	0.58±0.08
Phosphorus	P	0	1.42±0.10	1.42±0.10
Aluminium	Al	1.16±0.05	0	1.16±0.05
Silicon	Si	6.23±0.06	0	6.23±0.06
Sulphur	S	0.21±0.07	0.49±0.08	0.70±0.15
Chlorine	Cl	0.45±0.05	2.24±0.12	2.69±0.17
Potassium	K	0.74±0.06	0.98±0.09	1.72±0.15
Calcium	Ca	6.03±0.12	18.31±0.23	24.34±0.35
Titanium	Ti	0.27±0.06	0	0.27±0.06
Iron	Fe	1.92±0.21	0	1.92±0.21
Gold	Au	46.22±4.23	34.10±4.41	80.32±8.64

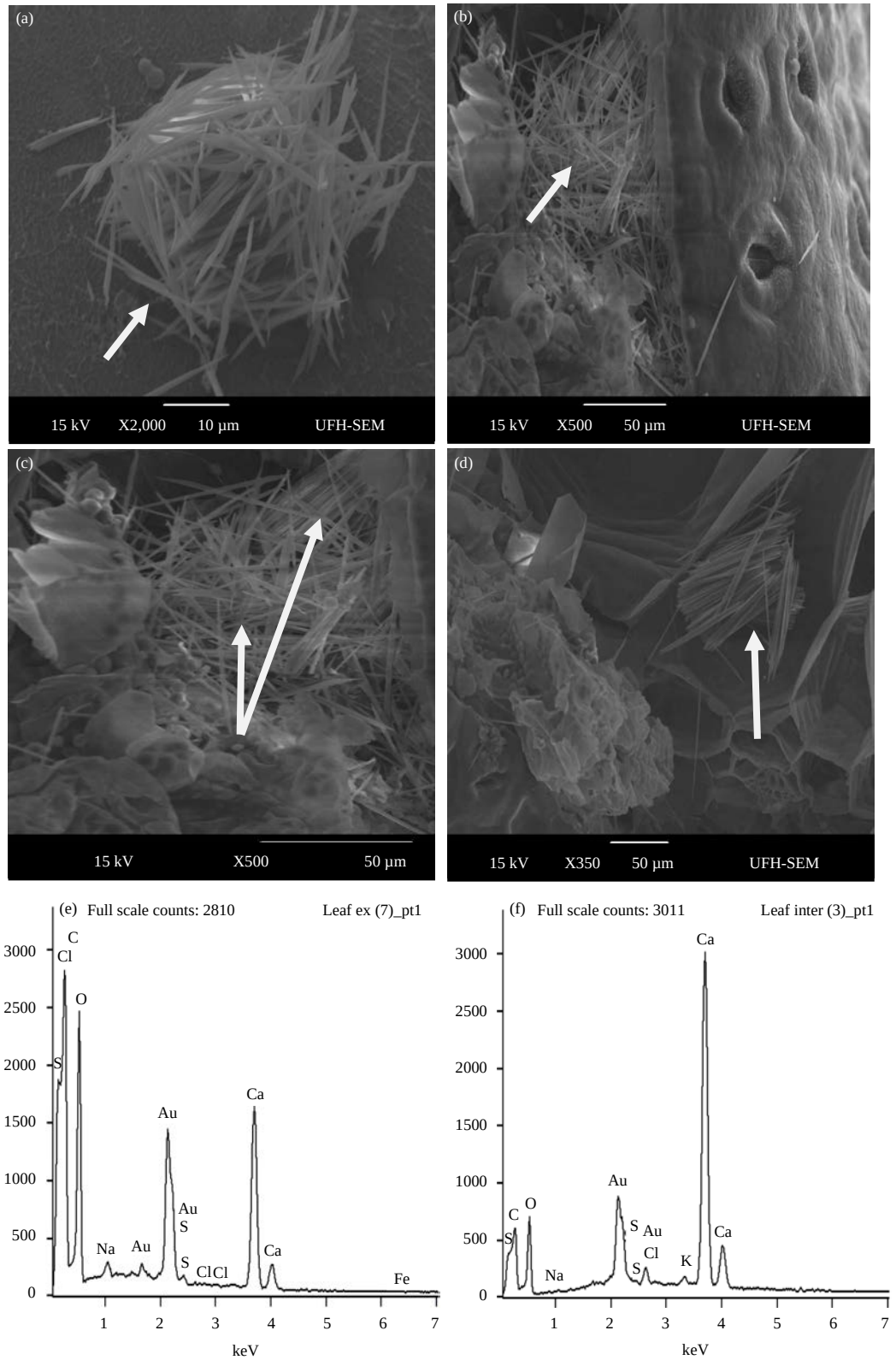

Fig. 5(a-f): Deposits of raphides crystals and X-ray micrograph

a-d: Appearing as dual-pointed needles, arranged and packed in bundles of about 2-3 mm long around the adaxial regions of stomata and GTs area, e and f: X-ray micrograph showing the mineral elements (C, Ca, Cl, O, S, Au, Na and K) present in both adaxial and abaxial surfaces of raphides crystals

Mineral elements: Natural minerals needed for adequate plant growth and reproduction are carbon, oxygen and calcium[31]. Deficiency in any one of these micro-minerals may reduce plant growth, yield and quality[32]. Based on the elemental X-ray micro-analysis, the mean values of the total mineral elements on both adaxial and abaxial crystals were carbon (37.53±0.54 wt.%), oxygen (45.94±0.72 wt.%), silicon (6.23±0.06 wt.%), calcium (24.34±0.35 wt.%) and gold (80.32±8.64 wt.%). Nitrogen (N), sodium (Na), magnesium (Mg), aluminium (Al), sulphur (S), chlorine (Cl), potassium (K),titanium (Ti) and iron (Fe) were present in trace amounts (Table 1). The elemental peaks of carbon and silicon present on the druses crystals were predominantly higher on the adaxial epidermal surface (Fig. 4a, b). Calcium and gold peaks were higher on both the adaxial and abaxial surfaces of the raphides crystals (Fig. 5). Mineral formation in plants is common[33,34]. They play vital role in the overall health of plants development, in cellular ion balance, in plant defence against herbivore, in cell growth and membrane tissue rigidity and support and in detoxification of oxalic acid or aluminium. It could be assumed that mineral contents found in the leaves of *Carpobrotus edulis* are responsible for its sustain ability in and out of seasons.

CONCLUSION

The present study summarizes the current information of the micro-morphological features of *C. edulis* leaf. The anatomical features identified include cuticularised epidermal cells with numerous paracytic stomata, bulbous glandular trichomes, druses and raphides rod-like crystals stored in the adaxial and abaxial surfaces. The highest mineral content analyzed was gold followed by oxygen, carbon, calcium and silicon. Sodium, magnesium, aluminum, sulphur, chlorine, potassium, titanium and iron were present in trace amounts. The study provides for the first time the micro-morphological information of the leaf of *C. edulis* L. bolus. However, further studies using *in vivo* staining techniques are needed to correlate the glandular morphology and chemical functionality.

SIGNIFICANCE STATEMENT

Species of *Carpobrotus edulis* are of great value in the cure and treatment of diseases but the genus has been difficult to classify into distinct species due to broad similar morphology of its members. Present micro-taxonomic confusion in this genus necessitates its proper identification. This study is a positive step in resolving the accurate taxonomic confusion that exists among the genus.

ACKNOWLEDGMENT

This research was supported by grants (2013SA02331) from National Research Foundation and Govan Mbeki Research and Development Centre, University of Fort Hare. We also thank to the Applied Microbial and Health Biotechnology Institute, Cape Peninsula University of Technology for assistance and moral support.

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