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Full Length Research Paper

Morphological and chemical composition of the essential oil of the leaf of *Schistostephium heptalobium*

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The morphology of *Schistostephium heptalobium* was investigated by using electron microscopical examination. The scanning electron microscope revealed that the leaves of this plant are characterized by short, multicellular glandular trichomes and long hairy-fibrous tubular non-glandular trichomes. These trichomes are associated with crystal-like deposits. The Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDX) showed that, Fe, Al, K, Mg and Si are the major components of the crystals-like deposits. The area of the non-glandular trichomes where these crystals-like substances are produced showed Al, Ca, K, Mg, Mo, Na, Pi and Si as major residue when analysed by SEM-EDX. However, the SEM-EDX analysis of glandular trichome sac and the content of the sac showed different results. The anatomical investigation also showed that the ends of the non-glandular trichomes are fibrous and have a tubular base while the glandular trichomes contain cuticular sac. The sac contains by products which are referred to as essential oil. These by-products are regarded as the first line of defence in this plant. Hydro-distillation of fresh leaves of *S. heptalobium* yielded 2.5% (v/v) of the essential oil. The GC-MS analysis of the oil revealed that the essential oil of *S. heptalobium* is characterized by α-pinene, sabinene, myrcene, α-phellendrene, 1.8-cineole, γ-terpinen, α-thujone, cis-sabinene hydrate, camphene, terpinen-4-ol, α-terpineol, 2-cyclohexen-1one, geranial, phenol, neryl acetate, β-caryophyllene, β-selinene and caryophyllene oxide.

Key words: Glandular trichomes, non-glandular trichomes, SEM-EDX analysis, essential oil composition, *Schistostephuim heptalobium*.

INTRODUCTION

The world orientation towards the use of natural products from organically grown plants is the most common phenomenon in the 21st century. Several companies make use of plant products or plant derived phytochemical to treat medical related illness, nutrition, fragrance and aromatherapy (Roddick, 1991; Koschier and Sedy, 2003; Valaint-Vetschera et al., 2003; Lima et al., 2004; Burt, 2004). In the past, most plants were well known for their herbs, essential oil, fragrance and medicinal values by traditional healers and indigenous people of South Africa (Bell, 1981; Rabe and van Staden, 1997; Also et al., 2000; Sader et al., 2002; Tan et al., 2002). These plants which are of herbs, essential oils, fragrance and medicinal value are common in diverse families such as Asteraceae, Lamiaceae, Cannabinaceae, Solanaceae, Poaceae etc, where many studies have been conducted on their secondary metabolites, anatomy and morphology in relation to their essential oil production (Vermeer and Peterson, 1979; Werker and Fahn, 1981; Bosabalidis and Tsekos, 1982; Bruni and Modenesi, 1983; Peterson and Vermeer, 1984; Ascensão and Pais, 1985; Werker et al., 1985a, b; Dudai et al., 1988; Antunes and Sevnate – Pinto, 1991; Fahn and Shimony, 1998; Bisio et al., 1999; Tan et al., 2002; Auge et al., 2003; Nguefack et al., 2004).

Schistostephium heptalobium (Umhlonyane for Africans) belongs to the Asteraceae. In the past, the plant was used as a remedy for flu, cough, cold and other diseases by indigenous people of the Eastern Cape of South Africa (Rabe and van Staden, 1997; Grierson and Afolayan, 1999).

The indigenous people use hot or boiling water to extract useful compounds in leaves and stems of the plant to treat or cure various ailments. However, this plant is unpalatable to herbivores and it has been stated that the exudates of some plant are believed to be not suitable

for a variety of livestock (Mayekiso, et al., 2008). These extracts which are produced by trichomes of the leaves, stems of the plant may function as plant growth regulators and also act as a defence mechanism for the plant against insects, other pathogens and possibly livestock (Croteau, 1977; Bell, 1981; Wagner, 1991; Duke and Paul, 1993; Werker, 1993).

This plant is a shrub which grows up to 1.2 – 1.5 m tall, characterized by bright yellow fragrant flowers. In spite of the pharmaceutical importance of this plant, there is a very little information with regard to its anatomy and essential oil composition. The aim of this study was to investigate the anatomical structure of the leaf in relation to chemical composition of the essential oil.

MATERIALS AND METHODS

During the spring (September – November, 2007) the vegetative shoots of *S. heptalobium* were collected from a farm in Fort Beaufort in the Eastern Cape Province, South Africa and placed in a fixative as described below. The plant was identified by the curator of the Schonland Herbarium at Rhodes University, Grahamstown. A voucher specimen (Mayekiso 16) was deposited at the Griffen Herbarium, University of Fort Hare, Eastern Cape.

Scanning electron microscopy

Fresh sections of leaves and stem (0.1 x 0.5 mm thick) were picked randomly and immediately fixed in 6% glutaraldehyde in 0.05 mM Sodium cacodylate buffer (pH 7.3), washed in 0.05 mM Sodium cacodylate for 12 h. Sections were then dehydrated in an ethanol series (10 – 100%). The leaf sections were dried in a Hitachi HCP-2 critical point dryer, and then coated with gold using a sputter coater (Hummer V-sputter coater).

The sections were then viewed at 15 KV with a Hitachi S-450 Scanning Electron Microscope equipped with a Polaroid 454 camera at 10 KV, to which an energy dispersive spectroscopy (EDX- FEI QUANTA 200 OXFORD) was attached.

Transmission electron microscopy – light microscopy

Young and mature leaves of *S. heptalobium* were randomly picked from the growing plant. The leaf portions were cut into small segments approximately 2-3 x 5 mm in cold 50 mM Sodium cacodylate buffer, (pH 7.3). They were fixed in a buffered 6 % glutaradehyde (50 mM Na-cacodylate, pH 7) and stored overnight in a refrigerator.

After rinsing in a 50 mM Na-cacodylate buffer, the sample were then postfixed in 2% Osmium tetroxide (OsO_4) in 50 mM Na-cacodylate buffer, pH 7.3, overnight at 4°C, infiltrate in a graded series resin (Spurr, 1969).

Thin sections (0.5 - 2.0 mm) were cut with glass knives on an LKB Ultramicrotome, stained with uranyl acetate followed by lead citrate and observed in a Hitachi Transmission electron microscope at 75 - 100 kV. (Some of the sections were stained with 0.05% toludine blue and examined with a Zeiss photo-microscope III).

Gas chromatography and mass spectroscopy analysis

GC-MS analysis was done after the extraction of the essential oil (water distillation). Fresh leaves were removed from stem and branches, and were weighed. The weighed mass of sample of leaves was 550 g, and the sample was subjected to hydro-distillation for 3 h using a Cleavenger Unit according to British Pharmacopoeia. This process was repeated three times using consistent mass in order to obtain statistical accepted results.

The fresh essential oil was collected, allowed to cool and was analyzed immediately on a Hewlet Packard HP 5973 mass spectrometer interfaced with an HP-6890 Gas Chromatograph. The column consisted of a cross-linked 5% pH ME Siloxane on 30 m x 0.25 mm x 0.25 µm film thick and the column head pressure was 55 Kpa. The carrier gas used was Helium and the flow was 35 cm/s-split flow 30 - 40:1. The temperatures were programmed at initial temperature of 50°C and accelerated to a temperature of 240°C at an acceleration of 3°C /min. Identification of chemical compounds was achieved by mass spectroscopy.

RESULTS

The morphology of the leaf surface of *S. heptalobium* is characterized by oval-club shape glandular and hairy-fibrous non-glandular trichomes (Figures 1, 2, 6 and 7). A high distribution of non-glandular trichomes compared to glandular trichome on both the adaxial and abaxial surfaces of the leaf has been observed (Figures 1 and 2). These non-glandular trichomes appeared to be shielding and protecting the glandular tichomes and the epidermis. The anatomical investigation has shown that non-glandular trichomes are characterized by digitiform shape with 3 – 4 cells. They appeared to be fleshy and tubular at the basal ends indicating that the basal cells seem to be alive at maturity (Figures 6B and 7A). However, the distal end of the non-glandular trichome is composed of dead cells which form a long fibrous thread (Figures 6B and 7A). The glandular trichome is composed of basal cell which is in line with the epidermal cells, the stalk cell and the oval shape glandular head which is characterized with cuticular sac (Figures 1A and B, 2B, 3, 6A and 7B). The cuticular sac is believed to store essential oil and once it ruptures due to external pressures it releases the essential oil (Figures 1B and 5).

Another common characteristic feature associated with both trichomes was the appearance of crystalline deposits (Figure 3). The SEM-EDX results showed that the crystalline deposits were predominately composed of Al, Fe, K, Mg, and Si (Figure 3). The ruptured area of non-glandular trichomes where these crystalline deposits were associated with was analyzed by EDX and the area was composed of Al, Ca, Mg, Mo, Pi and P (Figure 4). The SEM-EDX analysis of the cuticular sac of the glandular trichome revealed the following components, Ca, K, Mg, Na and Si. However it content was predominately composed of Ca, Mg, and Na (Figure 5).

The anatomical structure of the leaf has shown that *S. heptalobium* leaf was dorsi-ventral flattened and contained a layer of palisade parenchyma cells on both lower and upper surface (Figures 6A and 7A). These parenchyma cells occupied almost the entire space of the leaf and appeared to be the main photosynthetic tissues which form the palisade mesophyll. Within the mesophyll

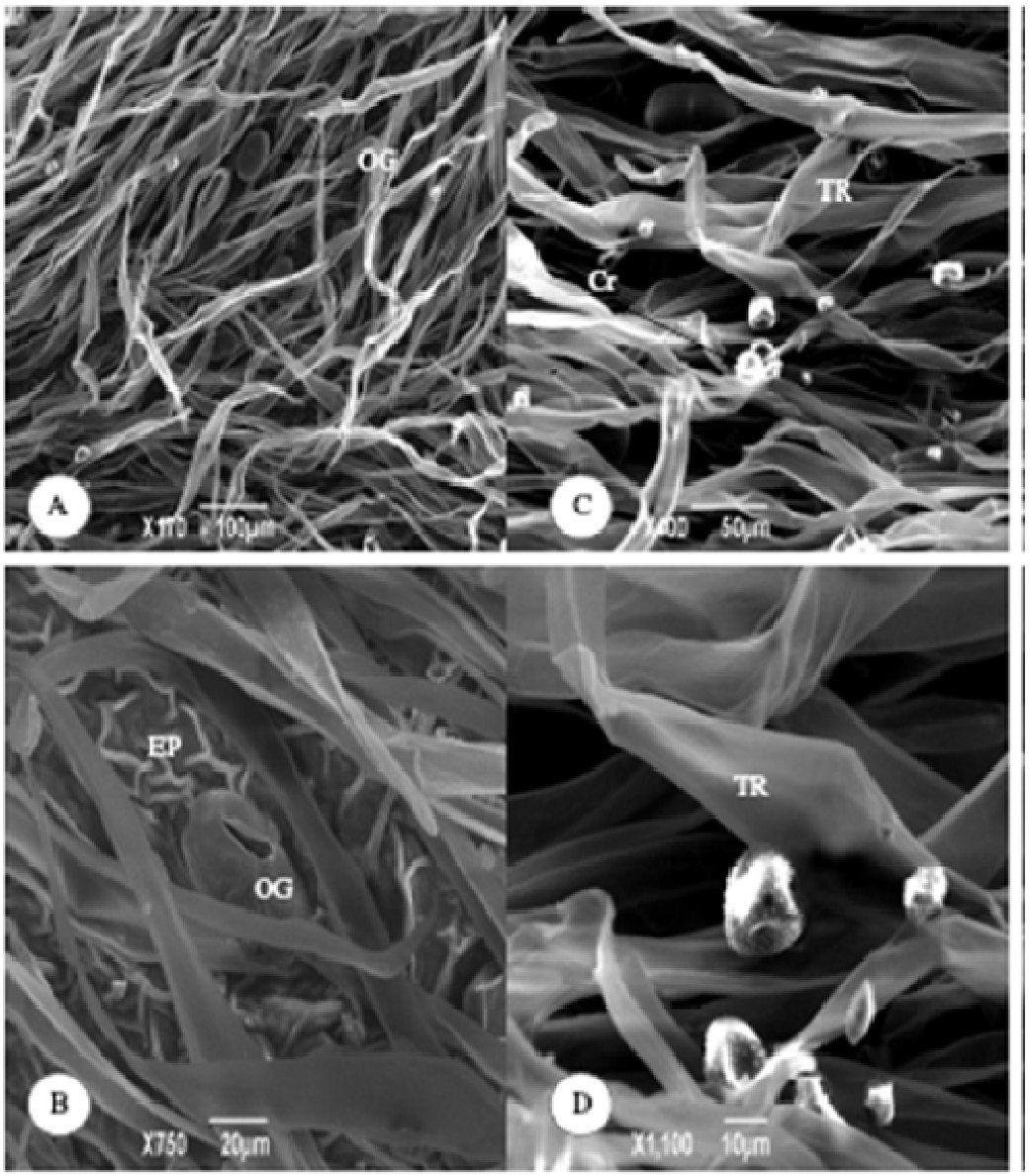

Figure 1. Abaxial surface of *S. heptalobium leaf* showing a high distribution of trichomes. (A) Fibrous-hairy like non-glandular trichome. (B) Epidermal surface covered with non glandular trichome.and a raptured glandular trichome. (C) Crystalline deposits on the surface of non-glandular trichome. (D) Higher magnification of the non-glandular trichome showing the crystalline deposits surface. OG = Oil gland, and TR = trichome.

cell the main vascular bundle is associated with the intermediate small vascular bundles of which they are all enclosed by the epidermal layer. This epidermal layer is characterized by thick wall cutinized cells, non-glandular and glandular trichomes and the stomatal pores with thick subsidiary cells (Figures 6 and 7).

The hydro-distillation of the leaves of *S. heptalobium* provided 10 ml of the essential oil per 500 g of plant specimen which is equivalent to 2.5% (v/w). The colour of the oil was cream-whitish but at the end of the process the colour turned brown. The GC-MS was used to analyze the essential oil and the characterized essential oil was dominated by monoterpenes which counted for 97.41% oil the oil (Table 1). The main compounds were α-thujone (79.3%), sabinene (4.58%), 1.8-cineole (3.1%), terpinen-4-ol (1.19%) and myrcene (1.43%). The other classes of compounds present in the essential oil were sesquiter-penes which comprised of β-caryophyllene (0.75%), β–selinene, (0.18%), and caryophyllene oxide (0.18%). The oxygenated monoterpenes were represented by α-terpinolene (0.98%), 2-cyclohexen-1-one (0.97%), geranial (0.22 %), phenol (0.29%) compounds.

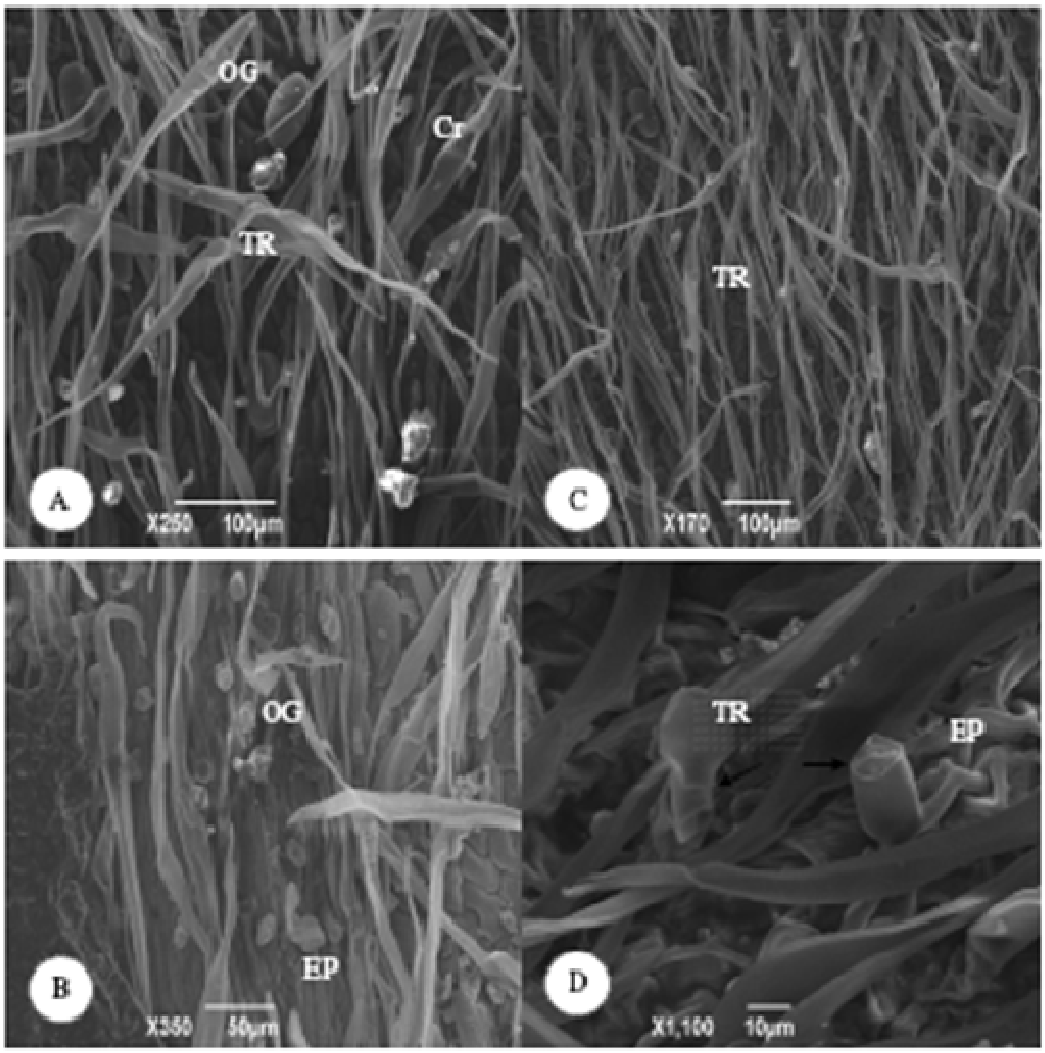

Figure 2. Adaxial surface of *S. heptalobium leaf* showing a high distribution of trichomes. (A) Non-glandular trichome with glandular trichome. (B) Surface layer of the epidermis. (C) A high distribution of the non-glandular trichome with some interesting features. (D) Higher magnification of the tubular trichome of the leaf. OG = Oil gland, TR = trichome, and EP = epidermis.

DISCUSSION

The *S. heptalobium* leaf was characterized by glandular trichome and a dense non-glandular trichome which obscure the epidermal layer of the adaxial and abaxial surface. The glandular trichomes were club to oval shape and were composed of basal cell which was in line with the epidermal layer, the stalk cells and globose unicellular gland head. This type of glandular trichome is a capitate type which is a common type in most genera of Compositae (Fahn, 1988; Ascensão et al., 1998). Their distribution pattern was difficult to observe because they were covered by non-glandular trichome. However, other authors suggested that, there is a high distribution of the glandular trichome throughout the life span of the plant (Vermeer and Peterson, 1979; Bosabalidis and Tsekos, 1982, 1984; Bosabalidis, 1990) and they further suggested that there are two types of glandular trichome. There are those that are found throughout the life span of a plant which are referred to as long term glandular trichome and those that have a short life span of which they occur during the development of a plant. The glandular trichome that have a short life span and play short role in plant are referred to as short term glandular trichome. The long term glandular trichomes are characterized by gland cell and cuticular sac in which the secretory material appeared to accumulate gradually and consist under elevated cuticular sac during the development and growth of the aerial parts of the plant.

The material within the cuticular sac is stored in the form of essential oil and when released to the outside of the cuticular sac it is kept within in the surface layer of the plant by the fibrous non-glandular trichome.

These toxic or secondary metabolites are regarded as

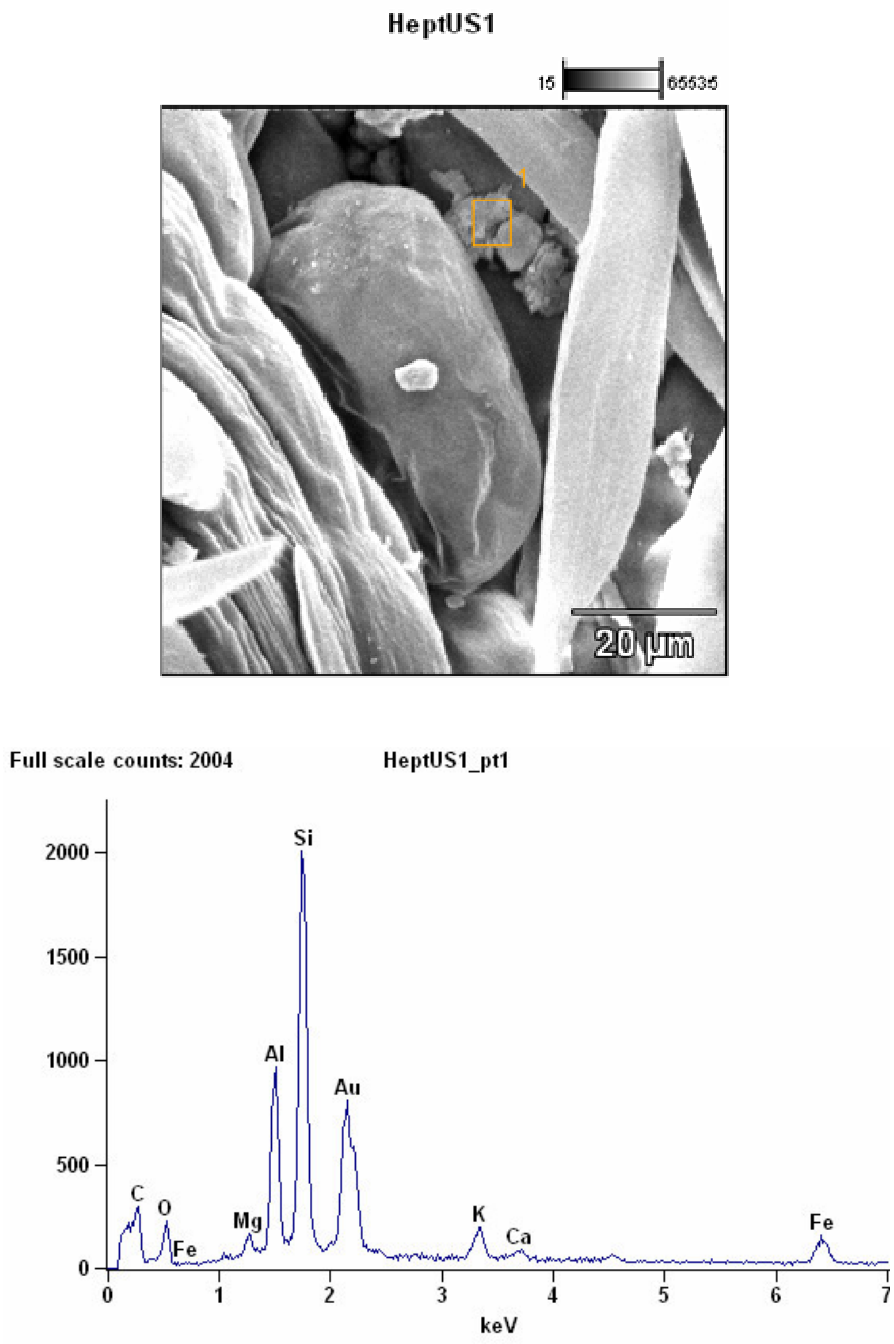

Figure 3. EDX-SEM of *S. heptalobium* on the surface of the epidermis. The arrows shows the crystalline deposits analyzed.

the first line of defence by the plant. In most species, the source of toxic exudates has been attributed to these glandular trichomes (Harborne, 1990; Lee et al., 2003).

The non-glandular trichomes on the other hand can also be associated with protection of the aerial parts of the plant possible against foraging insects and airborne propagules of fungi (Ascensão and Pais, 1985; Harborne, 1990; Antunes and Sevinate-Pinto, 1991; Afolayan and Meyer, 1995; Delamare et al., 2005). It has also been suggested that, the resistance to changing temperature appeared to depend solely on tissue morphology and on mechanical properties of cell wall (Harborne, 1990; Antunes and Sevinate-Pinto, 1991). Such proposition is supported by the characteristic nature of the anatomy, morphology of the leaf and highly specialized mechanical properties of the epidermal cell wall.

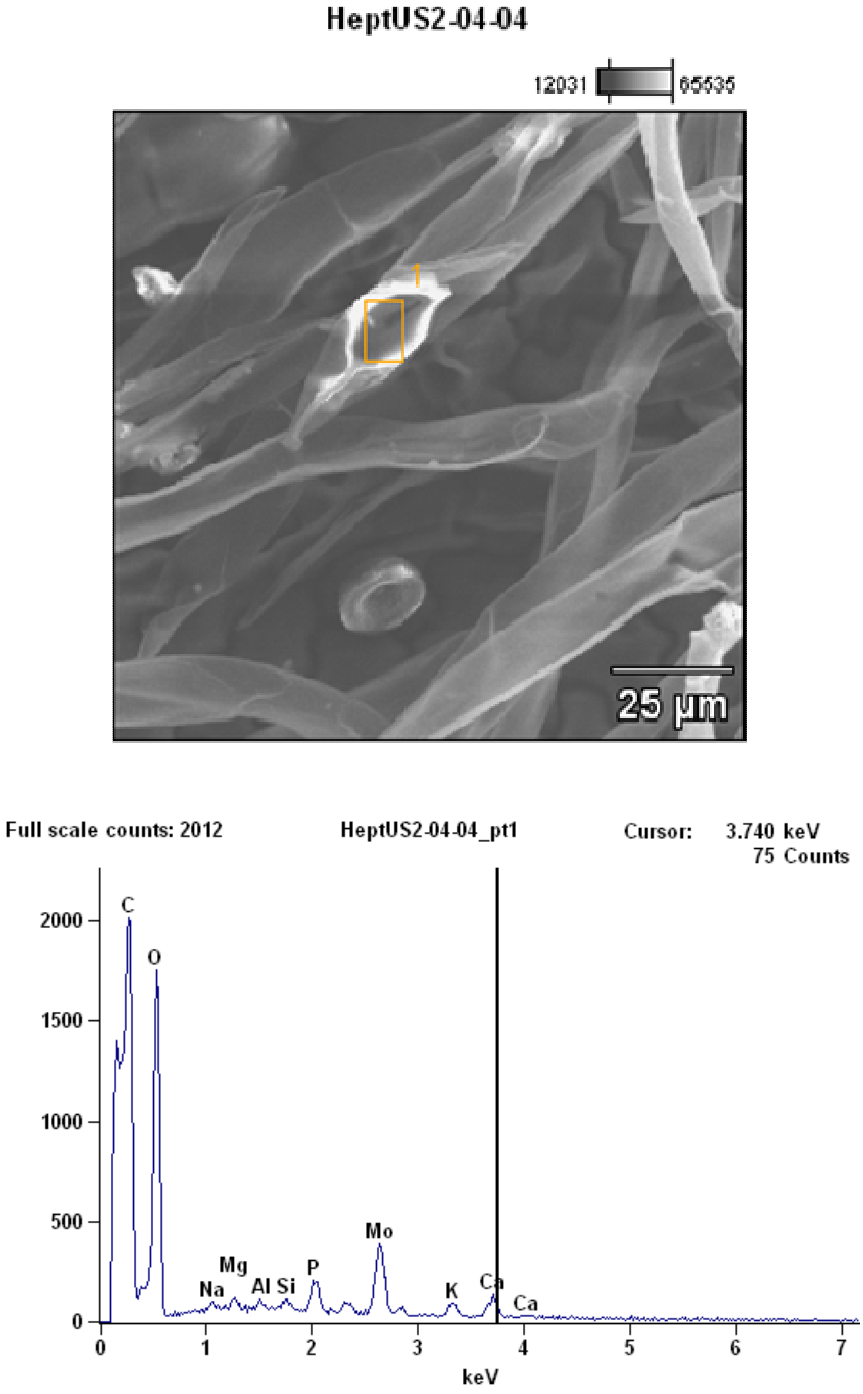

Figure 4. EDX-SEM of *S. heptalobium* on the trichome. The arrows shows the crystalline deposits analyzed within the trichome.

The crystalline deposits observed in *S. heptalobium* leaf are secreted by the trichomes to the surface of the epidermis, trapped by non-glandular trichome not to evaporate to the outside environment and their purpose is believed to aid in defense mechanism. The trichomes have been reported to secrete substances containing ions such as Na, Cl, Ca, Cd, Zn, Mo, Pb, Pi, Si, S and others which contributes to the toxic effect of the substance formed by that species and this might be the case in *S. heptalobium* (Salt et al., 1995; Choir et al., 2001; Aliyu Aliero et al., 2006).

The GC-MS results indicated that α-thujone, 1.8-cineole and terpinen-4-ol appeared to be the dominant compounds. These compounds are regarded as being to-

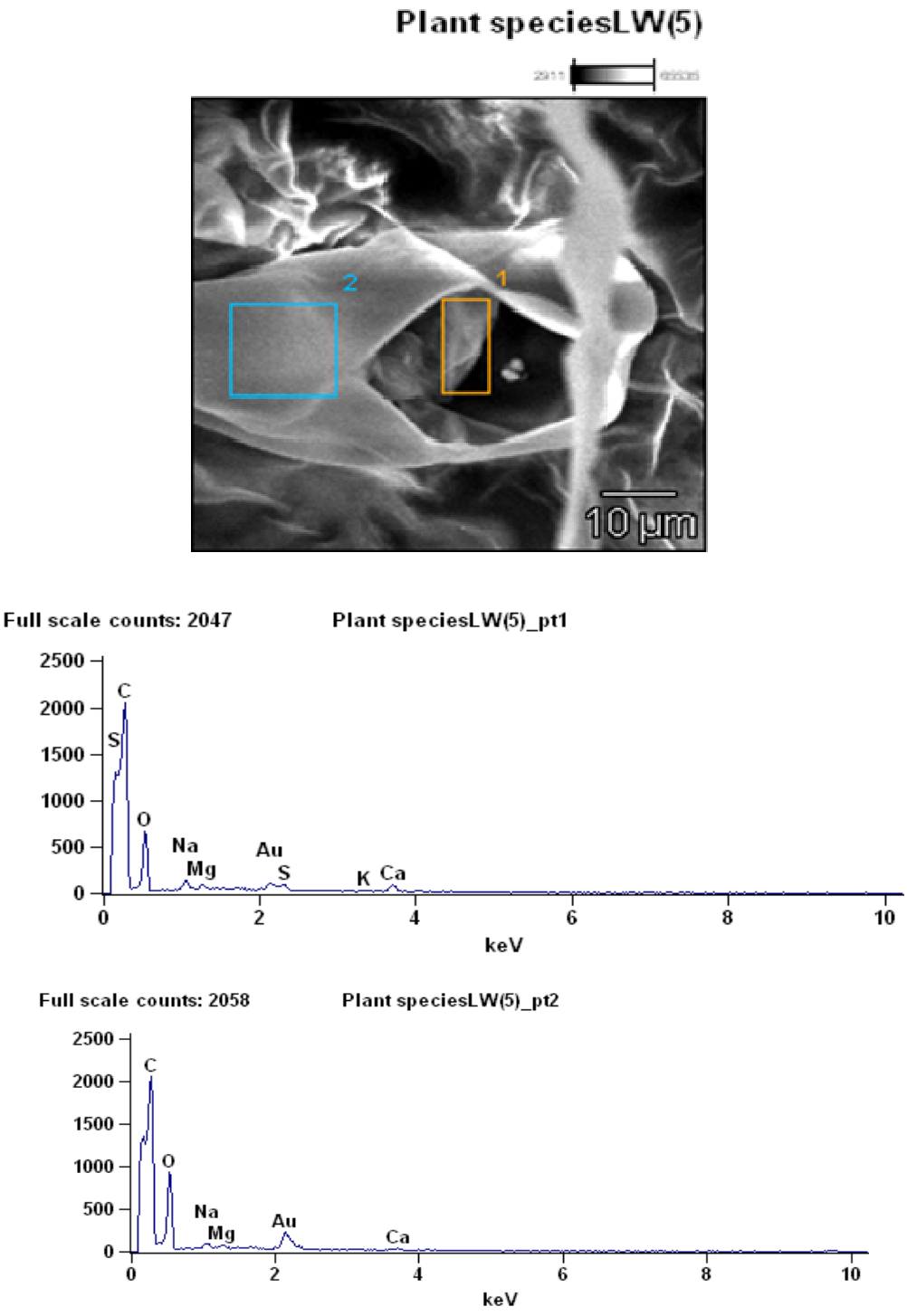

Figure 5. EDX-SEM of *S. heptalobium*. The boxes shows the contents analyzed. Box 1 the inner cuticular sac, and box number 2 the cuticular layer.

xic and are known for their antimicrobial property (Gundidza, 1993; Grierson and Afolayan, 1999; Lee et al., 2003; Magwa et al., 2005; McGaw et al., 2005). Their presence can therefore be regarded as assisting the plant in defense. However, the presence of other compounds which are recommended as active ingredients to herbal medicine, good ingredients for food like α-pinene, geranial, terpenen-4-ol and sabinene, while others have antiseptic properties to protect the plant against pathogens and herbivores makes the plant to be accessible to

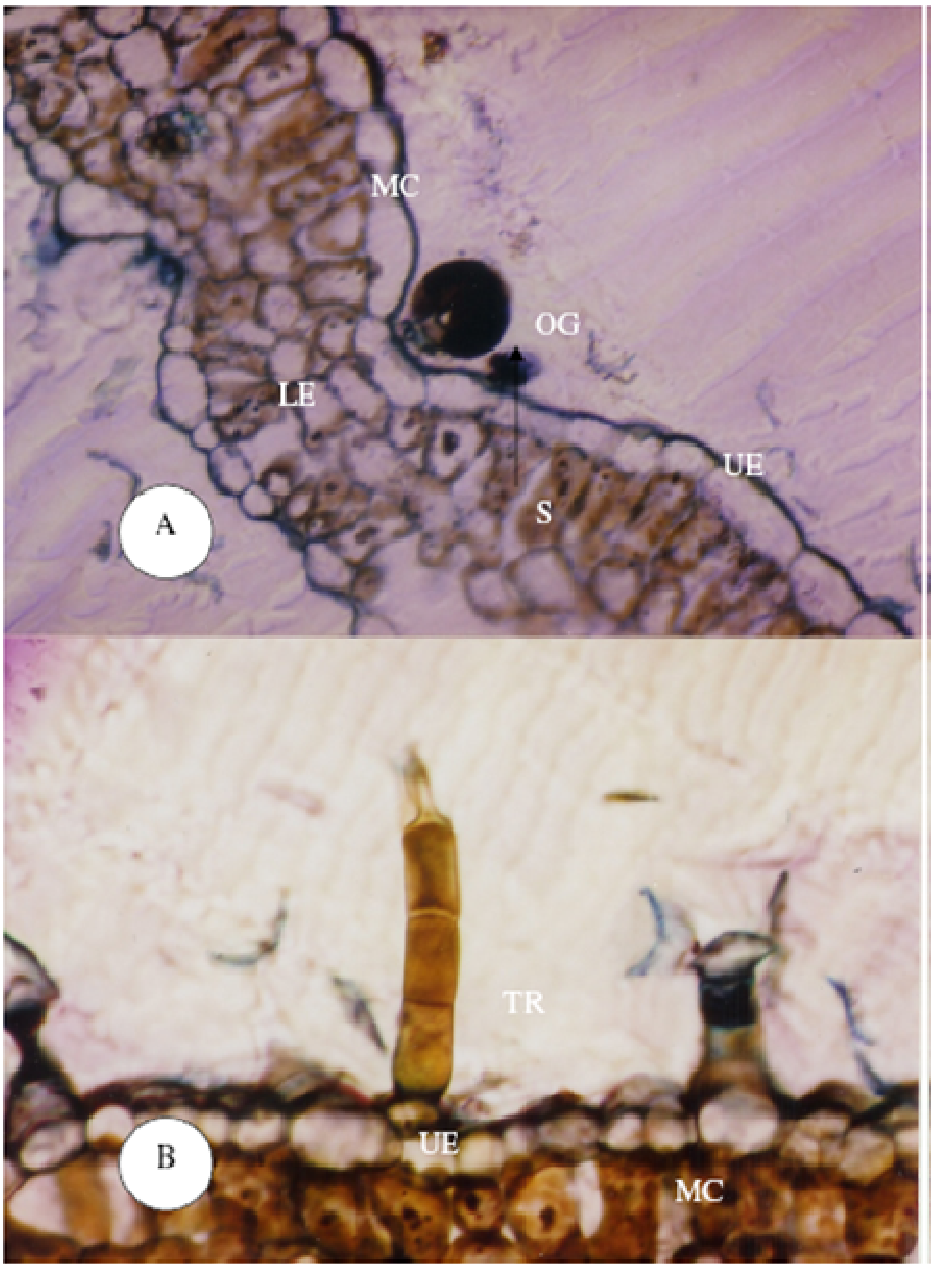

Figure 6. An electron micrograph of *S. heptalobium* leaf showing (A) an oil gland on the upper surface of the epidermal layer and (B) the characteristic feature of a non-glandular trichome at developmental stage. OG = Oil gland, TR = trichome, UE = upper epidermis, MC = mesophyll cells, and S = stalk cells

human race (Theimer, 1989; Moujir et al., 1993; Rabe and van Staden, 1997; Webber et al., 1999). Consequently, *S. heptalobium* has been able to protect itself from herbivores, pathogens, even though, the indigenous people of the Eastern Cape of South Africa are able to utilize the plant for cold and flu related illness.

The pharmaceutical application is based on its morphological adaptation to its harsh habitats thus it enables to defend itself against the micro-organisms, pests such as bacteria, fungi and nematodes. However, other ingredients within the secondary metabolites produced by this plant for defense are of importance to herbal and medicinal arena. Mills et al. (2000), for example, presented some findings of clinical guides in relation to these plants as well as related species for their effects in fever and flu. They stated that these plants have a stronger reduction in febrile temperature. It is assumed that effects in hot infusion, seen only in a febrile

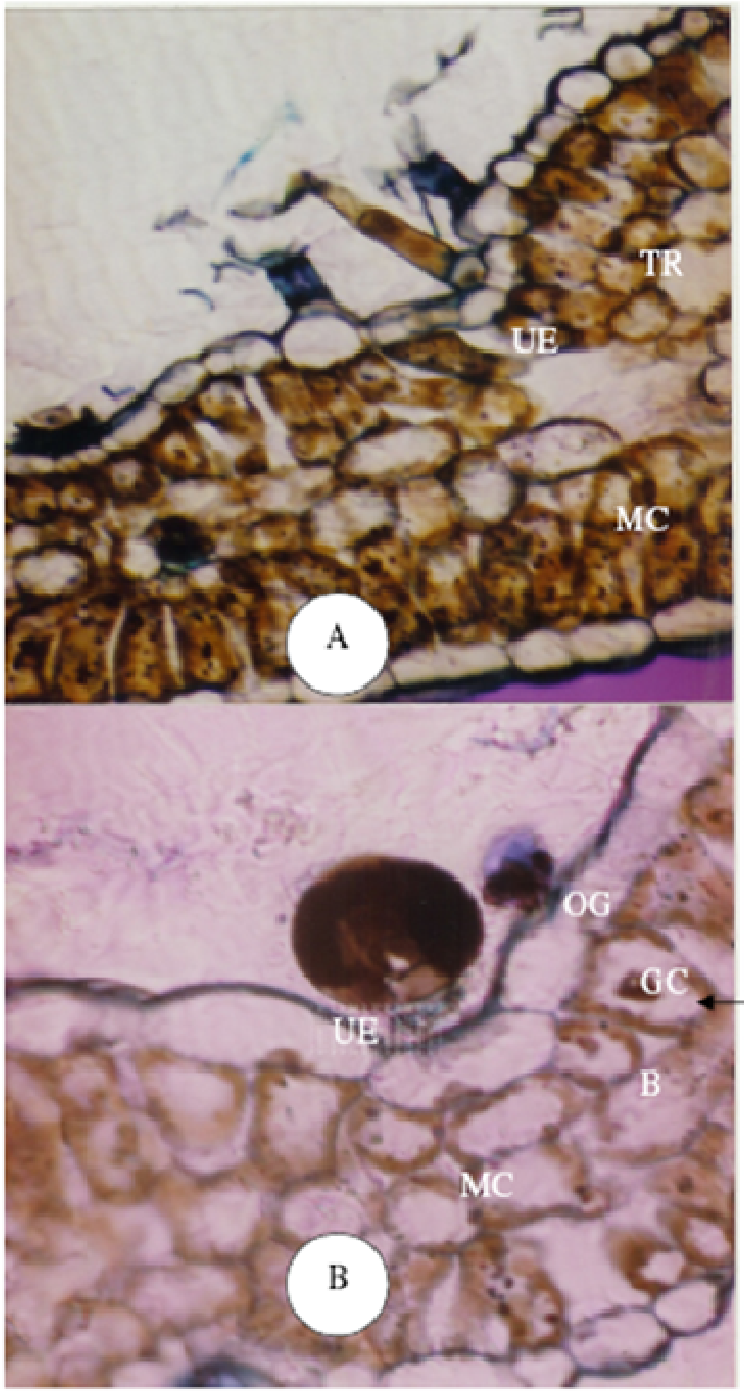

Figure 7. Higher magnification of the tranverse section of the leaf of *S. heptalobium* characterized with mesophyll cells of the leaf, (A) non-glandular trichome and (B) an oval-club shape glandular trichome. OG = Oil gland, GC = gland cells B = basal cell, S = stalk cell, UE = upper epidermis, LE = lower epidermis, MSC = mesophyll cells.

state, is subjectively reduce chill and encourage cooling perspiration. They also have a variety of other useful benefits for the digestion, mucous membranes and neuromuscular system.

In the past plants were favoured for their properties which reduce fever and related illness by the indigenous people of the Eastern Cape South Africa and these plants were notably differ in taste as well as having a even

Table 1. The chemical Composition of the essential oil of the leaf.

Compound	% Composition	Retention time
α-pinene	0.99	4.1
Sabinene	4.58	4.7
Myrcene	1.43	4.9
α-phellendrene	0.91	5.2
1.8-Cineole	3.23	5.7
γ-terpinen	0.46	6.2
α-thujone	79.3	7.8
Cis-sabinene hydrate	0.39	6.2
Camphene	1.04	8.2
Terpinen-4-ol	1.19	8.9
α-terpineol	0.98	9.2
2-cyclohexen-1one	0.97	10.6
Geranial	0.22	11.1
Phenol	0.29	11.9
Neryl acetate	0.32	13.4
β-caryophyllene	0.75	14.9
β-selinene	0.18	16.5
Caryophyllene oxide	0.18	18.8
Total identified	97.41	
Total unidentified	2. 51	

range of antipathogenic and anti-inflammatory properties. *S. heptalobium* has long been used and the research have been conducted on the antimicrobial activity which produced positive results by Mayekiso et al. (2008). However, these plants should be used with caution and with a closer supervision.

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