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Full Length Research Paper

The foliar micromorphology of *Arctotis arctotoides* (L.f.) O. Hoffm

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The foliar micromorphology of *Arctotis arctotoides* (L.f.) O. Hoffm. a medicinal plant in the Eastern Cape Province, South Africa was investigated using the Scanning Electron Microscope (SEM). The leaf surfaces of the plant were characterized by anisocytic stomata, glandular and non-glandular trichomes. The abaxial surfaces had more stomata and glandular trichomes than the adaxial surfaces of the leaf. Crystal deposits were also observed around the stomata and near the glandular trichomes. Energy dispersive X-ray spectroscopy showed that Na, Mg and Ca were the major constituents of the crystals. Naturally, distinct morphological variations existed among the ultra structures on the leaf surfaces of the plant. The distributions of the stomata and the two types of trichomes on both surfaces also differed both in shape and in structure. The presence of glandular trichomes on the leaf surfaces of this herb may serve as secretory sites where secondary metabolites are produced.

Key words: *Arctotis arctotoides,* foliar micromorphology, stomata, trichomes, crystals.

INTRODUCTION

Naturally occurring phytochemicals produced by plants are stored in various organs including the leaves, stems and roots (Ashrafi et al., 2008). The aerial surfaces of most plants also contain hairs which are functionally classified as glandular (GTs) or non-glandular (NGTs) trichomes. GTs are specialized secretory structures varying in size, form, location and function in different plant species (Robbles-Zepeda et al., 2009). Secretory structures such as stomata, GTs and NGTs are noted for transport of gasses, transpiration, storage and secretion of different metabolic products (Merkulov et al., 2000). These structures are of scientific interest due to their functional attributes and economic importance (Aliero et al., 2006; Ashafa et al., 2008). GTs have been reported to possess higher levels of secondary metabolites than those produced from other plant tissues (Macia et al., 2000; Marimoto and Komai, 2005). For example, the phytotoxin (artemisinin) a novel compound in pharmacological industries is produced only in the peltate glands of *Artemisia anna* (Duke and Paul, 1993; Duke et al., 1994).Many species in the family *Asteraceae* are economically important as weeds, ornamentals, medicinal plants and wild vegetables. One important species in this family is *Arctotis arctotoides* (L.f.) O. Hoffm., locally known as African daisy (English) and Ubushwa (Xhosa). It is a perennial, fast-growing, soft and herbaceous shrub growing up to 60 cm in height. The leaves are simple and pinnately lobed with alternate arrangement. The plant is indigenous to the coastal districts and summer rainfall areas of southern Africa (Pooley, 1998). It is widespread in disturbed areas such as road verges, lawns and cultivated fields. The shoot of the plant is characterized by the presence of aromatic odour typical of a medicinal plant. Several medicinal uses of the plant have been attributed to its leaves which are used for the treatment of epilepsy, gastritis, indigestion and in wound healing by the traditional healers (Afolayan, 2003). Both the root and the shoot of the plant possess antimicrobial and antioxidant (Afolayan et al., 2007). The pharmacological property of *A. arctotoides* has also been attributed to the two novel compounds nepetin and zaluzanin D isolated from its leaves. Other bioactive compounds isolated from the plant's essential oils are sesquisterpene (β-caryophyllene and γ-curcumene) and monoterpenoids (1,8-cineole, terpinen-4-ol and myrtenol) (Oyedeji et al., 2005;

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Sultana and Afolayan, 2007).

Generally, most plants of the Asteraceae family are noted for the production of secondary metabolites such as flavonoids, alkaloid, phenolic acids, saponnins and tannins in their leaves. Several sesquiterpenes and monoterpenes from plants are of scientific interest as potential herbicide leads due to their plant growth inhibiting properties (Cantrell et al., 2007). Phytotoxic natural products may be utilized directly or as lead compounds for the development of herbicides (Duke et al., 2000). Sesquestered compounds from the GTs are speculated to possess high levels of compounds than phytochemicals from other plant parts (Morimoto and Komai, 2005). Therefore, the presence of sesquiterpenes and monoterpenes isolated from *A. arctotoides* plant parts was worth studying. Despite the pharmacological profiles of *A. arctotoides* little or no information is available on its foliar ultra structures. Yet, such information can shed light on the micromorphological features and possible functional attributes of its foliar appendages. In this paper, we present the micromorphology of foliar ultra structures of *A. arctotoides* and relate our observations to their possible functional roles in the production of essential oil and other bioactive secondary metabolites.

MATERIALS AND METHODS

The general procedure for Scanning Electron Microscope (SEM) was adopted for this study. The leaves of *A. arctotoides* were collected from a natural habitat around the University of Fort Hare Research Farm, Alice campus in September, 2009. Fresh leaves were cut into segments of about 4 - 6 mm in length and fixed for 24 h in 6% glutaraldehyde in 0.05 M sodium cacodylate, rinsed in 0.05 M cacodylate buffer (pH 7.5) and dehydrated in a graded series of ethanol (20 -100% X 3) at 20 min per rinse. This was followed by critical point drying with liquid CO_2 in Hitachi HCP-2 Critical Point Dryer. Each dried sample was mounted unto aluminum 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 JEOL (JSM-6390LV) SEM, operated at 10 - 15 kV acceleration voltage. All the representative features examined were captured digitally using Microsoft image programme for windows. The energy dispersive X-ray spectroscopy-SEM, involved both the fixing and dehydration procedures as in SEM, while a FEI QUANTA 200 Oxford EDX Analyzer was used for the analysis.

RESULTS AND DISCUSSION

The foliar ultra structures of *A. arctotoides* are presented in (Figures 1 - 3). The SEM confirmed the presence of numerous anisocytic stomata which were more on the abaxial than adaxial surfaces (Figures 1A - B). This is a general phenomenon in most angiosperms (Koduru et al., 2006; Ashafa et al., 2008). The two types of trichomes observed on the leaves of the plant were glandular (GTs) or non-glandular (NGTs) trichomes. The abaxial surface had more GTs that were densely distributed over the entire leaf surfaces (Figure 1A) than NGTs which were highly clustered and linearly arranged only along the midrib (Figure 2B). The occurrence of numerous stomata and GTs on the abaxial surface of *A. arctotoides* leaf could be a specific adaptation in the ability of the plant to successfully inhabit the often extremes (summer-winter) environmental conditions of the Eastern Cape Province of South Africa. These features were also observed on the leaf surfaces of *Felicia muricata* and *Hippobromus pauciflorus* (Ashafa et al., 2008; Pendota et al., 2008).

Morphologically, GTs observed on *A. arctotoides* leaves were peltate with globular heads positioned in a depression due to invaginations of the adjacent epidermal cells (Figure 1D). The NGTs were cylindrical and filamentous with a tapered terminal cell attached to a stalk of variable number of cells in the basal portion (Figure 2C). The forms and structures of foliar appendages often develop as a result of the accumulation of secretory products in different organelles (Turner et al., 2000). For example, accumulation of organic terpenes and diterpenes were reported in the GTs of *Helichrysum tenax* and *Nicotiana tabacum* (Keene et al., 1985; Guo et al., 1994; Drewes et al., 2006). Several classes of sesqiuterpenes and inuloidin sequestered from the GTs of many plants have been associated with phytotoxicity on seed germination and seedling growth of other plants (Bohlmann and Zdero, 1979; Duke et al., 1988; Cantrell et al., 2007). The bioactive chemicals isolated from the essential oils and leaves of *A. arctotoides* might be secreted products of its glandular trichomes (Oyedeji et al., 2005; Sultana and Afolayan, 2007).

The ability of NGTs to reduce heat stress in plants, increase tolerance to freezing, seed dispersal and protection to plant tissues against ultraviolet (UV) light, biotic factors such as insect herbivores and airborne propagules of fungi have been documented (Afolayan and Meyer, 1995; Robbles-Zepeda et al., 2009). Therefore, the presence of hairy NGTs on the leaves of *A. arctotoides* might serve to regulate heat and /or moisture and possibly protect the plant against herbivory attack. Some crystal deposits were observed on the leaf epidermis and near the globular head of the GTs (Figures 3A - B). The spectra of the energy dispersive X-ray spectroscopy revealed the occurrence of Na, Mg and Ca as major constituents of the crystals (Figure 3C). Crystal formation is a common phenomenon in many higher plants. They are present in more than 215 plant families and distributed in various organs such as stems, roots, leaves, seeds and floral structures (Horner and Wagner, 1980, 1992; Prychid and Rudall, 1999; Horner et al., 2000; Lersten and Horner, 2006). They are often classified as sand, drusa, prismatic, raphides or styloides (Maiti et al., 2002; Franceschi and Nakat, 2005). However, the criteria for these classifications are not clearly defined; therefore, it was difficult to relate any of the crystals listed to those observed on *A. arctotoides* leaves.

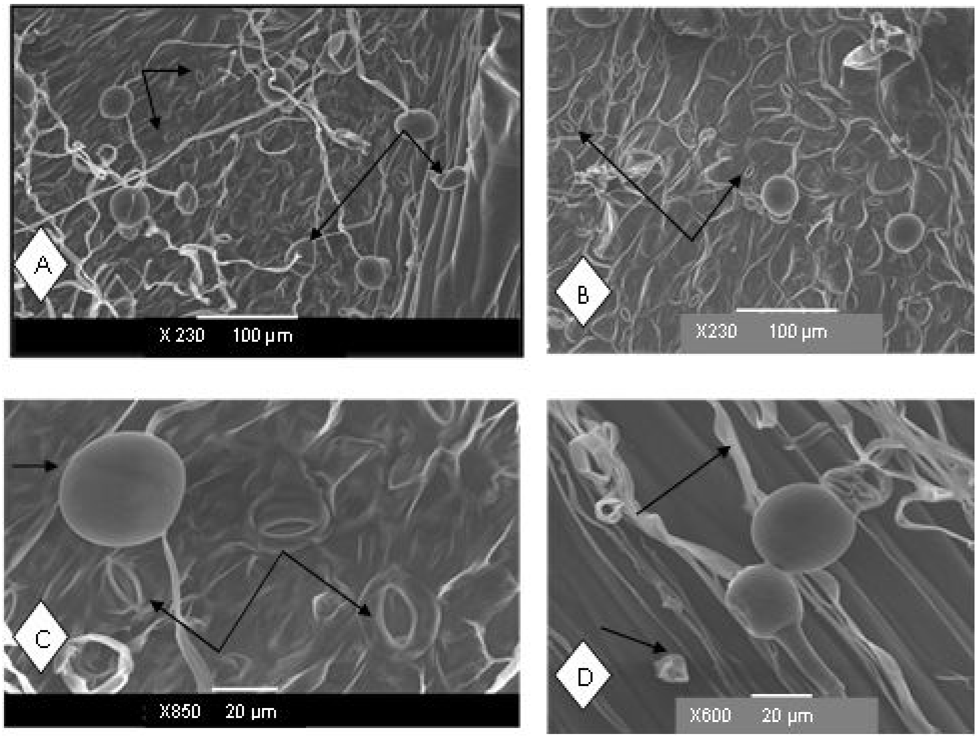

Figure 1. (A) Stomata and GTs distribution and their proximity on the abaxial and (B) Adaxial surfaces of *A. arctotoides.* (C) Magnified view of the globular head of GT and stomata pores on the abaxial surface. (D) Crystal deposit and connection of GTs with fibre-ends of NGTs.

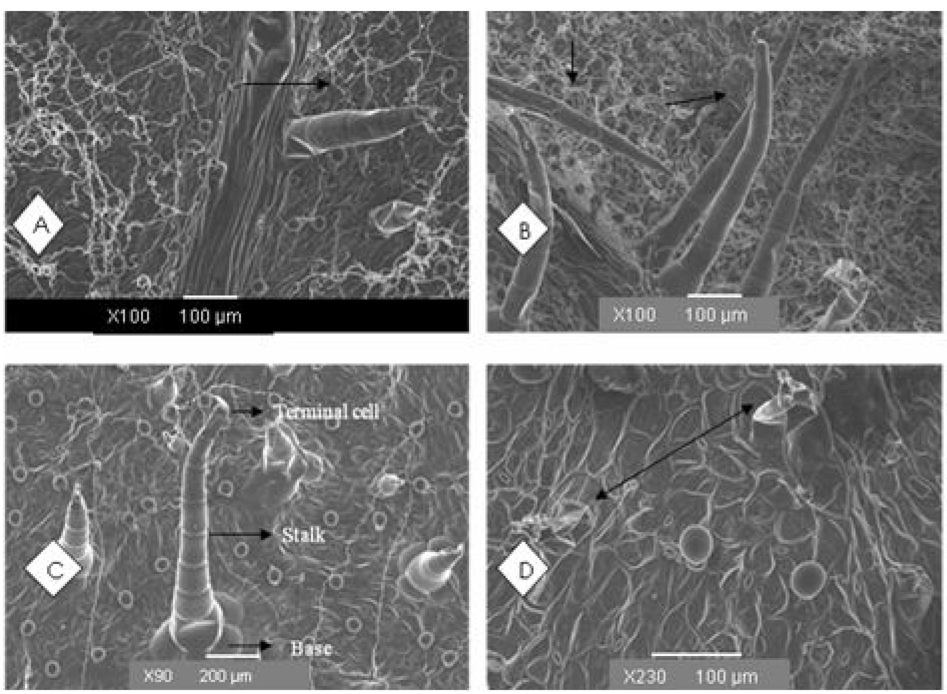

Figure 2. (A) Note the location of NGT on the midrib. (B) Distribution and arrangement of NGT and GTs on the midrib and leaf surfaces of *A. arctotoides*. (C) Morphology of a NGT showing the terminal cell, the stalk and the base. (D) Glandular (GT) either collapsed naturally or during preparation for SEM.

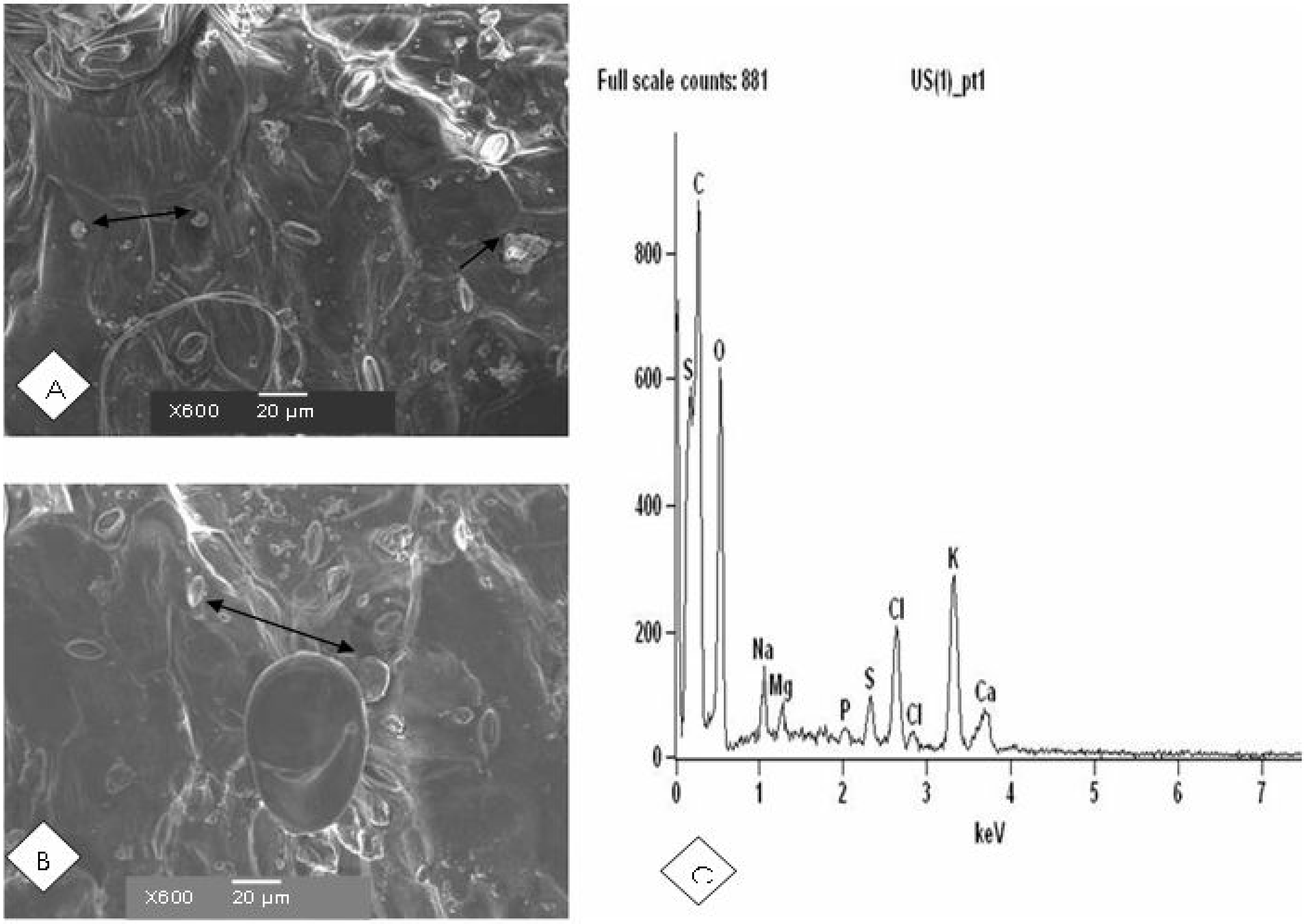

Figure 3. (A and B) Crystal deposits around the stomata and near the GTs on the leaf surfaces of *A. arctotoides*. (C) Spectra of energy dispersive X-ray spectroscopy of the crystals.

Although, the significance of crystal and its formation in plants is largely unknown, various functions have been attributed to their presence. These include regulation of Calcium (Ca) levels in plant tissues and organs, involvement in photosynthetic processes and detoxification of heavy metals or oxalic acid in plant (Franceschi and Nakata, 2005; Kuo- Huang et al., 2007).

In conclusion, the SEM has demonstrated the occurrence of anisocytic stomata, GTs and NGTs as well as deposition of crystals on the leaf surfaces of *A. arctotoides*. These structures might be the sites of production or storage of the various bioactive secondary metabolites present in this herb. Considering the high number of GTs on the lower epidermis of the leaves of the plant and the pharmacological importance of phytochemicals from Asteraceae family, compounds earlier isolated from this herb could serve as foundation for further research.

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