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FOLIAR MICROMORPHOLOGY OF *LIPPIA JAVANICA* (BURM.F) SPRENG

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Abstract

Background: *Lippia javanica* (Burm.F.) Spreng is an aromatic indigenous South African plant with culinary and medicinal values. This study investigated the foliar morphology and elemental composition of the plant because not much data concerning the anatomical and micro-morphological features can be found in literature
Materials and Methods: Fresh leaves of the plant were investigated using light and scanning electron microscopy (SEM). The elemental composition of the leaf was determined by energy dispersive X-ray spectroscopy.
Results: The leaves of *L. javanica* were amphitrichomic while the stomata distribution was amphistomatic. These stomata were more or less randomly distributed over the epidermis lying almost close to each other and were fewer in number in between the veins and over the finer veins. The major constituents of crystals found in the plant were Ca, Na, S, Al, P, Cl, K, Mg and Fe. The presence of long glandular trichomes on the leaf surfaces of the plant could indicate secretory sites where secondary metabolites are produced.
Conclusion: Secretions from the glandular trichomes and the presence of these elements, which are vital in maintaining good health, are probably responsible for the culinary and medicinal properties of *L. javanica*.

Key words: *Lippia javanica*, electron dispersive x-ray, scanning electron microscope, amphitrichomic leaf

Introduction

Foliar micro-morphological features are useful in the identification and authentication of plants. Advances in light and scanning electron microscopy have increased the capability of microscopy as a veritable means of botanical identification in foliar micro-morphological studies. Plants communicate with their external environment, protect and maintain essential internal physiological and biochemical processes through specialized epidermal structures (Da Silva et al., 2009, Sonibare & Osiyemi, 2012, Otang et al., 2014).

Lippia javanica is the largest group of the indigenous Lippia shrubs and it belongs to the family Verbenaceae. The height is about 5 m, and it grows at altitudes as high as 2000 m above sea level (Nomfundo et al., 2014). The species is widely distributed throughout South Africa with the exception of the Western Cape Province. It is also found in Botswana, Malawi, Swaziland, Mozambique, Tanzania, Zambia and Kenya (Nomfundo et al., 2014). This plant commonly grows on hillsides, forest edges, stream banks, grasslands and roadsides (Retief & Herman, 1997). The leaves of the plant are very hairy and strongly aromatic, protecting the plant against browsing by animals (Mwangi et al., 1991). The flowers are white, cream, greenish white, yellowish white or yellow with bracts which are never broader or longer than the flowers (Retief & Herman, 1997). The plant has been reported to possess analgesic, anti-inflammatory and antipyretic activities (Morton, 1981). Teas prepared from aerial parts of the plant are used by the Zulu and Xhosa people of South Africa to treat coughs, colds and bronchial problems, as well as to disinfect meat infected with anthrax (Hutchings et al., 1996). Tea infusions of the leaves have been used against common symptoms of HIV and AIDS in KwaZulu Natal and for the treatment of lung infections, dysentery and diarrhoea (Palgrave et al., 2003). *L. javanica* is one of the five medicinal plants used to prepare the herbal tonic, Imbiza, used for the management of HIV/AIDS infection in South Africa (Nomfundo et al., 2014). Tea from the plant is commercially sold as a caffeine-free health tea under the name “musukudu tea” in South Africa (Shikanga et al., 2010). Information gathered about this plant from traditional healers in the Eastern Cape Province revealed that the leaf of the plant is also commonly used as a spice.

Trichomes are mainly seen on the surfaces of leaves and the scientific interest is based on the economic importance of their products (Valkama et al., 2003; Koduru et al., 2006). Histochemical studies of some trichomes indicate that their secretions contain essential oils (Afolayan and Meyer, 1995; Ascensao et al., 1999; Koduru et al., 2006). To the best of our knowledge, there is little or no information on the morphology and ultrastructure of the leaf appendages of *Lippia javanica*. The objective of this study therefore, was to investigate the structure and distribution of different types of trichomes, stomata, crystals and epidermal cells in *L. javanica*.

Materials and Methods

Collection and Identification of Plant Materials

Mature leaves of *Lippia javanica* were collected from their natural habitat in the wild (GPS Coordinate- 32.7770° S, 26.8460° E) in Alice, Eastern Cape Province of South Africa. The plant was identified in Rhodes University Herbarium and voucher specimen (ASO 2014/1) was prepared and deposited in the Giffen Herbarium at the University of Fort Hare, Alice.

Scanning Electron Microscopy (SEM)

The ultra-structural examination of the mature leaves of *Lippia javanica* was carried out using the following method. Fresh leaves of *L. javanica* were pre-treated to remove atmospheric deposits by carefully washing in distilled water and then immersed in a

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fixative solution of 2.5% glutaldehyde in 0.1 M phosphate buffer for 24 hours. Samples were washed for 15-30 minutes with the buffer and dehydrated in graded ethanol series (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%). Samples were then critically point dried using CO_2. Each dried sample was mounted unto aluminium stubs with double-sided carbon and adhesive discs, then sputter-coated with gold-palladium (Eiko IB-3 Ion Coater) under vacuum. The samples were viewed using the Scanning Electron Microscope (SEM) equipped with an Energy Dispersive X-ray (Oxford ISIS 300) micro analytical system and the necessary software for point micro analysis. Images were captured digitally with an image saver. The major microscopic structures observed in the leaves were: outline of epidermal cells, types of stomata, trichomes and crystals.

Light Microscopy

Light microscopic examinations of the epidermal sections of the leaf were carried out as described by 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 placed on clean glass slides with 1-2 drops of sterile distilled water and covered with a cover slip. Prepared slides were observed under a motic light microscope and the microphotographs were taken with a digital camera fitted to the light microscope. The images were captured digitally using Microsoft Image Programme software for windows.

Results and Discussion

Appearance and Distribution of Trichomes

Both the lower and upper epidermis of *Lippia javanica* observed under scanning electron microscopy (SEM) contains unicellular, uniseriate, spine-like, long glandular trichomes with few stomata which are densely distributed on both surfaces. Under the light microscope, spine-like trichomes density was higher on the abaxial relative to the adaxial leaf surface (Figure 1 a, b, c & d). Trichomes are unicellular or multicellular outgrowths originating from the epidermis of plant parts. Their morphological features, location and mode of secretion are varied (Werker, 2000). It has been reported that the type and density of trichomes differ among species and can vary in organs of the same plant (Uphof, 1962; Bhat et al., 2010). Non glandular trichomes are generally classified according to their morphology. They range from unicellular to multicellular structures that can be uniseriate, biseriate or multiseriate, branched or unbranched (Werker et al., 1994). In this study we observed that *Lippia javanica* possessed spine-like, non-glandular, uniseriate trichomes.

According to Werker and Fahn (1981), glandular trichomes limit the transpiration rate and reduce leaf temperature. Withering and falling leaves with intact glandular hairs may also provide a phytotoxic environment for germinating seeds and growing seedlings while non-glandular trichomes often act as a physiological barrier against herbivores and contribute to plant adaptation to environmental conditions, particularly in dry environments (Afolayan and Meyer, 1995; Ascensao et al., 2001; Aneta, 2013). These attributes may explain the wide distribution of *L. javanica* in various climatic environments in Southern Africa. The glandular and non-glandular trichomes observed for Lippia species are distributed widely in the Verbenaceae family (Combrinck et al., 2007; Passos et al., 2009).

Histochemical studies of some trichomes indicate that secretions from some species of *Helichrysum aureonitens*, *Helichrysum stioechas* and *Plectranthus ornatus* contain essential oils which are mostly characterized by monoterpenoids (Afolayan and Meyer, 1995; Ascensao et al., 1999; Koduru et al., 2006). Terpenes are stored by plants in the form of glycosides, when required the aglycones can be mobilized for their respective functionality in the plant (Lalel et al., 2003). Long glandular trichomes (L-GST) are the most abundant trichome type and terpenoids isolated through distillation most probably originated from these structures (Combrinck et al., 2007). This implies *L.javanica* may have high terpenoid content due to the presence of long glandular trichomes.

Appearance of Crystals

In this study, crystals of different sizes and irregular shapes were observed in the leaves of the species investigated (Figure 2 a, b, c & d). This is similar to the observations of Adedeji (2012) on Stachytarpheta Vahl., belonging to family Verbenaceae. Rectangular, prism, triangular and spherical shaped crystals in aggregates were confirmed in *L. javanica*. According to Adedeji (2012), crystals in the form of small needles or prisms are widely distributed in all the parenchymatous tissues of leaves axis of the family Verbenaceae. This was observed in this study for *L.javanica*. Crystallization is the most common way by which plants neutralise abundant calcium absorbed in ionic solutions and these crystals remain even when water vapour transpires and are a common phenomenon in higher plants (Lerstern and Horner, 2006). The crystals are often classified as drusa, sand, prismatic, raphides or styloid (Maiti et al., 2002; Franceschi and Nakat, 2005; Lerstern and Horner, 2006; Badmus and Afolayan, 2010). They are always formed within cells, which are remarkably diverse among angiosperms (Wintola and Afolayan, 2014).

Stomata Distribution

Stomata are minute apertures bounded by two guard cells. They can be interpreted as intercellular spaces between the highly specialised guard cells. Regarding the position of guard cells in relation to the epidermal cells, they are found to be at the same level. The two guard cells of a stoma are almost equal in size, bean-shaped and surrounded by 2 to 5 subsidiary cells (Akhil and Subhan, 1997). The diacytic, anisocytic, anomocytic, circular to elliptic are different shapes of stomata in plants. Leaves of *L. javanica* are characterized by amphistomatic stomata which are more or less randomly distributed over the epidermis lying almost close to each other and are fewer in number in between the veins and over finer veins (Figure 2 a, b, c & d and Fig.3a & b).

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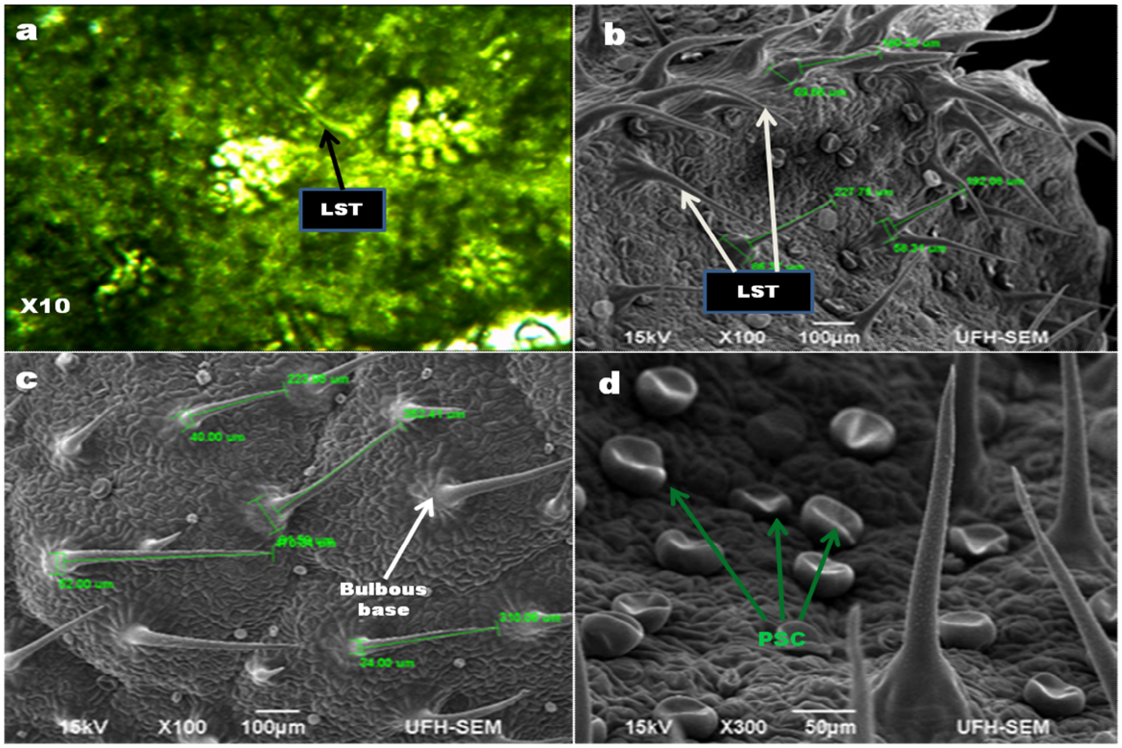

Figure 1: Light micrograph showing a) light microscope image of long stalk trichomes (LST) and guard cells (x10); and SEM micrographs showing b) image of long stalk trichomes (LST) (x100); c- bulbous base of trichomes (x100); d- peltate secretory cells (PSC) and conical long stalk (x300) on the abaxial surfaces of *L. javanica* leaf.

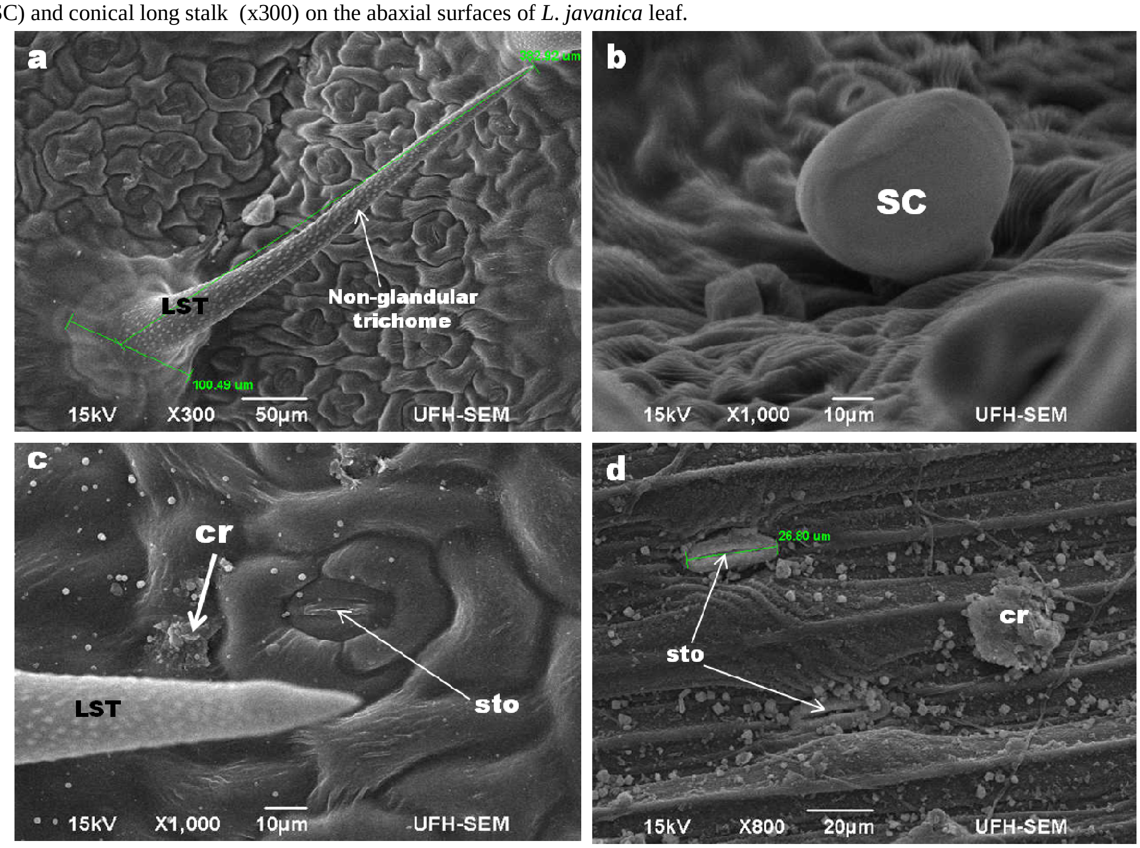

Figure 2: SEM micrographs showing a) spine like non-glandular trichome (x300); b) secretory cells (sc) (x1000); c) stomata-sto and crystals-cr (x1000) on the abaxial surfaces (cr); d): Different shapes of crystals (rectangular, prism, triangular and styloid) on adaxial surface (x800)

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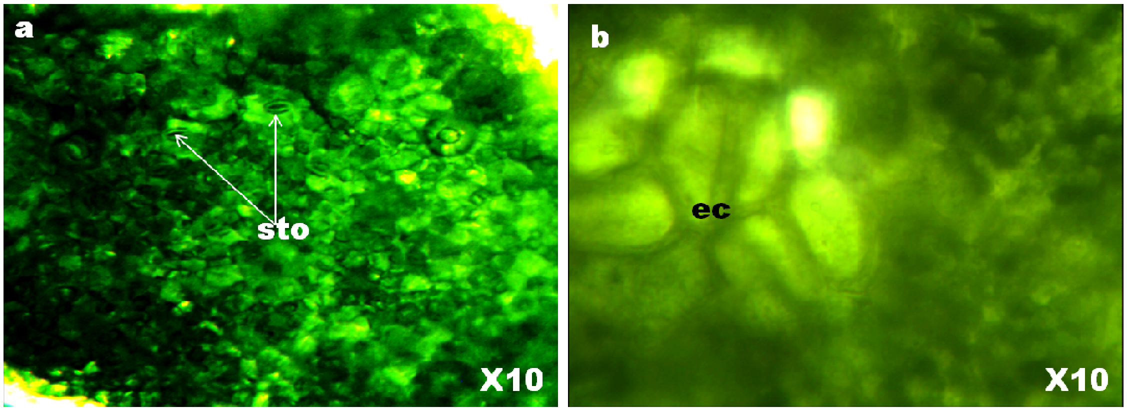

Figure 3: Light microscope micrographs of a) stomata (st) in the adaxial epidermal surfaces. b) adaxial surfaces showing the hexagonal shape of the epidermal guard cells (ec).

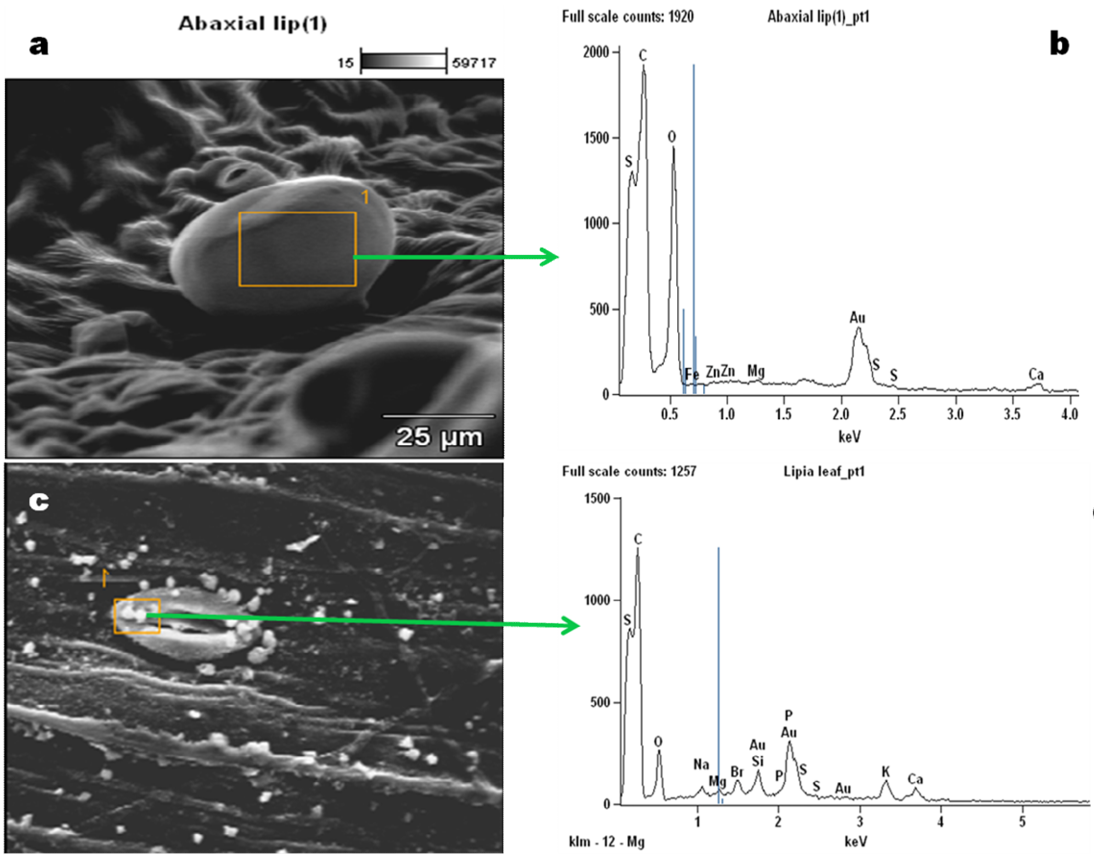

Figure 4: SEM micrographs a and c; and EDX spectra of elemental composition of crystal deposits on leaf b and d.

Elemental Composition

The chemical nature of the crystal deposits on *L.javanica* leaves (Figure 4 a & b) revealed that they were predominantly composed of Ca, O, Na, S, Al, S, P, Cl, K, C, Mg and Fe (Table 1). These elements (micro and macro) are very essential for plant growth. Their important functions include regulation of calcium levels in plant tissues and organs, involvement in photosynthetic processes and detoxification of heavy metals or oxalic acid in plants (Franceschi and Nakata, 2005; Kuo- Huang et al., 2007; Badmus and Afolayan, 2010). The high percentage of oxygen observed in the plant confirmed the use of oxygen in the transport of other nutrients into plants but calcium is required to make nutrients mobile within the plant tissue. In humans, the presence of 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; Afolayan and Otunola, 2014). Therefore, these elements and their various functions as well as other bioactive compounds present in the secretory trichomes, could account for the therapeutic actions of *L .javanica.*

Table 1: Elemental composition (%) of *Lippia javanica* leaves as shown by Energy Dispersive X-ray (EDX) analysis.

Elemental composition	*Lippia javanica* leaf
Calcium (Ca)	6.98
Oxygen (O)	36.46
Sodium (Na)	0.56
Aluminium (Al)	0.74
Silicon (Si)	25.81
Phosphorus (P)	0.27
Sulphur (S)	0.11
Chlorine (Cl)	0.23
Potassium (K)	1.89
Iron (Fe)	1.49
Bromine (Br)	1.37
Carbon (C)	-
Magnesium (Mg)	-

Conclusion

The foliar epidermal surfaces and trichomes of this plant species were investigated using light and scanning electron microscopy (SEM) connected with energy dispersive x-ray spectroscopy (EDX). Results showed that the leaves of *L. javanica* were amphitrichomic while the stomata distribution was amphistomatic, randomly distributed over the epidermis lying almost close to each other. The major constituents of crystals found in the plant were Ca, O, Na, S, Al, S, P, Cl, K, C, Mg and Fe. The presence of non-glandular trichomes on the leaf surfaces of this plant may serve as secretory sites where the aromatic secondary metabolites are produced. Of more than 200 known species in Verbenaceae, only about 46 have been chemically examined (Pascual, 2001; Combrinck et al., 2007). In addition, investigations concerning the non-volatile components of the genus Lippia have been described as scarce and fragmented (Pascual, 2001). Therefore, a further study to elucidate the chemical nature of the content of such internal secretory cells with transmission electron microscopy will be undertaken in future studies.

Declaration of Interest: The authors declare that they have no competing interest.

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