



COMPARATIVE LM AND SEM STUDIES OF GLANDULAR TRICHOMES ON THE CALYX OF FLOWERS OF TWO SPECIES OF *PLUMBAGO* LINN.

Smita S. Chaudhari and G. S. Chaudhari¹

Department of Botany, Dr. A. G. D. Bendale Mahila Mahavidyalaya, Jalgaon (Maharashtra), India.

¹P. G. Department of Botany, M. J. College, Jalgaon (Maharashtra), India.

Abstract

LM and SEM investigation of calyx of flowers of *Plumbago zeylanica* Linn. and *Plumbago auriculata* Lam. has shown two types of trichomes-glandular trichomes and unicellular trichomes. Basic structure of glandular trichomes in both taxa is same. Each trichome show multicellular stalk and head. The stalk penetrates the head. Heads of glandular trichomes in *Plumbago zeylanica* are colourless and translucent but in *Plumbago auriculata* colourless translucent as well as purple heads are noticed. In *Plumbago zeylanica* glandular trichomes have higher density, present throughout the length of calyx, distributed in random manner, oriented in different directions, show much more variation in lengths while in *Plumbago auriculata* glandular trichomes have lower density, present only in the upper part of calyx, arranged in linear fashion, trichomes in one line are oriented in the same direction, show less variation in lengths. EDAX analysis on the head of glandular trichomes of *Plumbago zeylanica* revealed only C, O, Mg, Al and Si but in *Plumbago auriculata* in addition to these elements Na, S, Cl, K, Ca, Ti, Fe were also found. Presence of glandular trichomes secreting mucilage (which is considered as adhesive trap for prey) supports the protocarnivorous nature of *Plumbago*.

Key words : *Plumbago zeylanica* Linn., *Plumbago auriculata* Lam., glandular trichomes, LM, SEM.

Introduction

Plumbago is commonly called leadwort and in Sanskrit it is called Chitrak. Anonymous (1989) reported 3 species from India - *Plumbago zeylanica* Linn., *Plumbago rosea* Linn. (*Plumbago indica* Linn.), *Plumbago auriculata* Lam. (*Plumbago capensis* Thunb.). *Plumbago zeylanica* Linn. (wild variety) is very popular plant in ayurveda because of its good medicinal potential. It is listed as threatened medicinal plant (Parthipan, 2006; Mittal and Sharma, 2010).

छायाशुष्कं ततो मूल मासं चूर्णीकृतं लिहन् ।
सर्पिषा मधुसर्पिभ्यां पिबन् वा पयसा यतिः ॥६३॥
अम्भसा वा हितान्नाशी शतं जीवति नीरुजः ।
मेधावी बलवान् कान्तो वपुष्मान् दीप्तपावकः ॥६४॥

This Sanskrit verse in ancient Indian literature (Vagbhata's Astangahrdayam) depicts the importance of chitrak as rejuvenator. Consumption of fine powder of shade dried roots of chitrak with ghee or mixture of ghee and honey or milk or water along with healthy or proper

food make the person to live for hundred years without any diseases and empowered with intelligence, strength, radiance, pleasant look and good digestive power (Murthy, 2012).

Another species *Plumbago auriculata* is ornamental plant with least medicinal use. It is cultivated variety and widely available. As both these plants are taxonomically closely related, it is essential to identify and authenticate them correctly. For precise identification, taxonomic trends like external morphology, anatomy, palynology, LM and SEM studies play important role. The objectives of present study were to identify both plants and describe features of calyx (including calyx surface, trichomes on calyx) that can be used as taxonomic tools to delineate two species of *Plumbago*.

Trichome characters were considered for taxonomic delimitation firstly by Hanstein as back as 1868, who studied glandular hairs on the leaf bud of *Azalea indica* L. and now this consideration has been stressed by many workers. Trichomes on both vegetative as well as floral parts have been studied (Pandey, 1989).

Morphologically trichomes are of two types – simple or non-glandular and glandular secreting trichomes (GST) with copious secretion (Wagner *et al.*, 2004). Non glandular trichomes may be unicellular or multicellular. Unicellular trichomes may be branched or unbranched. Multicellular trichomes may be uniseriate or multiseriate. Multicellular trichomes often show peculiar branching giving dendroid (tree like) appearance or stellate appearance (star like) (Pandey and Chadha, 2005; Verma, 2011). Sometime all the branch cells interconnect along their sides and form a disc or shield shaped structure. If these structures have a stalk, they are peltate hairs but if stalk is absent or extremely short, they are scale or squamiform hairs (Mauseth, 1988).

According to Maffei and Codignola (1990) morphologically glandular trichomes are of two types – capitate and peltate. Morphologically both have same basic structure consisting of basal region, stalk and head. Capitate trichome is with head like the head of pin or floral stigma (Combrinck *et al.*, 2007). Peltate trichome is with discoid head (Esau, 1993). Head is actual secretory organ or gland (Haberlandt, 1965) and is composed of glandular cells (Gangulee *et al.*, 2014). Glandular trichomes with multicellular stalk and multicellular head are referred as colleters. The term derived from the Greek *colla* means glue referring to sticky excretion from these structures (Esau, 1993).

According to Johnson (1975) and Lewin (1973), the functions of trichomes are extremely diverse. Glandular trichomes can secrete water, salt, nectar, mucilage, terpenes, adhesives, digestives, enzymes and irritants that sting. According to Benzing and Pridgeon (1983), Pridgeon (1981) some trichomes absorb water and salt. Trichomes are deterrent against insects, they can tangle the feet or impale the insects. According to Hooker (1875), Lloyd (1976) sometime trichomes are very short and curved in the same direction to make insect walk easy toward digestive glands. Trichomes also protect the plant from excessive sunlight (Mauseth, 1988). According to Bosabalidis (2002) they also control transpiration and temperature of organ on which they occur (Combrinck *et al.*, 2007). The list of functions and modifications of trichomes is almost endless (Mauseth, 1988).

Metcalf and Chalk (1950) reported the long stalked glandular shaggy hairs on the calyx of *Plumbago capensis* and *Plumbago zeylanica*. Solereder (1908) reported about occurrence of mucilage glands in the family Plumbaginaceae. The name mucilage gland is given by Wilson, who noticed them in eight genera of family Plumbaginaceae but not in all species, involved in his investigation (Solereder, 1908). Solereder (1908) divided mucilage glands into two types *viz.* those which are developed as hairs and those which are not so. The

mucilage glands of latter type are only found in *Aegialitis* where structure approaches nearest to chalk glands. In the remaining 7 genera mucilage glands are hair like structures.

Wilson (1890) reported occurrence of stalked glands on the exterior of calyx of *Plumbago*. These glands have also been studied by Maury, Martinet and others. Many authors remarked the extreme viscosity of the mucilaginous secretion (Wilson, 1890). Fahn (1969) also reported about presence of glandular trichomes on the calyx of *Plumbago capensis*. Joel and Fahn (1975), Rachmilevitz and Joel (1976) studied calyx glands of *Plumbago capensis* (<http://medplants.blogspot.in/2013/12/plumbago-auriculata-plumbago-capensis.html>). Stoltzfus *et al.* (2002) studied secretion of digestive enzymes in *Plumbago* (*P. auriculata* and *P. indica*) in response to chemical stimuli. LM and SEM studies of sepals of *Plumbago auriculata* and *Plumbago indica* have been carried out by Kettering *et al.* (2003).

Materials and Methods

Collection and identification of plants

Plumbago zeylanica was collected from botanical garden of Chaitanya Ayurved Mahavidyalaya, Sakegaon. *Plumbago auriculata* was collected from Ratnakar Nursery, Jalgaon. Both the plants were identified and authenticated by taxonomist Dr. G. S Chaudhari.

Cultivation of plants

Collected plants were grown and cared in the garden. Material for investigation was procured from plants cultivated in the garden.

Trichome preparation for LM study

A small piece of calyx of fresh flowers was placed on slide and few drops of lactic acid were applied for 10-20 minutes (Khokhar *et al.*, 2012). Then trichomes were mounted in glycerine and observed under light microscope

Scanning Electron Microscopy (SEM) and EDAX Analysis

The calyx of *Plumbago zeylanica* and *Plumbago auriculata* were directly mounted on metallic stub using double sided carbon tape. Dirt on the material was removed with the help of blower. Then the sample is viewed with FEI Quanta 200 Environmental Scanning Electron Microscope with EDAX System and photomicrographs were taken at different magnifications. EDAX analysis on the head of glandular trichome on calyx was carried out with EDAX system of FEI Quanta 200 Environmental Scanning Electron Microscope.

Measurements and calculations

Lengths of glandular trichomes on calyx were

measured by using FEI Quanta 200 Environmental Scanning Electron Microscope. Randomly length of 15 trichomes was measured for each taxon.

Observations

Glandular trichomes exude a sticky substance. Ants entangled in glandular trichomes were observed in both taxa. Terminology used to explain features of glandular trichome follows, Serrato-valenti *et al.* (1997), Mykesio *et al.* (2008), Martinez-Nataren *et al.* (2011). To explain calyx surface terminology used by Radford (1986) is adopted. The species wise observations are given below.

Plumbago zeylanica

The LM as well as SEM studies of the calyx revealed presence of glandular trichomes and unicellular trichomes on the surface of calyx. The unicellular trichomes are shorter and less dense than that of *Plumbago auriculata*. In SEM study the calyx surface appears areolate (Radford, 1986) with small polygonal epidermal cells having convex periclinal wall. The features of glandular trichomes as revealed by LM (fig. 1) and SEM (fig. 2) study are:

1. Density

Trichomes form dense covering *i.e.* have higher density.

2. Distribution

Glandular trichomes are present throughout the length of the calyx. They are distributed on calyx in random manner.

3. Orientation

Orientation of glandular trichomes is not uniform. Orientation in different directions has been observed.

4. Length

Length of glandular trichomes varies from 224.21 μm to 1.35 mm. Lengths of glandular trichomes on the calyx show much more variations as compared to those on *Plumbago auriculata*.

5. Structure

It consists of glandular head elevated from epidermis by multicellular stalk. The stalk penetrates the head. LM study has shown that head of all trichomes are colourless the translucent (fig. 4). The sticky mucilage secreted by glandular trichome usually forms a clearly distinct droplet at the tip of glandular trichome. The secreted mucilage glitters in light.

6. EDAX analysis

EDAX analysis on head of glandular trichome (fig. 5) revealed presence of following elements -

C (Wt % = 90.74, At % = 93)

O (Wt % = 8.85, At % = 6.81)

Mg (Wt % = 0.13, At % = 0.07)

Al (Wt % = 0.09, At % = 0.04)

Si (Wt % = 0.19, At % = 0.08)

Plumbago auriculata

The LM as well as SEM studies of the calyx revealed presence of glandular trichomes and unicellular trichomes on the surface of calyx. The unicellular trichomes are longer and denser than that of *Plumbago zeylanica*. In SEM study, the calyx surface appears striate (Radford, 1986) with elongated epidermal cells having almost flat periclinal wall. The features of glandular trichomes as revealed by LM (fig. 1) and SEM (fig. 3) study are

1. Density

Trichomes are scattered *i.e.* have lower density.

2. Distribution

Glandular trichomes are present only in upper part of calyx. They are arranged in linear fashion.

3. Orientation

Glandular trichomes in one line are orientated in the same direction.

4. Length

Length of glandular trichomes varies from 514.59 μm to 780.70 μm . Length of glandular trichomes on the calyx show less variation as compared to those on *Plumbago zeylanica*.

5. Structure

Structure of glandular trichome on the calyx of *Plumbago auriculata* is same as described in the calyx of *Plumbago zeylanica*. LM study revealed colourless translucent as well as purple coloured heads of trichomes (fig. 4).

6. EDAX analysis

EDAX analysis on the head of glandular trichomes (fig. 5) revealed presence of following elements.

C (Wt % = 71.21, At % = 80.60)

O (Wt % = 16.95, At % = 14.40)

Na (Wt % = 0.19, At % = 0.11)

Mg (Wt % = 0.57, At % = 0.32)

Al (Wt % = 0.98, At % = 0.49)

Si (Wt % = 5.56, At % = 2.69)

S (Wt % = 0.16, At % = 0.07)

Cl (Wt % = 0.29, At % = 0.11)

K (Wt % = 0.39, At % = 0.14)

Ca (Wt % = 1.58, At % = 0.54)

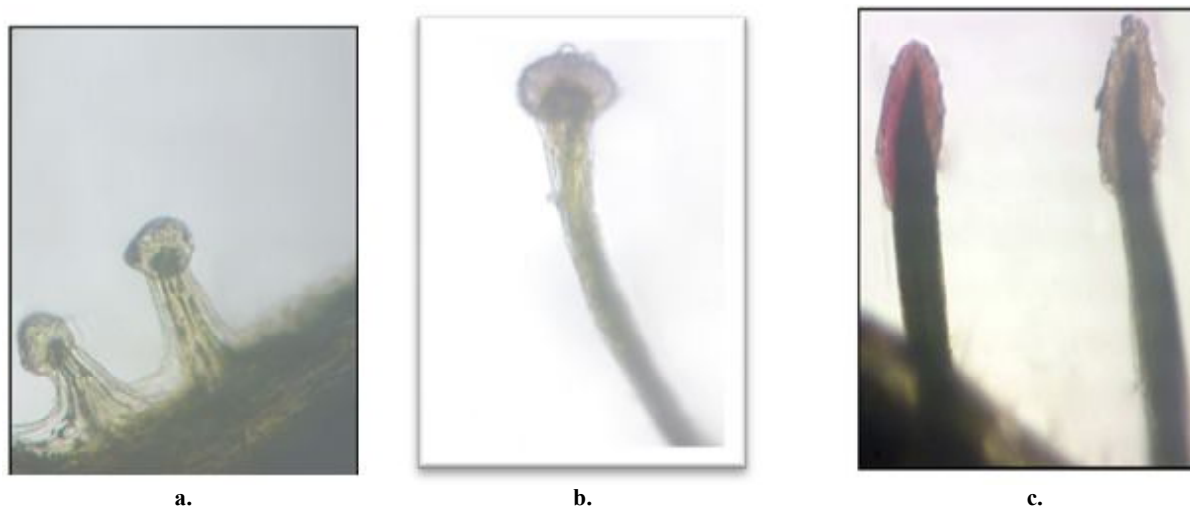


Fig. 1 : LM photomicrographs of glandular trichomes on the calyx of flowers of (a) and (b) *Plumbago zeylanica* (c) *Plumbago auriculata*.

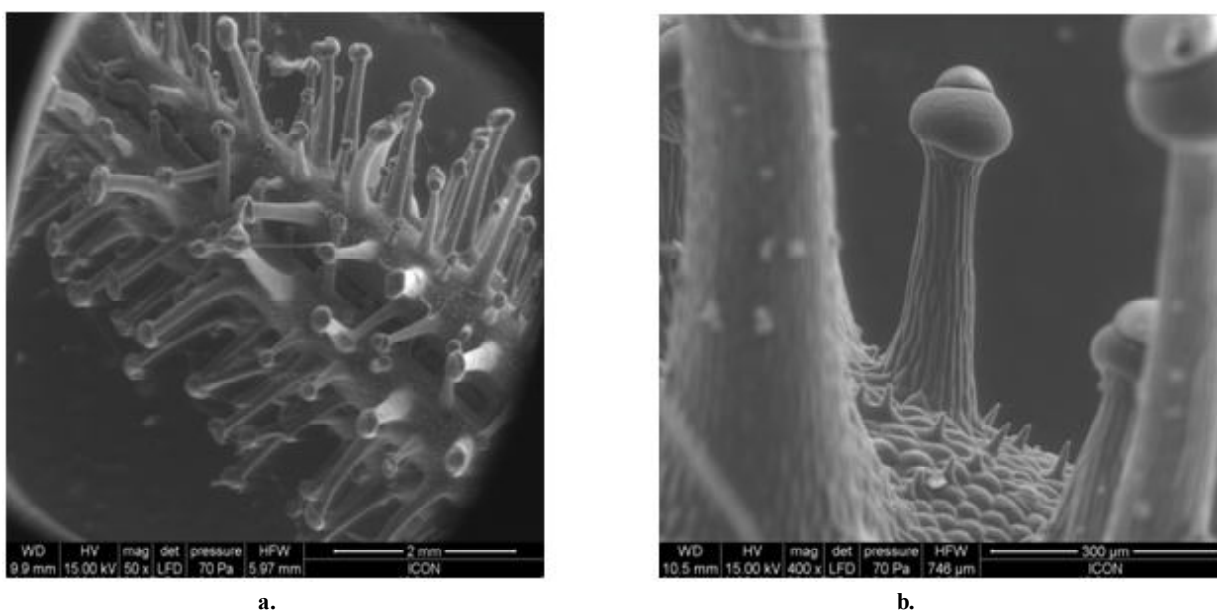


Fig. 2 : SEM Photomicrographs showing glandular trichomes on the calyx of flowers of *Plumbago zeylanica* (a) $\times 50$ (b) $\times 400$.

Ti (Wt % = 0.36, At % = 0.10)

Fe (Wt % = 1.76, At % = 0.43)

Discussion

The structure of glandular trichome (head and stalk) is same in both the species but density, distribution, orientation, length of glandular trichomes differ in both species. Trichomes on the calyx of flowers of *Plumbago zeylanica* are more dense; present throughout the length of calyx and distributed in random manner; oriented in different directions and show much more variation in length while trichomes on the calyx of flowers of *Plumbago auriculata* are less dense; present only in the upper part of calyx and arranged in a linear fashion; trichomes in one line are oriented in the same direction

and show less variation in length.

Our observations of translucent head of glands, penetration of stalk into head and viscosity of its secretion are in the conformity of observations of Wilson (1890). Glandular head is responsible for secretion (Adlassing *et al.*, 2010).

EDAX analysis on head of glandular trichome revealed more elements in *Plumbago auriculata*. In *Plumbago zeylanica* carbon, oxygen, magnesium, aluminium, silicon were found in the secretion while in *Plumbago auriculata*, in addition to these elements sodium, sulphur, chlorine, potassium, calcium, titanium, iron were also found.

The function of glandular trichomes in *Plumbago* is

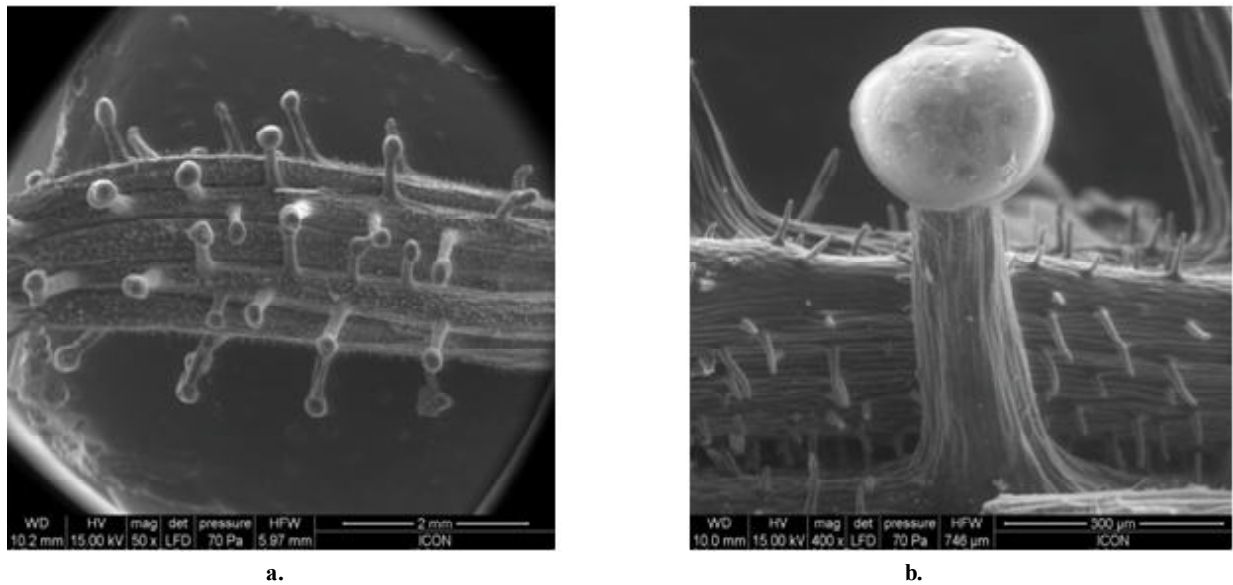


Fig. 3 : SEM Photomicrographs showing glandular trichomes on the calyx of flowers of *Plumbago auriculata*. (a) $\times 50$ (b) $\times 400$.

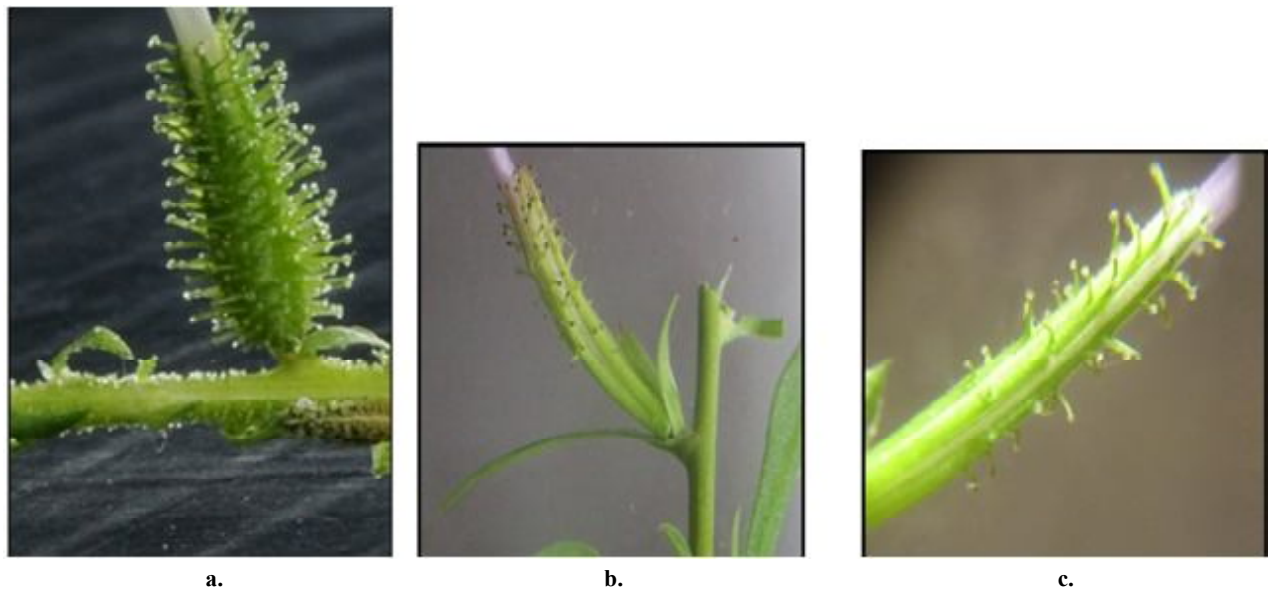


Fig. 4 : Photographs of calyx of (a) *Plumbago zeylanica* (b) *Plumbago auriculata* Lam. showing glandular trichomes with purple head and (c) *Plumbago auriculata* Lam. showing glandular trichomes with colourless translucent head.

disputed. The secreted mucilage acts as barrier against crawling insects like ants because ants can damage flowers or reduce pollen fertility or reduce the rate of visits of flying insects (pollinators) either by attacking them when they land or just by their presence. Ants do not have access to interior of flower but flying insects have access to interior of flower (Whitney *et al.*, 2009). Thus, glandular trichomes exclude crawling insect predators and favor flying pollinators for greater seed dispersal (http://en.wikipedia.org/wiki/Protocarnivorous_plant).

The mucilage secreted by glandular trichomes can be considered as glue, which is adhesive trap for prey

retention in carnivorous plants. It supports the protocarnivorous nature of *Plumbago*. According to Schlauer (1997), Nepenthes are thought to be derived from *Plumbago* like ancestors that were already equipped with sticky glands (Adlassning *et al.*, 2010). Plumbaginaceae and Polygonaceae are families sister to carnivorous plants of Carophyllates. According to Morrissey (1964), Amagase (1972), Juniper *et al.* (1989), Owen *et al.* (1999), Eilenberg *et al.* (2006), Hatano and Hamada (2008) homologous glands have apparently evolved in carnivorous plants to function in secretion of digestive enzymes and absorb amino acids and other organic nutrients. (Renner and Specht, 2011).

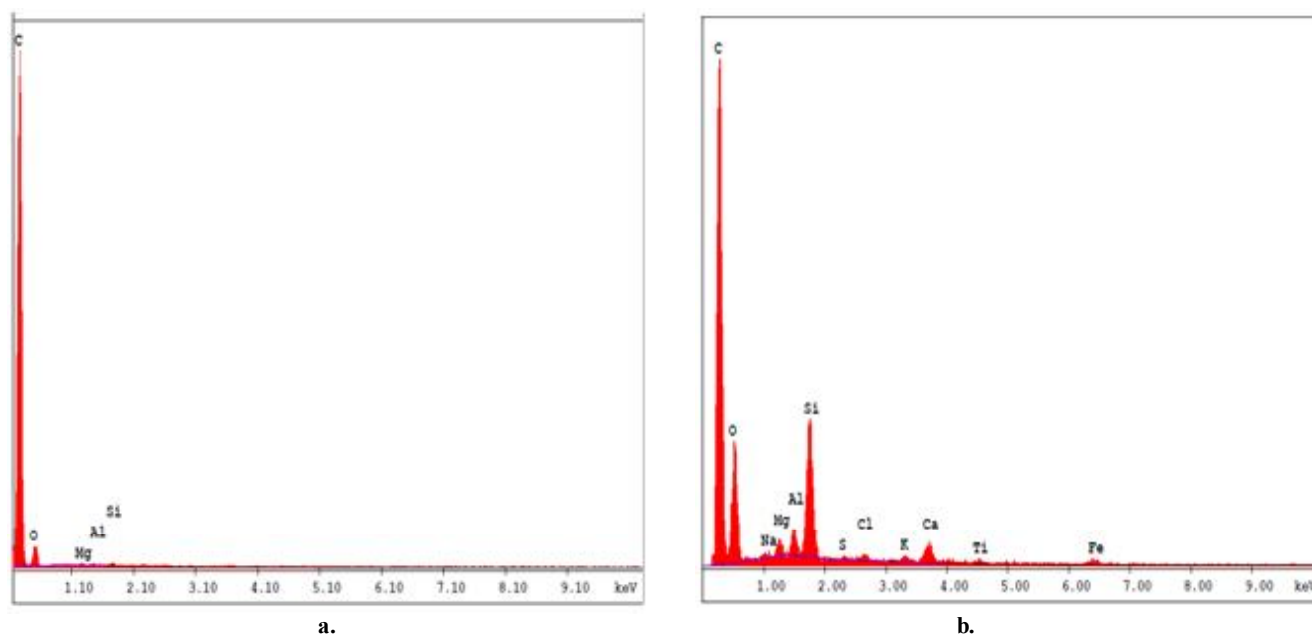


Fig. 5: EDS spectrum showing elemental analysis of secretion of glandular trichome on the calyx of flower of (a) *Plumbago zeylanica* (b) *Plumbago auriculata*.

According to Ellison (2006) the benefit of carnivory is improved supply with minerals especially nitrogen and phosphorus. Carnivorous syndrome comprises four steps— 1) attraction of animals 2) their retention 3) their degradation by digestive enzymes 4) uptake of soluble compounds. Plants lacking one of these features are protocarnivorous and regarded as ancestors of carnivorous plants (Adlassing *et al.*, 2010).

Stoltzfus *et al.* (2002) stated that phylogenetic studies using molecular characters indicate that *Plumbago* is related to *Drosera* and *Drosophyllum*. Glandular trichomes on sepals of *Plumbago* resemble those of *Drosophyllum*. Glandular trichomes of *Plumbago* are induced to secrete digestive enzymes in response to chemical stimuli but do not respond identically to all chemical stimuli effective with *Drosera* (true carnivorous plant). These glandular trichomes are capable of capturing insects (Stoltzfus *et al.*, 2002). As stated earlier, we also observed ants entangled in glandular trichomes on calyx of *Plumbago*.

Stoltzfus *et al.* (2002) considered that probably *Plumbago* is carnivorous during flowering like *Stylidium*. During this period plants have maximum demand for minerals because they are drained from plant for seed production. *Plumbago* is close relative to several genera of carnivorous plants including sundews (<http://www.carnivorousplants.org/cp/EvolutionCaryophyllales.php>). Thus, *Plumbago* is close relative of carnivorous plant and can be considered as protocarnivorous.

Taxonomic significance of study of calyx bearing

glandular trichomes

Sepals of *Plumbago* bear glandular trichomes and can be used as taxonomic tool to delineate two species of *Plumbago*.

1. Key based on calyx surface

Calyx surface areolate, epidermal cells small polygonal with convex periclinal wall. - *Plumbago zeylanica*

Calyx surface striate, epidermal cells are elongated with almost flat periclinal wall - *Plumbago auriculata*

2. Key based on unicellular trichomes on calyx surface

Unicellular trichomes on calyx surface are shorter and less dense. - *Plumbago zeylanica*

Unicellular trichomes on calyx surface are longer and denser - *Plumbago auriculata*

3. Key based on glandular trichomes

Glandular trichomes have high density, present throughout the length of calyx, distributed on the calyx in random manner, oriented in different directions, show much more variation in length and have colourless translucent head. - *Plumbago zeylanica*

Glandular trichomes have lower density, present only in upper part of calyx arranged in linear fashion, trichomes in one line are oriented in the same direction, show less variation in length, have colourless translucent or purple heads. - *Plumbago auriculata*

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