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Research Article

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Comparative LM, SEM and EDAX study of chalk glands on leaf and stem of two species of *Plumbago* Linn.

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ABSTRACT

Stem and leaves of two species of *Plumbago* Linn.viz. *P. zeylanica* Linn. and *P. auriculata* Lam. were investigated for the structure and chemical composition of chalk glands. Light Microscopy (LM) and Scanning Electron Microscopy (SEM) revealed the presence of chalk glands on both lower as well as upper surface of leaf and stem of both species. Chalk glands are abundant on lower surface and sparse on upper surface of a leaf. Chalk glands are approximately hemispherical glands with an oval or almost circular outline. It is composed of 8 cells arranged in two circles – central circle of 4 secretory cells and outer circle of 4 adjoining cells. Each secretory cell has depression which corresponds to pore. Each gland is surrounded by 4 subsidiary cells. No significant difference in the structure of chalk glands in both species was noticed. Chalk glands occupy three different positions with regard to epidermal cells– at the same level of the epidermis, slightly sunken in the epidermis and slightly raised above the epidermis. Common elements found in EDAX analysis of all chalk glands are carbon, oxygen, magnesium, sulphur, potassium and calcium. Differences in the presence of elements silicon, chlorine, aluminium, sodium, phosphorus were observed. The presence of a significant amount of calcium in chalk glands and their dried deposits and absence of sodium and chlorine from dried deposits and even in some chalk glands appealed to use the term 'Chalk gland' instead of 'Salt gland' in *Plumbago*.

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INTRODUCTION

Genus *Plumbago* belongs to the family Plumbaginaceae, a cosmopolitan family but most abundant in dry or saline habitats of Central Asia and Mediterranean region. Fossils of the family have been recorded from Middle Miocene deposits (Sambamurty, 2005). According to Solereder (1908), Metcalfe and Chalk (1950) characteristic feature of Plumbaginaceae is occurrence of epidermal glands. They described two types of glands in Plumbaginaceae – Chalk glands or Mettenius glands or Licopoli glands and Mucilage glands. Mettenius (1856) first described chalk secretion by epidermal glands in Plumbaginaceae. He also recorded variation in chalk secretion by these glands. Some species secreted more while others secreted little (Sakai, 1974). Thus the presence of chalk gland is old discovery of 19th century. Braconnot (1836) have noted chalk secretion and deposits on the surface of these glands many years back before the detection and description of glands. Deposits appeared as small parasitic fungus to Braconnot. He was the first who tried to analyse minerals secreted by these glands of different species of *Satice* and *Plumbago*. But his work remained unknown to many future botanists for many years (Grigore & Toma, 2016). Licopoli (1879), de Bary (1884), Volkens (1884), Maury (1886), Vuillemin (1887), Wilson (1890), de Fraine (1916) described the structure of these glands and also noticed differences in the amount of secretion of these glands which are dependent upon environmental as well as specific differences (Sakai, 1974).

Initially in 1800's these glands were referred to as 'chalk glands' because of the presence of carbonate salts in their secretion but later presence of sodium chloride was discovered in secretion of glands of some species. Hence the term 'salt gland' was used (Faraday & Thomson, 1986) and subsequent workers also used the term salt gland. Hence no descriptions of chalk glands occur in literature since the early 1900's. The majority of later investigators thought that all chalk glands are functional salt glands. Thus chalk glands and salt glands are not separate (Sakai, 1974).

Thomson (1975) defined salt glands as glands secreting both ions and minerals, including chalk glands. (Thomson, 1975). Haberlandt (1918) called chalk glands 'epidermal hydathodes' or 'active hydathodes' (Fahn, 1988). Haberlandt classified hydathodes into two types- 1. Passive hydathodes having direct

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communication with conducting system and secretion of water by these hydathodes is simple filtration under pressure. 2. Active hydathodes where energy for secretion is supplied by hydathode cells. There is no direct connection between active hydathodes and the conducting system (Haberlandt, 1965; Fahn, 1988).

Two species of *Plumbago* Linn. namely *P. zeylanica* Linn. and *P. auriculata* Lam. were investigated in the present study. The objectives of the present study were (1) to study structure of chalk glands with the help of light and scanning electron microscopy (2) to determine chemical composition of secretory products of chalk glands with the help of EDAX (Energy Dispersive X ray analysis) (3) to compare structure and chemical composition of chalk glands of *P. zeylanica* Linn., *P. auriculata* Lam

MATERIALS AND METHODS

Collection and Identification of Plants

Plumbago zeylanica Linn. was collected from botanical garden of Chaitanya Ayurved Mahavidyalaya, Sakegaon. *Plumbago auriculata* Lam. was collected from Ratnakar Nursery, Jalgaon. Both the plants were identified and authenticated by taxonomist Dr. G. S. Chaudhari. Collected plants were grown and cared in the garden. Material for the investigation was procured from plants cultivated in the garden

Light Microscopy

Epidermal peels of both (upper and lower) surface of mature leaves and stem were taken by direct hand peel method or simple blade scratching of leaf and stem (Khan, 1977) The epidermal peels were stained with 1% aqueous safranin (Kothari & Shah, 1974; Khan, 1977), washed with water and mounted in glycerine (Sheela, 1994) to make them semi-permanent. Slides were examined under a light microscope at different magnifications to observe different qualitative as well as quantitative characters of chalk glands on the epidermis.

Scanning Electron Microscopy (SEM)

SEM was carried out at ICON Analytical Equipment Pvt. Ltd., Worli. The piece of leaf of *P. zeylanica* and *P. auriculata* were mounted directly on metallic stub using double sided carbon tape. Dirt on the leaf was removed with the help of a blower. Then the sample was viewed with FEI Quanta 200 Environmental Scanning Electron Microscope with EDAX system and photomicrographs were taken at different magnifications. Both upper and lower epidermises of the leaf were observed. The same procedure was carried out for stem.

EDAX Analysis

EDAX analysis on chalk glands on the lower surface of leaf and stem was carried out with EDAX system of FEI Quanta 200 Environmental Scanning Electron Microscope. The naturally dried salt crystals on the lower surface of leaf were collected from leaves with the help of a brush. EDAX analysis of 6 randomly selected salt crystals was carried out with EDAX system of FEI Quanta 200 Environmental Scanning Electron Microscope.

Measurements and Calculations

Measurements were taken by using LM 52-1712 Digiscope (LCD Digital Microscope) of Lawrence and Mayo. Mean values of 10 observations with standard deviation were taken for size and abundance of chalk glands.

RESULTS

Chalk Glands

LM (Fig.1 A-F) and SEM study (Fig.2 A-B; Fig.3 A-B; Fig.4 A-B; Fig.5 A-B; Fig.6 A-B; Fig.7 A-B) revealed presence of chalk glands on both lower as well as on upper surface of leaf and on the stem of both species

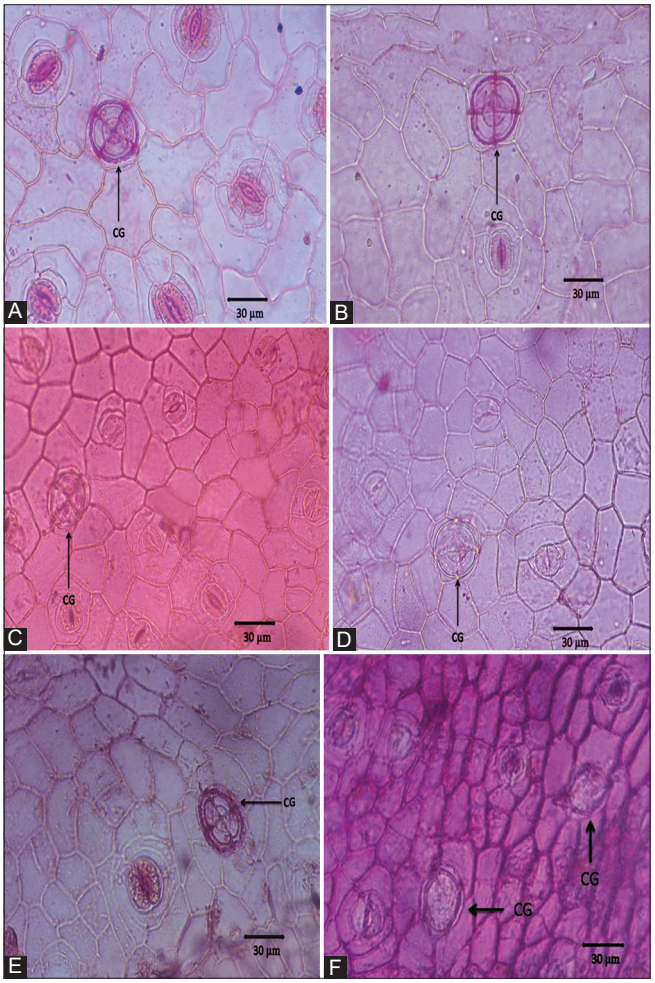

Figure 1: LM photomicrograph of epidermal peeling showing chalk gland (CG) on A. lower leaf surface of *P. zeylanica* (×400x). B. upper leaf surface of *P. zeylanica* (×400x). C. lower leaf surface of *P. auriculata* (×400x). D. upper leaf surface of *P. auriculata* (×400x). E. stem of *P. zeylanica* (×400x). F. stem of *P. auriculata* (×400x)

1. Shape
   Chalk glands are approximately hemispherical glands with oval or almost circular outline

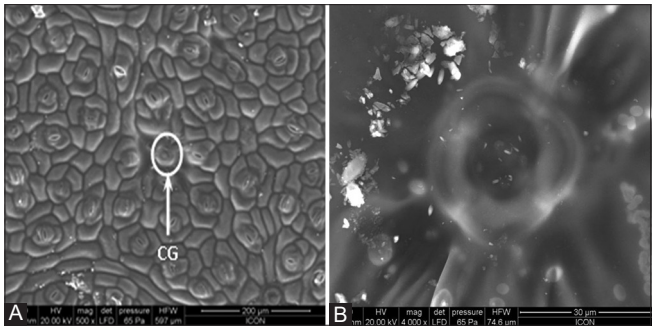

Figure 2: SEM photomicrograph showing chalk gland on lower leaf surface of *P. zeylanica* A. ×500x B. ×4000x

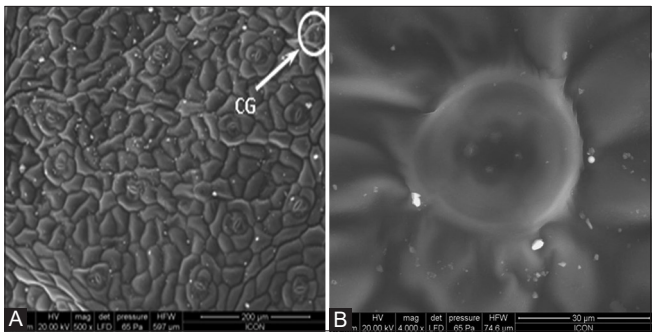

Figure 3: SEM photomicrograph showing chalk gland on upper leaf surface of *P. zeylanica* A. ×500x B. ×4000x

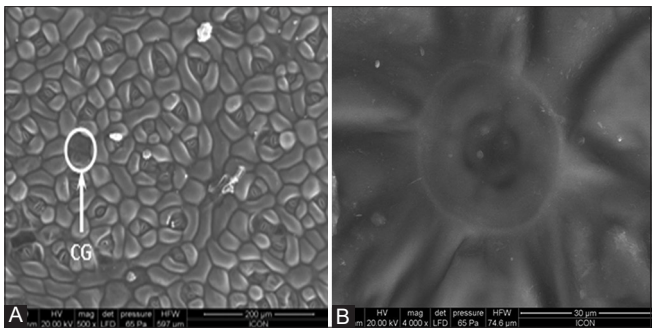

Figure 4: SEM photomicrograph showing chalk gland on lower leaf surface of *P. auriculata* A. ×500x B.×4000x

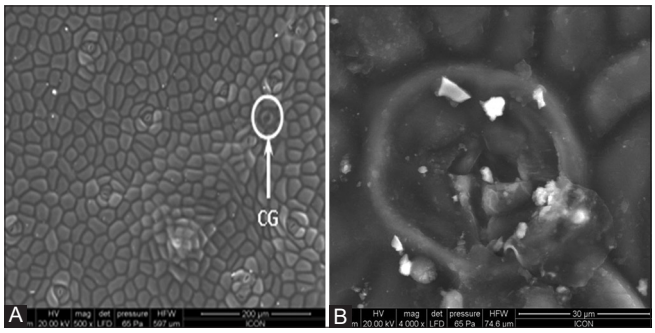

Figure 5: SEM photomicrograph showing chalk gland on upper leaf surface of *P. auriculata* A. ×500x B.×4000x

2. Position
   Chalk glands appeared slightly sunken in epidermis on the lower surface of leaf and at the level of epidermis on the upper surface of leaf of both plants and on the stem of *P. auriculata*. Chalk glands appeared slightly raised above the epidermis on the stem of *P. zeylanica*.
3. Distribution
   Chalk glands are found to be distributed on both the surfaces of leaf as well as stem. They occur abundantly on the lower side of leaves (about 13.600 ± 5.400 per mm^2 in *P. zeylanica and* 14 ± 3.399 per mm^2 in *P. auriculata*) and sparsely on the upper side of leaves of both plants. This was also confirmed by observing more number of dried salt crystals forming almost a crust on lower surface of leaf as compared to upper surface of leaf.
4. Structure
   Chalk gland is composed of 8 cells arranged in two circles. Each circle consists of 4 cells. 4 secretory cells are arranged in a central circle. On the outer side this central circle is accompanied by second circle of 4 adjoining cells. Each of the four central secretory cells occupies one quarter of the circle and has a depression on its surface which apparently corresponds to pore, the site of secretion. Secretory cells in chalk glands of stem appear sunken (slightly sunken in *P. zeylanica* and more sunken in *P. auriculata)* than adjoining cells. Each gland is surrounded by 4 subsidiary cells.
5. Dimensions

Chalk glands vary in their sizes. The size of chalk gland

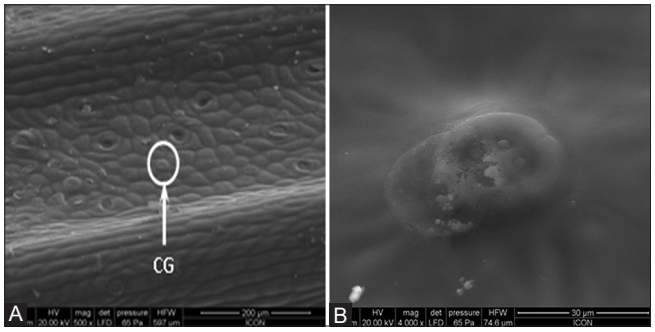

Figure 6: SEM photomicrograph showing chalk gland on stem epidermis of *P. zeylanica* A. ×500x B. ×4000x

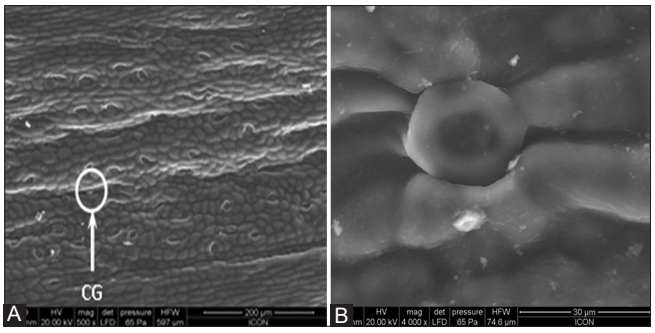

Figure 7: SEM photomicrograph showing chalk gland on stem epidermis of *P. auriculata* A. ×500x B. ×4000x

1. On *P. zeylanica* leaf is
   a) 39.986 ± 4.539 µm × 38.122 ± 2.944µm on upper surface

   b) 39.405 ± 4.482 µm × 36.389 ± 4.784µm on lower surface

2. On *P. auriculata* leaf is
   a) 35.267 ± 4.535 µm × 34.079 ± 3.509µm on upper surface

   b) 30.354 ± 2.656 µm × 30.620 ± 3.973µm on lower surface

3. On *P. zeylanica* stem is 30.342 ± 4.927 µm × 28.477 ± 7.304µm
4. On *P. auriculata* stem is 29.061 ± 2.664 µm × 26.607 ± 4.799µm

EDAX Analysis

EDAX analysis of chalk glands

Chalk glands on leaves as well as on stem were analysed by EDAX microanalysis. The species wise EDAX analysis is given below

Plumbago zeylanica Linn.

I. Leaves –
EDAX analysis on chalk gland of lower surface of leaf of *P. zeylanica* (Fig. 8) revealed presence of nine elements namely, C (Carbon), O (Oxygen), Mg (Magnesium),Si (Silicon), P (Phosphorus), S (Sulphur), Cl (Chlorine), K (Potassium), Ca (Calcium).

II. Stem-
EDAX analysis on chalk gland on stem of *P. zeylanica* (Fig. 9) revealed presence of nine elements namely C, O, Na, Mg, Si, S, Cl, K, Ca.

Plumbago auriculata Lam.

I. Leaves –
EDAX analysis on chalk gland of lower surface of leaf of *P. auriculata* (Fig.10) revealed presence of seven elements namely C, O, Mg, Al, S, K, Ca.

II. Stem
EDAX analysis on chalk gland on stem of *P. auriculata* (Fig.11) revealed presence of following nine elements namely C, O, Na, Mg, Si, S, Cl, K, Ca.

Quantitative proportion of elements present in chalk glands of leaves and stem of both species of *Plumbago* is summarised in the Table 1.

EDAX analysis of Salt Crystals

Salt deposits collected from lower leaf surface (Fig.12 A-B) were analysed by EDAX microanalysis. The SEM photomicrographs of salt crystals revealed various sizes and irregular shapes of crystals. Impressions of chalk glands on the crystals were also observed. (Fig. 13 and 14).The species wise EDAX analysis is given below.

Plumbago zeylanica Linn.

EDAX analysis of six randomly selected salt crystals of *P. zeylanica* Linn. (Fig.15) revealed the presence of 4 elements namely oxygen (O), Magnesium (Mg), Calcium (Ca) and Silicon (Si). The results of EDAX analysis of 6 salt crystals are summarised in the Table 2.

Plumbago auriculata Lam.

EDAX analysis of six randomly selected salt crystals of *P. auriculata* Lam. (Fig. 16) revealed the presence of 4 elements namely oxygen (O), Magnesium (Mg), Calcium (Ca) and Potassium (K). The results of EDAX analysis of 6 salt crystals are summarised in the Table 3.

DISCUSSION

Salinity of soil is one of the most serious environmental factors limiting the growth of plants. To cope with excess salinity, salt glands are developed in halophytes to excrete excess salt from

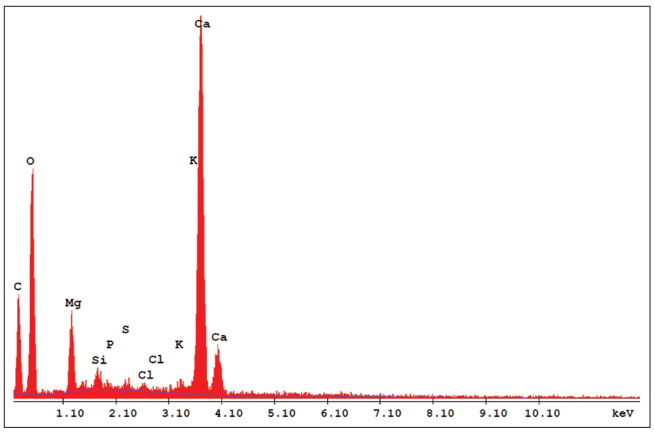

Figure 8: EDS spectrum showing elemental analysis of chalk gland on lower surface of leaf of *Plumbago zeylanica* Linn.

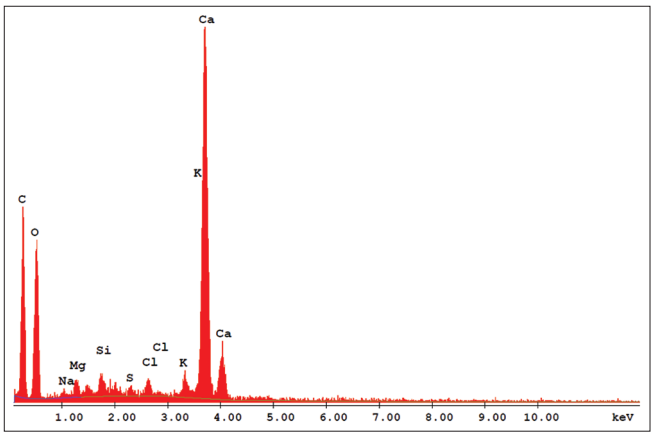

Figure 9: EDS spectrum showing elemental analysis of chalk gland on stem of *Plumbago zeylanica* Linn.

plants to regulate ion balance (Oi *et al*., 2012). Thus salt glands are specialized adaptive structure of halophytes which are efficient desalination devices (Tan *et al*., 2010). But salt glands are also present in plants occupying non-saline environment today (Liphschitz & Waisel, 1982). Presence of salt glands in *Plumbago* which is not hydrophyte could be suggested as an ancestral character. The structure of salt glands may vary greatly in different plant species or may be similar within the same genus or even within same family (Salama *et al*., 1999).Solereder (1908), Haberlandt (1965) reported the hemispherical shape of these glands. Our observations are also in accordance with these authors. Chalk glands in *P. zeylanica* are larger than those in *P. auriculata*. Glands on upper surface of leaf are larger than those on lower surface of leaf. We observed chalk glands on both sides of leaf- abundant on under side and sparse on upper side of leaf. Our observations are somewhat contradictory to those of Sakai (1974) who observed chalk glands only on underside of leaves and not on upper side of leaves of *P. capensis*.

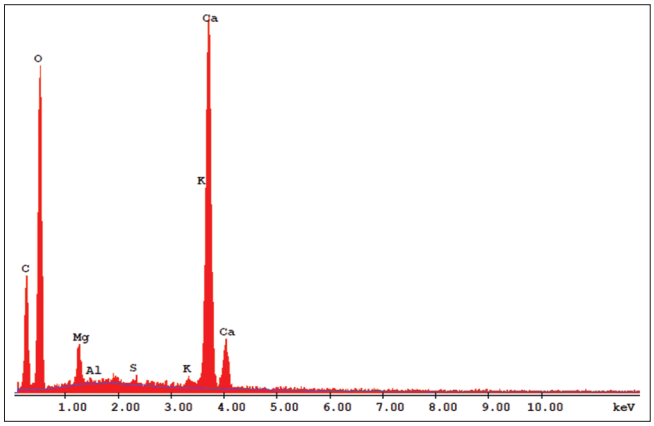

Figure 10: EDS spectrum showing elemental analysis of chalk gland on lower surface of leaf *Plumbago auriculata* Lam.

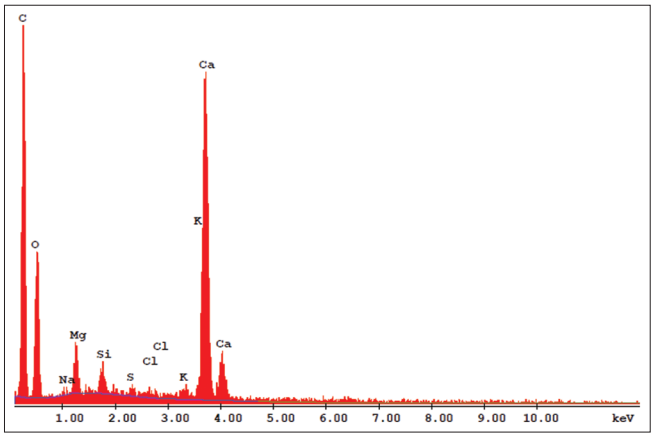

Figure 11: EDS spectrum showing elemental analysis of chalk gland stem of *Plumbago auriculata* Lam

We noticed no significant differences in structure of chalk glands in both species. Chalk glands occupy three different positions with regard to epidermal cells

1. At the same level of the epidermis
2. Slightly sunken in the epidermis
3. Slightly raised above the epidermis

In Plumbaginaceae, Metacalfe and Chalk (1950) reported about dilemma of number of cells forming chalk glands whether it is 4 or 8. Solereder (1908) stated that chalk glands in Plumbaginaceae are made up of 8 cells and not 4 cells as stated by Mettenius and then by Maury. As stated in the result, we also observed 8 cells in LM as well as SEM study – central 4 secretory cells and peripheral 4 adjoining cells. According to Solerder (1908), in Plumbaginaceae group of 8 glandular cells is cut off from internal tissue by double cap. Each layer of cap is composed of four subsidiary cells. The cells of upper cap directly enclose the group of glandular cells and have suberized wall and occasionally reach to the level of glandular cells so that they appear in the surface view of gland as four celled ring. These two characteristics are not shown by subsidiary cells of inner cap layer.

Salama *et al*. (1999) also stated that salt glands in Plumbaginaceae (*Limonium axillare*, *L. pruinosum*, *Lamoniastrum monopetalum*) are composed of 16 cells arranged in four circles and four sub-basal collecting cells. The four central excretory cells form the first circle, at their outer side four adjoining cells form second circle. Both circles are surrounded by two cup shaped layer- outer and inner layer each composed of 4 cells. The same 16 celled structure of the gland was reported in *Limonium* by Ruhland,

Table 1. Comparative EDAX Analysis of chalk glands of two species of ***Plumbago*** Linn.

Sr. No.	Name of element	Name of plant							
		Plumbago zeylanica				***Plumbago auriculata***			
		Leaf		Stem		Leaf		Stem	
		Wt%	At%	Wt%	At%	Wt%	At%	Wt%	At%
1	C	22.72	33.58	35.98	50.17	21.56	30.92	50.96	64.57
2	O	46.14	51.18	35.68	37.35	53.70	57.82	28.28	26.90
3	Na	-		0.47	0.34	-	-	0.18	0.12
4	Mg	4.25	3.10	0.87	0.60	2.04	1.45	1.72	1.08
5	P	0.08	0.05	-	-	-	-	-	-
6	S	0.36	0.20	0.38	0.20	0.16	0.09	0.27	0.13
7	Si	0.77	0.49	0.93	0.55	-	-	0.78	0.42
8	Cl	0.36	0.18	0.72	0.34	-	-	0.27	0.11
9	K	0.67	0.31	1.14	0.49	0.47	0.21	0.54	0.21
10	Ca	24.64	10.91	23.83	9.96	21.91	9.42	17.01	6.46
11	Al	-	-	-	-	0.16	0.10	-	-

1915; de Fraine, 1916; Ziegler and Luttage, 1966 (Waisel, 1972). According to Scassellati *et al*. (2016) salt glands of *Armeria canescens* of Plumbaginaceae is made up of 16 cells arranged in four quadrants including 4 subsidiary cells and 12 gland cells. Thus they counted subsidiary cells as cells of chalk glands while Solereder (1908) and Metacalfe and Chalk (1950) counted subsidiary cells separately.

Faraday and Thomson (1986 a) observed the salt glands of eleven species belonging to six different genera of family Plumbaginaceae and found the same ultrastructure in all species indicating that salt glands of members of Plumbaginaceae are extremely similar (Salama et al, 1999). Haberlandt (1965) termed these glands as 'water glands' of Plumbaginaceae and stated that these hemispherical glands consist of four central and four peripheral cells and are enclosed by four epidermal subsidiary cells. Observations in the present investigation are also in accordance with Hyberlandt. Freitas and Breckle (1992), Marcum and Murdoch (1992) reported that there is dilemma about salt glands, whether they functions to secrete, excrete or recreate (Dajic, 2006). Some investigators e. g. Metacalfe and Chalk, 1950; Fahn, 1988; Sakai, 1974 used the term of secretory cells while other e.g. Salama *et al*., 1999 used the term excretory cells for the cells in salt glands

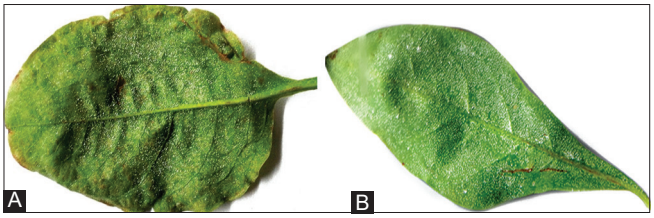

Figure 12: Dried salt crystals on the lower surface of leaf of A. *P. zeylanica* Linn. B. *P. auriculata* Lam.

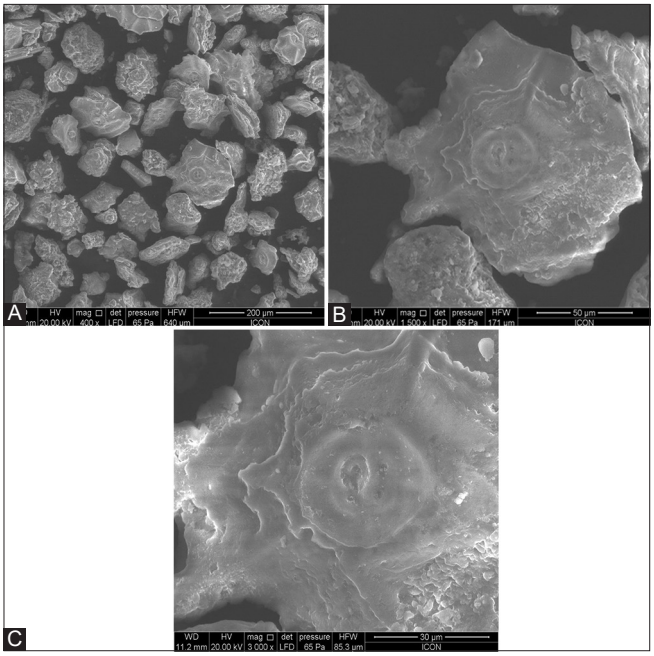

Figure 13: SEM photomicrographs of dried salt crystals collected from lower surface of leaf of *P. zeylanica* Linn. A. Salt crystals (×400x) B. Salt crystals (×1500x) C. Salt crystals (×3000x)

EDAX Analysis

EDAX analysis of chalk glands revealed the presence of Carbon, Oxygen, Magnesium, Sulphur, Potassium and Calcium. Silicon and Chlorine were also found except in chalk glands on leaves of *P. auriculata* Lam. Instead of Silicon and Chlorine the chalk glands of a leaf of *P. auriculata* Lam. showed the presence of Aluminium. Sodium and phosphorus were found only in chalk glands on the stem of both species and leaf of *P. zeylanica* respectively (Table 1). After evaporation of water form secretion of chalk glands crystals are formed which form crust on leaves and stems. These crystals are easily visible to naked eye. The

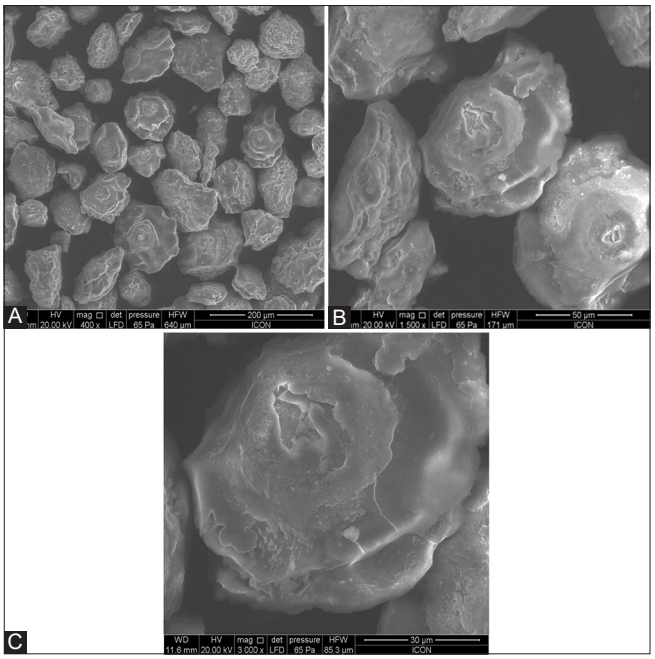

Figure 14: SEM photomicrographs of dried salt crystals collected from lower surface of leaf of *P. auriculata* Lam. A. Salt crystals (×400x) B. Salt crystals (×1500x) C. Salt crystals (×3000x)

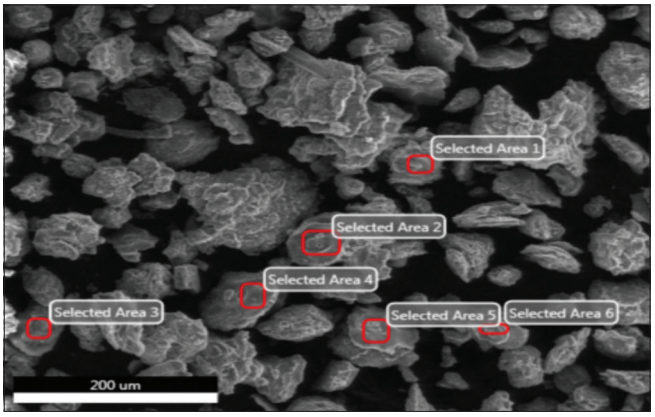

Figure 15: SEM photomicrograph of salt crystals of *P. zeylanica* marked with selected crystals for EDAX analysis

Table 2: EDAX Analysis of salt crystals collected from lower surface of leaf of ***P. zeylanica*** Linn.

Element	Crystal 1		Crystal 2		Crystal 3		Crystal 4		Crystal 5		Crystal 6		Average ± S. D.	
	Wt %	At%	Wt %	At%	Wt %	At%	Wt %	At%	Wt %	At%	Wt %	At%	Wt %	At%
O	55.60	74.50	59.20	76.56	61.90	78.79	57.38	75.34	63.78	80.44	55.00	74.46	58.8 ± 3.50	76.68 ± 2 0.45
Mg	5.07	4.47	6.56	5.59	5.63	4.71	6.22	5.37	4.07	3.38	3.10	2.76	5.11 ± 1.32	4.38 ± 1.11
Ca	39.33	21.04	33.44	17.26	32.47	16.50	35.48	18.60	32.14	16.18	41.34	22.34	35.70 ± 3.83	18.65 ± 2.53
Si	-	-	0.80	0.59	-	-	0.92	0.69	-	-	0.56	0.43	0.38 ± 0.43	0.29 ± 0.32

Table 3: EDAX Analysis of salt crystals collected from lower surface of leaf of ***P. auriculata*** Lam.

Element	Crystal 1		Crystal 2		Crystal 3		Crystal 4		Crystal 5		Crystal 6		Average±S. D.	
	Wt %	At%	Wt %	At%	Wt %	At%	Wt %	At%	Wt %	At%	Wt %	At%	Wt %	At%
O	56.39	75.74	66.47	79.06	58.81	76.64	58.39	76.21	58.32	76.42	63.74	80.28	60.35 ± 3.87	77.39 ± 1.83
Mg	2.53	2.23	16.25	12.72	5.72	4.90	6.26	5.38	5.25	4.53	4.58	3.80	6.77 ± 4.82	5.59 ± 3.66
Ca	41.08	22.03	15.81	7.51	35.48	18.46	35.35	18.42	36.43	19.06	31.67	15.92	32.64 ± 8.78	19.90 ± 5
K	-	-	1.47	0.71	-	-	-	-	-	-	-	-	0.25 ± 0.60	0.12 ± 0.29

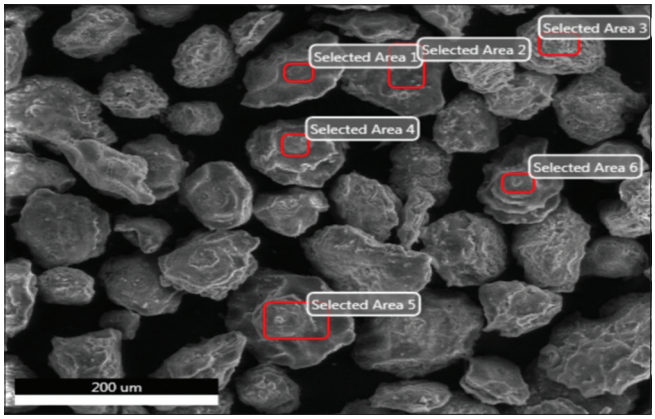

Figure 16: SEM photomicrograph of salt crystals of *P. auriculata* marked with selected crystals for EDAX analysis

lower surface of the mature leaf is heavily encrusted with white salt crystals. The white salt residue on the lower side of the leaf resembles powdery mildew disease. According to Marloth saline crust reduce the effect of insolation and also produce a cooling effect due to evaporation of water and thus counteracting the heating effect of sun. Salt glands represent devices for obviating the accumulation of excessive quantities of mineral matter within the plant body (Haberlandt, 1965). Saline crust protects against excessive loss of water through transpiration (Solereder, 1908).

EDAX analysis of dried deposits of chalk glands revealed the presence of Oxygen, Calcium and Magnesium. Some crystals of *P. zeylanica* Linn. showed the presence of a trace amount of Silicon whereas some crystals of *P. auriculata* Lam. showed the presence of a trace amount of Potassium (Table 2 and 3). Our results of investigation of elemental analysis of chalk glands and their dried deposits on the leaf of *P. auriculata* Lam. showed slight variation with the finding of Sakai (1974). He found Calcium, Magnesium and trace of Silicon while the present study revealed the presence of Oxygen Calcium, Magnesium and trace of Potassium in dried deposits on the leaf of *P. auriculata* Lam. In EDAX analysis of leaf epidermal cell, Sakai (1974) investigated Magnesium, Silicon, Phosphorus, Sulphur, Chlorine, Potassium and Calcium while the present investigation of EDAX analysis on chalk glands on the lower surface of a leaf revealed the presence of Carbon, Oxygen, Magnesium, Aluminium, Sulphur, Potassium and Calcium.

As stated earlier, chalk glands and salt glands are not separate secretory structures. Our study revealed a significant amount of calcium in both chalk glands and dried salt crystals. Na and Cl were not found as major elements in the secretion of chalk glands. Na is even absent in chalk glands on the leaf of both taxa and Cl is absent in chalk glands on the leaf of *P. auriculata*. Na and Cl were not found in even dried deposits of both plants. Hence I thought in *Plumbago* the term chalk gland is proper instead of salt gland.

Taxonomic significance

1. Key based on size of chalk gland on leaf and stem

Chalk glands larger (39.986 ± 4.539µm × 38.122 ± 2.944µm on upper surface and 39.405 ± 4.482µm × 36.389 ± 4.784µm on lower surface of leaf, 30.342 ± 4.927 × 28.477 ± 7.304µm on stem)

-Plumbago zeylanica

Chalk glands smaller (35.267 ± 4.535µm × 34.079 ± 3.509µm on upper surface and 30.354 ± 2.656µm × 30.620 ± 3.973µm on lower surface of leaf, 29.061 ± 2.664 µm × 26.607 ± 4.799µm on stem)

-Plumbago auriculata

2. Key based on SEM characteristics of chalk glands on stem

Chalk glands slightly raised above the epidermis, central circle of secretory cells slightly sunken

-Plumbago zeylanica

Chalk glands at the level of epidermis, central circle of secretory cells more sunken

-Plumbago auriculata

CONCLUSION

The results of this descriptive study showed that chalk glands in both studied species of *Plumbago* have same the structure; differences were noticed in their sizes and position with respect to epidermal cells. Chalk glands can be used as a supplementary taxonomic tool to differentiate two species of *Plumbago*. EDAX analysis studies suggested using the term chalk gland instead of salt gland in *Plumbago.*

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