

A COMPREHENSIVE REVIEW ON THE GENUS *PLUMBAGO* WITH FOCUS ON *PLUMBAGO AURICULATA* (PLUMBAGINACEAE)

Karishma Singh^{1*}, Yougasphree Naidoo², Himansu Baijnath³

School of Life Sciences, University of KwaZulu Natal, Westville Campus, Durban, South Africa.

*Corresponding Author's Email: k1008.singh@gmail.com

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Abstract

Background: The genus *Plumbago* distributed in warm tropical regions throughout the world is the largest genus in Plumbaginaceae. Medicinal plants are characteristic to the genus *Plumbago* and are cultivated and utilized worldwide. *Plumbago auriculata* Lam. is common in South Africa and is often cultivated for its ornamental and medicinal uses throughout the world.

Materials and Methods: A comprehensive review of the genus *Plumbago* with focus on *Plumbago auriculata* was carried out and information was gathered using scientific publications, conference proceedings, the internet and books. Articles based on the morphology, pharmacological and medicinal uses of *Plumbago auriculata* was analysed thoroughly.

Results: *Plumbago auriculata* plant parts possess a wide range of phytochemicals with plumbagin being the marker compound showing various pharmacological activities. Different plant parts are claimed to be used for the treatment of human and animal ailments, however they do exhibit toxic properties and need to be administered with caution. Salt secreting glands and trichomes are characteristic of Plumbaginaceae.

Conclusion: This study reveals new insights on the genus *Plumbago* and the potential use of species in the genus as medicinal plants. *Plumbago auriculata* possess the bioactive compound plumbagin and secondary metabolites, thus, it is of high medicinal importance. *P. auriculata* is a poorly nor favourite studied species in the genus *Plumbago* and further research needs to be carried out to explore specific details of the species.

Key words: *Plumbago*, salt glands, trichomes, pharmacological activities, plumbagin, micropropagation.

Introduction

Over the years, human health problems have been increasing at an alarming rate and are now becoming life threatening. Conventional medicine used to control health problems is often too expensive and has many side-effects. Therefore, many people have turned to the use of medicinal plants for the control and treatment of health problems. Plant extracts have been used for hundreds of years to cure ailments (Balunas and Kinghorn, 2005). The vast resource of thousands of medicinal plants and their contribution to human health are to be discovered. Exploiting traditional medicine systems has served as a promising approach in an era of increasing demand for drug production and rising costs of western medicine, globally.

It has been reported that many plants naturally produce secondary metabolites, commonly referred to as phytochemicals or biologically active compounds which are essential for plant metabolism but play a great role in the plants' protection mechanism (Ascensao et al., 1997). In addition to plant protection, these bioactive compounds also serve as precursors for the development of natural, environmentally friendly and low toxicity pharmaceuticals, flavourants, fragrances, cosmetics and pesticides due to their therapeutic and aromatic properties. Bioactive compounds are either produced, excreted or stored in small amounts in specialized cells, termed secretory structures in the form of salt glands, trichomes, resin ducts, idioblasts, laticifers, colleters and nectaries located in various reproductive and vegetative organs of the plant (Thomas, 1991). In order to understand the biological activity and composition of the secreted exudate, it is vital to know the morphology, distribution and secretory processes of the main secretory structures involved. However, these structures vary in form, distribution, function and type of secretion across different plant families.

The family Plumbaginaceae Juss.(a.k.a the leadwort family) has about 24 genera and about 400 species (The Plant List, 2017). This is debatable because some publications reported that there are 700-800 species in the family (APG II, 2003; Simpson, 2010; Renner and Specht, 2011). Plumbaginaceae was first described in 1789 by Antoine Laurent de Jussieu and it was the only family in the order Plumbaginales. However, in 2003 the APG system of plant classification placed Plumbaginaceae in the order Caryophyllales due to certain species having carnivorous characteristics and is sister to the family Polygonaceae (Kubitzki, 1993; Perveen and Qaiser, 2004; Simpson, 2010). Plumbaginaceae comprises mainly herbs, lianas and shrubs, often occurring in saline habitats. The presence of secretory glands is characteristic of the family (Wilson, 1980; Faraday and Thomson, 1986a). Species of the Plumbaginaceae are of ornamental and medicinal importance. *Plumbago auriculata* Lam. an evergreen shrub is indigenous to South Africa (Figure 1) but is distributed in other parts of the world in tropical and subtropical regions (Foden and Potter, 2015). *P. auriculata* occurs in thicket and shrub valley bushveld. It is often called Cape *Plumbago* or Cape Leadwort because it is widely found in the Cape regions of South Africa (Batten, 1986; Kubitzki, 1993; Foden and Potter, 2015). *P. auriculata* is an easy to manage plant as it is tolerant to high humidity, high temperature and diseases and can be treated as an ornamental as well as a medicinal plant as it contains many potent bioactive compounds (Chen and Gao, 2013). *P. auriculata* bears a close resemblance to *Drosera*, a carnivorous plant, which plays a role as an attractant as well as a repellent to many insects (Plachno et al., 2006). The genus *Plumbago* comprises of 18 species, three commonly studied species that is *Plumbago rosea* L. syn *indica* L., *Plumbago zeylanica* L., and *Plumbago auriculata*. In all these three species, *P. auriculata* is the least-studied especially in South Africa. This review focuses on information about the morphology, biology, chemical composition, pharmacology, medicinal uses and toxicity of the genus with a particular emphasis on *Plumbago auriculata*.

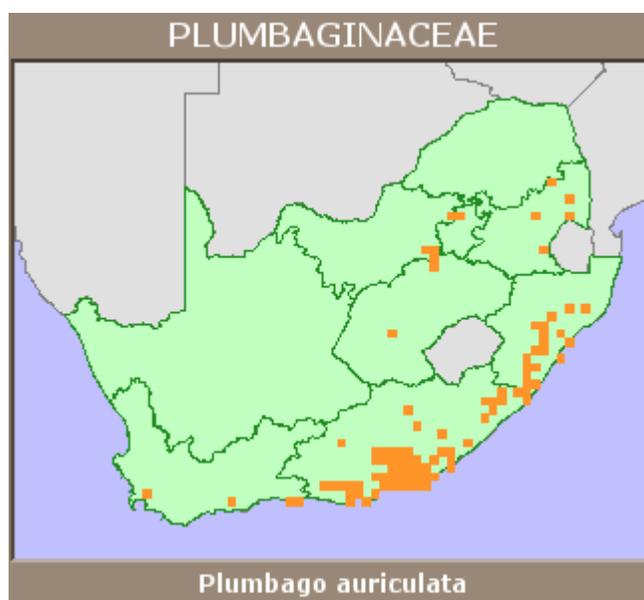


Figure 1: Distribution of *Plumbago auriculata* in South Africa (After Foden and Potter, 2015).

An overview of the genus

The genus *Plumbago* comprises 18 species distributed and utilised throughout the world in warm regions (APG, 2009; The Plant List, 2017). It is the largest genus in the family (APG, 2003, 2009). In South Africa 5 species are native and 2 are cultivated (Glen, 2002). Species are cultivated and utilized worldwide mostly for their medicinal and pharmacological properties due to the presence of plumbagin amongst other phytochemical compounds (Table 1) (Craven and Craven, 2000; Galal et al., 2012; Jose et al., 2014; Saha and Paul, 2012, 2014; Omwenga and Paul, 2012; Purger et al., 2012; That et al., 2012; Thamaraj and Antonysamy, 2013). The genus comprises shrubs or perennial herbs and the presence of a hairy calyx is characteristic of the genus (Batten, 1986) The hairy calyx is associated with insect entrapment amongst other functions. The most commonly cultivated and utilized species is *Plumbago zeylanica* with several publications (Abera et al., 2008; Chauhan, 2014; Demma et al., 2009; Jeyachandran et al., 2009; Tyagi and Menghani, 2014).

Table 1: Species List of the genus *Plumbago* (The Plant List, 2017).

Species Name	Common Name	Locality	Description	Traditional uses	Pharmacological uses
<i>Plumbago amplexicaulis</i> Oliv.		Tanzania	Erect herb or subshrub, with glabrous stems and bright deep blue flowers.		
<i>Plumbago aphylla</i> Bojer ex Boiss.		Madagascar, Aldabra Islands and Tanzania	Slender shrub with erect stems, leaves simple, entire, and alternate. Flowers white with hairy calyx.	Used to treat diarrhoea, mouth infections and is applied as an eyewash for cataract.	Anti-bacterial and antifungal.
<i>Plumbago auriculata</i> Lam	Cape leadwort	South Africa, Africa, America, Asia, India	Bushy perennial evergreen shrub, trusses of pale blue and white flowers, leaves have minute dots and persistent hairy calyx.	Has many therapeutic properties and can be used for warts, fractures, oedema, headaches, skin lesions, piles, rheumatism, diarrhoea and malaria and as emetics.	Antimicrobial, antiulcer, antimalarial, antifungal, anticancer.
<i>Plumbago ciliata</i> Engl.		Tanzania			
<i>Plumbago caerulea</i> Kunth	chileno	Chile	Shrub, with erect stem and blue flowers.	Ornamental value.	Roots yield plumbagin.
<i>Plumbago dawei</i> Rolfe		Ethiopia, Kenya, Uganda	Perennial herb with erect stems and white flowers.	Used to treat diarrhoea, stomach ache..	Antimicrobial, antimalarial
<i>Plumbago europaea</i> L.	Common leadwort/ European leadwort	Europe, Turkey	Herbaceous half shrubby, multi-branched plant with trusses of pink or purple flowers.	Used for treatment of blisters, inflammation, itching, toothache, skin disorders.	Antimicrobial, anticancer, antifungal, antimutagenic and insecticidal.
<i>Plumbago glandulicaulis</i> Wilmot-Dear		Kenya, Tanzania	Herb, erect hairy and sticky stem with leaves glabrous and white flowers.	Ornamental value.	
<i>Plumbago indica</i> L. syn. <i>Rosea</i> L.	Scarlet leadwort	Asia Africa, India, Indonesia ,Phillippines	Perennial herbaceous plants with erect half woody stems and red flowers.	Used to treat gastric acidity, skin disease, constipation, , haemorrhoids, rheumatoid arthritis, paralysis, also used in horses to expel worms, also abortifacient.	Anticancer, anti-inflammatory, antiatherogenic.
<i>Plumbago madagascariensis</i> M. Peltier		Madagascar	Shrub, erect stem and variations of blue, white and		

			purple flowers.		
<i>Plumbago montis- elgonis</i> Bullock		Ethiopia, Kenya, Tanzania	Stout short perennial herb with pink flowers.		
<i>Plumbago pearsonii</i> (L.) Bolus		Namibia	Erect shrub, slightly branched with pink and purple- violet flowers.		Roots yield plumbagin.
<i>Plumbago pulchella</i> Boiss.	Cola de iguana	Mexico	White flowers.	vesicant and caustic effect, used for body aches and pains, skin disorders, used as veterinary medicine..	Anticancer, antiulcer.
<i>Plumbago stenophylla</i> Wilmot-Dea		Kenya, Tanzania	Small erect shrub or woody herb, white flowers.	Skin disorders and orally for hookworms.	Antimalarial
<i>Plumbago scandens</i> L.	Louco; caataia; caapomonga	Brazil	Subshrub with white flowers.	Leaves used as nape compresses in mentally ill patients also used as local anesthetic. Soothes toothaches and earaches. Reduces joint in flammation. ..	Antimicrobial, anti- cancer and antimalarial treatments.
<i>Plumbago tristis</i> Aiton	South Africa	Dark- flowered leadwort	Perennial shrub, dark-pink fowers at the end of pink hairy stems.		Roots yield plumbagin.
<i>Plumbago wissii</i> Friedr.	Brandberg <i>Plumbago</i>	Namibia	Multi-stemmed shrub, with violet to maroon purple flowers.	Ornamental value, used as a cure for lead disease or lead poisoning.	
<i>Plumbago zeylanica</i> L.	Ceylon leadwort, White leadwort	Africa, Asia, Australia, Et hopia, India, China	Herbaceous shrubby plants with climbing or erect stems, petiolate leaves and white flowers.	Used to treat anemia, bronchitis, rheumatism, skin disorders, internal and external trauma, toxic swelling, ulcers.	Antibacterial, antimalarial, antiplasmodial, anti- inflammatory, antiatherosclerotic, antidiabetic, hypolipidaemic, antifungal.

Blank spaces= no available information.

Classification of *Plumbago auriculata* (Fonden and Potter, 2015).

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Caryophyllales

Genus: *Plumbago*

Species: *Plumbago auriculata* Lam.

Common names: Cape *Plumbago*, Cape Leadwort, Blue *Plumbago*.

Morphology

Plumbago auriculata is a perennial, bushy evergreen shrub (Figure 2a), up to 3m high with erect, climbing or trailing stems that are glabrous below becoming pubescent above (Batten, 1986). The leaves are simple, elliptic to obovate, slightly discolorous, greyish green beneath and often with whitish scales seemingly for light reflection (Aubrey, 2001). Leaves are thin in texture (Figure 2b), having miniscule glandular dots, with a winged petiole at the base and auriculate. The glands found on the both surface of the leaves are reported to be salt glands (Wilson, 1890; Sakai, 1974). *P. auriculata* is covered with trusses of pale, sky blue flowers, however, there are variations of deep blue or white flowers (Figure 2b) (Batten, 1986; Ferrero et al., 2009). Flowers are salver-shaped (Figure 2d), actinomorphic and grouped in terminal inflorescences 2.5-3 cm long and flowering all year round (Luteyn, 1990; Aubrey, 2001; Ferrero et al., 2009). Corolla is pale blue with the tube twice or more than twice the length of the calyx. Glandular and non-glandular hairs, often known as trichomes, are found on the calyx (Figure 2c). Stamens are free from the corolla, included or exerted and style exerted with 5 linear stigmas and superior 1-celled ovary. Fruit is a capsule, long-beaked, the valves coherent at the apex. The seed one, dark brown or black, oblong 7mm long and slightly flattened (Luteyn, 1990; Aubrey, 2001).

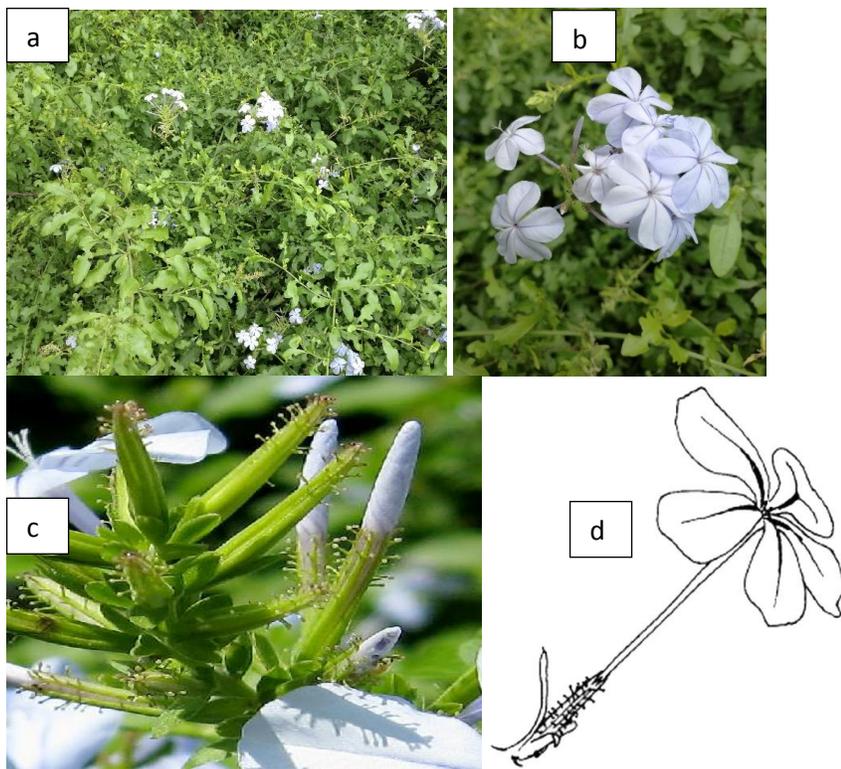


Figure 2: *Plumbago auriculata*: a) Bushy evergreen shrub image captured at the University of Kwazulu-Natal, Westville, Durban, South Africa; b) Variation of pale-blue flower and leaves (UKZN); c) Calyx showing trichomes; d) Illustration of flower showing calyx with trichomes (After Batten, 1986; ICPS, 1986).

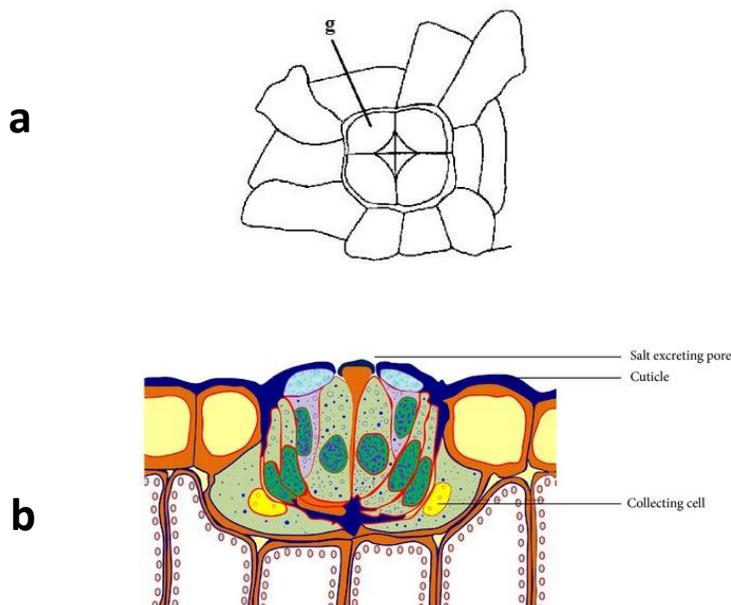
Secretory Structures

Secretory structures are specialized plant structures associated with the release of substances produced in the cytoplasm and moved outside the cell (Cutter, 1978). Secretory structures appear in the form of trichomes, resin ducts, salt glands, idioblasts, laticifers, colleters and nectaries located in various parts of the plant (Thomas, 1991). It has been reported that the primary function of secretory structures is related to defense responses against both herbivores and pathogens. (Fahn, 1988; Lange, 2015). These structures have the ability to produce or sequester secondary (or specialized) metabolites. Literature reports that salt glands occur sunken in the epidermis of leaves and are associated with ion secretion whereas trichoms protrude from the surface of leaves or stems and are often responsible for the secretion of exudates (Cutter, 1978, Fahn, 1988; Naidoo and Naidoo, 1998). Although some researchers report salt glands to be specialized trichomes, based on available literature for the purpose of this review salt glands and trichomes are classified independently as secretory structures in Plumbaginaceae (Metcalfe and Chalk, 1972; Faraday and Thomson, 1986b, Grigore and Toma, 2016).

Salt glands

A major constraint to plant growth is salinity, which causes plants to adapt to saline environments via specialized epidermal structures known as salt glands (Kobayashi, 2008). Salt glands are highly specialized organs consisting of several cells intended to excrete salt or shift ions from mesophyll tissue to the leaf surfaces where a layer of salt crystals is formed (Figure 3b). This serves an important role in regulating salt concentration in plant tissue (Fahn, 1988; Kobayashi, 2008; Hasanuzzaman et al., 2014). Salt glands are known to secrete a wide variety of ions, especially metal ions which contribute to metal tolerance in plants once these metal ions are eliminated (Fahn, 1988, Salama et al., 1999). The most commonly secreted ions via salt glands in flowering plants are sodium, potassium, calcium and magnesium (Kobayashi, 2008). These glands also secrete other salts in addition to sodium chloride that has a composition related to that of the root environment (Storey and Thomson, 1994). Salt glands occur in halophytic species, which include several families that are not taxonomically related, thus, providing a clear example of convergent evolution on a common adaptive device (Fahn, 1988; Naidoo and Naidoo, 1998). Salt glands occur on any aerial organ of the plant but are most abundant on leaves and are inserted in the epidermis (Fahn, 1988).

Salt glands function to eliminate salts to the outside of the plant or within the plant into vacuoles (Thomson, 1975). Structurally there are three types of salt glands described (Thomson, 1975); the bicellular glands of the grasses, the bladder cells of the Oxalidaceae, Chenopodiaceae and Mesembryanthemaceae and the multicellular gland found only in dicotyledons. Regardless of the fact that the structure of salt glands varies greatly among different species, it is, very similar in plants within the same family or genus (Salama et al., 1999). Salt glands of the family Plumbaginaceae are multicellular and sunken in the leaf epidermal cells, consisting of basal and secretory cells with the cells varying in number from six up to forty (Thomson, 1975, Faraday and Thomson, 1986b). Glands were first described in the 1800's and were referred to as chalk glands due to the presence of insoluble carbonate salts found above the gland on the surfaces of stems and leaves (Grigore and Toma, 2016). However, when it was later discovered that the glands of some species secrete sodium chloride (NaCl) these glands were then referred to as salt glands (Faraday and Thomson, 1986a; Grigore and Toma, 2016). The salt glands (Mettenius glands or Licopoli glands), as described by Grigore and Toma (2016), based on historical facts by Metcalfe and Chalk (1972), occur inside the cavities on the inner side of stems and leaves, sometimes surrounded by simple hairs or groups of elongated cells. The glands are made up of 4 to 8 epidermal cells arranged in palisade surrounded by accessory cells made up of one or two layers (Figure 3a). Cutinized walls exist between the secreting cells and the accessory cells. The salt-secreting glands of the species within Plumbaginaceae are similar in ultrastructure and morphology and the primary pathways and basic mechanisms of salt movement through the glands is the same in all species of the family (Figure 3c) (Faraday and Thomson, 1986a). These glands are able to secrete a wide variety of ions and the secretions are similar in species of the family (Storey and Thomson, 1994). Salt glands are common in the family Plumbaginaceae and sometimes these glands secrete mucilage in addition to calcium carbonate (Fahn, 1988; Grigore and Toma, 2016). Salt glands of *P. auriculata* are found on the epidermal axials of the leaves and are exo-recreto (excrete salt that form crystals on leaf surfaces) (Ceccoli et al., 2015).



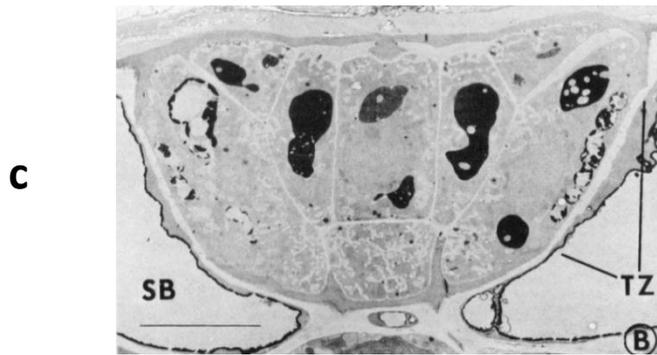
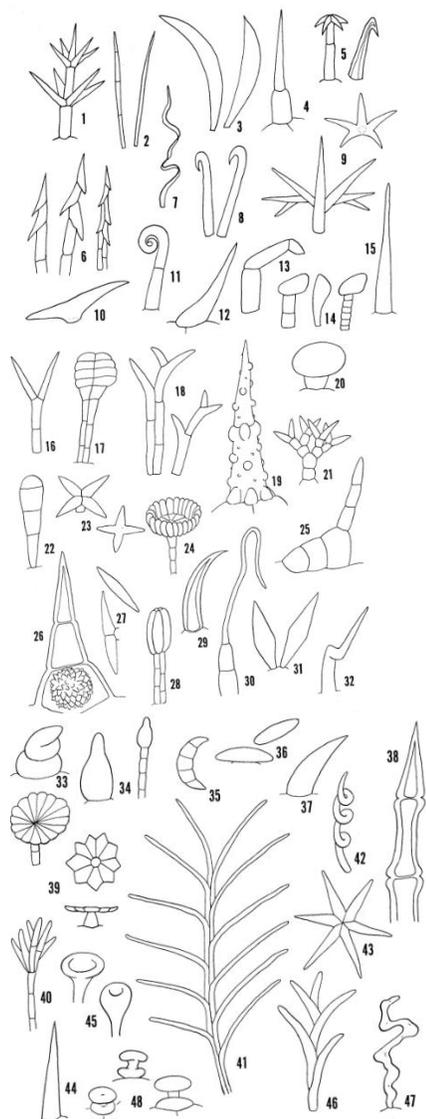


Figure 3: Structure of salt glands in various species: a) Salt glands (g) in the leaf of *Plumbago europaea* (After Grigore and Toma, 2016); b) Cross section of a generalized salt gland (After Hasanuzzaman et al., 2014); c) Electron micrograph showing the ultrastructure of an almost median longitudinal section from a secreting *Plumbago auriculata* salt gland (After Faraday and Thomson, 1986a); TZ-Transfusion zone; SB- subbasal cell; Scale bar 80 μ m.

Trichomes

Trichomes are often referred to as epidermal appendages that can either be singular or multicellular, developing outwards on the surface of plant organs (Payne, 1978; Fahn, 1988). Their morphology varies greatly among tissue and species and according to botanical literature there are over 300 descriptions to characterize various morphological types, outlining a few in (Figure 4) (Payne, 1978; Wagner, 1991). Trichomes occur in all major groups of terrestrial plants and are needed to carry out the following functions: light reflectance; reduction of water loss through transpiration; thermoregulation; herbivory; defence against radiation, pathogen attack. However, the role of trichomes can be considered species specific (Wagner, 1991; Bauer et al., 2015). As outlined by Payne (1978), trichomes can be either glandular or non-glandular and are often found occurring on different parts of the plant. Glandular trichomes are the primary secretory structures in most flowering plants and distributed over the vegetative aerial part of plants (Kaya et al., 2006). They vary in their structure, function and location as well as chemical composition in the substances they secrete (Payne, 1978; Rusydi et al., 2013). Morphologically, glandular trichomes are classified as either peltate or capitate and generally consist of a foot, a stalk that is usually unicellular or bicellular and a secretory head (Payne, 1978; Ascensao et al., 1997; Salmaki et al., 2009). Capitate trichomes vary widely in stalk length, head shape and secretion type but a general rule is that the stalk length measures more than half the height of the head whereas peltate trichomes are usually made up of a short wide stalk, several head cells and one basal epidermal cell (Ascensao and Pais, 1998; Kaya et al., 2006). Capitate trichomes secrete a small amount of essential oils and some polysaccharides and these exudates are mostly excreted to the surrounding environment via pores in the cuticle of the head cell (Kamatou et al., 2007). The peltate trichomes are most important for essential oil production because most of the secretory cells are in the head and the exudates are stored in the subcuticular spaces between the head cell walls and the cuticle. They function as storage structures for the secreted exudates (Salmaki et al., 2009).

Non-glandular trichomes are also found occurring on the aerial vegetative parts of the plant as well as within plant tissue and can be described as follows (Fahn, 1988; Maclachlan and Carlquist, 1992): a) simple unicellular or multicellular, non flattened hairs; b) squamiform hairs which are conspicuously flattened, multicellular hairs. These trichomes are termed 'scales' if they are sessile or peltate hairs if they are stalked; c) branched, multicellular hairs which may be stellate; d) shaggy hairs which consist of a base and two or more contiguous rows of cells. The function and location of non-glandular trichomes are species-specific. Non-glandular trichomes of some *Kalanchoe* spp demonstrated how diverse these type of trichomes are in terms of length, number of cells in the stalk, shape of the upper part, density of occurrence, occurrence of wax on their surface and cuticle ornamentation (Weryszko-Chmielewska and Chernetsky, 2005). Trichome viability also acts as a determinant for the function of the trichome i.e. dead non-glandular trichomes found on the surface of *Kalanchoe* leaves provide a protective structure to the leaves enabling surface moisture. The function of trichomes is dependent on the species as well as the environment.



1. Abietiform; candelabra; orthocladous.
2. Acerate; acicular.
3. Acinaciform.
4. Acuminate terminal cell; belemoid.
5. Anchor hairs, uncinata and with terminal fluke cells; barbed.
6. Ancistrus; anchor; barbed; glochids.
7. Aduncate; anfractuose; curly; ribbon-hair; serpentine.
8. Angler; hamate; hooked; uncinata.
9. Antler; pedate (sometimes termed stellate).
10. Anvil; malpighiaceus.
11. Apicirrinatus; circinate.
12. Arrect.
13. Arthrocladous.
14. Asciform; hatchet-shaped.
15. Attenuate.
16. Bifid; dichotomous; forked; furcate.
17. Biseriate; colleter; gland.
18. Bootjack.
19. Bosselated; nodose; postulate.
20. Brevicollate.
21. Brevifurcate.
22. Clavate.
23. Cruciate; stellate.
24. Cup-shaped.
25. Cushion hair.
26. Cystolith hair (with cystolith in basal cell)
27. Dolabrate.
28. Doliform head of colleter.
29. Falcate.
30. Flagelliform; whip-like.
31. Fusiform.
32. Geniculate.
33. Heliciform; snail-shaped.
34. Lageniform; pulvinate.
35. Lunate; solenoid.
36. Limaciform.
37. Ornithorhynchous.
38. Osteolate cells of uniseriate hair.
39. Peltate.
40. Penicillate.
41. Plumose.
42. Spiral.
43. Stellate.
44. Subulate.
45. Surculate.
46. Sympodial.
47. Torulose.
48. Trochlear; trochleariform.

Figure 4: Trichome types (After Payne, 1978)

The calyx of *P. auriculata* bear large mucilage secreting trichomes (Figure 5a), which resemble those of the genus *Drosera* (Droseraceae) and *Drosophyllum* (Droserophyllaceae) that also occur in the order Caryophyllales (Stoltzfus et al., 2002; Panicker and Haridasan, 2016). The trichomes on the calyx can also be termed “colleters” meaning it secretes a sticky substance and usually consist of a multicellular stalk and head. However due to its close resemblance to *Drosera* the trichomes can also be considered as digestive glands due to their function in insect entrapment (Figure 5b) (Fahn, 1952). Naidoo and Heneidak (2013), studied the glandular hairs of *Drosera capensis* L. and reported that the leaves are characterized by eight types of glandular hairs with a red-coloured stalk and stalk head making it more attractive to insects. These trichomes function to capture and entrap insects, absorb nutrients, produce mucilage and digestive enzymes, as well as secrete proteases in response to stimulation by certain salts (Stoltzfus et al., 2002; Naidoo and Heneidak, 2013; Bauer et al., 2015). Trichomes of Droseraceae were also found to exhibit phosphatase activity in the external glands (Plachno et al., 2006). Studies by Rashmievitz and Joel (1976), mentioned in Stoltzfus et al. (2002), that trichomes of *Plumbago* have a resinous secretion and showed a positive response when stained with Sudan IV, therefore proving it has a substantial hydrophobic constituent. It was reported that trichomes of the genus *Plumbago* exclude crawling insects from the flowers, therefore favouring flying insects for cross pollination (Jose et al., 2014). Trichomes of *P. auriculata* produce a sticky transparent exudate which traps winged insects which resembles a cobweb. The exudate is known to turn brown upon maturity of the plant in *Plumbago* species, however nothing has been reported with regards to *P. auriculata*. (Panicker and Haridasan, 2016). Non-glandular trichomes are also found on the calyx of *P. auriculata* (Figure 5c), but the function of the

trichomes is unknown in this species. However, it has been reported in other species that these trichome types provide shade to the plant and serve as a mechanical barrier to prevent insects from piercing the leaf (Gonzales et al., 2008).

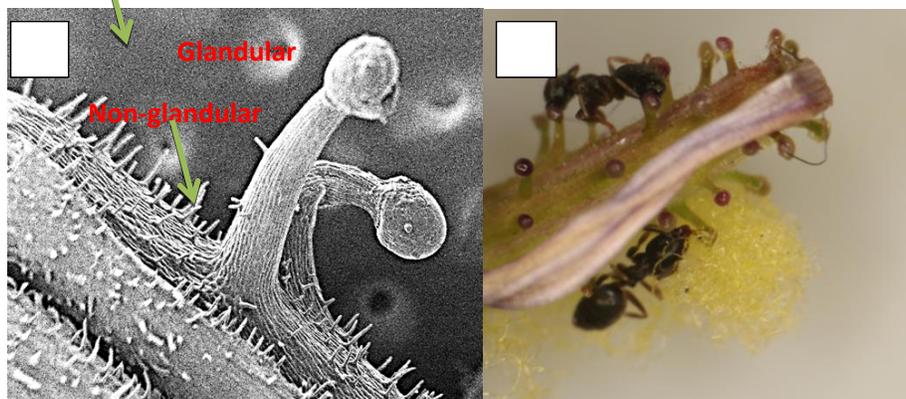


Figure 5: Types of trichomes on the calyx of *Plumbago auriculata*: a) Scanning electron microscopy (SEM) micrograph of glandular trichomes and non-glandular trichomes; b) SEM micrograph of an ant captured on calyx. Scale bar 100 μ m.

Chemical Composition

Plants are of great importance to humans for use as ornaments, food preparations and medicines.. The use of herbal medicines has always been in great demand (Balunas and Kinghorn, 2005). There is an intense interest in medicinal plants in which phytochemical constituents can have long-term health promoting properties (Kennedy and Wigthman, 2011). Plants naturally produce secondary metabolites, commonly referred to as phytochemicals or biologically active compounds that exhibit a holistic healing approach (Kennedy and Wigthman, 2011). These include alkaloids, saponins, tannins, glycosides, flavonoids, terpenoids, naphthaquinones, carbohydrates, proteins, phenolic compounds, fixed oils and fats (Katsoulis et al., 2000; Pourmorad et al., 2006). Alkaloids and phenolic compounds are often the two major phytochemical compounds that are of medicinal importance. A wide range of medicinal properties of *P. auriculata* are attributed to plumbagin and other secondary metabolites.

Phytochemical Screening

Methanolic leaf extract of *P. auriculata* revealed the presence of the following phytochemicals- tannins, flavonoids, phenols, alkaloids, saponins, proteins, and carbohydrates with phenols being the most abundant compound (Lakshmanan et al., 2016). Aerial parts of *P. auriculata* extracted in the following solvents: acetone, chloroform, petroleum ether, ethanol and ethyl acetate showed positive results when tested for steroids, carbohydrates, phenolics, tannins, saponins, flavonoids and terpenoids, however the aqueous extract only showed positive for the presence of tannins (Tharmaraj and Antonyamy, 2013). It can be concluded that aqueous extractions are not as effective as other solvents for phytochemical screening. Systematic fractionation and phtochemical examination of the methanolic root extract of

Bioactive Compounds

P. auriculata revealed the presence of the following compounds: α -amyrin, capensisone, α -amyrin acetate, isoshinanolene, β -sitosterol, diomuscinone, plumbagin and β -sitosterol-3 β -glucoside (Table 2)(Padhye et al., 2010; Ariyanathan et al., 2011; Saeidnia et al., 2014; Khan and Hossain, 2015). Capensisone is a novel quinone that was first to be characterized from the roots of *P. capensis* and, in their study, isoshinanolone and diomuscinone were reported for the first time for the genus *Plumbago* (Ariyanathan et al., 2011).

Table 2: Bioactive compounds reported in plant extracts of *Plumbago auriculata*

Compounds	Medicinal/ pharmacological uses
Plumbagin	Anticancer, antifungal, anti-inflammatory, antibacterial, antifertility, antimalarial, antidiabetic and antioxidant properties.
α -amyrin and α -amyrin acetate	
capensisone	
isoshinanolene	Antibacterial and antifungal properties.
β -sitosterol (Beta-sitosterol)	Heart disease and high cholesterol, gallstones, common

	cold and flu, rheumatoid arthritis, asthma, migraine headaches, menopause symptoms, enlarged prostate, chronic fatigue syndrome, cervical cancer. It also boosts the immune system and prevents colon cancer as well as enhances sexual activity.
β -sitosterol-glucoside	Antioxidant, hypertension and antidiabetic properties (used to control menstrual bleeding).
diomuscinone	Wound healing, antimicrobial and anti-inflammatory properties.

Plumbagin

In the genus *Plumbago*, a highly potent and broad spectrum biological compound known as plumbagin is commonly found (Tyagi and Menghani, 2014). Plumbagin is a 5-hydroxy-2-methyl-1,4-naphthoquinone-(C₁₁H₈O₃) (Figure 7) present in the roots, stems and leaves of various *Plumbago* species; with the roots predominantly having the highest concentration of plumbagin (Padhye et al., 2010). Plumbagin is naturally a yellow pigment that was first isolated in 1829 via solvent extraction of powdered plant material and has since been of interest for research due to its high medicinal value (Tyagi and Menghani, 2014). Plumbagin is soluble in a variety of solvents such as acetone, benzene, alcohol, methanol, acetic acid and chloroform. Plumbagin exhibits anticancer, antifungal, anti-inflammatory, antibacterial, antifertility, antimalarial, antidiabetic and antioxidant properties (Mallavadhani et al., 2002; Checker et al., 2009; Padhye et al., 2010; Jose et al., 2014). It is also reported to have other therapeutic properties such as stimulant action on the intestine, nervous system and heart as well as rheumatic pain relief (Galal et al., 2012). All parts of *P. auriculata* contain plumbagin with the highest amount of plumbagin accumulating in the leaves and stems of the plant in comparison to *P. zeylanica* and *P. rosea* (Jose et al., 2014). This shows that different species accumulate plumbagin in different parts of the plant.

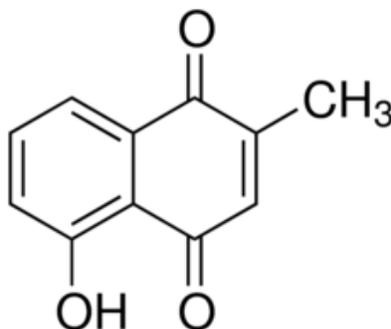


Figure 6: Structure of Plumbagin (After Padhye et al., 2010).

Mode of Action of Plumbagin

Anticancer activities

Plumbagin displays anticancer activities over a wide range of tumors such as breast cancer, ovarian cancer, prostate cancer, pancreatic cancer, lung cancer, skin cancer, leukemia, liver cancer, renal cancer, cervical cancer. Studies have revealed that plumbagin is an effective inhibitor of cell growth and when administered in combination with radiotherapy plumbagin has the ability to augment cell growth inhibition very effectively in comparison to a higher dose of radiation alone. (Padhye et al., 2010). Extensive publications exist on the anticancer effect of plumbagin outlined in Padhye et al (2010).

Antifungal activities

The findings of the antifungal activity of plumbagin by Dzoyem et al. (2007) suggested that the naphthoquinone delayed germination of the fungus and was capable of inhibiting growth when administered at higher concentrations.

Anti-inflammatory

Plumbagin has been reported to be a topoisomerase-II inhibitor by displaying inhibitory activities of corresponding enzymes involved in rheumatoid arthritis (RA) (Jackson et al., 2008). Checker et al (2009) reported that plumbagin suppresses NF- κ B (ubiquitous transcription factor) activation in tumor cells, and hence might have an effect on biological functions of leukocytes actively participating in various immune responses.

Antibacterial activity

Extensive publications reported the antibacterial activity of plumbagin as outlined by Padye et al (2010). However to summarize these findings, plumbagin displays potent antibacterial activity against a range of bacterial strains such as: *Staphylococcus aureus*, *Myobacterium smegmatis* and various *Myobacterium tuberculosis* strains and *Escherichia coli*. However, plumbagin does display cytotoxic activity and needs to be administered at the appropriate concentration.

Antifertility activity

Plumbagin has been reported to be an irritant to the smooth muscle of the uterus when administered orally at high concentrations. Therefore it needs to be administered with caution. Madhavan et al (2009) reported that antifertility effects were found to be effective with doses of 1 mg/100 gm of body weight in female rats. Plumbagin also displays a significant effect on estrogen cycle and hormonal levels of female rats.

Antimalarial activity

Antimalarial activity of plumbagin was reported by Sumsakul et al (2014) by investigating In vitro antimalarial activity of plumbagin against K1 and 3D7 Plasmodium falciparum and In vivo antimalarial activity in Plasmodium berghei-infected mouse model (a 4-day suppressive test). Their study concluded that plumbagin exhibited efficient antimalarial activity in both In vivo and In vitro experiments and displayed a fairly low toxicity at the dose levels up to 100 (single oral dose) and 25 (daily doses for 14 days) mg/kg body weight for acute and subacute toxicity.

Antidiabetic

It was reported that orally administered plumbagin significantly reduced the blood glucose levels and altered all other biochemical parameters to near normal. Further, it increased the activity of hexokinase and decreased the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase in diabetic rats. Plumbagin also enhanced GLUT4 mRNA and protein expression. It also contributed to glucose homeostasis. (Sunil et al., 2012).

Antioxidant

Plumbagin contributes to EGFR activation in ROS-related mechanisms (Padhye et al., 2010). It was also found to significantly reduce the catechol-induced DNA damage, and inhibit ascorbate and NADPH-dependent lipid peroxidation against mouse lymphoma cells (Demma et al., 2009).

Medicinal Uses and Pharmacology of *Plumbago auriculata* Extracts

Plumbago auriculata has a strong medicinal value and for many years has been used in the traditional medicinal market as an alternate remedy for the prevention and control of certain human ailments. All parts of the plant are reported to have therapeutic properties for example, the leaves and roots have cardiotoxic, neuroprotective, anti-atherogenic and hepatoprotective properties (Deshpande et al., 2014). Roots and leaves are also reported to be used for warts, as emetics, fractures, oedema, headaches, skin lesions, piles, rheumatism, diarrhoea and malaria (Elgorashi et al., 2003; Chen and Gao, 2013). In Mozambique where malaria is endemic, aerial parts of *P. auriculata* are used for the treatment of symptoms associated with malaria such as vomiting and fever (Ramalhete et al., 2008). In Manchale, a rural village in India, it was reported that herbal healers use the root of the plant for the treatment of piles by forming a paste with water and applying directly (Poornima et al., 2012). Certain indian tribes also make use of the root extract by preparing fresh roots in rainwater and drinking for a week to combat acidity (Jain et al., 2010). Plants are not only used for the treatment and control of human ailments but it has been reported that plant extracts can also be used in ethnoveterinary as low-cost treatments for diseases of livestock and other animals (McGaw and Eloff, 2008). Root extracts of *P. auriculata* are used in as a treatment for diarrhoea in cows (Dold and Cocks, 2001). A fair amount of studies have been conducted to isolate and evaluate the pharmacological activities of the extracts of *P. auriculata*.

Antiulcer Activity

Different species of *Plumbago* plants were collected from different regions of Kerala, India. Roots of plants were dried and coarsely powdered, soaked in limewater and then extracted using ethanol as a solvent (Ittiyavirah and Paul, 2016). Goat intestines were used to test for antiulcer activity of the ethanolic root extracts by treating small pieces of the intestines (1.5-2 cm long) with 1ml *P.auriculata* extract. The results of this study reported that *P. auriculata* showed

significant acid neutralizing capacities as well as excellent antioxidant properties observed from the DPPH assay. The use of goat intestine can be used as a standard test for in vitro antiulcer activities of plant extracts.

Antifungal Activity

Fresh plant material of *P. auriculata* such as roots, leaves, stems and flowers were extracted using distilled water as a solvent (Rajasekaran et al., 2015). Each extract was then mixed with a 1mM silver nitrate solution in a 1:10 ratio to form a silver nanoparticle. Only the root, flower and stem extracts were effective in the synthesis of silver nanoparticles and were further used in the application of the newly synthesized nanoparticles for antifungal activity. Antifungal activity was tested by spore germination assay on the following fungi: *Aspergillus fumigatus*, *Fusarium oxysporium*, *Aspergillus flavus*, *Curvularia lunata* and *Trichoderma* sp. Results of this study reported that the efficacy of the particle reflects on its size i.e. bigger particles were effective on larger spore such as *Curvularia lunata* and ineffective against smaller spore bearing fungi (Rajasekaran et al., 2015)

Antibacterial Activity

Roots of *P. auriculata* were dried and ground to a fine powder and, active components were extracted using the following solvents water, chloroform, methanol and ethanol (Muringani and Makwikwi, 2017). Antibacterial activity was tested using the Kirby-Bauer disc diffusion method. Bacterial strains used were about 150 *E.coli* isolated from water and patient stool samples from the Mthata Region, Eastern Cape, South Africa. The results of this study reported that the ethanolic root extract showed the highest rate of activity against all the examined strains of the *E. coli* samples. Antibacterial activity of aerial parts of *Plumbago* species have also been reported. Aerial parts of the plants were dried and extracted using various solvents and antibacterial assay was determined using the agar-well diffusion method (Tharmaraj and Antonysamy, 2015). The following bacterial strains used were *Streptococcus pyrogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Morganella morganii* and *Bacillus subtilis* and antibiotic amikacin (30 µg/ disc) was used as a standard to compare its effect on the bacterial strains with the plant extracts. The results of the study for *P.auriculata* reported that the ethanolic extract showed the highest activity (70%) and the highest zone of inhibition (23 ± 0.3 mm), followed by petroleum ether and chloroform extracts. The ethanolic extract of *P. auriculata* showed above 50% activity against Gram positive bacteria and 26% against Gram negative bacteria. The overall results of the study showed that *P. zeylanica* and *P. rosea* had the highest activity against Gram positive bacteria whilst *P. auriculata* had the least activity and the antibacterial potentials of *Plumbago* species is probably due to the presence of alkaloids and phenolic compounds (Tharmaraj and Antonysamy, 2015).

Plasmid-mediated multiple drug resistance is a serious and emerging problem in the treatment of infectious diseases because bacteria have become resistant to most of the drugs available. Scientists have developed a way to combat this problem by combining herbal extracts with antibiotics to inhibit the development and spread of R-plasmids (Patwardhan et al., 2015). *P. auriculata* root extracts were used to cure plasmid-mediated antibiotic resistance, The plasmids curing activity of the root extract was determined by growing *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumonia*, in the root extract. Ethanol root extract demonstrated the highest antibacterial activity as well as the maximum plasmid curing activity (13-32%) compared to chloroform, acetone or petroleum ether root extracts. *P. auriculata* root extracts can be used as plasmid curing agents in the treatment of nosocomial infections.

Cytotoxic activity

Gastric ulcer is one of the most common gastrointestinal disorders in recent times (Paul et al., 2013). Anti-*Helicobacter* and cytotoxic activity of detoxified ethanol root extracts of *P. auriculata* were assessed by preparing the roots in lime water and extracting them using ethanol as a solvent (Paul et al., 2013). Cell viability was assessed by Microculture tetrazolium (MTT) Assay in the presence and absence of different plant extracts. The study reported that the ethanol root extract has possible activity against *H.pylori*, cytotoxicity with MTT assay HGE-17 cell lines and the zone of inhibition test of *P. auriculata* ethanol root extracts showed significant activity. Paul et al. (2013), also revealed in their study that *Plumbago* species show cytotoxic activity, even in the absence of bioactive plumbagin.

Methanolic leaf extracts also showed significant cytotoxic activity when assayed against human lung cancer (A549) and ovarian cancer (PAI) cell lines using MTT assay (Lakshmanan et al., 2016). For A549, it showed a minimum cytotoxic activity at 45µg/ml and 10µg/ml cell line and for PAI 10µg/ml and 60µg/ml cell line at 24 hours and 48 hours, respectively.

Anticancer Activity

Cancer is a common, most dreadful life threatening disease reported worldwide and is often difficult to control and cure (Balunas and Kinghorn, 2005). Bioactive compounds extracted from plants are being extensively researched and utilized as a possible anticancer agent. *In vitro* *P. auriculata* leaves were dried and extracted using methanol as a solvent

(Lakshmanan *et al.* 2016). The leaf extract was tested against ovarian (PAI), lung (A549) and malignancy cell lines for apoptotic and anti-proliferative activities. The GC-MC analysis of the methanolic extract revealed the presence of the compound sitosterol, hence showing good anticancer activity at minimal concentration 10-40µg. The study was successful in reporting that *P. auriculata* constitutes novel anticancer compounds.

Toxicity

Bioactive compounds found in plants often have many therapeutic uses and are of high medicinal value. However, these compounds can exhibit toxic effects which can result in detrimental side-effects. Plumbagin, often referred to as the marker compound in the genus *Plumbago* is also toxic, as it generates superoxide anion reactive oxygen species that can damage various biomolecules (Jose *et al.*, 2014). It is a powerful irritant in low doses that inhibits cell mitosis and, in higher than recommended doses, it can cause death from respiratory failure, paralysis as well as nucleotoxic and cytotoxic effects. Other commonly known side effects of plumbagin include skin rashes, diarrhoea, increase in neutrophil counts and white blood cells, hepatic toxicity and increase in acid phosphatase and serum phosphatase levels (Padhye *et al.*, 2010). *P. auriculata* is assigned to toxicity class 4 meaning the juice sap or thorns of the plant can have adverse effects on the skin, resulting in dermatitis (Stone and King, 1997). It has been reported that traditional tribes in India that make use of roasted *P. auriculata* roots for wound healing use very small quantities and for a limited time period because prolonged exposure and large quantities can lead to death by irritation (Jain *et al.*, 2010).

Propagation Techniques

Plumbago auriculata makes a good ornamental plant that can be planted indoor or outdoors. Plant management is quite simple because of tolerance to high humidity, high temperatures and diseases and even though the plant secretes salt it is not limited to saline conditions (Joy *et al.*, 1998; Aubrey, 2001). The common propagation method of this species is seed sowing. However, seed germination rate is very low and seed prices are often too expensive (Batten, 1986; Chen and Gao, 2013). Due to its high medicinal value, the conventional method of propagating *P. auriculata* is often difficult and cannot meet the growing demand for the plant in the traditional medicine and pharmaceutical markets due to poor germination and death of young seedlings under natural conditions. Therefore, researchers have developed plant tissue-culture as a method to produce seedlings which have certain advantages over conventional propagation methods. Chen and Gao (2013), micropropagated 1 year old plants of *P.auriculata* using the nodals and leaves. They concluded that young nodals are best to use as explants because leaves have a high differentiation grade and the ability of regeneration is weak. Deshpande *et al.* (2014), reported that *P.auriculata* increased callus *in vitro* mass production by using various growth hormones such as indoleacetic acid (IAA), 1-Naphthaleneacetic acid (NAA) and Indole-3-butyric acid (IBA). IAA is a simple compound (auxin) that assists in plant growth and development (Davies, 2004). IBA is most commonly used commercially for plant propagation due to its efficacy to stimulate adventitious root growth and is often more stable than IAA against *in vivo* catabolism (Davies, 2004). NAA is a synthetic plant hormone widely used in agriculture, horticulture and plant tissue culture to increase cellulose fiber formation (Davies, 2004). However, it is toxic to the plant in high concentrations. Growth hormone NAA exhibited higher production of callus formation in all the explants; combination of hormones IBA and NAA showed best callusing from leaf explants. Combinations of BAP and NAA showed best callusing from stem and shoot apex explants. BAP are synthetic cytokinins used in tissue culture to promote cell division, bud formation and stem branching (Zhang *et al.*, 2005; Madhavam *et al.*, 2009). It has been reported that this species can also be propagated vegetatively by placing 15cm long stem cuttings in polybags and applying IAA and IBA treatments to improve rooting (Joy *et al.*, 1998; Lakshmanan *et al.*, 2016).

In related studies plumbagin production was enhanced using root cultures of *Plumbago indica* L. through precursor feeding using L-alanine followed by in situ adsorption of plumbagin on the nonpolar copolymer adsorbent, styrene-divinylbenzene resin (Diaion® HP-20) (Jaisi and Panichayupakaranant, 2017) Roots were fed with L-alanine (concentration 5 mM) for 14 days followed by the sequential addition of Diaion® HP-20 (10 g L⁻¹) after 36 hours of L-alanine-feeding. Plumbagin production was significantly increased. Productivity levels obtained were higher than that achieved using untreated root cultures or L-alanine feeding alone. Jaisi and Panichayupakaranant (2017) concluded that their study suggests the use of precursor feeding in combination with in situ adsorption as an easy and cost effective tool for the commercial production of medicinally important bioactive compounds like plumbagin. Overall tissue culture is a beneficial propagation technique for increasing plumbagin production in *Plumbago* spp as well as the production of other medicinal plant compounds.

Conclusion

The genus *Plumbago* yields many medicinally important species throughout the world. There is a lack in knowledge on some species within the genus thus providing an opportunity for future research. This review clearly shows the importance of *Plumbago auriculata* as a useful medicinal plant and also its level of toxicity. This species is used throughout the world, however limited research exists in South Africa where it is native. While the morphology of

trichomes has been studied, the detailed trapping mechanisms of insects are still unknown. The structure and function of secretory structures occurring in this species are not well documented. Although much pharmacological studies have been conducted, studies on the secretory structures are limited to only a small number of publications dealing with secretory structures, especially the types of trichomes present and ultrastructure of the leaf glands. However, the nature of the secretions from these structures are unknown. Micropropagation results viewed in this study are aimed at encouraging and attracting researchers to promote rapid regeneration of *P. auriculata* to ensure easy availability of the plant for horticulture, medicinal and pharmacological uses. The use of plant hormones in tissue culture techniques proved to be effective in optimizing the production of medicinal plants, thus increasing the availability of bioactive compounds. Other techniques such as genetic engineering has not yet been explored for the genus and could prove useful in commercializing this medicinally important genus.

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