



Morphological and elemental analysis of multipurpose medicinal plant *Plumbago*

Smita Chaudhari

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

Abstract

Plumbago is multivalent medicinal plant in Ayurveda. Two species of *Plumbago* investigated during present work are *P. zeylanica* and *P. auriculata*. The objectives for the present work were to study external morphological features of *Plumbago* for identification and taxonomic significance and to investigate elemental composition of different parts of *Plumbago*. Morphological study revealed distinctive differences in morphological characteristics of vegetative as well as reproductive parts which are of taxonomic value and can be used in identification and authentication of both species of *Plumbago*. Nitrogen, phosphorus, potassium, sodium, calcium, magnesium, manganese, iron, copper, zinc, sulphur which are nutritionally important minerals were found in all parts of both plants. Hence, both the plants would be used as supplementary source of dietary minerals. Leaves of *P. auriculata* seem to be more nutritious because they contain maximum amount of nitrogen, sodium, calcium, magnesium, manganese, zinc and sulphur in the investigated plants.

Keywords: morphology, elemental analysis, *Plumbago zeylanica* linn, *Plumbago auriculata* lam

Introduction

Plumbago which is commonly called as 'leadwort' or 'Chitrak' belongs to family Plumbaginaceae. The paper presents morphological and elemental analysis of two species of *Plumbago* namely *P. zeylanica* and *P. auriculata*. *P. zeylanica* Linn. is very popular multivalent medicinal plant in Ayurveda because of its good medicinal potential while *P. auriculata* Lam. is ornamental plant with least medicinal use. The major block in use of herbal drugs is confusion in the identification of medicinal plants [1]. Hence proper identification and authentication of medicinal plant is important. According to Radford (1986) the morphological characters of plants are most frequently used in taxonomic studies because they are easily observable and obtainable. According to him external morphological evidence provides 'basic language for plant characterization, identification, classification and relationship'. Angiosperms are identified by morphological characters. According to Ogura (1964), Holttum (1968), Cronquist (1975) morphology is closely related to taxonomy and would continue to prevail for many years to come [2].

Morphological characters of plants have contributed to the foundation and framework for taxonomy. Both vegetative and floral characters are used in taxonomy. But in general floral characters have been preferred over vegetative characters because vegetative characters are often environmentally controlled while reproductive features are conservative against fluctuating environmental pressures. But reproductive characters are subjected to much lesser degree of evolutionary pressure than vegetative parts because sexual reproductive parts are produced only for a brief period in most species. According to Clifford and Lavarack (1974) reproductive characters are liable to strong selection pressures originating from various aspects of reproductive biology e.g. the pollinator relationship. Hence both vegetative and reproductive characters are equally important as both are subject to different types of selection pressures. Vegetative morphological characters used in

taxonomy are growth habit, growth pattern, seedling characters, characters of underground part, leaf characters, pubescence etc. Reproductive morphological characters used in taxonomy are inflorescence, flower, fruits and seed [3]. Morphology of *P. zeylanica* plant is studied extensively by many previous workers because of its good medicinal potential.

Inorganic components of food that leave ash as residue when burned are called minerals [4]. Plants form a major portion of the diet and hence their nutritional value is very important. The inorganic elements are of immense importance in the biological world. The inorganic elements in plant have very significant role in human and livestock nourishment [5].

Twenty six different elements are normally present in human body. They are divided into 3 groups - major elements, lesser elements and trace elements [6]. Minerals have various functions in human body. Abundant research work has been carried out on the organic constituents of medicinal plants but relatively little attention has been paid on the inorganic elements in medicinal plants.

The objectives for the present work were- 1. To examine external morphological features of *Plumbago* for identification and taxonomic utility 2. To investigate elemental composition of different parts of *Plumbago*. Vagbhata and Sushrut have recommended Chitrak as *Rasayana* or rejuvenator [7, 8]. It is also recommended as *Katupaushatika* (Bitter tonic) in Ayurveda [7, 8, 9, 10, 11]. Hence current study has been undertaken to investigate elements present in different parts of plant *Plumbago* which will validate its medicinal as well as nutritional value.

Materials and Methods

P. zeylanica Linn. and *P. auriculata* Lam were collected from botanical garden of Chaitanya Ayurved Mahavidyalaya, Sakegaon and Ratnakar Nursery, Jalgaon respectively. Taxonomist Dr. G. S. Chaudhari identified and authenticated both the plants. The external morphological

details were studied using fresh material. Macroscopic studies of root, stem and leaf were done by naked eye. Detailed study of flowers was done by observing dissected floral parts.

Fresh plant parts were collected, washed thoroughly with water and then shade dried. Dried plant parts were pulverized separately using electric grinder. The pulverized plant parts were analysed in Jain Research and Development

Laboratory, Jain Irrigation Systems Limited, Jalgaon for elemental analysis.

Results and Discussion

Morphological Analysis

Two species of *Plumbago* have distinct morphological characters. Comparative morphological characters of both species are summarised in Table 1 and 2.

Table 1: Comparative account of external morphology of leaf of two species of *Plumbago* Linn.

Sr. No.	Morphological characters	<i>Plumbago zeylanica</i>	<i>Plumbago auriculata</i>
1	Condition when observed	Fresh	Fresh
2	Type	Simple	Simple
3	Phyllotaxy	Alternate	Alternate
Size			
4	Length	3.8 cm to 17.5cm	3 cm to 9.5 cm
	Width	2.5 to 9 cm	1.2 cm to 3.2 cm
5	Stipules	Absent	Absent, auricles present
6	Shape	Ovate or elliptic	Elliptic or elliptic ovate or elliptic spatulate
7	Apex	Acute	Obtuse or acute
8	Margin	Entire	Entire
9	Venation	Unicostate reticulate	Unicostate reticulate
10	Base	Attenuate	Attenuate
11	Petiole	Leaf shortly and abruptly attenuated into long petiole (length 1-3 cm), petiole often dilated at base, petiole with incurved margin	Leaf attenuating into very short petiole with large auricles at the base
Colour			
12	Upper surface	Dark green	Grass green
	Lower surface	Grass green	Light grass green
13	Texture	Membranous	Membranous
14	Surface	Glabrous. Lower surface of mature leaf heavily encrusted with salt crystals which resembles powdery mildew disease	Glabrous. Lower surface of mature leaf heavily encrusted with salt crystals which resembles powdery mildew disease

Table 2: Comparative account of external morphological characters (except leaf) of two species of *Plumbago* Linn.

Sr. No.	Morphological characters	<i>Plumbago zeylanica</i>	<i>Plumbago auriculata</i>
1	Habit	Perennial, vigorous, evergreen, subscandent shrub	Perennial, less vigorous, evergreen, subscandent shrub
2	Height	Upto 9 feet	Upto 5.5 feet
3	Root	Stout, cylindrical, branched, have strong characteristic odour	Stout, cylindrical, branched, have strong characteristic odour
4	Stem	Erect or trailing, diffusely branched, glabrous, striate, jointed, zig zag, thicker, young stem show presence of purple coloured ring surrounding node	Erect or trailing, diffusely branched, glabrous, striations are observed on mature stem only, not jointed, straight, thinner, no purple coloured ring observed
5	Inflorescence	Simple spike or compound spike, peduncle or rachis with glands	Simple spike, peduncle without glands
6	Flower	Bracteate, sessile, complete, bisexual, pentamerous, hypogynous, white coloured	Bracteate, sessile, complete, bisexual, pentamerous, hypogynous, dimorphic, blue coloured
7	Calyx	5 sepals, gamosepalous tubular, ribbed, persistent, dark green, densely covered by stalked sticky glands throughout the length, calyx tube length 9-12 mm, calyx lobes minute triangular	5 sepals, gamosepalous tubular, ribbed, persistent, dark green, sparsely covered by stalked sticky glands in the upper part, calyx tube length 10-13 mm, calyx lobes minute triangular
8	Corolla	5 petals, gamopetalous, hypocrateriform, white coloured Corolla tube – slender, cylindrical, 20-22 mm long, mostly two times or less than two times the length of calyx tube Corolla lobe- obovate, twisted, acuminate apex, smaller in size (8-10 mm in length and 4-5 mm in width)	5 petals, gamopetalous, hypocrateriform, blue coloured Corolla tube – slender, cylindrical, 30-38 mm long, more than two times the length of calyx tube Corolla lobe- obovate, twisted, rounded apex, larger in size (11-15mm in length and 8-10 mm in width)
9	Androecium	5 stamens, all are of equal length, opposite to corolla lobe, stamen as long as corolla tube Anther – dorsifixed, dark purple	5 stamens, all of equal length, opposite to corolla lobe. In ‘pin’ morph flowers stamens inserted, in ‘thrum’ morph flowers stamens exerted Anther- dorsifixed, bluish
10	Gynocium	5 carpels, syncarpous Style- filiform with 5 stigmatic branches and protruding outward from corolla tube Ovary – superior, unilocular, with single basal ovule	5 carpels, syncarpous Style- filiform with 5 stigmatic branches. In ‘pin’ morph flowers style is long protruding outwards from corolla tube, in ‘thrum’ morph flowers style is short and remain included within corolla tube Ovary – superior, unilocular, with single basal ovule
11	Fruit	Capsule enclosed in persistent calyx, one seeded	Fruits not observed

Heterostyly is mentioned in *P. zeylanica* by Jose *et al* (2014) [12]. I examined flowers of *P. zeylanica* Linn. from different locations but did not find heterostyly. Heterostyly

was observed only in *P. auriculata*. Heterostyly (styles of different lengths) is one of the contrivances which promote cross pollination. It is characterized by reciprocity in sex

organ levels (reciprocal herkogamy). ‘Pin’ morph is long styled with stigma above anthers and a ‘Thrum’ morph is short styled with reverse positioning. According to Barnett *et al* (2000) heterostyly is known to occur in at least 28 families of flowering plants including Plumbaginaceae [13]. Kumar (1993) [14], Yadav and Sardesai (2002) [15], Tharmaraj

(2013) [16] reported fruit formation in *P. auriculata* Lam. But I did not observe fruit formation in *P. auriculata* Lam. Patil (2003) [17] also reported that fruits were not seen in *P. auriculata* Lam. Chen and Gao (2013) [18] reported low fruiting rate in *P. auriculata* Lam.

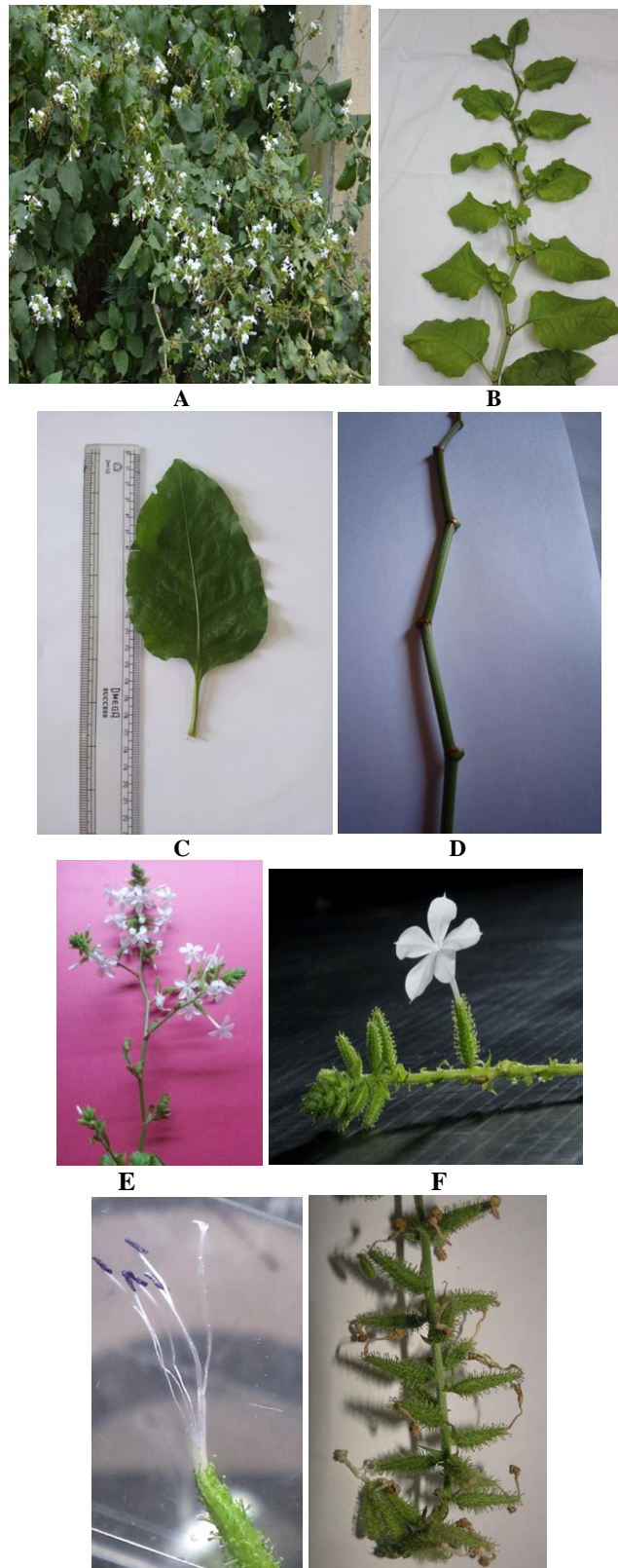


Fig 1: (A-H) Vegetative morphology of *P. zeylanica* Linn. A. Natural habit in flowering stage, B. Twig C. Single leaf, D. Zig zag young stem with striations and purple coloured ring around the node, E. Inflorescence, F. Single flower, G. Dissected androecium and gynoecium, H. Twig bearing fruits with persistent calyx.

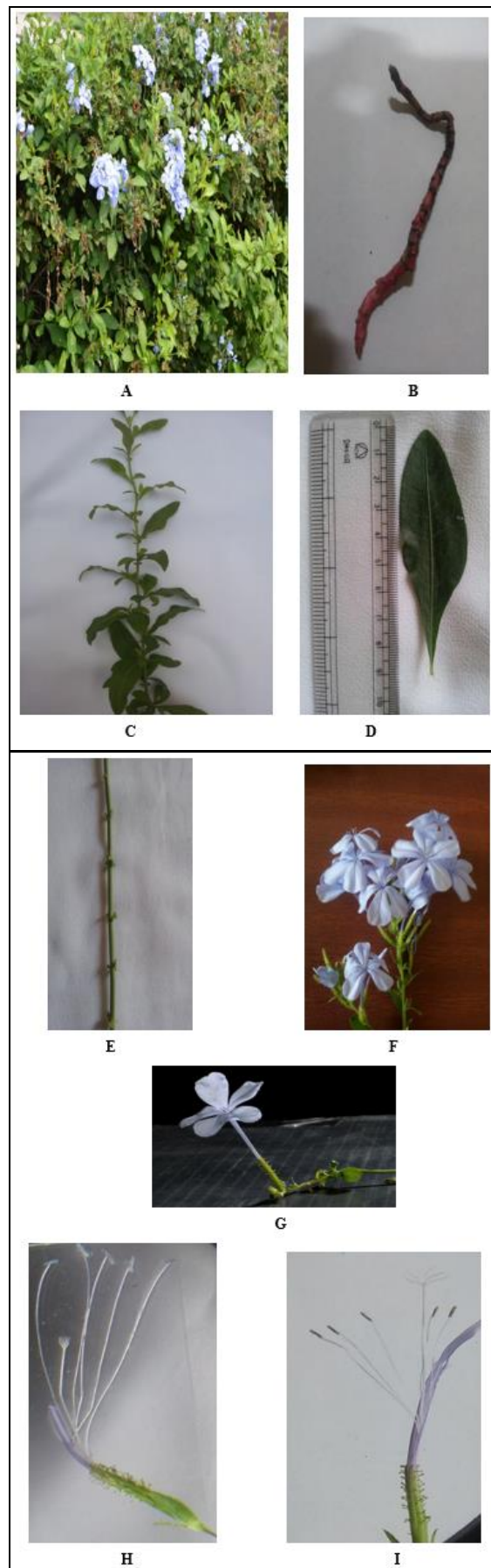


Fig 2: (A-I) morphology of *P. auriculata* Lam, A. Natural habit in flowering stage, B. Sucker. C. Twig., D. Single leaf, E. Young stem, F. Inflorescence, G. Single flower, H. Dissected androecium and gynoecium with short style which remain included in corolla tube, I. Dissected androecium and gynoecium with long style protruding outwards from corolla tube

Taxonomic significance of external morphology

External morphological characters studied are of taxonomic utility and help to delineate two species of *Plumbago*

Keys to *Plumbago* species**Key based on morphological characters of stem**

Stem jointed, striate, zig-zag, thicker, purple coloured ring surrounding node is observed in young stem

-*Plumbago zeylanica*

Stem not jointed, striations are observed in mature stem only, straight, thinner, no purple ring surrounding node is observed

-*Plumbago auriculata*

Key based on morphological characters of leaf

Leaves large (length 3.8 cm to 17.5 cm and width 2.5 to 9 cm),

Leaf shape – ovate or elliptic,

Leaf colour -dark green on upper surface and grass green on Lower surface,

Auricles – absent,

Petiole – long with incurved margin

- *Plumbago zeylanica*

Leaves small (length 3 cm to 9.5 cm and width 1.2 cm to 3.2 cm)

Leaf shape – Elliptic, elliptic ovate or elliptic spatulate,

Leaf colour –grass green on upper surface and light grass

Green on lower surface,

Auricles – present,

Petiole – very short and its margin not incurved

-*Plumbago auriculata*

Key based on morphological characters of Inflorescence

Inflorescence simple or compound spike, peduncle and rachis glandular, flowers shorter

-*Plumbago zeylanica*

Inflorescence simple spike, peduncle without glands, flower longer

-*Plumbago auriculata*

Key based on morphological characters of floral whorls

Calyx densely covered by stalked sticky glands throughout the length; corolla white, corolla tube mostly two times or less than two times the length of calyx tube, corolla lobe smaller (8-10 mm × 4-5 mm) with acuminate apex, anthers dark purple

-*Plumbago zeylanica*

Calyx sparsely covered by stalked sticky glands in the upper part only; corolla blue, corolla tube more than 2 times the length of calyx tube, corolla lobe larger (11-15 mm × 8-10 mm) with rounded apex, anthers bluish

-*Plumbago auriculata*

Elemental analysis

Elemental analysis of leaf, stem, and root of both species of *Plumbago* is presented in table 3.

Table 3: Elemental analysis of two species of *Plumbago* Linn.

Sr. No.	Name of element	Results					
		Leaf		Stem		Root	
		<i>P. z.</i>	<i>P. a.</i>	<i>P. z.</i>	<i>P. a.</i>	<i>P.z.</i>	<i>P. a.</i>
1	Total Nitrogen (%)	2.39	2.57	0.79	1.00	1.05	1.17
2	Total Phosphorus (%)	0.19	0.15	0.073	0.079	0.11	0.18
3	Total Potassium (%)	1.56	1.19	1.18	1.67	0.62	0.82
4	Total Sodium (ppm)	382.55	4323.39	1083.76	1037.20	339.41	257.87
5	Total Calcium (%)	0.26	2.76	0.35	0.93	0.86	0.72
6	Total Magnesium (%)	0.87	1.58	0.12	0.15	0.19	0.13
7	Total Manganese (ppm)	32.11	38.91	5.19	11.35	36.54	36.10
8	Total Iron (ppm)	522.35	528.09	98.40	313.12	1463.32	914.43
9	Total Copper (ppm)	4.07	11.21	3.36	5.97	14.27	162.04
10	Total Zinc (ppm)	39.70	67.75	10.47	17.52	19.02	39.39
11	Total Sulphur (%)	0.19	0.34	0.039	0.15	0.079	0.075

Minerals in plants have role in therapeutic effect of plants [5]. In the present investigation quantitative analysis of different elements viz. nitrogen, calcium, phosphorus, potassium, sodium, magnesium, manganese, iron, sulphur, copper, zinc was done

It is observed that all elements under investigation were present in both taxa of *Plumbago* and are accumulated to greater or lesser extent in different parts of both plants.

Nitrogen

Nitrogen is component of all proteins, amino acids and nucleic acid. Nitrogen enters the human body through nitrogen containing foods such as proteins in our diet.

Nitrogen content is found in following order- leaves > root > stem. Highest nitrogen content is found in leaf of *P. auriculata* and lowest nitrogen content is found in stem of *P. zeylanica*.

Phosphorus

Elemental analysis revealed presence of lesser amount of phosphorus as compared to amount of potassium and calcium. Highest phosphorus content is found in leaves of *P. zeylanica*. Phosphorus along with calcium forms the supporting structure of the body. It is second to calcium in abundance and constitutes 22% of the total minerals of the human body. About 80% of phosphorus is present as

calcium phosphate in bone and 20% is present in the blood and other cells as soluble phosphate ions and also in lipids, proteins and carbohydrates. Nucleic acids and the cell membrane also contain phosphorus. Energy transfer compounds contain phosphate. Phosphorus deficiency leads to weakness, anorexia, malaise and pain in the bones [4].

Potassium

Stem and leaf of both plants have higher amount of potassium than root.

Maximum potassium content is found in *P. auriculata* stem. Potassium constitutes 5 percent of the total mineral content of human body. It helps in maintaining osmotic equilibrium, acid base balance and conduction of nerve. Potassium is component of lean body tissue. Hence an adequate supply of potassium is required for growth and development of lean tissue. Deficiency of potassium cause muscular weakness or paralysis [4].

Sodium

Among the investigated plants parts the maximum sodium content (4323.39 ppm) is found in *P. auriculata* leaf and minimum sodium content (257.87ppm) is found in *P. auriculata* root. Sodium constitutes 2 percent of the total mineral content of the human body [4].

Like potassium, sodium also helps in maintaining osmotic equilibrium, acid-base balance and conduction of nerve impulse [4]. Sodium is important for absorption of monosaccharides and amino acids from small intestine; in initiating and maintaining the heart beat [19].

Calcium

Calcium content is highest in leaf of *P. auriculata* and lowest in leaf of *P. zeylanica*. Calcium is most abundant minerals in the body which comprises 39% of the total minerals present in human body. About 99% of human body's calcium is present in the skeleton. In bones calcium occur as calcium phosphate. About 1% of body's calcium is present in body's fluids and soft tissues. It is necessary for certain enzyme activities, excitability of peripheral nerves and muscles, myocardial function and muscle contraction, blood clotting. Calcium deficiency in children leads to rickets in children and osteomalacia and osteoporosis in adults [4].

Magnesium

Maximum magnesium content among the investigated plant parts is found in *P. auriculata* leaf. 20-28 gram of magnesium is present in the human body, more than half of which is stored in the bones. Magnesium is important part of many enzymes. It helps in maintaining the electrical potential of nerve and muscle membrane [4].

Manganese

Maximum (38.91ppm) and Minimum (5.91ppm) manganese content among the investigated plant parts are found in *P. auriculata* leaf and *P. zeylanica* stem respectively. Manganese is dispersed throughout the body tissue and fluids.

More manganese is present in bone, kidney, liver, pancreas and pituitary than other tissues [19]. Manganese is important for several enzymatic processes and elimination of fatigue [20]. Manganese deficiency leads in skeletal abnormalities, sterility [19].

Iron

Iron content is in the order of root > leaf > stem in both plants. Maximum (1463.32 ppm) and minimum (98.40 ppm) iron content among the investigated plant parts are found in *P. zeylanica* root and *P. zeylanica* stem respectively. Adult healthy body contains about 3-5 gram of iron. Out of this, about 70 percent is distributed in haemoglobin, myoglobin and many enzymes. The remaining 30 percent is stored in liver, bone marrow and spleen as ferritin and haemosiderin. Iron deficiency result in anaemia [4].

Copper and Zinc

Zinc content in each part is higher than its copper content except in root of *P. auriculata*. The maximum (162.04 ppm) and minimum (3.36 ppm) copper content is found in *P. auriculata* root and *P. zeylanica* stem respectively. Copper is present in combination with plasma protein, ceruloplasmin and is component of some enzymes. The deficiency of copper leads to anaemia (due to improper mobilization of iron from iron storage sites), skeletal defects, reproductive failure, Cardiovascular diseases [4].

The maximum (67.75 ppm) and minimum (10.47 ppm) zinc content is found in *P. auriculata* leaf and *P. zeylanica* stem respectively. Zinc is constituent of many enzymes and large part of it is stored in bones. Deficiency of zinc causes loss of appetite, skin changes and impaired regeneration of wounds, decreased taste activity [4].

Sulphur

Maximum (0.34%) and minimum (0.039%) sulphur content among the investigated plant parts are found in *P. auriculata* leaf and *P. zeylanica* stem. Sulphur is component of sulphur containing amino acids (cysteine, cystine, methionine), glutathione, vitamins (thiamine and biotin) [4].

Our observations of amount of minerals calcium, potassium, sodium, manganese, iron, zinc and copper in roots of both species showed variation with those recorded by Ariyanathan *et al* (2010) [21]. The presence and concentrations of different elements in plants is affected by the composition of the soil, water and fertilizers used; permissibility, selectivity and absorbability of plants for uptake of elements [22]; stage of growth (young or old) [23].

Conclusions

The present work revealed distinctive differences in morphological characteristics of vegetative as well as reproductive parts which are of taxonomic value and can be used in identification and authentication of both species of *Plumbago*

Nutrition is pre-requisite for healthy life. Nitrogen, phosphorus, potassium, sodium, calcium, magnesium, manganese, iron, copper, zinc, sulphur which are nutritionally important minerals were found in all parts of both plants. Hence, both the plants would be used as supplementary source of dietary minerals. Leaves of *P. auriculata* seem to be more nutritious because they contain maximum amount of nitrogen, sodium, calcium, magnesium, manganese, zinc and sulphur in the investigated plant parts.

This validate its use as bitter tonic as mentioned in Ayurveda

The presence of phytochemicals along with minerals makes *Plumbago* not only the potential drug but source of nutrients also (Neutraceuticals).

Acknowledgement

The author is grateful to Dr. G. S. Chuadhari (Retd.), Reader, P. G. Department of Botany, M. J. College, Jalgaon for his guidance during my research work. Author is also thankful to Jain Research and Development Laboratory, Jain Irrigation Systems Limited, Jalgaon for help in elemental analysis

References

- Anandanayaki S. Comparative Pharmacognostical studies on selected plants (*Pedaliium murex* Roen ex. L. and *Martynia annua* L.) Ph. D. thesis, Tamil University, Thanjavur, Tamil Nadu, 2010.
- Sharma OP. Plant Taxonomy, Second edition, Tata McGraw Hill Education Private Limited, New Delhi, 2011.
- Mondal AK. Advanced Plant Taxonomy, New Central Book Agency (P). Ltd., Kolkata, 2009.
- Manay NS, Shadaksharaswamy M. Foods-Facts and principles, Third revised edition, New Age International (P) Limited Publishers, New Delhi, 2008.
- Paul ET. Taxonomic and phytochemical studies on selected species of family Apocynaceae, Ph. D. thesis., Mahatma Gandhi University, Kottayam Kerala, India, 2008.
- Tortora GJ, Derrickson B. Principles of Anatomy and Physiology Vol. 1- Organization, support and movement, and control, system of the human body, International student version, John Wiley and Sons, Inc, 2011.
- Deshpande AP, Jawalagekar RR, Ranade S. Dravyagun vidnyan (Part -1 and 2), Anmol Prakashan, Pune (Marathi Book), 1997.
- Deshpande AP, Ranade S. Dravyaguna vijnyana (Part I and II), Proficient Publishing House, Pune, 2010.
- Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in Ayurveda, Volume I, Documentation and publication division, Central council for research in Ayurveda and Siddha, New Delhi, 2000.
- Sharma PV. Dravyaguna vijñāna Vol. II, Caukhambha Bharti Academy, Varanasi (Hindi book).
- Pandey G. Dravyaguna vijñāna, Part I, Krishnadas Academy, Varanasi, 2002.
- Jose SS, Joselin J, Florence AR, Brintha TSS, Jeeva S. Phytochemical constituents of leaf of *Plumbago zeylanica* L. a common understory species of homestead Agroforests of Kanyakumari district, Tamilnadu, International Journal of Pharmacy, 2014;4(3):162-165.
- Ferrero V, de Vega C, Stafford GI, Staden JV, Johnson SD. Heterostyly and Pollinators in *Plumbago auriculata* (Plumbaginaceae), South African Journal of Botany, 2009;75:778-784.
- Kumar A. Plumbaginaceae In: Verma D. M., Balkrishnan N. P., Dixit R. D. (Editors), Flora of Madhya Pradesh Vol. I, Published by Director, Botanical survey of India, Calcutta, 1993.
- Yadav SR, Sardesai MM, Flora of Kolhapur District, Shivaji University, Kolhapur, 2002.
- Thramaraj RJJM. Studies on Interspecific and Intraspecific variation in the genus *Plumbago* (Plumbaginaceae) from the South India, Ph. D. thesis, Manonmaniam Sundaranar University, Tirunelveli, 2013.
- Patil DA. Flora of Dhule and Nandurbar District, Published by Bishen Sing Mahendra Pal Singh, Dehradun, 2003.
- Chen Yi, Gao Suping. Preliminary report of PGR'S influence to multiple shoots induction and Plant regeneration on *Plumbago auriculata*, American Journal of Plant Sciences, 2013;4:23-29.
- Swaminathan M. Advanced text book on Food and nutrition Volume 1, Second edition, Bappco, Bangalore, 2009.
- Babu NG, Raju TP, Shrinivasu CC, Ramanamam V, Ram SS, Sudershan M *et al.* Estimation of elemental concentrations of Indian medicinal plants using Energy dispersive X-ray fluorescence (EDXRF), International Journal of Scientific and Engineering Research, 2015;6(7):1379-1387.
- Ariyanthan S, Saraswathy A, Rajamanickam GV. Quality control standards for the roots of three *Plumbago* species, Indian Journal of Pharmaceutical sciences, 2010;72(1):86-91.
- Zafar M, Khan MA, Ahmad M, Jan G, Sultana S, Ullah K *et al.* Elemental analysis of some medicinal plants used in traditional medicine by atomic absorption spectrophotometer (AAS), Journal of Medicinal Plants Research, 2010;4(19):1987-1990.
- Piper CS. Soil and plant analysis: A laboratory manual of methods for the examination of soils and the determination of inorganic constituents of plants, Scientific Publishers, Jodhpur, 2010.