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Research Note

Morphological, yield and quality variability among the accessions of *Plumbago zeylanica*

L. Nalina[1], K. Shanthanu[2], K. Rajamani[1], A. John Joel [3], Tushar Dhanani[4], Raghuraj Singh[4] and Satyanshu Kumar[4]

[1]Department of Medicinal & Aromatic Crops, HC & RI, TNAU, Coimbatore-3
[2] Graduate Research Assistant, Department of Plant Agriculture, University of Guelph
[3]Centre for Plant Breeding and Genetics, TNAU, Coimbatore
[4]ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi-387310, Gujarat, India
E-mail: lnalina_hort@rediffmail.com

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Abstract
Forty two accessions of *Plumbago zeylanica* were collected and evaluated for morphological, yield and quality traits. The results revealed that the accession *Pz* 27 registered the highest mean value for plant height (178.33cm) and fresh weight of root (518.60g).The same accession registered the maximum number of roots (32.33). Plumbagin content ranged from 0.19 - 0.34 % and was maximum in *Pz* 15 (0.34%) followed by *Pz* 17 (0.33%). Correlation between dry root weight and morpho - economic characters revealed that dry root weight exhibited positive and highly significant correlation with fresh root weight, root number and plant height. Based on the dry root weight and plumbagin content the accessions in Part III (*Pz* 19 and *Pz* 20) and Part IV (*Pz* 25, *Pz* 27, *Pz* 39, *Pz* 40 and *Pz* 41) were identified as best performing accessions

Key words
Germplasm, *Plumbago zeylanica*, morphological, yield and quality variation

Plumbago zeylanica L. which is commonly known as Ceylon leadwort, Chita, Chitra and Chitramoolam is a perennial, sub-scandent shrub .It is one of the common plants used in Indian traditional systems of medicine. Plumbago belongs to the family Plumbaginaceae which consists of 10 genera and 280 species. The genus *Plumbago* includes three species, namely *Plumbago indica* (Syn :*P. rosea*) *Plumbago capensis* and *Plumbago zeylanica*, which are distributed in several parts of India. The roots of *P. zeylanica* and *P. indica* are credited with potential therapeutic properties including antiatherogenic, cardiotonic, hepatoprotective and neroprotective properties (Tilak *et al*. 2004). The chief active principle is plumbagin (5-Hydroxy-2-methyl-1,4 naptho quinone), the bioactive compound responsible for various medicinal properties of *Plumbago* species. It is obtained as golden yellow needle shaped crystals. Plumbagin possesses anticancer and bactericidal activities (Melo, 1974; Durga *et al*,. 1990, Kini *et al*., 1997). It is also effective against insects. At present, the roots are collected from wild without any proper information about its growth and plumbagin content. Hence, the present study was conducted to assess the variability in plant growth, root yield and quality among *Plumbago* genotypes and to identify an elite genotype for yield and plumbagin content.

The experimental material comprised of 42 accessions of *Plumbago zeylanica* collected from different parts of Tamil Nadu, Kerala and Maharashtra. The accessions were evaluated in a Randomized Block Design with two replications at Department of Medicinal and Aromatic Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. Subsequently, cuttings of all entries were submitted to the National Active Germplasm Site at Directorate of Medicinal and Aromatic Plants Research, Anand Gujarat and IC number for the accessions were obtained from NBPGR, New Delhi (Table 1).

The field was thoroughly ploughed with addition of organic inputs in the last plough. Beds of $12m^2$ were formed and rooted cuttings of the accessions were planted at a spacing of 1.20m x 0.9m. Regular irrigation and intercultural operations were carried out. Observations on morphological and yield characters were recorded. Plant height was measured from base to tip of the plant. Stem girth was measured at the collar region. Total numbers of roots were counted and recorded. Root length, root girth, rind diameter and core thicknesses were also measured in centimeters. Fresh root weight was recorded and expressed in gram. For the estimation of plumbagin content, the harvested roots were cut into small pieces, dried under shade and then dried in a hot air oven. Dry weight was recorded and the roots were powdered for estimation of plumbagin content.

To estimate the plumbagin content, HPLC grade solvent methanol was purchased from Merck, Mumbai, India. Deionized water was used throughout the experiment which was obtained using a Millipore water purification system (Millipore, gradient- 0.22 0m). Plumbagin was purchased from M/S Sigma-Aldrich, Bangalore, India. For quantification of plumbagin, extract of

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the samples was prepared by mixing powdered sample with methanol in 1:20 ratio. The samples were replicated thrice and the mean values were taken as concentration of plumbagin content in each genotype. The mixture was kept at room temperature for overnight. The extract was filtered and the solvent was evaporated using a vacuum evaporator. The dried extract was reconstituted in methanol. Standard stock solution of plumbagin (1000 µg mL^{-1}) was prepared in HPLC grade methanol and working solutions of lower concentration were prepared by appropriate dilution of the stock solution and stored at 4^{o}C.

HPLC system for chromatographic analysis consisted of a separation module (Waters 600E) equipped with Empower software (Waters), a quaternary pump, an in-line vacuum degasser and a photodiode array detector (Waters 2996). The chromatographic separation was carried out in an isocratic elution mode on RP-18 column (250 × 4 mm, 50m Merck, India). The mobile phase was a mixture of the solvents methanol and sodium dihydrogen phosphate (5 mM) in the proportion of 9:1 v/v. The solvent flow rate was 0.8 ml/min and the injection volume was 20 µL. Column temperature was 25 ^{o}C. The photo diode array detector wavelength was set at 265 nm (Unnikrishnan *et al*., 2008). Identification and quantification of plumbagin in the sample extracts was done on the basis of retention time of peak (Figure 1). Concentration of plumbagin in extract samples was calculated by comparing the integrated peak areas of the individual samples with that of a standard curve prepared from the corresponding standards.

Genetic resources of medicinal and aromatic plants have assumed a great significance to plant breeders, foresters and conservation scientists in identification of elite lines. Characterization of the genetic diversity and examination of the genetic relationship among *P. zeylanica* genotypes are essential for the sustainable conservation and increased use of plant genetic resources. In any selection programme, the *per se* performance serves as an important criterion for selection of desirable genotypes. This offers a better scope for the germplasm to be used as the potential source and also for selecting the high yielding accessions. Hence, the accessions chosen for the present study were assessed for their *per se* performance.

The mean performance and spectrum of genetic variation would help to identify the superior genotypes among the existing populations. Allard (1960) suggested that selection should be applied mainly in lines exhibiting high mean and variability. According to Finker *et al.* (1973), crosses or families with the highest mean could be effectively utilized to identify the superior segregates. The mean performance served as a primary criterion for selecting desirable plants (Kumar *et al.*, 1979).

The mean performance of forty two accessions in the present study revealed that certain accessions exhibited their superiority over others for different traits. The growth parameters *viz*., plant height, stem girth, number of branches and yield characters namely number of roots, root length, root girth, root thickness, core diameter and root weight (fresh, dry and total) are presented in table 2 and 3.

The accession *Pz*-27 registered the highest mean value for plant height (178.33 cm). Plant height is an important component for growth and vigour of the plants. The robust vegetative growth is an essential prerequisite for higher yield in Plumbago, wherein the economic part is the root. The same accession registered the highest mean value for root fresh weight (518.60 g).

The values for stem girth ranged from 28.00 cm to 50.33 cm. The accession *Pz* 14 recorded maximum girth of 50.33 cm followed by *Pz* 10 (49.00cm). The lowest girth was recorded in *Pz* 23 (11.33 cm) *Pz* 23 (11.33cm). The number of branches ranged from 20.00 to 41.67 .00 to 52.00, with the maximum number (41.67) in *Pz* 30.

The fresh root weight ranged from 255.87 g to 518.60 g/plant. The accession *Pz* 27 recorded maximum weight of 518.60 g/plant and was followed by *Pz* 40 (510.50). The lowest weight was recorded in *Pz* 13 (255.87g). The dry root weight ranged from 199.58g to 394.14 g/plant which was maximum in *Pz* 27 (394.14g) and was followed by *Pz* 40 (387.98g). The lowest weight was recorded in *Pz* 13 (199.58g).

The number of roots per plant is considered as an important yield component in tuber crops. The final root yield in root crops depends on the number of roots initiated and subsequent development. In this study, number of roots ranged from 17.33 to 32.33 .The accession *Pz* 27 recorded maximum number of roots (32.33) and was followed by *Pz* 19 (30.67). The lowest number of roots was recorded in *Pz* 1 (17.33) (Table 2b).

Root length and girth have higher contribution for root yield among other yield components, since they provides a better sink for effective utilization of assimilates. *Pz* 4 registered superior performance for root length (86.24 cm) followed by *Pz* 7 (84.54 cm). The same accession, *Pz* 7 (9.66 cm) showed superior mean performance for root girth. Lowe and Wilson (1974) observed that the final tuber girth was related to mean tuber weight and yield in sweet potato. Thamburaj (1975) reported that the tuber girth appeared to be

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the most important single dimensional determinant for high yield in sweet potato.

The core diameter of the root ranged from 0.40 to 0.81 cm, which was maximum in *Pz* 9 (0.81 cm) followed by *Pz* 27 (0.79cm). The core diameter was minimum in *Pz* 23 (0.34cm).

The plumbagin content ranged from 0.19-0.34% (Table 3) and was maximum in *Pz* 15 (0.34%) followed by *Pz* 17 (0.33%) and the content was lowest in *Pz* 13 (0.19%). Unnikrishnan *et al*. (2008) reported 0.2001 and 0.1601 per cent plumbagin content in *P. indica* and *P. zeylanica* respectively.

The data on correlation between dry root weight and morpho-economic characters are presented in Table 4. The dry root weight exhibited positive and highly significant correlation with fresh root weight (0.966), root number (0.827) and plant height (0.667). It had significant association with stem girth (0.543), total root weight (0.520), core diameter (0.485) and total plant weight (0.474). The number of branches, stem weight, collar weight, root length and rind thickness did not have any correlation with dry root weight.

Kurian *et al*. (2001) assessed the variability in morphological and yield related characters and plumbagin content in 45 accessions of *Plumbago rosea* collected from different parts of Kerala. Highly significant variation was recorded for all characters studied except leaf size. Shoot weight showed the highest positive correlation (0.96) with root weight. Positive correlations were also recorded for number of branches (0.63), number of leaves (0.61), plant spread (0.59), root length (0.53), number of roots (0.52) and root girth (0.41).

The accessions were grouped into four parts based on the dry root weight and plumbagin content (Fig.2) The accessions in Part 1 and part II had dry root weight from 199.58 g to 250 g and plumbagin content from 0.19-0.34%. The accessions in part III and part IV had dry root weight more than 250 g and plumbagin content from 0.19-0.33%. Hence, based on the dry root weight and plumbagin content the accessions in Part III (*Pz* 19 and *Pz* 20) and Part IV (*Pz* 25, *Pz* 27, *Pz* 39, *Pz* 40 and *Pz* 41) were identified as best performing accessions.

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Table 1. List of accessions in *Plumbago zeylanica*

S.No	Collection number	IC number	Place of collection
1.	*Pz*-1	IC- 0614421	Coimbatore
2.	*Pz*-2	IC- 0614422	Patikkad
3.	*Pz*-3	IC- 0614423	Rahuri
4.	*Pz*-4	IC- 0614424	Odakalli
5.	*Pz*-6	IC- 0614426	Arasanatham
6.	*Pz*-7	IC- 0614427	Karamadai
7.	*Pz*-8	IC- 0614428	Anaikatti
8.	*Pz*-9	IC- 0614429	Kottayam
9.	*Pz*-10	IC- 0614430	Kottakkal
10.	*Pz*-11	IC- 0614431	KAU, Trissur
11.	*Pz*-12	IC- 0614432	Chalakudi
12.	*Pz*-13	IC- 0614433	Rayirath, Pattikkad
13	*Pz*-14	IC- 0614434	Aryavaidya Sala, Kottakkal
14.	*Pz*-15	IC- 0614435	Periyakulam
15.	*Pz*16	IC- 0614436	ABS, Salem
16.	*Pz*-17	IC- 0614437	Karipatti-1
17.	*Pz*-18	IC- 0614438	Karipatti-2
18.	*Pz*-19	IC- 0614439	Karipatti-3
19.	*Pz*-20	IC- 0614440	Karipatti-4
20.	*Pz*-21	IC- 0614441	Peciparai
21.	*Pz*-22	IC- 0614442	Kalluvanthittai
22	*Pz*-23	IC- 0614443	Kollangodu
23	*Pz*-24	IC- 0614444	Babanasam
24	*Pz*-25	IC- 0614445	Pudhukottai
25	*Pz*-26	IC- 0614446	Navavurprivu
26	*Pz*-27	IC- 0614447	Virudhachalam
27	*Pz*-28	IC- 0614448	Solamadevi, Trichy
28	*Pz*-29	IC- 0614449	Silambavelangadu, Pattukottai
29	*Pz*-30	IC- 0614450	Maruthamalai
30	*Pz*-31	IC- 0614451	High school road,Kallanguthukadu
31	*Pz*-32	IC- 0614452	Periyavayalthottam
32	*Pz*-33	IC- 0614453	West Rajapuram
33	*Pz*-34	IC- 0614454	Semmatty
34	*Pz*-35	IC- 0614455	Mottur
35	*Pz*-37	IC- 0614457	Kalveerampalayam
36	*Pz*-38	IC- 0614458	Odakalli-2
37	*Pz*-39	IC- 0614459	Yercaud
38	*Pz*-40	IC- 0614460	Avanam, Thanjavur
39	*Pz*-41	IC- 0614461	Thiruchitrambalam Thanjavur,
40	*Pz*-42	IC- 0614462	Kamudhi, Ramanathapuram
41	*Pz*-43	IC- 0614463	Nainarpatty,Sivagangai
42	*Pz*-44	IC- 0614464	Vellingiri, Vaidehi falls

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Table 2. Growth and yield characters of *P. zeylanica* accessions

Accessions	Plant height (cm)	Stem girth (cm)	No. of branches	Fresh root weight (g/plant)	Dry root weight (g/plant)	Root number	Root length (cm)	Root girth (cm)	Core diameter (cm)	Plumbagin content (%)
Pz-1	119.33	30.33	22.67	266.47	205.18	17.33	52.65	7.89	0.45	0.21
Pz-2	160.00	30.50	26.33	300.53	225.40	19.33	83.42	8.25	0.70	0.20
Pz-3	120.00	28.50	21.50	279.17	209.38	18.25	51.33	8.14	0.50	0.21
Pz-4	142.67	34.33	28.50	330.70	248.03	26.67	86.24	8.54	0.45	0.21
Pz-6	170.67	33.67	25.33	423.67	317.75	26.67	77.22	9.15	0.56	0.20
Pz-7	152.67	30.33	23.00	405.00	303.75	19.10	84.54	9.66	0.64	0.20
Pz-8	146.67	38.33	34.00	399.77	299.83	18.67	50.50	6.02	0.66	0.20
Pz-9	149.67	45.33	33.00	425.50	319.13	18.67	51.99	5.87	0.81	0.20
Pz-10	153.33	49.00	36.67	388.63	291.47	18.00	51.25	5.74	0.55	0.19
Pz-11	153.33	46.00	33.67	400.33	300.25	25.33	50.17	7.25	0.57	0.19
Pz-12	160.18	45.67	31.33	415.67	311.75	23.33	51.67	6.00	0.78	0.20
Pz-13	120.00	32.50	32.33	255.87	199.58	18.25	50.25	5.92	0.78	0.19
Pz-14	130.67	50.33	27.67	424.63	318.47	19.33	48.67	6.23	0.60	0.19
Pz-15	120.00	29.50	24.00	325.40	244.05	18.25	48.85	6.12	0.64	0.34
*Pz*16	120.00	31.50	27.33	287.90	215.93	20.33	49.27	5.90	0.50	0.28
Pz-17	124.33	38.00	27.00	365.70	274.28	19.67	50.67	8.09	0.47	0.33
Pz-18	127.67	36.33	28.33	286.43	214.82	23.67	50.00	5.77	0.74	0.25
Pz-19	148.33	42.00	28.33	420.87	315.65	30.67	49.82	5.69	0.75	0.32
Pz-20	147.00	40.67	29.00	439.17	329.38	26.67	56.59	5.77	0.68	0.24
Pz-21	130.00	32.60	36.67	282.80	212.10	18.67	47.63	5.44	0.45	0.22
Pz-22	120.00	32.00	35.50	336.95	252.71	18.00	48.50	5.59	0.46	0.21
Pz-23	140.00	28.00	20.33	257.97	201.22	17.50	46.99	5.13	0.40	0.20
Pz-24	119.50	28.50	20.33	276.73	207.55	17.99	46.79	8.33	0.47	0.21
Pz-25	142.33	34.00	26.33	445.63	356.50	27.33	48.33	5.97	0.56	0.19
Pz-26	156.67	33.00	29.33	354.63	265.97	19.00	48.99	5.64	0.57	0.20
Pz-27	178.33	47.33	40.33	518.60	394.14	32.33	54.95	6.05	0.79	0.19
Pz-28	158.33	36.33	39.33	425.00	340.00	19.50	50.33	7.43	0.78	0.21
Pz-29	136.33	35.00	29.67	378.23	283.67	19.67	47.25	5.54	0.50	0.24
Pz-30	125.50	44.50	41.67	405.70	304.28	24.50	48.25	5.51	0.50	0.27
Pz-31	122.00	28.50	26.67	280.00	210.00	17.67	48.00	5.29	0.46	0.26
Pz-32	120.00	28.00	27.00	280.70	210.53	18.13	47.10	5.54	0.47	0.29
Pz-33	120.00	32.50	27.67	339.00	254.25	18.99	47.77	5.82	0.60	0.30
Pz-34	143.00	39.00	38.67	415.43	311.57	19.67	47.50	5.77	0.77	0.26
Pz-35	150.00	29.00	27.67	289.30	216.98	18.50	46.89	5.00	0.52	0.31
Pz-37	152.67	32.67	28.50	419.17	314.38	22.67	49.97	5.82	0.62	0.28
Pz-38	142.67	32.60	26.67	422.33	320.97	23.67	49.00	5.25	0.62	0.21
Pz-39	156.67	33.00	26.67	429.87	326.70	25.67	49.10	5.64	0.52	0.20
Pz-40	150.00	37.33	29.00	510.50	387.98	25.67	51.54	6.10	0.64	0.20
Pz-41	134.33	33.67	27.33	451.03	342.78	26.67	51.00	6.07	0.51	0.19
Pz-42	158.00	28.00	20.00	284.35	227.48	17.75	47.33	5.51	0.45	0.19
Pz-43	119.50	29.50	26.00	315.97	252.78	18.67	48.13	5.69	0.68	0.19
Pz-44	120	31.5	31.00	280.89	224.71	18	47.00	6.05	0.55	0.19
Mean	139.58	35.22	29.10	362.91	275.32	21.30	52.70	6.34	0.59	0.23
STDEV	16.54	6.34	5.40	72.41	54.80	3.99	10.18	1.18	0.12	0.04
CV	11.85	18.01	18.55	19.95	19.90	18.72	19.31	18.55	19.96	19.62
Range	119.33 - 178.33	28.00 - 50.33	20.00 - 41.67	255.87 - 518.60	199.58 - 394.14	17.33 - 32.33	46.79 - 86.24	5.00- 9.66	0.40 - 0.81	0.19 - 0.34

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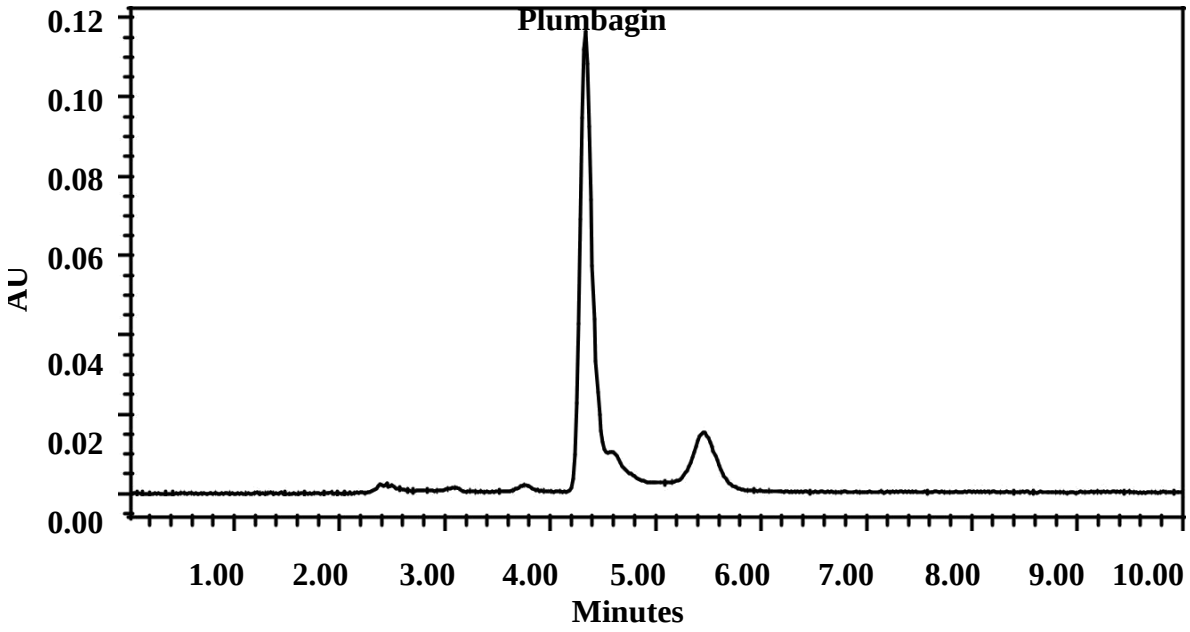
Table 3. Correlation between morpho-economic characters and dry root weight

Traits	SG	BR	TPW	SW	TRW	FRW	CW	RN	RL	RG	RT	CD	DRW
PHT	0.702	0.417	0.701	0.481	0.666	0.727	0.511	0.679	0.329	0.219	0.321	0.512	0.667**
SG	1.000	0.721	0.782	0.588	0.842	0.606	0.718	0.618	0.224	-0.090	0.113	0.528	0.543*
BR		1.000	0.619	0.545	0.634	0.378	0.591	0.325	-0.090	-0.329	-0.122	0.341	0.309
TPW			1.000	0.824	0.802	0.560	0.599	0.561	0.513	0.152	0.168	0.467	0.474*
SW				1.000	0.472	0.390	0.396	0.540	0.404	0.042	-0.045	0.297	0.345
TRW					1.000	0.587	0.695	0.432	0.206	-0.094	0.169	0.491	0.520*
FRW						1.000	0.365	0.840	0.214	0.064	0.476	0.472	0.966**
CW							1.000	0.342	0.149	-0.084	0.022	0.528	0.302
RN								1.000	0.352	0.182	0.431	0.365	0.827**
RL									1.000	0.651	0.300	0.225	0.139
RG										1.000	0.392	-0.061	-0.001
RT											1.000	0.009	0.433
CD												1.000	0.485*

*, ** Significant at 5 and 1 per cent level, repectively

PHT: Plant height, SG: Stem girth, BR: Number of Branches TPW: Total Plant Weight, SW: Stem weight, TRW: Total Root Weight, FRW: Fresh Root Weight, CW: Collar Weight, RN: Root Number, RL: Root Length, RG: Root Girth, RT: Root Thickness, CD: Core Diameter, DRW: Dry Root Weight.

Fig. 1. HPLC chromatogram of standard plumbagin 20 ppm



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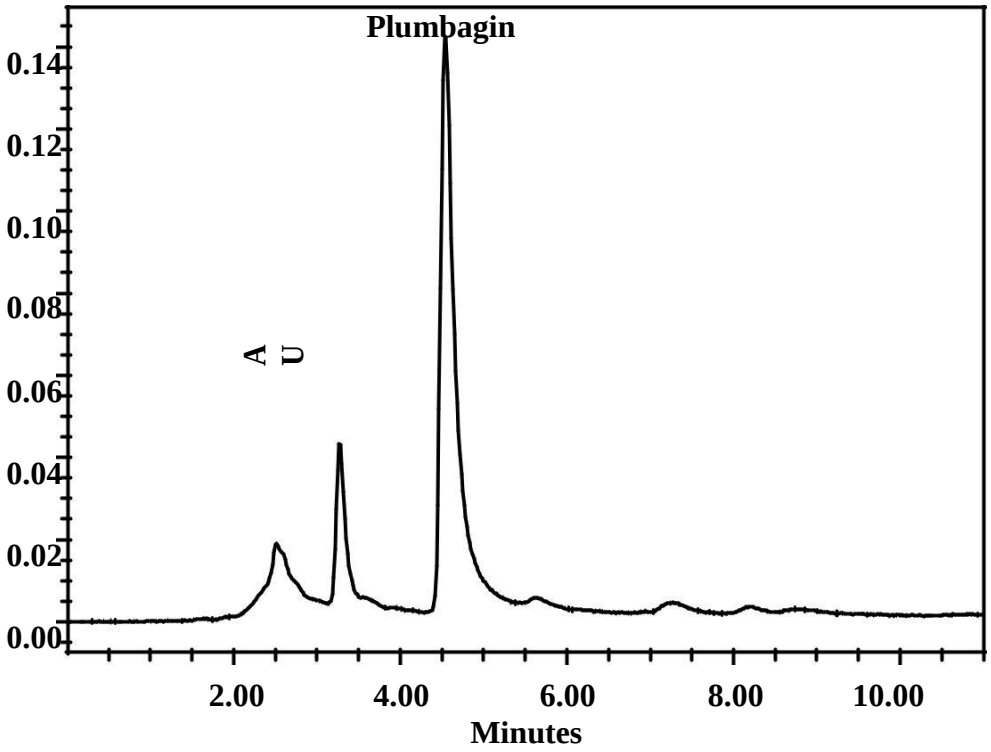
HPLC chromatogram of methanol extract of of *P. zeylenica* (R-80)



HPLC chromatogram of methanol extract of of *P. zeylenica* (R-85)

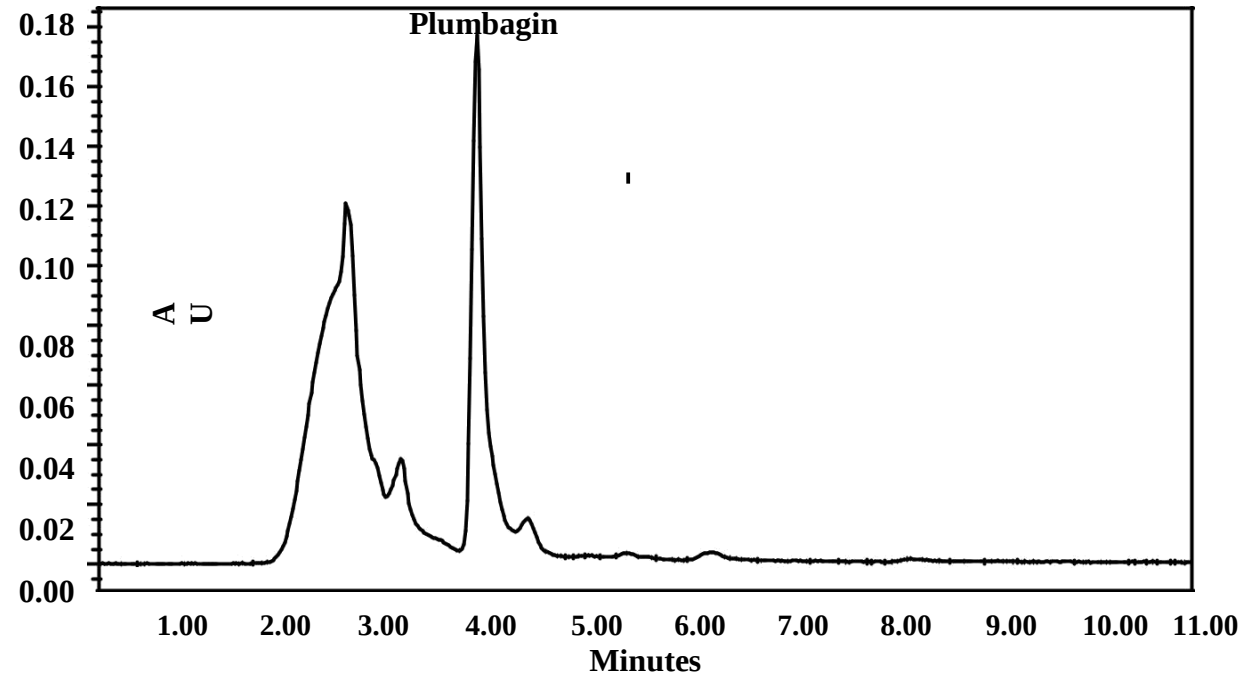


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Fig. 2. Grouping of *Plumbago* accessions based on yield and quality