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Effect of *Śodhana* (An āyurvedic purification technique) on *Citraka* (*Plumbago zeylanica* Linn. and *Plumbago rosea* Linn.) with special reference to plumbagin content

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Abstract
Citraka (2 species - *Plumbago zeylanica* Linn. and *Plumbago rosea* Linn.) is a medicinal plant used extensively in various Āyurvedic formulations, with great therapeutic significance. Even though it is not placed in the poisonous plant category, *Āyurveda* - The traditional system of medicine of India advocated *śodhana* (purification) for this drug. In the present study, the effect of *Śodhana* (purification) of root of both species of *Citraka* in lime water ($CaCO_3$) with special reference to Plumbagin content was analysed using HPTLC. Changes in the physico-chemical parameters, phytochemical analysis and quantification of Plumbagin was observed after *śodhana.* The Plumbagin content was reduced from 0.026% to 0.009% in *Plumbago rosea* and from 0.015% to undetectable quantity in *Plumbago zeylanica* after *śodhana* .The study establishes the significant role of *Śodhana* in the phytochemical as well as the toxicological profile of the drug.

Keywords: *citraka, śodhana, āyurveda, cūrṇodaka,* $CaCO_3$, plumbagin

Introduction
According to World Health Organization (WHO), 80% of the world's population is estimated to be using Herbal medicines. Generally Medicinal plants are considered to be safe, but some of these plants contain toxic constituents. These toxic elements has to be removed or processed to make it suitable for consumption. *Āyurveda* - The Science of life; emphasizes on administration of these plants containing toxic elements only after purification technique called *Śodhana*. The purification of the drug (grinding, trituration etc...) to remove the toxicities - unwanted or harmful constituents is termed as *Śodhana* [1]. Different types of *śodhana* is being specified for the drugs mentioned in *Āyurveda*; API (Āyurvedic Pharmacopoeia of India) has authenticate *śodhana* techniques for the respective drugs. The reviewed literature suggests that *śodhana* enables the drug to be non-toxic, easily digestible and absorbable. This in turn enhances the therapeutic potential of the drug [2]. *Citraka* is one such plant which is used in the *Āyurveda* classics after *śodhana*. It is used in treating dyspepsia, helminthiasis, cough, colic, inflammations, bronchitis, elephantiasis, haemorrhoids, leprosy, chronic and intermittent fever, leukoderma, scabies, ring-worm, hepatosplenomegaly, anaemia and amenorrhoea [3]. Root of *Plumbago zeylanica* Linn. (*Śveta Citraka*) and *Plumbago rosea* Linn. (*Rakta Citraka*) of Plumbaginaceae are used in *Ā*yurveda as source plants of *Citraka*. But Āyurvedic Pharmacopoeia of India and Quality Standards of Indian Medicinal Plants specifies white-flowered *Plumbago zeylanica* Linn. as the source plant in contradictory to the use of red-flowered *Plumbago rosea* Linn. as the source plant of *citraka* in Kerala [4]. It contain Plumbagin as the chief chemical constituent belonging to the class naphthoquinone, which is responsible for its corrosive effects. The red variety (*Rakta Citraka*) is considered to be more corrosive than white (*Śveta Citraka*) one [5].
Specific *Śodhana* (Purification) procedures have been adopted for the purification of *Citraka* (*Plumbago zeylanica* Linn. and *Plumbago rosea* Linn.) root and these methods are either mentioned in the classics of *Ā*yurveda or practiced traditionally [6]. The *śodhana* method mentioned in the text Arogya kalpadruma as well as API (Āyurvedic Pharmacopoeia of India) was adopted.
The purpose of the present study is to evaluate the role of *Śodhana* (purification) in the quantitative reduction of toxic naphthoquinone - Plumbagin of *Citraka* (2 Species - *Plumbago zeylanica* Linn. and *Plumbago rosea* Linn.) root by the high-performance thin layer chromatography (HPTLC) technique.

Materials and Methods

Collection of drugs

Fully matured *Citraka* (2 Species - *Plumbago zeylanica* Linn. and *Plumbago rosea* Linn.) root were collected from Pathanamthitta district, Kerala, India during the month of June, 2018, and were botanically authenticated by pharmacognosists. The root of the plants were cut into smaller pieces thoroughly washed in tap water, and shade dried for 15-20 minutes.

Preparation of media

Cūrṇodaka (Lime water - $CaCO_3$) was freshly prepared following the method mentioned in the Āyurvedic Pharmacopoeia of India (Part I, Vol I). *Cūrṇodaka* is the filtrate obtained from the mixture of 250mg of lime powder and 60ml of water, kept for 9 hours.

Equipments for *Śodhana* (Purification)

Stainless steel vessel having a capacity of 10 L, stainless steel spatula (length 30 cm), stainless steel filter, thick cotton cloth were used for purification of *Citraka* (both species).

Procedure

The purification procedure was carried out at Changampally Ayurveda Vaidyasala, Malappuram, Kerala under the strict monitoring of expert āyurvedic physicians. *Śodhana* of *Citrakamula* (Root of 2 species of *Citraka*) were carried out by one of the classical methods. The cut pieces of 1 kg root of *citraka* (both species) were kept immersed in lime water ($CaCO_3$) for 24 hours. There was a significant colour change from beetroot red to a pale pink after the mentioned time period. Later the roots are taken out, washed with hot water, dried and preserved as '*Śuddha Citraka*' - purified form for further pharmaceutical use.

Fig 1: Step wise Pharmaceutical procedure of *Citraka Śodhana*

Fig: 1a Drug Collection Fig: 1b Crushing of the Drug Fig: 1c *Cūrṇodaka* preparation ($CaCO_3$) Fig: 1d Drug is immersed in *Cūrṇodaka* (Colour changed to beetroot red after 1 hour) Fig: 1e Colour change observed after 24 hours Fig: 1f Drug washed with pure water Fig: 1g & 1h Drug after *Śodhana* (Purification) Fig: 1i Final stage of drying

Preparation of sample

Roots of *Plumbago rosea* Linn. and *Plumbago zeylanica* Linn. before and after *śodhana* were powdered with mechanical grinder and passed through mesh no. 60.

Physico-chemical evaluation

The Physico-chemical parameters such as Loss on drying (LOD), Total ash, Acid insoluble ash, Water soluble ash, pH with Eutech Instruments pH Tutor, Alcohol soluble extractive value and Water soluble extractive value were carried out following standard procedures recommended by Āyurvedic Pharmacopoeia of India.

Preliminary phytochemical screening

The coarse power of the root of *Citraka* (both species) was made in to decoction as per Āyurvedic Pharmacopeia of India

(Part I, Vol I).The decoctions were used for preliminary phyto-chemical screening with a set of various chemical tests viz., Dragendroff's, Wagners's, Mayer's, Hager's tests for alkaloids; Molisch's, Fehling's, Benedict's tests for carbohydrates; Libermann-Burchard, Salkowski tests for steroids; Magnesium test for saponins; Ferric chloride test for tannins; Shinoda's test for flavonoids; Alcoholic ferric chloride test for phenol; 2 N Sodium hydroxide test for coumarins; thionyl chloride test for triterpenoids; Sodium bicarbonate test for carboxylic acid; Acetone test for resin; 0.5% of Sodium hydroxide test for quinone. These parameters were performed following the standard procedure.

HPTLC

Standard preparation - The standard Plumabagin marker compound was prepared in different concentration range (10-100ng) in ethanol absolute 99.9% (Changshu Hongsheng Fine Chemicals Co. ltd)

Sample preparation - 1g each of root powders of *Plumbago rosea* Linn. and *Plumbago zeylanica* Linn. before and after *śodhana* were extracted with 10 ml of alcohol. 2µl (sample) of each of the above extract were applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (9.9: 0.1) solution. The developed plates were visualized under short UV, long UV and then derivatised with vanillin sulphuric acid (observed under white light). Scanned under UV 272nm. R_f, colour of the spots and densitometric scan were recorded. Marker Plumbagin (P7262 - 100mg) was obtained from Sigma Aldrich, USA.

Method

Standard Plumbagin, *Plumbago rosea* Linn. and *Plumbago zeylanica* Linn.(both before and after *śodhana*) were applied in different concentrations. Marker concentration was ranging from 1 -10 µl (10-100ng). *Plumbago rosea* Linn. (before and after *śodhana*) and *Plumbago zeylanica* Linn. (before and after *śodhana*) were applied in 2 µl concentration. Densitometric scanning was carried out at 272 nm, where dark green colour band appeared. The calibration curve was plotted for the above mentioned concentration of Plumbagin Marker.

Results and Discussion

Pharmaceutical study

1000 g of *Plumbago zeylanica* Linn. was taken for *śodhana*. The percentage loss of sample after *śodhana* was 59 %; similarly 1000 g of *Plumbago rosea* Linn. was taken for *śodhana* and the loss percentage was 46.5 %. (Table 1)

Table 1: Pharmaceutical study of *Citraka* root

Batch	Weight of *Citraka* Root (g)			% of the *Citraka*
	Initial (Before *Śodhana*)	Obtained (After *Śodhana*)	Loss/gain	Loss/gain %
Plumbago zeylanica Linn. (*Śveta Citraka*)	1000	410	590	59
Plumbago rosea Linn. (*Rakta Citraka*)	1000	535	465	46.5

Analytical study

The parameters such as physico-chemical analysis, qualitative tests of raw and *śodhita citraka* (both species) were carried out as a part of analytical study and was systematically presented in Table 2, 3, 4 and 5 respectively.

In the HPTLC chromatogram for quantification of Plumbagin (marker) which was identified at Rf of 0.50±0.02, for different standard concentrations range of 10-100ng/spot with R^2±SD = 0.97±23.75 (regression via height) and R^2±SD = 0.99±11.50 (regression via area). In *Plumbago rosea* Linn. before *śodhana* via height; the concentration obtained was 55.99 ng in 2µl (200µg) concentration and via area was 50.74 ng. *Plumbago rosea* Linn. after *śodhana* in 2µl (200µg) concentration it was 17.63 ng via height and 17.65 ng via area. The before *śodhana* of *Plumbago zeylanica* Linn. in 2µl (200µg) concentration was 30.67 ng via height and 28.19 ng via area. In *Plumbago zeylanica* Linn. in 2µl (200µg) concentration after *śodhana* it was not detected. This shows that after *śodhana* (Processing) the toxic naphthoquinone - Plumbagin content was significantly negligible in quantity. A secondary finding from the present study suggest that the toxic naphthoquinone - Plumbagin was not detected in *Plumbago zeylanica* Linn. after *śodhana* (purification). (Table 6).

The HPTLC method development was precise, specific, accurate and robust for determination of plumbagin in *P. rosea* Linn. and *P. zeylanica* Linn. before and after *śodhana*. The comparison of bio marker compound has shown that it is present in higher amount before *śodhana* in *P. rosea* Linn. and the concentration was lowered after *śodhana* process, while in *P. zeylanica* Linn. The concentration was in detectable quantity before *śodhana* and after *śodhana* it was absent. The biological activity of a plant extract is influenced by quantity of active principle present in the extract. Since Plumbagin used as inhibitor in hormone refractory prostate cancer and many other ailments, it is essential to develop a chromatography method for plumbagin quantification.

Table 2: Physico-chemical parameters for *Plumbago rosea* (before and after *śodhana*)

	before	after
pH	5.28	7.80
Loss on drying	11.56±0.01	11.06±0.02
Total ash	6.57±0.01	8.11±0.26
Acid insoluble ash	0.49±0.00	0.09±0.00
Water soluble ash	3.30±0.01	3.27±0.00
Alcohol soluble extractive value	11.24±0.01	5.75±0.01
Water soluble extractive value	51.42±0.00	44.50±0.01

Table 3: Physico-chemical parameters for *Plumbago zeylanica* (before and after *śodhana*)

	before	after
pH	5.55	8.45
Loss on drying	10.80±0.01	9.87±0.01
Total ash	2.20±0.00	7.06±0.00
Acid insoluble ash	0.19±0.00	0.00±0.00
Water soluble ash	0.69±0.01	0.48±0.01
Alcohol soluble extractive value	15.85±0.00	1.28±0.00
Water soluble extractive value	26.11±0.01	10.24±0.01

Table 4: Results of preliminary phytochemical screening of Root of *Plumbago rosea* before and after *śodhana* process

Test	Inference	
	before	***after***
Alkaloid	+	+
Steroid	-	-
Carbohydrate	+	+
Tannin	-	-
Flavanoids	-	-
Saponins	+	+
Terpenoid	-	-
Coumarins	-	-
Phenols	+	+
Carboxylic acid	-	-
Amino acids	-	-
Resin	+	+
Quinone	+	+

(+) - present; (-) – negative

Table 5: Results of preliminary phytochemical screening of Root of *Plumbago zeylanica* before and after *śodhana* process

Test	Inference	
	before	***after***
Alkaloid	+	+
Steroid	-	-
Carbohydrate	+	+
Tannin	+	+
Flavanoids	+	+
Saponins	+	+
Terpenoid	+	+
Coumarins	-	-
Phenols	-	-
Carboxylic acid	-	-
Amino acids	-	-
Resin	+	+
Quinone	+	-

(+) - present; (-) – negative

Table 6: Percentage of Plumbagin in test extracts by HPTLC

Sample	X (Calc) via Height (µg)	X (Calc) via Area (µg)	Average (µg)	Percentage (%)
Plumbago rosea before *śodhana*	0.027	0.025	0.026	0.026%
Plumbago rosea after *śodhana*	0.0088	0.0088	0.0088	0.009%
Plumbago zeylanica before *śodhana*	0.0153	0.0140	0.0146	0.015%
Plumbago zeylanica after *śodhana*	Not detected	Not detected	Not detected	Not detected

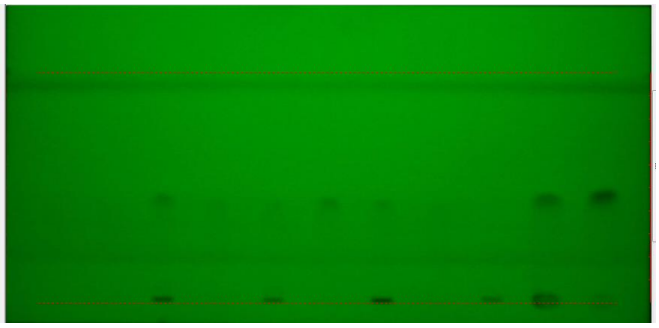


Fig 2a. At 254nm

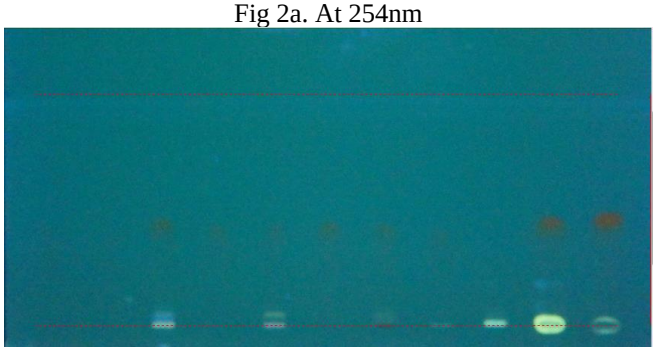


Fig 2b. At 254nm

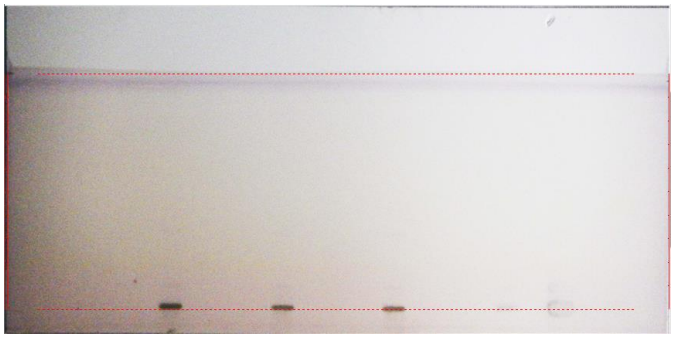
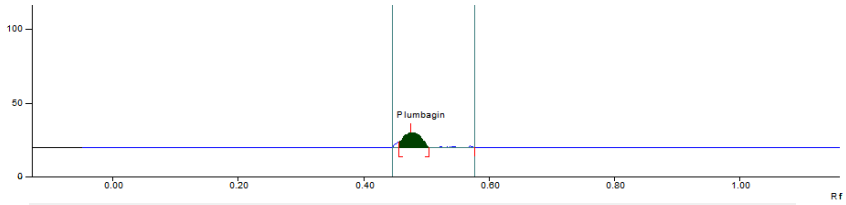


Fig 2c. At 254nm

Fig 2: HPTLC Photo documentation of sample of root of *Plumbago rosea and plumbago zeylanica* before and after *śodhana*

Track 1: *Marker plumbagin* (Standard 1) - 1µl
Track 2: *Marker plumbagin* (Standard 1) - 1µl
Track 3: *Plumbago rosea before śodhana* - 2µl
Track 4: *Marker plumbagin* (Standard 2) - 2µl
Track 5: *Plumbago rosea after śodhana* - 2µl
Track 6: *Marker plumbagin* (Standard 3) - 4µl
Track 7: *Plumbago zeylanica before śodhana* - 2µl
Track 8: *Marker plumbagin* (Standard 4) - 8µl

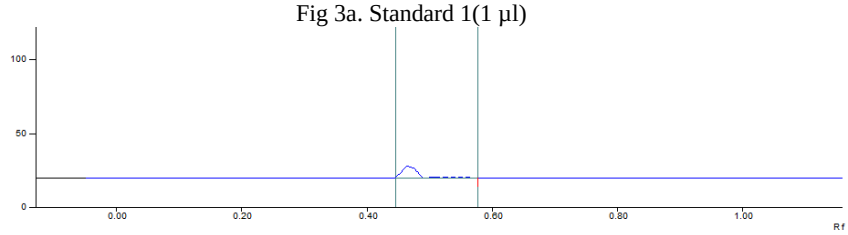
Track 9: *Plumbago zeylanica after śodhana* - 2µl
Track 10: *Marker plumbagin* (Standard 5) - 10µl
Track 11: *Marker plumbagin* (Standard 5) - 10µl
Solvent system- Toluene: Formic acid (9.9: 0.1)



Track 1, ID: Standard1

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.46 Rf	3.9 AU	0.48 Rf	10.3 AU	100.00 %	0.50 Rf	0.1 AU	210.7 AU	100.00 %

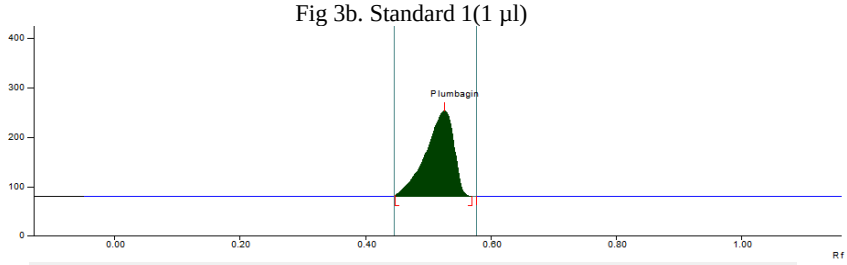
Fig 3a. Standard 1(1 µl)



Track 2, ID: Standard1

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %

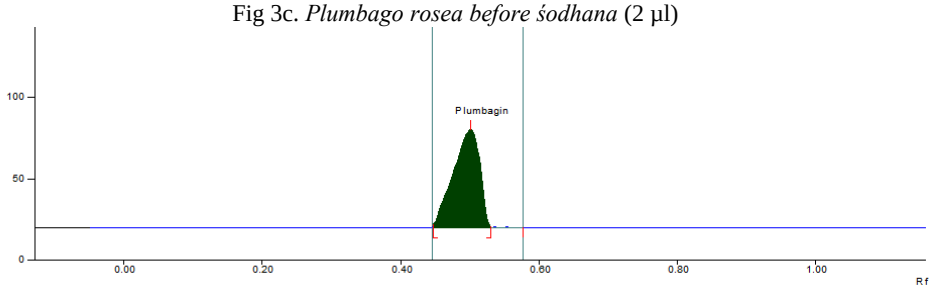
Fig 3b. Standard 1(1 µl)



Track 3, ID: plumbago rosea before shodhana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.45 Rf	1.5 AU	0.53 Rf	172.3 AU	100.00 %	0.57 Rf	0.4 AU	5522.3 AU	100.00 %

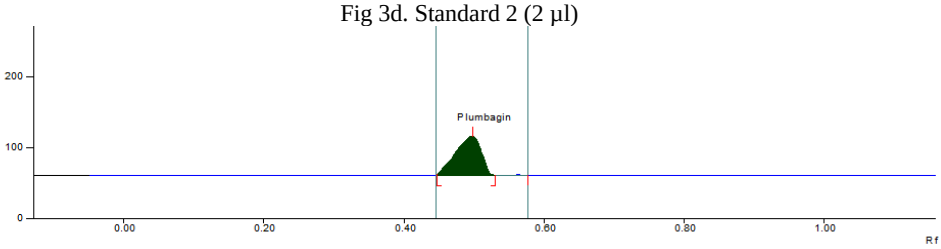
Fig 3c. *Plumbago rosea before śodhana* (2 µl)



Track 4, ID: Standard2

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.45 Rf	1.6 AU	0.50 Rf	60.2 AU	100.00 %	0.53 Rf	0.4 AU	1672.9 AU	100.00 %

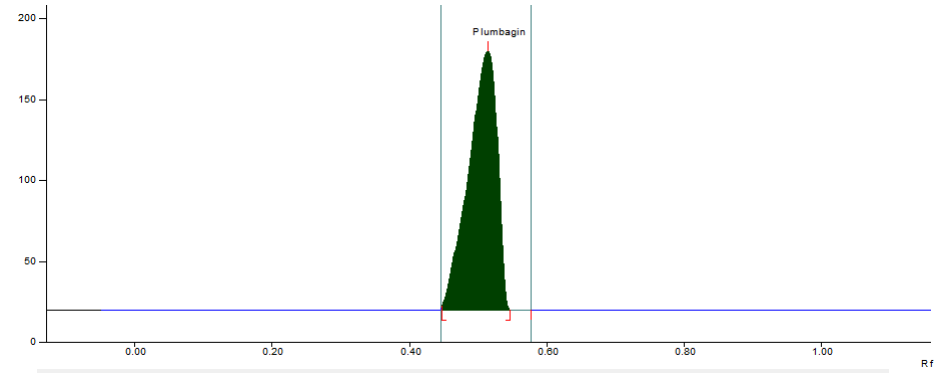
Fig 3d. Standard 2 (2 µl)



Track 5, ID: plumbago rosea after shodhana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.45 Rf	1.7 AU	0.50 Rf	55.5 AU	100.00 %	0.53 Rf	0.4 AU	1477.5 AU	100.00 %

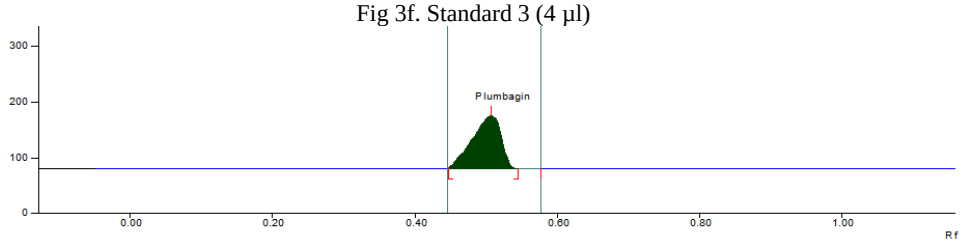
Fig 3e. *Plumbago rosea after śodhana* (2 µl)



Track 6, ID: Standard3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.45 Rf	2.3 AU	0.51 Rf	159.8 AU	100.00 %	0.55 Rf	0.3 AU	4828.0 AU	100.00 %

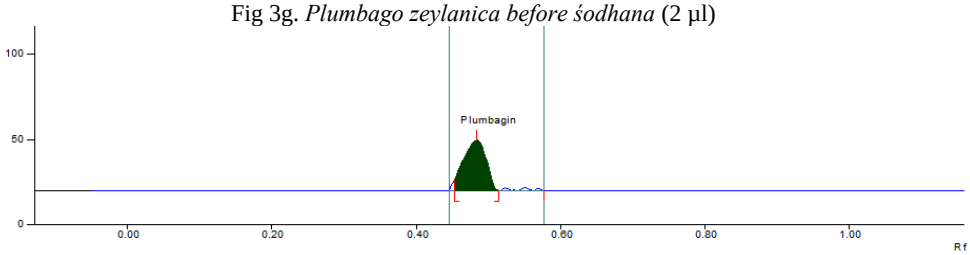
Fig 3f. Standard 3 (4 µl)



Track 7, ID: plumbago zeylanica before shodhana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.45 Rf	1.4 AU	0.51 Rf	95.2 AU	100.00 %	0.55 Rf	0.2 AU	2765.2 AU	100.00 %

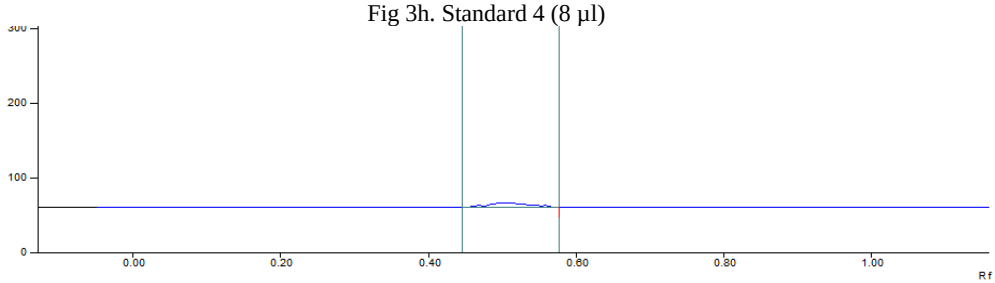
Fig 3g. *Plumbago zeylanica before śodhana* (2 µl)



Track 8, ID: Standard4

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.45 Rf	6.4 AU	0.48 Rf	29.7 AU	100.00 %	0.51 Rf	0.0 AU	676.7 AU	100.00 %

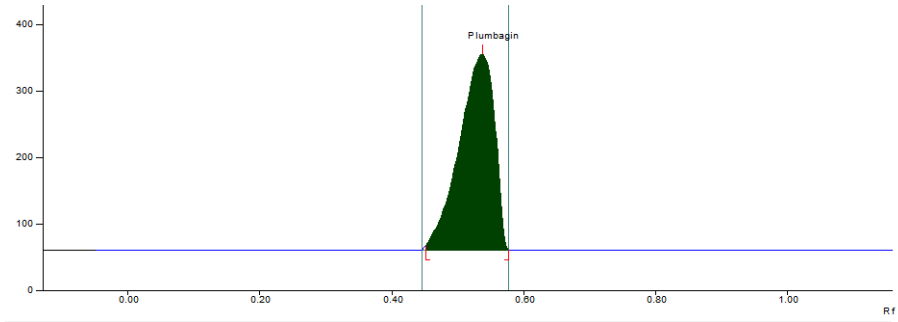
Fig 3h. Standard 4 (8 µl)



Track 9, ID: plumbago zeylanica after shodhana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %

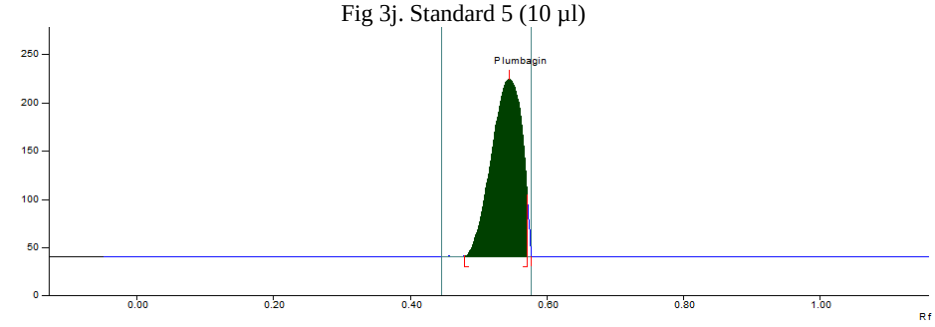
Fig 3i. *Plumbago zeylanica after śodhana* (2 µl)



Track 10, ID: Standard5

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.45 Rf	6.3 AU	0.54 Rf	294.4 AU	100.00 %	0.58 Rf	0.0 AU	11347.4 AU	100.00 %

Fig 3j. Standard 5 (10 µl)

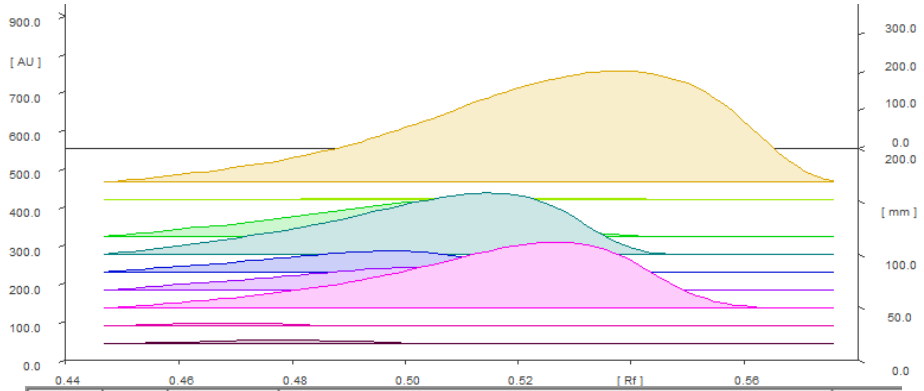


Track 11, ID: Standard5

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.48 Rf	1.2 AU	0.55 Rf	183.6 AU	100.00 %	0.57 Rf	64.1 AU	5911.7 AU	100.00 %

Fig 3k. Standard 5 (10 µl)

Fig 3: Densitometric scan of the sample at 272nm



Track	Scan	Integrate	Sample ID	Color
1	✓	✓		
2	✓	✓		
3	✓	✓	plumbago rosea before shodhana	
4	✓	✓		
5	✓	✓	plumbago rosea after shodhana	
6	✓	✓		
7	✓	✓	plumbago zeylanica before shodhana	
8	✓	✓		
9	✓	✓	plumbago zeylanica after shodhana	
10	✓	✓		
11	✓	✓		

At 272nm

Fig 4: 3-D Chromatogram

Substance: Plumbagin @ 272 nm Regression mode: Linear

Regression via height Y = 1.867 + 3.043 * X r = 0.97917 sdv = 23.75 %
area Y = -680.5 + 122.2 * X r = 0.99632 sdv = 11.50 %

Track	Vial	Rf	Amount Fraction	Height	X(calc)	Area	X(calc)	Remark
1	1	0.48	10.00 ng	10.35		210.68		Std Level 1
2	1							Std Level 1: No peak detected or peak deleted
3	2	0.53		172.27	55.99 ng	5522.31	50.74 ng	Sample plumbago rosea before shodhana
4	1	0.50	20.00 ng	60.24		1672.88		Std Level 2
5	3	0.50		55.52	17.63 ng	1477.48	17.65 ng	Sample plumbago rosea after shodhana
6	1	0.51	40.00 ng	159.85		4827.99		Std Level 3
7	4	0.51		95.21	30.67 ng	2765.19	28.19 ng	Sample plumbago zeylanica before shodhana
8	1							Std Level 4 not evaluated
9	5							Sample plumbago zeylanica after shodhana: No peak detected or peak deleted
10	1	0.54	100.00 ng	294.39		11347.40		Std Level 5
11	1							Std Level 5 not evaluated

Fig 5a. Quantification via linear regression

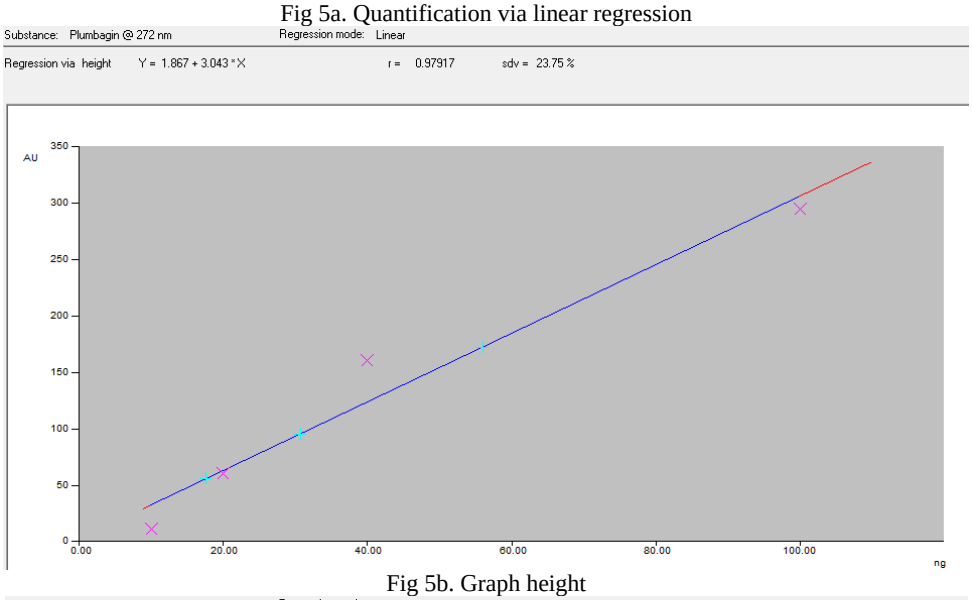


Fig 5b. Graph height

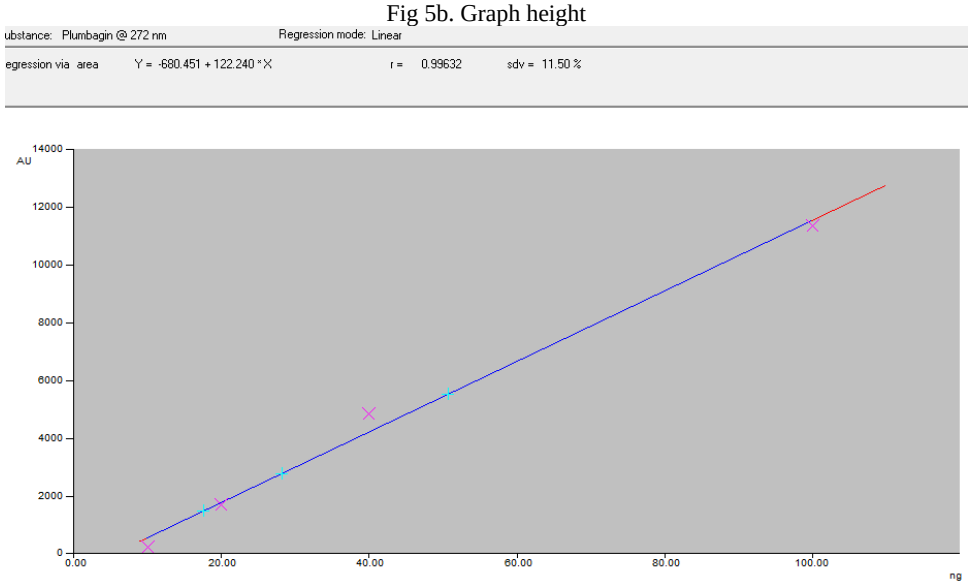


Fig 5c. Graph area

Fig 5: Quantification of Plumabagin Via peak height and Area

Conclusion

From the comparative analysis of both species of *Citraka* (*Plumbago rosea* Linn. and *Plumbago zeylanica* Linn.) pre and post purification as specified in *Arogya Kalpadruma* and API (Āyurvedic Pharmacopoeia of India) reveals that when subjected to *Śodhana* (Purification) there is a decrease in quantity of plumbagin (toxic-naphthoquinone). In *Plumbago zeylanica* Linn. (*Śveta Citraka*), Plumbagin becomes totally undetectable in comparison with *Plumbago rosea* Linn. (*Rakta citraka*) with plumbagin in traceable amounts. Hence it can be concluded that system of purification (*Śodhana*) can influence phytochemical, pharmacological and toxicological profile of the herbal drugs thereby promoting its potency, safety and efficacy.

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