Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 14 (4), 2023; 1087-1092

Physicochemical and Phytochemical analysis of Wild and Cultivated Chitrak (Plumbago zeylanica Linn.)

Research Article

Payal Kamdi[1*], Rajkumar Gupta[2], Pramod Khobragade[3], Gayatri Bahatkar[4]

1. Department of Dravyaguna, Bhausaheb Mulak Ayurveda College & Research Hospital, Butibori, Nagpur. India.
2. Department of Dravyaguna, Mahatma Gandhi Ayurveda College Hospital & Research Centre, Datta Meghe Institute of Higher Education & Research (DU), Sawangi (M), Wardha. India.
3. Department of Dravyaguna, DMAMCH&RC, Wanadongari (H), Nagpur. India.
4. Department of Quality Assurances, Vidyabharti College of Pharmacy, Amravati (M), India.

Abstract

Chitrak (*Plumbago zeylanica* Linn.) is a perennial shrub that grows slowly. Its roots become mature enough for medicinal use after a long time. The major source of *Chitrak* is wild and mainly roots are used in medicine preparation, so it needs to be uprooted and plant sustainability can't be maintained. Therefore, this study was conducted to evaluate the physicochemical and phytochemical parameters of wild and cultivated root, stem, and leaves of *Chitrak*. Wild and cultivated *Chitrak* was collected from *Kharangana* forest and Herbal garden, MGACH&RC, Dist. Wardha respectively in an appropriate season as per the guidelines mentioned in *Charak Samhita.* Collected plant material was cleaned properly, shed-dried, ground, stored in an airtight container, and labeled for further study. For the Chromatographic study, a standard Plumbagin marker was procured from Sigma Aldrich Company, Bengaluru. This study concluded that all test samples contain Plumbagin but cultivated *Chitrak* root contains more Plumbagin than leaves and stem. So, according to ancient *Ayurveda* texts, the use of *Chitrak* root for various diseases as compared to leaves and stem is justified and ideal as far as the Plumbagin is concerned. But as leaves and stems also contain some amount of Plumbagin then it may be used for less chronic diseases or in patients who cannot tolerate the *Tikshnatva* and *Ushnatva* of *Chitrak* root. In this study, cultivated *Chitrak* root showed more Plumbagin than the wild source. So we can say that cultivated sources can be used in therapeutics instead of the wild sources after confirmation by clinical trials thereby minimizing the unauthorized collection practices and motivating the cultivation of this plant to some extent.

Keywords: *Chitrak,* HPTLC, Physicochemical, Phytochemical, *Plumbago zeylanica*.

Introduction

Chitrak, one of the frequently used important medicinal plants in *Ayurveda* has been included in *Agryadravya* (1) by *Acharya Charaka and* considered as *Rasayana* by *Acharya Sushrut* (2) and *Acharya Vagbhata* (3). Mostly *Chitrak* root or root bark is used in the *Ayurvedic* formulations (4). *Chitrak* grows slowly and after long time, its roots become mature enough for medicinal use. Generally, this plant is propagated and grown by seeds and semi-ripe cuttings which are mainly preserved by growth regulators. But traditional approaches for proliferation are less efficient and problematic due to long time sprouting of seeds for 21 to 30 days and degradation in germination rate by extended storage. Currently the demand *Chitrak* root is increasing because of its uses in various types of *Ayurvedic* formulation. But due to continued overexploitation of forest, disappearance of many medicinal plant species from the flora and escalating worldwide demand of herbal medicine all over the world created gap between demand and supply of medicinal plants (5).

There are many of the wild medicinal plant species which are facing serious threat due to indiscriminate harvesting, there by leading to dangerous and unethical practice of adulteration which has compromised the credibility and reliability of *Ayurvedic* medicine to serious extent. To maintain the standard of *Ayurvedic* medicine, it requires good qualities of medicinal plants. According to NMPB (National Medicinal Plant Board), major source of *Chitrak* is wild (6) and so there is a need of conservation, cultivation and propagation of this plant species and to search for the alternative sources.

Chitrak grows quite slowly and in medicine preparation mostly roots are used, so it needs to be uprooted and plant sustainability can't be maintained. But *Acharya Sushruta* has classified *Chitrak* in *Shaka varga* (7). So the question arises that can other parts of

*** Corresponding Author:**

Payal Kamdi

Assistant Professor, Department of Dravyaguna, Bhausaheb Mulak Ayurveda College & Research Hospital, Butibori, Nagpur. Maharashtra. India.

Email Id: dr.payalkamdi@gmail.com

the plant be used instead of its root in the medicine? *Chitrak* which is a perennial shrub, one has to compromise whole plant at the cost of its root. Therefore study regarding physico-chemical and phyto-chemical similarities and dissimilarities become the focal point for their use of stem and leaves alternative to the roots.

Materials and Methods

Collection, authentication and standardization of plant material are important steps to maintain quality, safety and efficacy of herbal medicine. Wild *Chitrak (Plumbago zeylanica* Linn.*)* was collected from Kharangana forest, Dist. Wardha (Maharashtra) with the help of forest department after extensive search and cultivated *Chitrak* was collected from Herbal garden, MGACH&RC, Salod (H), Wardha. During collection, it was observed that the *Chitrak* plants are not much available in the forest because of overexploitation and it is on the verge of extinction in some geographical areas where it was abundantly available earlier.

Roots of *Chitrak* were collected in *Greeshma ritu* (May 2020) and stem and leaves of *Chitrak* were collected in *Vasant ritu* (March 2020) as per the guidelines mentioned in *Charak samhita* regarding collection of different parts of plant in different seasons (8). After that the voucher specimen of collected plant got authenticated by FRLHT [Foundation for Revitalization of Local Health Traditions] Bengaluru with Voucher no 124312. Collected plant material then cleaned properly, shed dried, grinded, stored in an airtight container and labeled for further study. For Chromatographic study, standard Plumbagin marker was procured from Sigma Aldrich Company, Bengaluru. For comparative evaluation, six groups were formed viz. WR (Wild Root), WS (Wild Stem), WL (Wild Leaves), CR (Cultivated Root), CS (Cultivated Stem) and CL (Cultivated Leaves).

A) Panchbhautik parikashan (9-11) **-** According to *Ayurveda,* each *dravya* is made up of five basic elements i.e. *Akash, Vayu, Agni, Jala, Prithvi mahabhoot* and *rasadi* properties of *dravya* mainly depend on these *Mahabootas.* So, organoleptic study was done by five senses i.e *Shabda* (Fracture), *Sparsha* (Texture), *Rupa* (Colour), *Rasa* (Taste), *Gandha* (Smell) to determine the quality of dravya.

B) Physicochemical study (12-14) - Physicochemical studies were carried out as per the *Ayurvedic* Pharmacopoeia of India (API).

1. Foreign matter: Each powder drug was taken and spread uniformly into thin layer and examined for the detection of foreign substance with the help of hand lens. After that percentage of foreign matter was calculated and noted.

2. Loss on drying: The loss on drying was calculated by using 2 g of sample. Sample is taken in Petri dish (tarred evaporating dish) and dried in an oven at 110° C till constant weight. The weight after drying was noted and percentage was calculated on the basis of air-dried sample and expressed as % w/w.

3. Total Ash value: About 2g of accurately weighted crude drug was taken in previously weighted silica crucible and incinerated in muffle furnace. Temperature should not exceed 450^{o}C until free carbon. After that drug was kept in the desiccator for self cooling and weighted again. From this weight of residue, ash value was calculated in the percentage on the basis of air dried sample.

4. Acid insoluble ash: To get acid insoluble ash, obtained ash value from above method is treated with diluted HCL to detect silica, calcium oxalate and carbonate content. The ash of the drug was boiled along with 25 ml of 6 N HCl for at least 5 min. The insoluble matter was collected on ash less filter paper (Whatman filter paper no. 40)

5. Extractive Value: The amount of extractives give information about quality of a drug.

a) Water soluble extractive: 5 g of sample was taken and macerated with 50 ml of distilled water. This solution was kept aside for 24 hours. It was stirred intermittently and after 24 hr residue was filtered by Whatman filter paper and precaution was taken for prevention of loss of solvent. After filtration, 25 ml of filtrates was taken and evaporated over water bath and then at 110^{o}C in hot air oven till the constant weight was observed.

b) Alcohol soluble extractive: This method is useful for the raw drugs which contain alcohol soluble contents. For the determination of alcohol soluble extractive, similar procedure of water soluble extractive was repeated, but instead of distilled water, 90% methanol is used.

6. Determination of pH value – 10 gm of sample was weighed and transferred to a clean flask. 100ml distilled water added and shook it continuously with glass rod for 45 min. The pH value was measured with pH meter by calibrating it previously with standard buffer solution of pH 4 and 7. Then pH of the sample was calculated on dipping the electrode in the sample solution.

C) Preliminary Phytochemical screening (15, 16) **-** Phytochemical investigation is an important part before pharmacological evaluation. It provides basic idea regarding drug's probable pharmacological activity as plant biosynthesizes many compounds that exert therapeutic effect in the human body.

1. Estimation of total Alkaloids by Dragendorff's reagent - The filtrates were taken in test tubes and treated with 0.5ml Dragendorff's reagent and few drops of diluted 2N HCL. Appearance of orange to reddish colour precipitate confirms the positive test.

2. Estimation of Glycosides by Keller-Kiliani test: 0.5 ml of glacial acetic acid, conc. H_2SO_4, 1 drop of 5% $FeCl_3$ was added to a test tube containing 2ml of extract. At the junction of 2 liquids, reddish brown colour was seen. Presence of glycosides was confirmed as upper layer turned bluish in colour.

3. Estimation of Saponin by Foam test: For 30 seconds, test tube containing 1ml extract and 5ml of

distilled water, was vigorously shaken and then kept aside for 20 minutes. Formation of persistent foam indicated the presence of Saponin in the sample.

4. Estimation of Amino acids by Ninhydrin test: Non Appearance of grey blue colour confirmed the absence of amino acids after adding 2 drops of Ninhydrin solution into the test tube containing alcohol and water extracts.

5. Estimation of Flavonoids by Shinoda test: In test tube, 5ml extract was taken along with 95% ethanol and 0.5 g magnesium turnings (ribbon). On adding few drops of conc. HCL in it, Crimson black colour was not appeared which means test was negative.

6. Estimation of Tannin: The extract of sample when treated with diluted solution of ferric chloride, blue colour changed to olive-green which means test was positive.

7. Estimation of Starch: Extract of sample was treated with iodine solution. Blue colour appearance indicates the presence of starch in the sample.

8. Estimation of total Carbohydrates by Molisch test: To 1 ml of extract, 2 drops of fresh 10 % *a* -naphthol solution was added followed by gentle addition of Sulphuric acid. Carbohydrate presence was confirmed by purple colour ring between two layers of liquid.

9. Estimation of Proteins by Biuret test: Few drops of 1% $CuSO_4$ were added to test tube having, 2ml of extract and 1ml of 40% NaOH. Proteins were confirmed by presence of violet colour.

10. Estimation of Steroids by Salkowski reaction: In a test tube 2ml extract, 2ml Chloroform and 2 ml conc. H_2SO_4 was taken. Presence of steroids was confirmed by reddish brown colour.

D) Chromatographic study (17-20): Chromatography is an analytical method appropriate and suitable for the separation and quantitative determination of considerable number of compounds. HPTLC is quality assessment tool for the evaluation of plant material.

Preparation of Standard Plumbagin solution: Accurately weighed 25mg standard Plumbagin was dissolved in 25 ml of methanol to prepare 1mg/ml solution.

Preparation of Test samples: Each 1 gm of sample was taken in conical flask along with 10 ml methanol and kept it overnight. Next day all samples are sonicated and centrifuged. Supernatant liquid is collected and stored in test tube for further use.

Application of standard and sample: For spot application, Linomat V sample applicator (CAMAG, 4132 Muttenz, Switzerland) was used and samples solution applied to Silica Gel 60 F_{254} plate as sharp bands. After that this plate kept aside till the spots get dried. After that the mobile phase, Toluene: Formic acid (9.9:0.1) was poured into Twin trough chamber and chamber covered with lid for 45 minutes to allow saturation. Then lid was removed and plate was inserted into the chamber carefully. Hereafter the plate was allowed to develop until the solvent front had travelled at the distance of 70mm above the base of the plate. After this the plate was taken off from the chamber and dried by using blow dryer and then at 266nm wavelength, detection was done by using CAMAG TLC Scanner III & Win cats 4.02 software. This study was done at CRL (Central Research Laboratory), DMIHER (Datta Meghe Institute of Higher Education & Research), Sawangi (M), Wardha, Maharashtra.

Observations and Results

Organoleptic study: The test drugs were subjected to following organoleptic characters viz. touch, colour, odor, taste and observations are recorded in Table no 1 & 2.

Table 1: Result of Organoleptic study of crude drug

Test	WR	WS	WL	CR	CS	CL
Shabda Pariksha	Tough to break/ make *Churna* (*Kat* sound on breakage)	Hard (*Abhangur*)	-	Tough to break/ make *Churna* (*Kat* sound on breakage)	Hard (*Abhangur*)	-
Sparsha Pariksha	Rough	Rough	Smooth	Rough	Rough	Smooth
Rupa Pariksha	Dark Blackish Brown	Pale Green	Dark Green	Dark Blackish Brown	Pale Green	Dark Green
Rasa Pariksha	Acrid astringent Pungent	Bitter, astringent	Characteristic	Acrid astringent Pungent	Bitter, astringent	Characteristic
Gandha Pariksha	Disagreeable Irritant	Characteristic	-	Disagreeable Irritant	Characteristic	-

Table 2: Result of Organoleptic study of powdered drug

Test	WR	WS	WL	CR	CS	CL
Sparsha Pariksha	Smooth	Smooth	Rough	Smooth	Smooth	Rough
Rupa Pariksha	Dark Brown	Greenish cream	Dark Green	Dark Brown	Greenish cream	Dark Green
Rasa Pariksha	Pungent & Bitter	Characteristic	Characteristic	Pungent & Bitter	Characteristic	Characteristic
Gandha Pariksha	Disagreeable Irritant	Irritant	-	Disagreeable Irritant	Irritant	-

Physicochemical study: Physicochemical parameters of all the samples were tested by performing various physicochemical tests and observed results are stated in the table 3.

Table 3: Physicochemical analysis of *Chitrak* samples

Sr no.	Parameters	WR	WS	WL	CR	CS	CL
1	Foreign matter	Nil	Nil	Nil	Nil	Nil	Nil
2	pH (Aq. Extract for extractive value)	4.55	7	7.10	4.65	7.2	7.3
3	Loss on drying	7%	6.5%	8.4%	8%	6.9%	8%
4	Ash value	2.4%	4.4%	9%	1%	3.5%	8%
5	Acid insoluble ash	0.3%	0.3%	2.4%	0.5%	0.2%	0.8%
6	Water soluble extractive	17.6%	5.6%	6%	14.8%	6.8%	5%
7	Alcohol soluble extractive	24.4%	6.8%	14%	29.2%	15.2%	11.6%

Phytochemical study

Table 4: Preliminary Phytochemical Screening of *Chitrak* samples

Characters	Test	Results					
		WR	WS	WL	CR	CS	CL
Steroid	Salkowski reaction	+	+	-	+	+	-
Alkaloids	Dragendorff's test	-	-	-	-	-	-
Glycoside	Killer Kiliani test	+	+	+	+	+	+
Flavonoid	Shinoda test	-	-	-	-	-	-
Tannin	Ferric chloride reagent	+	+	+	+	+	+
Protein	Biuret test	+	+	+	+	+	+
Carbohydrate	Molisch's test	+	+	+	+	+	+
Starch	Iodine test	+	+	+	+	+	+
Saponin	Foam test	+	+	+	+	+	+
Amino acids	Ninhydrin test	-	-	-	-	-	-

HPTLC Observations and Results

Table 5: Comparative HPTLC profile of all the Samples with Standard Plumbagin Marker at 266 nm wavelength

Track no.	Sample code (Applied volume)	No of Spots	Rf values	Area covered
1	Std (1µl)	3	0.39, **0.45,** 0.51	7891.7
2	Std (2 µl)	3	0.36, **0.43,** 0.49	10206.1
3	Std (3 µl)	3	0.36, **0.43,** 0.50	12082.7
4	Std (4 µl)	3	0.35, **0.42**, 0.48	13093.1
5	Std (5 µl)	9	0.30, 0.35, 0.49, 0.31, **0.41**, 0.49, 0.32, 0.47, 0.50	87.7, 12961.9, 75.9
6	Std (6 µl)	9	0.30, 0.32, 0.48, 0.31, **0.39,** 0.49, 0.32, 0.45, 0.51	148.3, 13773.5, 104.7
7	Sample 1 (10 µl)	3	0.30, 0.37, **0.44**	9538.4
8	Sample 2 (10 µl)	9	0.32, 0.34, 0.42, 0.33, 0.37, **0.43,** 0.34, 0.41, 0.44	258.5, 927, 66.1
9	Sample 3 (10 µl)	3	0.31, 0.34, **0.39**	1292.7
10	Sample 4 (10 µl)	3	0.30, 0.35, **0.40**	13158.3
11	Sample 5 (10 µl)	9	0.31, 0.40, 0.48, 0.50, 0.35, 0.41, 0.49, 0.50, 0.39, **0.42,** 0.49, 0.52	2447.5. 171.8, 68.1, 375.1
12	Sample 6 (10 µl)	8	0.33, 0.44, 0.39, 0.45, **0.44,** 0.47	2471.3, 112.3

Table 6: Quantification of Plumbagin in all samples

Sample ID	Samples	Quantification of Plumbagin
STD	Standard (Plumbagin)	1%
Sample 1(WR)	Wild Root	0.24%
Sample 2 (WS)	Wild Stem	0.02%
Sample 3 (WL)	Wild Leaves	0.03%
Sample 4 (CR)	Cultivated Root	0.33%
Sample 5 (CS)	Cultivated Stem	0.004%
Sample 6 (CL)	Cultivated Leaves	0.06%

Discussion

Organoleptic study plays an important role in identification, authentication and quality assessment of the raw drug. Both wild and cultivated *Chitrak* root was dark blackish brown colour while powdered root showed dark brown colour. Taste of root was acrid astringent pungent and powder form of root has pungent & bitter taste. Both the form of roots had very strong disagreeable irritant smell. Powdered root had smooth texture while crude root had rough texture along with very tough fracture. Both wild and cultivated *Chitrak* stem was pale green in colour. It tastes bitter, astringent while powdered form of stem manifests characteristic taste. Crude stem had characteristic smell while *churna* form showed somewhat irritant smell. Texture of stem *churna* was smooth while crude drug had rough texture along with hard fracture. Both wild and cultivated *Chitrak* leaves showed dark green colour with characteristic taste. (Table -1&2)

Comparative Physicochemical study of wild and cultivated *Chitrak* root, stem and leaves were done by means of various physicochemical tests (Table -3). There was no foreign matter found in any sample as all samples were self collected from field, washed, dried and stored properly.

The percentage of moisture content depends upon air dried basis and it should be minimized to prevent decomposition of active chemical constituents and to increase the shelf life. The amount of moisture present in wild root was 7% and in cultivated root was 8%. Wild and cultivated stem had 6.5% and 6.9 % of moisture respectively, while 8.4% and 8 % of loss on drying was observed in wild and cultivated leaves respectively at 105° C.

Determination of ash value in the drug is important to assess the percentage of inorganic salts which are naturally occurring, adhering to it or intentionally added as an adulteration. In cultivated root 1% w/w and in wild root 2.4 % w/w ash value was recorded. Wild source has more content of ash compared to cultivated source which may be due to the presence of more minerals or salts in the forest soil. But still these values are acceptable as per the recommendation of API i.e. < 3%. Wild stem showed 4.4% w/w while cultivated stem showed 3.5% w/w of Ash value. In case of leaves, wild source had 9% w/w and cultivated source had 8% of ash value. The wild and cultivated root had 0.3% w/w and 0.5% w/w of acid insoluble ash respectively while wild and cultivated stem had 0.3% w/w and 0.2% w/w acid insoluble ash respectively. Wild and cultivated leaves had 2.4% w/w and 0.8% w/w of acid insoluble ash respectively. The acid insoluble ash values observed for wild and cultivated root are within the limits, given by API i.e. not more than 1%.

API has suggested that the water soluble extractive value of *Chitrak* root should not be less than 12%. The wild and cultivated root showed 17.6% w/w and 14.8% w/w of water soluble extractive value respectively which is acceptable as per API. Wild and cultivated stem had 5.6% w/w and 6.8% w/w of water soluble extractive respectively. Wild and cultivated leaves had 6% w/w and 5% w/w of water soluble extractive respectively. As per the API, alcohol soluble extractive value of *Chitrak* root should not be less than 12% and it was observed that the wild and cultivated root contained 24.4% w/w and 29.2% w/w of alcohol soluble extractive respectively which are acceptable according to API. Wild and cultivated stem had 6.8% w/w and 15.2% w/w of alcohol soluble extractive respectively. Wild and cultivated leaves had 14% w/w and 11.6% w/w of alcohol soluble extractive respectively.

pH of wild root was 4.55 in comparison with to 4.65 of cultivated root. Wild and cultivated stem is 7 and 7.2 respectively. Wild and cultivated leaves showed 7.1 and 7.3 pH respectively. This showed that roots are more acidic in nature than stem and leaves.

In Preliminary Phytochemical study Glycoside, Tannins, Proteins, Carbohydrates, Starch, Saponin were present in all samples. While amino acids, Flavonoids and Alkaloids were absent in all samples. Steroids were present in root and stem of wild and cultivated samples but absent in leaves of both the samples (Table- 4).

In HPTLC, the plate was studied under the wavelength 266 nm. Cultivated root extract showed maximum area covered (13158) followed by wild Root sample (9538.4) while cultivated stem showed minimum area covered (171.8). Sample 1, 2, 3, 4, 5 and 6 showed spots at Rf 0.44, 0.43, 0.39, 0.40, 0.42 and 0.44 respectively which were nearly same as found in Standard Plumbagin Marker Rf i.e 0.43 (Table 5). All these spots detected in standard and samples showed same yellow colored bands in visible light. So, the same constituent (Plumbagin) was present in all the samples. As Plumbagin is detected in all samples, quantification was done by multilevel calibration method through CAMAG Wincat software. Wild root had 0.24 % Plumbagin while cultivated root had 0.33 % Plumbagin. Wild stem had 0.02% and cultivated stem had 0.004% of Plumbagin content while wild and cultivated leaves had 0.03 % and 0.06 % of Plumbagin content respectively (Table -6).

Conclusion

This study concluded that all test samples i.e. wild root, stem, leaves and cultivated root, stem, leaves contained Plumbagin. This study also revealed that root of *Chitrak* contained more Plumbagin than leaves and stem. Cultivated root showed highest Plumbagin among all six samples. So, according to ancient *Ayurveda* texts, use of *Chitrak* root for various diseases as compare to leaves and stem is justified and ideal as it contained highest amount of Plumbagin. But leaves and stem also contained some amount of Plumbagin, therefore it may be used for the less chronic diseases or in patients who have Gastritis, Peptic Ulcer, Deodenal Ulcer, Gratroesophageal Reflux Disease where they may not tolerate the *tikshnatva* and *ushnatva* of *Chitrak* root. *Acharya Sushruta* has also included *Chitrak* in *Shaka varga* which also justifies the internal use of it's another part of the plant apart from its root. In this study,

cultivated *Chitrak* root showed more Plumbagin than wild source. So we can say that cultivated source can be used in place of wild source in therapeutics after confirmation by clinical trials thereby minimizing the unauthorised collection practices and motivate the cultivation of this plant to some extent.

Funding Support - Nil
Conflict of Interest – None

References

1. Shukla V, Tripathi R. Charak Samhita of Agnivesha. Su25/40, Vol 1.Varanasi; Chaukhamba Sanskrit Pratishthan; 2013. 338p.
2. Shastry J. Dravyaguna Vigyan. 2 ed. Vol 2, Varanasi; Chaukhmbha Orientalia; 2005. 314p.
3. Lochan K. Ashtanghrudayam of Vagbhata.Vol-3.U/ 62.Varanasi; Chaukhamba Sanskrit Sansthan; 2017. 376 p.
4. Deshpande A, Ranade S. Dravyaguna Vigyan. Part 1& 2, Pune; Proficient Publishing House; 2010. 468-472 p.
5. Prajapati N, Purohit S, Sharma A, et al. A Handbook of Medicinal Plants. Jodhapur; Agrobios India; 2013.178 p.
6. National Medicinal Plant Board, Government of India, Nmpb.nic.in. Available at https:// www.nmpb.nic.in Dated 15-9-2023 at 19:00 IST
7. Sharma A (ed). Sushruta. Sushrutasamhita. Su/46. Vol I. Varanasi; Chaukhamba Surbharati Prakashana; 2010. 414 p.
8. Shukla V, Tripathi R (ed). Charak Samhita of Agnivesha. Ka/10. Vol 2.Varanasi; Chaukhamba Sanskrit Pratishthan; 2010. 808 p.
9. Kokate C. Analytical Pharmacognosy, 46ed. Pune; Nirali Prakashan; 2010. 6.18p.
10. Paradkar S. Identification and Standardization of Crude Drugs according to Ayurvedic and Modern Parameters. Pharmajournals.stmjournals.in.2021, http://pharmajournals.stmjournals.in/index.php/ RRJoPC/article/view/439 Dated 17-9-2023 at 20:15 IST
11. Committee members. General guidelines for Drug development of Ayurvedic Formulations. CCRAS, Ministry of AYUSH, Govt of India. 2018. http:// ccras.nic.in/sites/default/files/viewpdf/Publication/ CCRAS_Guideline%20of%20Drug%20Developme nt.pdf Dated 01-10-2023 at 15:40 IST
12. Anonymous. The Ayurvedic Pharmacopoeia of India, 1ed. Part 1, Vol.1. Govt. of India, Ministry of Health and Family Welfare, Dept of Ayush. New Delhi; 2011. 14p.
13. Baxi A, Shukla V, Bhatt U. Methods of Qualitative Testing of some Ayurvedic Formulations. Jamnagar; Gujarat Ayurvedic University; 2001. 5p.
14. Meena A, Singh B, Kaur R. Pharmacognostic & Physicochemical Studies on Plumbago zeylanica Linn. Root. Drug Invention Today; 2010. 217p. http://www.researchgate.net/publication/331398081 Dated 17-9-2023 at 20:45 IST
15. Khandelwal K. Practical Pharmacognosy, Preliminary Phytochemical Screening, 28ed. Pune; Nirali Prakashan; 2017. 25.3p.
16. Rajakrishnan R, Lekshmi R, Benil P., et al. Phytochemical evaluation of roots of Plumbago zeylanica L. and assessment of its potential as a nephroprotective agent. Saudi Journal of Biological Sciences; 2017. 760p. https:// pubmed.ncbi.nlm.nih.gov/28490944 Dated 20-9-2023 at 13:00 IST
17. William H. (ed). Official methods of analysis. 4ed Washington; Association of Official Agricultural chemists; 2006. 25p.
18. Ravishankar S. Text book of Pharmaceutical Analysis. Otcmund; Rx Publication; 2001.36p.
19. Kokate C. Analytical Pharmacognosy, 46ed. Pune; Nirali Prakashan; 2010. 6.26p.
20. Kasture A, Wadodkar S, Mahadik K, More H. Pharmaceutical analysis, Industrial Methods. Vol.II. Pune; Nirali Prakashan; 2016. 14p.

*****