Original Article

Standard operating procedure of Purification of *Chitraka* (*Plumbago zeylanica* Linn.) along with pharmacognostical and analytical profiles of Plumbagin

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Abstract

Introduction: *Shodhana* (purification) is the process by which one can remove the impurity or toxicity of the raw drug and make the drug suitable for therapeutic purpose. *Chitraka* (*Plumbago zeylanica* Linn.) is well known drug in Ayurveda and root of this plant is being used for therapeutic purpose and requires purification before used as a medicine. **Aims and objective:** There is no data available for pharmacognostical and analytical profile of processed *Chitraka*, hence it was planned to develop SOP of processed *Chitraka* for its identity, purity and strength through pharmacognostical and analytical profile. **Materials and methods:** *Chitraka* roots were procured from Pharmacy, Gujarat Ayurved University, Jamnagar. Purification was done in five batches with *Churnodaka* (lime water). Organoleptic characters, microscopic features, pH, loss on drying, ash value, water soluble extracts, methanol soluble extracts and plumbagin quantification through high-performance thin layer chromatography (HPTLC) were carried out, before and after the purification. **Results:** Average 98.07% yield of *Chitraka* was obtained after purification. Differences were found in the processed samples of *Chitraka* in organoleptic features, pharmacognostical characters and physicochemical parameters, which show the impact of purification procedure on *Chitraka*. In HPTLC profile, plumbagin content was 0.29% in unpurified *Chitraka* powder, where in it was noted 0.98% after purification. **Conclusion:** Increase in plumbagin content through pharmaceutical process of *Chitraka* purification with lime water indicates that, this operating procedure is simple, convenient and can be considered as standard procedure. The organoleptic features, pharmacognostical characters, values of physicochemical parameters and quantity of plumbagin of purified *Chitraka* powder may be utilized for quality assurance in future studies.

Keywords: *Chitraka,* high performance thin layer chromatography, plumbagin, *Plumbago zeylanica Shodhana*

Introduction

Ayurveda pharmaceutics mentions processing of drugs under the name "*Samskara"* (quality enhancing or toxin reducing procedure). *Shodhana* (purification) is one of such process used for *Samskara* of drugs. The process, which eliminates the blemishes from raw substances, is called *Shodhana*. According to Rasatarangini[1] (a book on pharmaceutical procedures for herbo-mineral Ayurveda drugs), it is the process intended for the removal of impurities from substances by various procedures such as *Mardana* (incineration), *Swedana* (sudation), and *Nirvapa* (metals to be burnt to red hot and dipped in liquids). This makes the substance nontoxic, easily absorbable, assimilable, and more effective therapeutically. It is used to remove toxic compounds or to reduce concentration of toxic constituents and to make it more potent. Many toxic drugs such as *Bhallataka* (*Semecarpus anacardium* Linn), *Vatsanabha* (*Aconitum ferox* Wall.) and *Karavira* (*Nerium indicum* Mill.) are being used in various Ayurvedic therapeutic formulations. To remove its toxic property by keeping its active ingredient intact, various types of stringent purification methods are mentioned. Rasatarangini though did not classify *Chitraka* (*Plumbago zeylanica* Linn) in *Visha Dravya* but has described mandatory process of *Rakta* (red variety of) *Chitraka* in

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Churnodaka (lime water) before internal administration. However, the duration for the process has not mentioned.[2] The text has mentioned *Nimajjana* (dipping) of *Chitraka* roots in *Churnodaka*. *Nimajjana* is a process of *Shodhana* in which, a drug is dipped in a particular media for certain time. Although this process is easy and convenient, standardization of this process is not available along with reference values of analytical parameters. Hence, this study was conceptualized to establish standard operative procedure (SOP) and to evaluate the changes that take place after purification of *Chitraka* in physical, microscopic and chemical constitution with respect to plumbagin.

Materials and methods

Raw *Chitraka* roots were procured from Pharmacy, Gujarat Ayurved University, Jamnagar and it was authenticated at Pharmacognosy Laboratory of the institute. Lime stone was procured from local market.

Powder (coarse; #40 number) of the small amount (25 g) of raw *Chitraka* was done once by mixture machine for the analysis purpose. After purification *Chitraka* roots were converted to powder (fine; #72 numbers) by pulverizer, such five batches were prepared.

Sample of raw *Chitraka* powder (RCP) was labeled as RCP and purified *Chitraka* powder was labeled as *Shodhita Chitraka* powder (PCP). Pharmacognosy and analytical study of both samples were carried out at Pharmacognosy laboratory and Chemistry laboratory of the institute, respectively. The quantification of plumbagin (main chemical constitution of *Chitraka*), was carried out of both samples through high-performance thin layer chromatography (HPTLC) at Vasu Research Center, Vadodara, Gujarat.

Pharmaceutical procedures were carried out in five batches while pharmacognosy, analytical parameter, and HPTLC for plumbagin marker were carried out in samples of 1st batch only.

Pharmaceutical procedure

Pharmaceutical procedure of purification of *Chitraka* was divided in two phases:

1. Preparation of *Churnodaka*: *Churnodaka* (lime water) was prepared with classical ratio 1:240 of lime powder and water.[3] For 10 L *Churnodaka*, 41.66 g lime powder was added in 10 L of water. It was kept stable for 12 h. After 12 h, it became clear water with lime powder sediment at the bottom. Then clear water was filtered through cotton cloth to obtain lime powder. pH of lime powder was taken by digital pH meter
2. Mandatory process: *Chitraka* roots were dipped into lime powder. It was kept immersed for 9 h (as per classical reference of three *Yama*; 1 *Yama* = 3 h). After that, *Chitraka* roots were washed with lukewarm water 3 times and was dried completely by keeping in sunlight [Figure 1]. Same process was repeated for each batch.

Figure 1: Process of *Chitraka* purification. (a) Lime water. (b) Immersing of *Chitraka* in lime water. (c) Physical impurity after purification. (d) Purified *Chitraka* kept for sun drying. (e) Purified *Chitraka* powder

Organoleptic parameters

Organoleptic characters such as consistency, color, touch, and odor of the samples of raw *Chitraka* powder (RCP) and PCP (of all five batches) were noted through the *Gyanendriya Parikshana* (examination by sensory organ) by researchers and experts of Pharmacognosy laboratory of the institute. All tests except taste test were also carried on lime water before and after purification (of all five batches).

Pharmacognostical characters

Samples of RCP and PCP (from batch 1) were examined with and without staining and microphotographs were taken under Carl zeiss trinocular microscope attached with camera.

Analytical parameters

Preliminary physicochemical parameters such as loss on drying at 110°C,[4] ash value,[5] acid insoluble ash,[5] pH value,[6] water soluble extractives[4] were carried out for RCP and PCP (from batch 1). These parameters were compared with the reference standard of *Chitraka* root given in Ayurveda Pharmacopeia of India (API).[7]

High-performance thin layer chromatography

HPTLC for plumbagin quantification was carried out at Vasu Research Center, Vadodara, for samples of RCP and PCP from batch 1. Test solutions (Track 1 and 3) were prepared by taking accurately weighed samples individually into iodine flask. 20 ml acetone was added to it. It was refluxed for 30 min, filtered and evaporated to 10 ml and then used for HPTLC profiling. Standard solution (Track 2) was prepared by taking 6 mg of plumbagin marker into 5 ml volumetric flask. Acetone was added into it up to mark. It was sonicated for 10 min and then used for HPTLC profiling [Table 1].

Results

During initial phase, pH of water was 7.004 ± 0.007 which turned to 11.644 ± 0.141 after it was converted into lime water [Table 2].

In all five batches, 1500 g of raw dry *Chitraka* root were immersed in 10 L of lime water. Average

yield of purified *Chitraka* from this procedure was 98.07 ± 0.23% [Table 3].

Organoleptic characteristics

Clear liquid consistency and white color of lime water turned to turbid consistency and had dark red color after purification process [Figure 1]. The color of RCP was light brown with acrid and astringent taste and characteristic smell. After purification, purified *Chitraka* powder became cadbury brown in color, gained slightly aromatic odor with lime effervescent followed by strong astringent taste [Table 4].

Table 1: Chromatographic conditions during high-performance thin-layer chromatography

Specifications	Details
Application mode	Camag linomat 5-applicator
Filtering system	Whatman filter paper number 1
Stationary phase	Merck-TLC/HPTLC silica gel 60 F254 on aluminum sheets
Application (Y axis) start position	10 mm
Development end position	80 mm from plate base
Space between band	10 mm
Sample application volume	5.0 µL standard and 10 µL sample
Development mode	Camag TLC twin trough chamber
Chamber saturation time	30 min
Mobile phase	Toluene: Ethyl acetate: Formic acid: Methanol (3: 3: 0.8: 0.2)
Visualization	@ 272

HPTLC: High-performance thin-layer chromatography, TLC: Thin-layer chromatography

Table 2: Batch wise pH of lime water

Batch	Lime water (L)	pH	
		Before (water)	After
1	10	7.01	11.50
2	10	7.00	11.66
3	10	7.02	11.88
4	10	6.99	11.48
5	10	7.00	11.70
Mean±SD	10±0	7.004±0.007	11.644±0.141

SD: Standard deviation

Powder microscopy

Sample of RCP shows fibers with brown contents, group of starch grains, compound starch grains, simple starch grains, cork in surface view, pitted vessels, oil globules, prismatic crystals, silica deposition, tannin content, lignified parenchyma cells, lignified fibers, lignified pitted vessels, and group of stone cells [Figure 2].

The sample of PCP had same prismatic crystals. Starch grains became free and split from compound. Hence, simple starch grain increased proportionately in comparison to compound. Reduction and emptiness in intra-cellular substances of stone cells and scleroids were observed. Tannin was absorbed by other cellular contents like starch grains. Pitted vessels become clear. Fibers were slightly loosen and had less brown color, which may be due to reduction in tannin content. Group of scleroids was seen. Oil globules were not observed. Well-defined lignified stone cells were observed and lignified fibers were seen [Figure 3].

Analytical study

Foreign matter was not found in any of the sample. Loss on drying, ash value, water soluble extractive, methanol soluble extractive and pH was 5.9, 19.4, 12.3, 11.4 and 4.77 in RCP and 5.25, 3.75, 3, 9.3 and 6.83 in PCP. [Table 5].

High-performance thin layer chromatography for plumbagin quantification

HPTLC was done to assess the change in concentration of principal chemical constituent of *Chitraka,* i.e. plumbagin. To establish fingerprinting profile PCP, standard plumbagin marker and RCP were kept in track 1, 2 and 3 respectively. All three tracks showed spot at 272 nm at 0.92 R_f value on given chromatographic conditions. RCP had 0.39% and PCP had 0.98% of plumbagin [Table 6].

Discussion

Pharmacognosy, pharmaceutics and analytical evaluation are the preliminary step for standardization of any medicinal drug. Standardization is essential for the drugs, which are mentioned to be purified in classics. Purification methods remain unique for every drug and its internal use might cause side effects if not purified properly.

Chitraka Shodhana process requires crude *Chitraka* root and hence all raw *Chitraka* were not powdered. Therefore, small

Table 3: Mandatory process of *Chitraka*

Batch	Raw *Chitraka* (weight in g)	Lime water (L)	pH of lime water after purification	Purified *Chitraka* (weight in g)	Yield after purification of *Chitraka* (%)
1	1500	10	6.4	1470	98.00
2	1500	10	6.5	1483	98.86
3	1500	10	6.6	1460	97.33
4	1500	10	6.3	1468	97.86
5	1500	10	6.5	1475	98.33
Mean±SD	1500	10	6.46±0.07	1471.2±3.53	98.07±0.23

SD: Standard deviation

Table 4: Organoleptic characters of lime water, raw *Chitraka* powder and purified *Chitraka* powder

Parameters	Lime water		Raw *Chitraka* powder	Purified *Chitraka* powder*
	Before purification*	After purification *		
Consistency	Liquid	Turbid liquid	Rough powder	Fine powder
Color	White	Dark red	Light brown	Cadbury brown
Odour	Characteristic smell	Slightly aromatic	Characteristic smell	Slightly aromatic
Taste	-	-	Acrid and astringent	Lime effervescent followed by strong astringent

*Results of organoleptic parameters of all 5 batches remained same and hence collective results are depicted in this table.

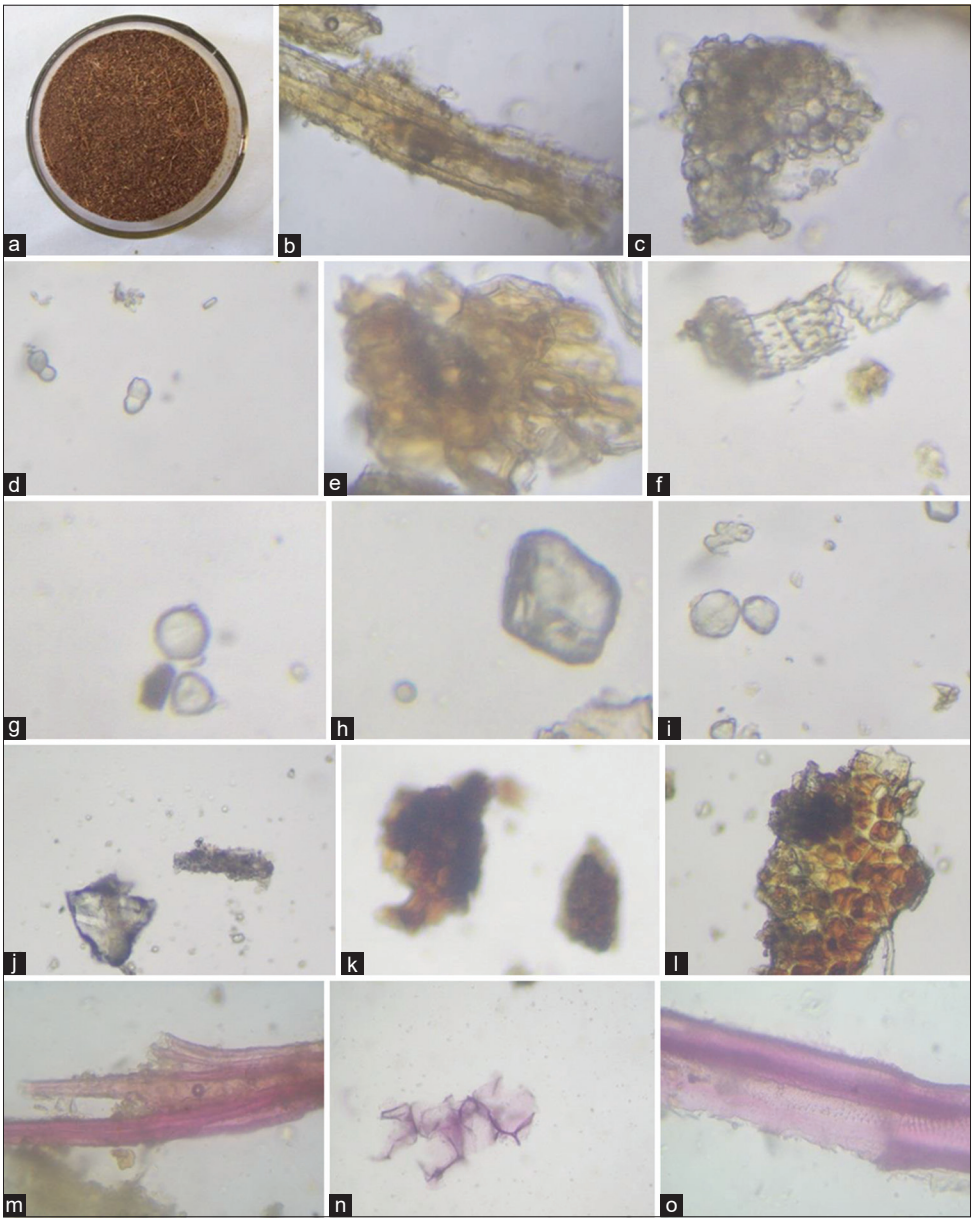

Figure 2: Microscopic feature of raw *Chitraka* powder. (a) Raw *Chitraka* powder. (b) Group of starch grains. (c) Fibers with brown content. (d) Compound starch grains. (e) Cork in surface view. (f) Pitted vessels. (g) Oil globules. (h) Prismatic crystals. (i) Simple starch grains. (j) Silica deposition. (k) Tannin content. (l) Lignified parenchymal cells. (m) Lignified pitted vessels. (n) Lignified fibers. (o) Group of stone cells

amount (25 g) of raw *Chitraka* was made to powder (RCP) for analytical purpose, through mixture machine and hence fine powder was not prepared and obtained consistency of powder was adequate to perform the analysis.

Color of media changed to dark red from white, it indicates that impurity remains in the media.

The astringent taste increased after purification. *Chitraka* roots were kept in lime water for 9 h and hence PCP sample

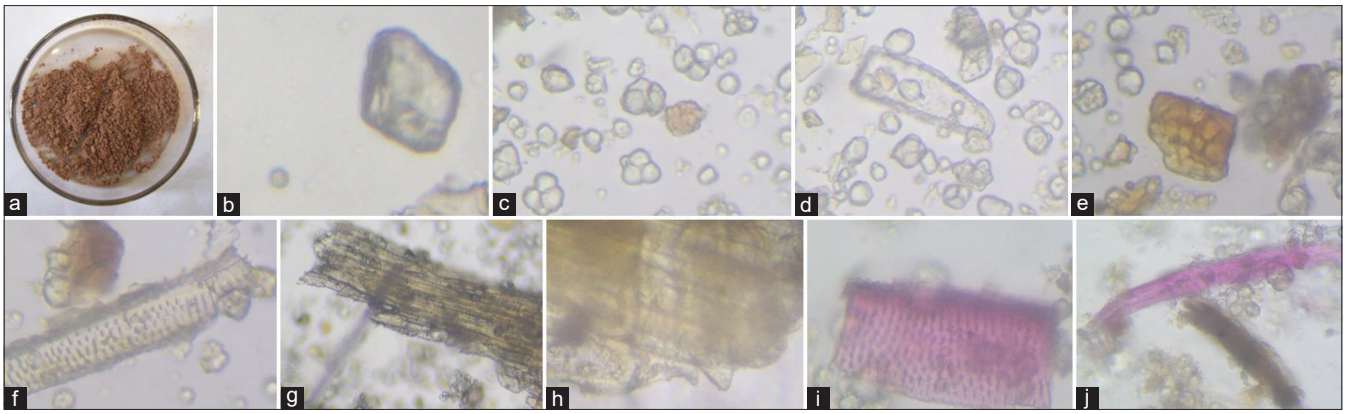

Figure 3: Microscopic feature of purified *Chitraka* powder. (a) Purified *Chitraka* powder. (b) Prismatic crystals. (c) Splitted and free starch grains. (d) Empty stone cells and scleroids. (e) Absorbed tanin content. (f) Clear pitted vessels. (g) Fibers with slightly loosened brown content. (h) Group of scleroids. (i) Lignified fibers. (j) Well defines lignified stone cells

Table 5: Physicochemical parameters of *Chitraka* samples and standards of *Ayurveda* Pharmacopeia of India (API)

Parameters	Raw *Chitraka* powder	Purified *Chitraka* powder*	API standards
Foreign matter	Nil	Nil	Not more than 3 percent
Loss on drying % w/w	5.9	5.25	-
Ash value % w/w	19.4	3.75	Not more than 3 percent
Water soluble extract % w/w	12.3	3	Not <12 percent
Methanol soluble extract %w/w	11.4	9.3	Not <12 percent
pH (5% aqueous)	4.77	6.83	-

*Sample of purified *Chitraka* powder was collected from the first batch of purification process; w/w=Weight by weight

Table 6: Result of plumbagin percentage through high-performance thin-layer chromatography

Parameters	Purified *Chitraka* powder* (track 1)	Plumbagin standard (track 2)	Raw *Chitraka* powder (track 3)
R_f value	0.92	0.92	0.92
Area (AUC)	13,588.4	27,524.6	21,638.3
Percentage of plumbagin	0.98	-	0.39

*Sample of purified *Chitraka* powder was collected from the first batch of purification process; R_f=ratio between the migration distance of a substance and the migration distance of the solvent front; PCP: purified *Chitraka* powder; AUC: Area under the curve

had mild tint of lime taste along with strong astringent taste. This increase in astringent taste might be due to increased percentage of plumbagin content [Table 6].

The powder microscopy shows that all the cells of the roots got affected during purification. During the time of purification, exchange of contents takes place. The cells of the roots absorb the media and also lose some contents into the media. Due to absorption of *Churnodaka,* the roots swell and enlarge. The starch dissolves due to the release of plumbaginic acid present in the cells and clumping of starch takes place due to reaction of sugar with acids [Figures 2 and 3].

All the parameters were campier with Ayurveda Pharmacopeia of India (API) standards.[8] Loss on drying decreased from 5.9 to 5.25. It indicates the parenchyma cells which possess the capacity to hold water get reduced due to this specific purification and sun drying process and causes decrease in loss on drying [Table 5].

Ash value was 19.4 before purification which decreased to 3.75 after purification. It may be due to impurities and more quantity of minerals or salts of soil in RCP. Although raw *Chitraka* was thoroughly washed prior to its use, results of this study indicates that lime water and dipping time of 9 h is definitely imparting its unique effect different than simple water wash. Ash value of PCP reaches near to API standard [Table 5] which was not more than 3%.[5] This indicates the importance of mandatory process in case of *Chitraka*.

Water soluble extract of sample RCP was 12.3 which is similar to API standards [Table 5] of *Chitraka*. In sample PCP, it decreased to 3. During purification procedure, *Chitraka* root were kept in lime water for 9 h. During this time, many water soluble component and impurities could have transferred to lime water that leads to decrease in value of water soluble extract. As, this result of PCP does not match API standards, it requires further studies.

Methanol soluble extractive was decreased from 11.4 to 9.3; it may be due to some methanol soluble phytoconstituents need time and medium (lime water) to dissolve during purification process.

The pH of purified *Chitraka* (6.83) is more than that of raw *Chitraka* (4.77). On the other hand, pH of lime water was changed to 6.46 from 11.64 [Tables 2 and 3], which indicates that, lime water neutralizes the acidic contents of the roots (plumbaginic acid) and hence pH of *Chitraka* increases. After these data, it could be inferred that, *Chitraka* purification reduces acidic substances from *Chitraka*.

HPTLC results indicate that, purification process has increased the purity of drug. Quantity of plumbagin was increased from 0.39% to 0.98%. [Figure 4] It is important to notice that plumbagin is soluble in alcohol, acetone, chloroform, benzene, and acetic acid[8] and classical method is having water based (*Churnodaka*) purification process and hence it spares plumbagin content and even increases the purity by removing impurities and unwanted water soluble contents of *Chitraka*.

Reduction in loss on drying, ash value, water soluble extracts, methanol soluble extracts and increase in pH and plumbagin value after purification could be utilized to track the reduction in toxicity and increase in purity.

Limitation of the study

Due to time and funding limitation, pharmacognosy, analytical, and HPTLC for plumbagin marker could not be performed on the PCP samples of all five batches. This study results could be applied to the *Chitraka,* which have same cultivation region and collection season. Variation in cultivation area, climate, and time of collection may show the variation from above findings. Deviation of the present analytical parameters of PCP from API standards indicates the need of further extensive study on *Chitraka* purification inclusive of multiple drug collection area and season.

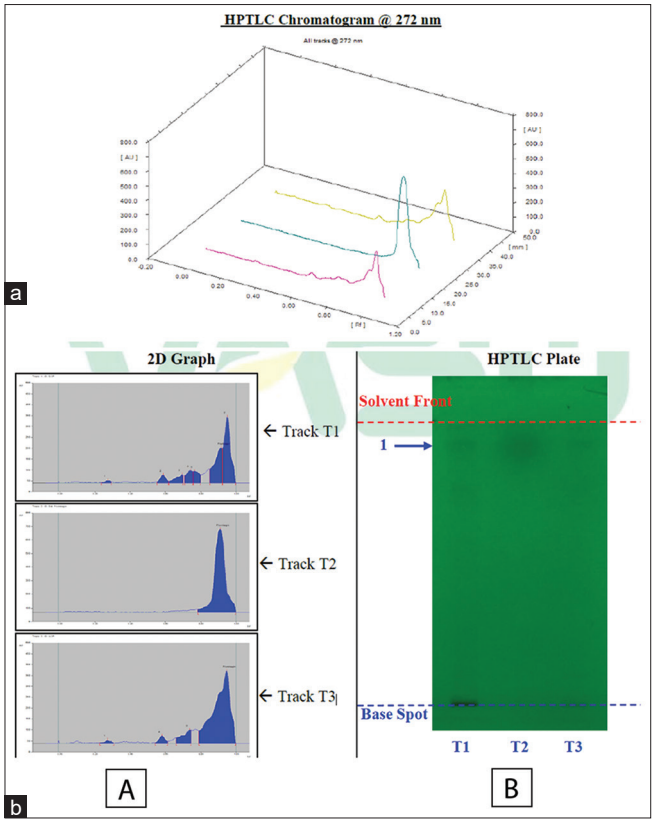

Figure 4: HPTLC of purified *Chitraka* powder, plumbagin standard and raw Chitraka powder (a) High performance thin layer chromatography 3D overlay chromatogram @ 272 nm. (b): high performance thin layer chromatography quantification @ 272 nm; A: 2D Graph, B: high performance thin layer chromatography plate

Conclusion

Alteration found in organoleptic, pharmacognostical and physico-chemical parameters after purification show the impact of purification procedure by lime water on *Chitraka*. In HPTLC profile, plumbagin content was 0.29% in raw *Chitraka* powder, whereas it is increased up to 0.98% after purification. Hence, the results of the present study could be used as reference for SOP of purification of *Chitraka* and standards of purified *Chitraka* for its identity and purity.

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Nil

Conflicts of interest

There are no conflicts of interest.

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