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 Rasatarangini1 (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), Vatsanabh (Aconitum ferox Wall.) and Karavira (Veratum 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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How to cite this article: Bhinde SS, Ravi AK, Patgiri BJ, Harisha CR, Shukla VJ, Standard operating procedure of purification of Chitraka (Plumbago zeylanica Linn.) along with pharmacognostical and analytical profiles of Plumbagin. AYU 2020;41:117-22.
Submitted: 22-Jul-2020 Revised: 19-Sep-2020 Accepted: 16-Feb-2021 Published: 23-Oct-2021

Quick Response Code:
Website: www.ayujournal.org
DOI: 10.4103/ayu.AYU_299_20

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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 by 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.[5] 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.

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 1].

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

Table 2: Batch wise pH of lime water

| Batch | Lime water (L) | pH Before (water) | pH 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       | 7.00±0.007        | 11.64±0.141 |

SD: Standard deviation

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

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 Rf 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 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              |
| Color            | White      | Dark red             | Light brown               |
| Odour            | Characteristic smell | Slightly aromatic   | Characteristic smell      |
| Taste            | -          | -                    | Acrid and astringent      |

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

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.

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.

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
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. 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.

**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.

**Financial support and sponsorship**

Nil

**Conflicts of interest**

There are no conflicts of interest.

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