ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

A PRELIMINARY INVESTIGATION OF THE *SHODHANA* (DETOXIFICATION) OF ROOTS OF *PLUMBAGO INDICA* L. IN AYURVEDA

Padumadasa C*, Abeysekera A M, Meedin S D K

Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda 10250, Sri Lanka.

ABSTRACT

Plumbago indica L. (Plumbaginaceae) is a medicinal herb, credited with a number of therapeutic properties. It is widely used in Sri Lankan traditional medicine and in Ayurveda. In Sri Lanka, Ayurveda formulations which incorporate the roots of *P. indica* L. are prepared using commercially available air dried material after subjecting it to a detoxification with limewater prepared from commercially available milk of lime. The detoxification process is referred to as "*Shodhana*". According to the Ayurveda, this process is done to remove toxicity associated with the roots and, it can be surmised that it is done to remove toxicity associated with plumbagin, the predominant toxic naphthoquinone in *P. indica* L.. *Shodhana* of roots results in a deep maroon coloured extract arising from the calcium salt of plumbagin. Here, we report a qualitative and quantitative study of the *Shodhana* of roots of *P. indica* L. using Ultra Violet-Visible spectrophotometric and chromatographic methods to give a scientific basis for this process. A method for the quantitative extraction of plumbagin from root samples was optimized. A calibration curve for plumbagin in hexane was developed using the absorption values at 258 nm. The plumbagin content of 8.7 ± 0.1 mg/g in a fresh root sample was lowered by 19.4% to 7.0 ± 0.1 mg/g upon subjecting to *Shodhana*. It was found that a commercial dried root sample of *P. indica* L. root contained a much lower level (0.55 ± 0.05 mg/g) of plumbagin. To better understand the changes in the level of plumbagin in roots of *P. indica* L. during drying, a study was carried out by drying the root samples for five weeks subjecting them to analysis periodically by TLC and Ultra Violet-Visible spectrophotometric methods. The amount of plumbagin (8.6 ± 0.1 mg/g) present in fresh untreated roots at the beginning of the study was reduced by 62.7% to 3.2 ± 0.1 mg/g after one week. There was no measurable change in the plumbagin level thereafter up to week 5. The amount of plumbagin in dried roots can be further reduced by *Shodhana.* The total reduction of plumbagin by drying and *Shodhana* was 87.7%. Our results show that *Shodhana* of the fresh undried roots does not reduce the plumbagin content substantially, and that air drying followed by *Shodhana* is the most effective method to reduce the plumbagin content to a non-toxic level, supporting the currently used processing method.

KEYWORDS: *Plumbago indica* L., Plumbagin, *Shodhana*, Limewater.

INTRODUCTION

Plumbago indica L. (Plumbaginaceae) syn. *P. rosea* L. is a medicinal herb (Figure 1), credited with a number of therapeutic properties. It is widely used in Sri Lankan traditional medicine and Ayurveda[1]. Popularly it is called "Rathnitul" in Sinhala. The chemical profile of roots is marked by the presence of naphthoquinones, flavonoids, tannins, alkaloids, and terpenoids[2]. Naphthoquinones are the major secondary metabolites in the roots of which plumbagin (2-methyl-5-hydroxy-1,4-naphthoquinone), a volatile compound is predominant[3]. In Sri Lanka, Ayurveda formulations which incorporate the roots of *P. indica* L. are prepared using commercially available air dried material after subjecting it to a detoxification with limewater[4]. The detoxification process is referred to as "*Shodhana*". According to the Ayurveda pharmacopoeia, this process is done to remove toxicity associated with the roots and based on anecdotal evidence, it can be surmised that it is done to remove toxicity associated with plumbagin. Previous investigations indicate that plumbagin at high doses causes skin irritations and is toxic to normal cells[5]. Here, we report a qualitative and quantitative study of the *Shodhana* of the roots of *P. indica* L. by using Ultra Violet- Visible (UV-VIS) spectrophotometric and chromatographic methods to give a scientific basis for this process.

MATERIALS AND METHODS

Plant material, roots of *Plumbago indica* L. were collected from six months old plants grown in the Botanical Garden of the Bandaranaike Memorial Ayurveda Research Institute (BMARI), Nawinna, Sri Lanka. A voucher specimen was deposited in the herbarium of the Botany division of the BMARI (reference number 638 e). Roots were washed, wiped and cut in to small pieces to prepare fresh homogenized

bulk, which was used immediately. A commercial sample of finely powdered dried roots was obtained from Link Natural Products (Private) Limited, Colombo 02, Sri Lanka and used as the dried sample. Commercially available milk of lime was used to prepare limewater. Solvents, chemicals and reagents were purchased from Sigma-Aldrich Chemie (Germany). Water was used after distillation through GFL distillation apparatus. Thin Layer chromatography (TLC) was performed on pre coated Kieselgel 60 F_{254} plates (0.2 mm, Merck KGaA, Germany) with benzene: hexane (9:1) as the solvent system. GC/MS analysis was carried out using Agilent 7890 A GC system (EI mode). UV-VIS absorption spectra were recorded on a Labomed, Inc., single beam scanning UV-VIS spectrophotometer (UV- 2602) equipped with optical cells photo diode detector and IR spectra were recorded on a Thermo Nicolet iS 10 FT-IR spectrometer.

Qualitative analysis of *Shodhana*

Fresh roots (4 g) were soaked in saturated limewater (100 cm^3) for 4 hours (using the same solid: liquid ratio used by Ayurvedic medical practitioners in Sri Lanka for *Shodhana* of these roots). The resulting deep maroon coloured solution was filtered, neutralized using a few drops of 2 mol dm^{-3} HCl and extracted in to hexane (100 cm^3). The hexane extract was labeled E_1. The roots after *Shodhana* were washed with distilled water and air dried (2 hours) and refluxed with hexane (100 cm^3). After filtering, the hexane extract obtained was labeled as E_2. Another fresh root sample (4 g) without subjecting to *Shodhana* was also refluxed with hexane (100 cm^3), filtered and the resulting hexane extract was labeled as E_3. The solvent was removed from E_1, E_2, and E_3 hexane extracts under *vacuum* and subjected to TLC alongside the plumbagin working standard which was isolated as given in section; extraction of plumbagin. Spots were visualized under UV (at 254 nm and 365 nm) and spraying 10% KOH in methanol, a reagent used for the identification naphthoquinones.

The detoxification process was also studied varying the soaking time. For that, fresh roots (4 g) were soaked in saturated limewater (100 cm^3) for 8 hours. An aliquot of extract (3 cm^3) was collected every hour, filtered, neutralized and extracted into hexane. After which the extracts were subjected to TLC alongside the plumbagin working standard and visualized under UV (at 254 nm and 365 nm) and by spraying with 10% KOH in methanol.

Quantitative analysis

Extraction of plumbagin.

Fresh roots (20 g) were refluxed with hexane (100 cm^3) for 15 minutes. The hexane layer was extracted with limewater (25 cm^3 x 2). The aqueous layer was neutralized with 2 mol dm^{-3} HCl and extracted with hexane (25 cm^3 x 2). The hexane layer was evaporated under *vacuum* and the crude plumbagin obtained was purified by recrystallization using hexane as the solvent[6]. The recrystallized product was obtained as orange coloured needles (0.12 g, 0.62 % on fresh weight basis). M. p. 76-77 ^{0}C.[7] The identity and purity of the product was further established by its GC/MS, UV, IR spectra. It was used as the plumbagin working standard in this study.

Developing a calibration curve of plumbagin

The UV-VIS spectra (190 nm to 800 nm) of a series of solutions of plumbagin in hexane, whose concentrations ranged from 2×10^{-3} to 2×10^{-2} mg/cm^3 were measured. Each concentration was measured in triplicate. To identify the best wavelength for the quantification of plumbagin in root extracts, calibration curves were developed at λ_{max} 210 nm, 258 nm, and 426 nm of plumbagin and evaluated statistically.

Optimization of extraction of plumbagin

Extraction of plumbagin from fresh roots was optimized considering optimum weight: volume ratio, refluxing time and number of repetitive extractions using hexane as the solvent.

Optimum weight: volume ratio of root sample: solvent: Fresh root sample (1.0 g) was refluxed with hexane (50.0 cm^3) and after every ½ hour the solvent was replaced with fresh hexane. Each extract removed was filtered and its absorbance measured at 258 nm using UV-VIS spectrophotometer until absorbance readings were insignificant. The experiment was repeated in triplicate.

Optimum time of refluxing: Fresh root sample (1.0 g) was refluxed with hexane (500 cm^3) for 2 ½ hours. Here the optimum weight: volume ratio of root sample: solvent was used. After every 0.5 hour an aliquot of extract (3.0 cm^3) was taken out, filtered and absorbance was measured at 258 nm using UV-VIS spectrophotometer. After which the solvent was replaced with fresh hexane (100.0 cm^3) and refluxed for another 2 ½ hours during which period an aliquot of extract (3.0 cm^3) was taken out every ½ hour as before and its absorbance at 258 nm measured. The experiment was repeated in triplicate

Optimum number of repetitive extractions: A solution of plumbagin working standard (8×10^{-3} mg /cm^{-3}) was prepared using hexane and absorbance was measured at 258 nm using UV-VIS spectrophotometer. The plumbagin solution (25.0 cm^3) was extracted repeatedly with limewater (25.0 cm^3) until the absorbance of organic layer was insignificant at 258 nm. The resulting aqueous extracts were combined, neutralized using few drops of 2 mol dm^{-3} HCl and extracted repeatedly into hexane (25.0 cm^3) until the absorbance of the current hexane layer was insignificant at 258 nm.

Quantification of plumbagin

The amount of plumbagin in fresh roots (1g) was quantified under optimized conditions obtained in section; optimization of extraction of plumbagin. Fresh roots (1 g) were refluxed with hexane (500 cm^3) for 2 hours. The resulting extract was filtered and washed with lime water (100.0 cm^3 × 2). Then the aqueous layer

was neutralized using a few drops of 2 mol dm^{-3} HCl and extracted into hexane (100.0 cm^3). The resulting hexane layer was labeled as H_1. The amount of plumbagin in commercial roots (1 g) was also quantified using the same optimized conditions. The resulting hexane extract was labeled as H_2. The absorbance of both H_1 and H_2 was measured at 258 nm and the amount of plumbagin in fresh and commercial roots were quantified from the calibration curve developed at 258 nm.

Variation of plumbagin content during the drying of roots

Twelve samples of fresh roots (1 g for each) were prepared and stored in glass containers exposed to air in the shade. Each week two samples were taken and one sample was refluxed with hexane (500 cm^3) for 2 hours. After filtering, the resulting hexane extract was labeled as H_3. The other sample was subjected to the *Shodhana* for 4 hours. The resulting deep maroon coloured solution was filtered, neutralized using few drops of 2 mol dm^{-3} HCl, extracted in to hexane (100.0 cm^3) and labeled as H_4. The roots after the *Shodhana* was washed with distilled water, air dried (2 hours) and refluxed with hexane (100.0 cm^3). Upon filtering, the resulting hexane extract was labeled as H_5. Absorbance of H_3, H_4 and H_5 hexane extracts was measured at 258 nm using UV-VIS spectrophotometer and the amount of plumbagin was quantified from the calibration curve developed at 258 nm. Solvent was removed from H_3, H_4, and H_5 under *vacuum* and subjected to TLC alongside the plumbagin working standard. Spots were visualized under UV (at 254 nm and 365 nm) and spraying 10% KOH in methanol.

RESULTS AND DISCUSSION

For the qualitative analysis of *Shodhana*, fresh roots were soaked in saturated limewater for 4 hours. This resulted in a deep maroon coloured extract in accordance with the literature that plumbagin gives a reddish coloured solution in alkali (Figure 2). All the three extracts E_1, E_2, and E_3 (E_1- hexane extract of the aqueous solution obtained after *Shodhana*, E_2-hexane extract of roots after subjecting to *Shodhana*, E_3-hexane extract of fresh roots without subjecting to *Shodhana)* were subjected to TLC against the plumbagin working standard using benzene: hexane (9:1) as the solvent system. Spots were visualized under UV (at 254 nm and 365 nm) and spraying 10% KOH in methanol. The resultant phytochemical profiles are shown in Figure 3 and the corresponding schematic diagram by considering all visualized spots using different visualization techniques is shown in Figure 4. According to the results, E_3 showed a very intense spot (S_7) with R_f - 0.63 which correspond to the plumbagin working standard and six other spots of which two were very intense (S_3 and S_6) while others were of low intensity. In the case of E_1, the S_7 spot was observed in low intensity. The spot S_6 was not observed at all while the other spots were observed in low intensity of which one was an extra spot (S_5). When considering E_2, the spots S_3, S_6 and S_7 were observed but not as intense as in the case of E_3. The spots S_1, S_4, and S_8 were observed in low intensity and spots S_2 and S_5 were not observed at all. These results show that the main effect of *Shodhana* is to reduce the amount of the naphthoquinones plumbagin and the compound represented by S_6 in the roots. It is interesting to note that the compound represented by S_6 is not found in the hexane extract of the *Shodhana* extract and appears to have decomposed in the aqueous alkaline medium.

In Ayurveda, the *Shodhana* is carried out by soaking roots in lime water for 4 hours. The extent of extraction of plumbagin with time during *Shodhana* was studied by varying the soaking time and analyzing the extract by TLC. The chemical profile of the extract did not change with the time but the intensity of the spots changed. For plumbagin the most intense spot was observed after 4 hours soaking time. For the other spots, the most intense spots were observed after 1 hour soaking time. If the *Shodhana* process is carried out to reduce plumbagin from the roots of *P. indica* L. then the traditional time period of soaking of 4 hours is justifiable.

Next the amount of plumbagin in roots was quantified. For this, plumbagin to be used as the working standard was extracted from fresh roots using hexane and characterized. In GC/MS studies, the gas chromatogram showed a single peak and corresponding mass spectrum with a molecular ion at m/z 188. IR and UV-VIS spectra were in accordance with published data [7] and confirmed the purity of isolated plumbagin. In the UV-VIS spectrum of plumbagin, three λ_{max} 210 nm, 258 nm, and 426 nm were observed. To identify the best wave length for the quantification of plumbagin in root extracts, calibration curves were developed at these three wavelengths. Due to the clear peak arrangement and the highest correlation coefficient (r^2) at 258 nm, the concentration of plumbagin in unknown root extracts was computed from the calibration curve at 258 nm (Figure 5).

Before the quantification of plumbagin, the extraction method was optimized. The weight/volume ratio of root sample/solvent, refluxing time and number of repetitive extractions were optimized. When considering optimization of weight/volume ratio of root sample/solvent, by the 10^{th} change of solvent of volume 50.0 cm^3, a constant, insignificant absorbance was obtained. In the case of optimizing the time of refluxing, the highest absorbance was obtained after 2 hours reflux and replacing the solvent with fresh hexane did not result in an extract with significant absorbance. The number of repetitive extractions was optimized using a solution of plumbagin working standard in hexane and extracting it repeatedly with limewater. At the end of the 3^{rd} extraction of the plumbagin solution with limewater an insignificant absorbance was obtained. Therefore, only the first two aqueous layers were combined, neutralized and extracted repeatedly with hexane. By the 2^{nd} extraction no significant absorbance was obtained. According to the obtained results, the optimum weight/volume ratio of fresh root sample/solvent and refluxing time were 1/500 g cm^{-3} and 2 hours respectively. Extraction of plumbagin solution twice with

limewater (1:1) and the extraction of the resulted aqueous layers once with hexane (2:1) were the optimum conditions for the quantitative extraction of plumbagin.

Employing these optimized conditions and the calibration curve at 258 nm, it was found that the fresh root sample contained 8.7 ± 0.1 mg/g of plumbagin which was lowered to 7.0 ± 0.1 mg/g (a reduction of 19.4%) upon subjecting to *Shodhana*. In contrast, commercially available air dried samples contained only 0.55 ± 0.05 mg/g of plumbagin. It is clear that most of the plumbagin in the roots of *P. indica* L. is lost during the drying process due to its volatility. It may be presumed that *Shodhana* will reduce this further to the required non-toxic therapeutic level.

To study the changes in phytochemical profile and the level of plumbagin in roots during the drying process further qualitative and quantitative studies were carried out by drying the samples in air at ambient temperatures for five weeks and subjecting them to periodic TLC and UV-VIS spectrophotometric analysis. The phytochemical profile of the roots did not change during drying but the intensity of the spots changed. The intensity of spots S_3, S_6, S_7 (plumbagin) and S_8 decreased clearly while the intensity of other spots did not change much during drying. When considering the spot S_7 the intensity reduced at week 1 and remained constant upon further drying. For the quantitative analysis of the drying process, absorbance of H_3, H_4, H_5 extracts were measured at 258 nm each week using UV/Vis spectrophotometer. According to the results the highest amount of plumbagin (8.6 ± 0.1 mg/g) was observed in fresh untreated roots at week 0 and this reduced to 3.2 ± 0.1 mg/g after one week. After that it remained constant upon further drying showing 62.7% reduction at the end of week 5. The variation of the amount of plumbagin in roots during drying is shown in Figure 6. The total reduction of plumbagin by drying for five weeks and *Shodhana* was 87.7%. When considering the value of 0.55 ± 0.05 mg/g obtained for a commercially obtained dried sample, it is clear that root samples will continue to lose plumbagin with storage time, with the most rapid rate of loss being during the first few days.

Ayurveda formulations are incorporated with commercially available roots of *P. indica* L. that have already been air dried, after subjecting them to *Shodhana*. According to the Ayurveda *Shodhana* is done to remove toxicity associated with roots. Plumbagin is the predominant and toxic naphthoquinone in the roots of *P. indica* L.[8]. Our results show that *Shodhana* of the fresh undried roots does not reduce the plumbagin content substantially, and that air drying followed by *Shodhana* is the most effective method to reduce the plumbagin content to a non-toxic level, supporting the currently used processing method.

REFERENCES

1. Jayaweera D M A. Medicinal plants (Indigenous and exotic) used in Ceylon: part II. NSC: Colombo; 1980. p. 279.
2. Dinda B, Chel G, Achari B. A dihydroflavonol from *Plumbago indica.* Phytochemistry 1994; 35 (4): 1083-1084.
3. Takashi N T, Takada N, Ueda M. Mechanism of antifeedant activity of plumbagin, a compound concerning the chemical defense in carnivorous plant. Tetrahedron Letters 2004; 45: 7115-7119.
4. Satheeshkumar K, Jose B, Pillai D, Krishnan P N. Prospects of *Plumbago rosea* L. hairy root culture in traditional preparations: a phytochemical comparison with tuberous roots. Plant Root 2014; 8 (0): 13-23.
5. Paul A S, Islam A, Yuvaraj P. Anti-Helicobacter Pylori and Cytotoxic activity of detoxified root of *Plumbago auriculata*, *Plumbago indica* and *Plumbago zeylanica.* The Journal of Phytopharmacology 2013; 2 (3): 4-8.
6. Chellampillai B, Prajakta P J, Ganesh Y D, Atmaram P P. Rapid method for isolation of plumbagin, an alternative medicine from roots of *Plumbago zeylanica*. Eur J Integr Med 2011; 3 (1): 39-42.
7. Thomson R H. Naturally Occurring. 2nd ed. Academic Press: London: 1971. p. 228-229.
8. Padhye S, Dandawate P, Yusufi M, Ahmad A, Sarkar F H. Perspectives on medicinal properties of plumbagin and its analogs. Medicinal Research Reviews. 2012; 32 (6): 1131-1158.

Cite this article as:
Padumadasa C, Abeysekera A M, Meedin S D K. A Preliminary Investigation of the *Shodhana* (Detoxification) of Roots of *Plumbago Indica* L. In Ayurveda. International Journal of Ayurveda and Pharma Research. 2015;3(12):1-6.
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr. Chayanika Padumadasa
Senior Lecturer, Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda 10250, Sri Lanka.
Email: chayanikapadumadasa@yahoo.com
Tel.: +94714492911/
Fax: +94112804206

Study Photographs of *Plumbago indica* L.

(A) **(B)** **(C)**

Fig. 1: *Plumbago indica* L. A- Shoot, B- Root, C- Flowers

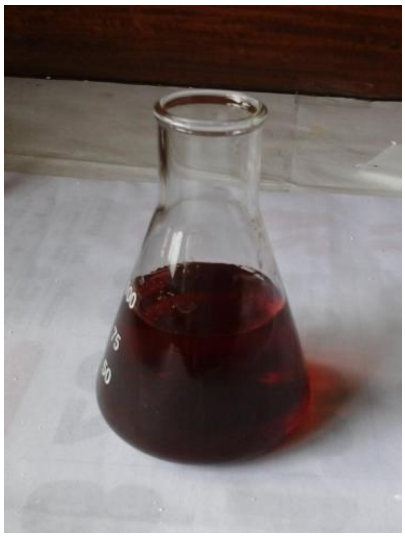
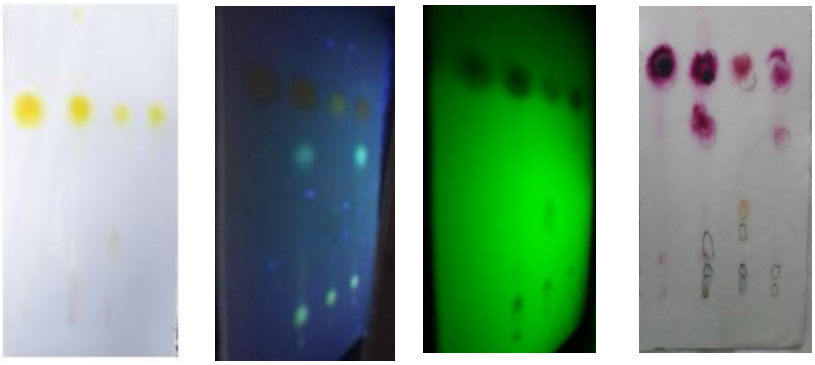


Fig. 2: Extract resulted from the *Shodhana* of fresh roots of *P. indica* L.



P E_3 E_1 E_2 P E_3 E_1 E_2 P E_3 E_1 E_2 P E_3 E_1 E_2

A B C D

Fig. 3: Chromoplates under different visualization techniques. A-visualizing under visible light. **B**-visualizing under UV at 365 nm, **C**-visualizing under UV at 254 nm (Images were captured in 45˚ angle). **D**-spraying 10% methanolic KOH. **P**-plumbagin working standrd, **E_1**-hexane extract of resulting solution of *Shodhana*, **E_2**-hexane extract of roots upon subjecting to *Shodhana*, **E_3**-hexane extract of fresh roots without subjecting to *Shodhana*.

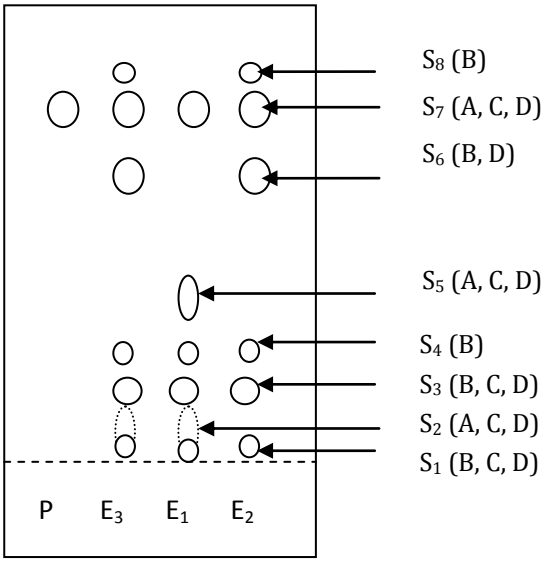


Fig. 4: Schematic diagram of phytochemical profiles of different root extracts of *P. indica* L.. P- plumbagin working standard, **E_1**-hexane extract of resulting solution of *Shodhana*, **E_2**-hexane extract of roots upon subjecting to *Shodhana*, **E_3**-hexane extract of fresh roots without subjecting to *Shodhana*. Visualization technique is given in next to the spot number. (Size of the spots is not related to the intensities).

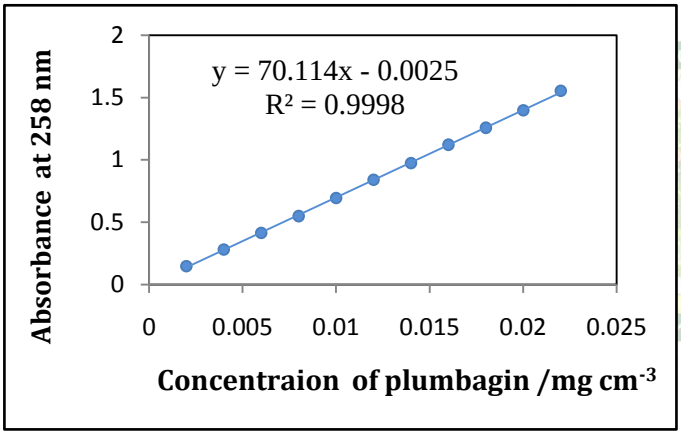


Fig. 5: Calibration curve of plumbagin

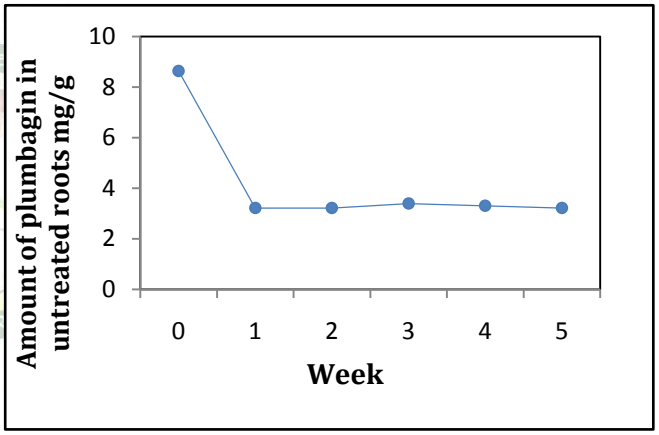


Fig. 6: Variation of the amount of plumbagin in roots of *P. indica* L. during drying