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Original Article

Pharmacognostic and Phytochemical Profile of Karavira (*Nerium indicum* Mill.) Roots with Reference to its Change after Shodhana (Purification Process as per Classics)

Veerappa Hugar[1], Daneshwari S Kanashetti[2,*], Kamal Nayan Dwivedi[2]

[1]Department of Rasashastra and Bhaishajya Kalpana, Ayurveda Mahavidhyalaya, Hubballi, Karnataka, INDIA.
[2]Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, INDIA.

ABSTRACT

Background: Standardization is a crucial method for establishing the identification, purity, safety, and quality of herbal medicines. Numerous morphological, physicochemical, and phytochemical investigations are performed in order to standardize a medication. For the purpose of establishing standards for unprocessed crude drug, the quantitative assessment of some phytochemical characteristics is necessary. **Objectives:** The current work discusses the standardization of *Nerium indicum* root based on several pharmacognostic characteristics, and the identification of these traits will help subsequent researchers to study this species pharmacology. **Materials and Methods:** Sectioning of fresh root sample of *N. indicum*, assessment of macroscopic, microscopic, and physicochemical parameters were done based on standard procedures as per API and WHO. Qualitative phytochemical screening, quantitative estimation of total polyphenolic content and cardiac glycoside content of ashodhita (non-purified) and shodhita (purified) hydroalcoholic extract were carried out through standard procedures. **Observation and Results:** Pharmacognostic analysis confirms the identity of genuine root sample of *Nerium indicum*. Phytochemical analysis of Ashodhita and Shodhita hydroalcoholic extract shows presence of Carbohydrate, Alkaloids, Flavonoids and Saponins and negative test for Tannins, Starch and Steroids. Quantitative estimation of total phenolic content was found to be is 4.14% w/w and 2.59% w/w of pre and post shodhita extract respectively. Total cardiac glycoside content was found to be 3.27% w/w and 3.05% w/w in Ashodhita and Shodhita extract respectively. **Conclusion:** The results of the current investigation indicate that the physico-chemical and phytochemical analyses of *Nerium indicum* root, produced a set of qualitative and quantitative pharmaco-botanical parameters that can be used as a valuable source of data to establish the identity and to assess the quality and purity of the plant material in subsequent studies.

Keywords: Karavira, Shodhita and Ashodhita Karavira, *Nerium indicum*, Phytochemical study.

Correspondence:
Dr. Daneshwari Kanashetti S
Department of Dravyaguna, Faculty of Ayurveda, IMS, BHU, Varanasi, Uttar Pradesh, INDIA.
Email: daneshwari@bhu.ac.in

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INTRODUCTION

In ancient times there was no need of drug identification because people were aware of plants surrounding them. There was *Guru shishya parampara*, the knowledge of medicinal plant identification was transferred from generation to generation. Industrialization leads to extinction of plants leading to end of this tradition. After this, during medieval period drug identification is explained based on morphological characteristics, origin, and action of drug (*Nama Rupa gyanam*).[1] In present scenario, deficiency and unavailability of authentic drug has resulted in adulteration and substitution. It has become a burning issue which hinders safety and efficacy of drug. So, there is need of standardization of raw drug using present day techniques.

Pharmacognostical and Phytochemical study of *Karavira* is carried out for identification of genuineness of drug. Quality control depends on identity, purity, and contents of drug. Identity can be achieved by organoleptic, macroscopic, and microscopic examination of drug. Purity of drug is assessed by analytical techniques like foreign matter contamination, loss on drying, extractive value, chromatographic techniques etc. Content of drug is known by phytochemical analysis. If the drug gives positive results in all the above three (identity, purity, and content) then it is called authentic drug and it is safe and effective for clinical use.

Nerium indicum is large profusely branched evergreen, xerophytic shrub with milky latex, up to 3 m tall. Leaves linear-lanceolate, thickly coriaceous. Flowers rose red or white, sweet scented, in terminal many-flowered polychasial cymes. Fruit paired follicles,

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cylindric, ribbed. Seeds tipped with coma of light brown hairs. Cultivated throughout India for its ornamental flowers, also cultivated near temples and gardens. In Ayurveda it is named as Karavira. As per ayurvedic classics it is indicated in various conditions such as Hridroga, Jvara, Krimiroga, Kandu, Kustha, Netraroga, Vrina and Tamaka shwasa.

It is cardio tonic and contains cardiac glycosides. A new cardenolide, isolated from the roots, revealed antibacterial and digoxin-like cardiac activities. The plant has been used as antidote against venomous snake bites, antibacterial, antileprotic, anticancer, cardiotonic and CNS depressant. The juice of tender leaves is reported to be good for ophthalmia. Root causes abortion. The plant is highly poisonous. Its toxicity primarily causes gastro intestinal and cardiac symptoms, much like digitoxin overdose. While the documented cardiac reactions include an irregular heartbeat, arrhythmia, auriculo-ventricular block, subendocardial and abomasal hemorrhages, the reported gastrointestinal reactions include nausea and vomiting, excessive salivation, abdominal pain, and diarrhea.[2] In Ayurveda, specific shodhana (purification) process for root of Karavira is mentioned to remove its toxic effect, where it involves swedana (fomentation) in cow milk for 3 hr. In this article phytochemical profile after shodhana process has also been studied. Various parts of the plant are known to yield cardenolides, pregnanes and triterpenes. The principal cardio-substance present in root is oleandrin. Chemical constituents include cardiac glycosides, oleandrin, digitalin, adynerin, neriantin, neriin, folinerin, rosagenin, cornerin, pseudocuramine, rutin and corterin.[3,4]

MATERIALS AND METHODS

Collection of plant material and authentication: The roots of *N. indicum* were collected from vicinity of Varanasi, Uttar Pradesh India in the month of May 2022. It was identified and authenticated by Prof. K.N Dwivedi, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Dried sample was used for macroscopic identification and its powder was used for microscopy. Fresh root sample was used for section cutting.

Procedure of shodhana

This treatment was performed according to the traditional methods detailed in the Ayurvedic classical text.[5] 300 g of coarsely powdered Karavira root was tied in dry clean muslin cloth making pottali (pouch form). This pottali was immersed in a vessel containing fresh cow milk in such a way that it was immersed completely in milk and not touch the base of the vessel. It was heated on medium flame continuously for 3 hr. Milk was stirred intermittently. Total 5000 mL of milk was utilized in this procedure which was procured directly from cow shelter (go shala) having domestic breed (desi). After 3 hr, root sample were taken out and dried in shade, further subjected for extraction. Weight of drug after shodhana was 370 g and remaining milk was 3400 mL. Color of milk was changed to woody gradually till the end of procedure.

Macroscopic and Microscopic Characteristics

Macroscopic

The macroscopical or morphological description of a *N. indicum* root sample includes size, shape, nature of outer and inner surfaces, type of fracture and organoleptic characteristics like color, odor, taste, consistency, etc.

Microscopic

Transverse section of root was cut with the help of sharp blade. Cut enough sections and select thin, fine uniform section. Then stain the section with safranin solution. Transfer the section from safranin to 50% alcohol containing watch glass, keep for 5 min. Transfer the section in watch glass containing water; keep for 5 min. This washing will remove the stain from cellulose part. Then it was mounted on glass slide and observed under microscope.

Powder microscopy

Reagents used

Saffranin (Dissolved 1 g saffranin in 100 mL distilled water) and Glycerol (Mixed equal amounts of glycerol and distilled water).

Enough powder was taken on a microscopic slide add 1-2 drops of saffranin. Spread the sample evenly over the slide. Mount in glycerin. Observe through the microscope. Repeat the procedure in 2-3 slides to get maximum characters. Transferred the images using the attached camera and software.[6]

Physico-chemical evaluation

All the procedures of physico-chemical analysis such as determination of foreign matter, loss on drying, total ash value, acid insoluble ash, water soluble ash and extractive value were followed as per Ayurvedic Pharmacopoeia of India (API).[7]

Qualitative phytochemical Screening of pre and post shodhana extracts of *N. indicum* root

Determination of primary metabolite or nutritional substance (carbohydrate, protein, fat, oil, vitamins, enzymes etc.) and secondary metabolites or medicinal substance (alkaloids, glycosides, tannins, resins, volatile oil, flavonoids, phenolic compounds etc.) in sample qualitatively through color change, precipitation, and any physical change. Standard procedures for various tests were followed as per described in pharmacognosy text.[8]

Table 1: Results of physico-chemical evaluation.

Sl. No.	Physico-chemical Parameters	Values
1	Loss on drying (w/w %)	4%
2	Foreign matter %	Nil
3	Total Ash value %	3.3
4	Acid insoluble Ash value %	1.0
5	Water soluble Ash value %	2.3
6	Extractive value (Ashodhita sample)	14.96% (13.47 g extract for 90 g crude drug)
7	Extractive value (Shodhita sample)	14.66% (13.66 g extract for 90 g crude drug)

Quantitative estimation of total polyphenolic content

Reagents and Chemicals

Methanol, Gallic acid, Folin-ciocalteau reagent, Sodium carbonate Distilled water.

20% w/v Sodium Carbonate Solution

Take 20 g Sodium Carbonate in 100 mL volumetric flask and make up to 100 mL with distilled water.

Gallic acid Standard Stock Solution (1 mg/mL)

Take 100 mg Gallic acid standard in 100 mL volumetric flask and make up to 100 mL with distilled water.

Intermediate Stock standard (0.1 mg/mL)

Take 1.0 mL Gallic acid Standard Stock Solution (1 mg/mL) in 10 mL volumetric flask and make up to 10 mL with distilled water.

Sample preparation: For raw material

Take 5.0 g sample in 100 mL volumetric flask and make up to 100 mL with methanol. For Extract: prepare 1.0 mg/mL solution in methanol. For liquid: prepare 1.0 mg/mL solution in methanol.

Procedure

Take 0.20, 0.40, 0.60, 0.80, 1.0 mL from Gallic Acid Intermediate stock solution (0.1 mg/mL) and sample stock solution and Blank in 25.0 mL volumetric flask. Add 10 mL distilled water and add 1.5 mL Folin-ciocalteau reagent. This was kept for 5 min at room temperature for incubation. Then add 4.0 mL 20% w/v Sodium Carbonate Solution and Make up the volume up to 25.0 mL with distilled water. This solution was kept in dark and cool place for 30 min for incubation. After incubation process take the absorbance at 765nm by UV-vis spectroscopy. Draw the linearity in-between concentration and absorbance and measure the sample concentration against standard calibration curve.

$$\% \text{ total polyphenol} = (A/B) \times \% \text{ purity of Gallic acid standard}$$

A-Concentration obtained from calibration curve (mg/mL)

B-Concentration applied on UV-vis spectroscopy (mg/mL)

Quantitative estimation of total cardiac glycoside content

8 mL of plant extract was transferred to a 100 mL volumetric flask and 60 mL of H_2O and 8 mL of 12.5% lead acetate were added, mixed, and filtered. 50 mL of the filtrate was transferred into another 100 mL flask and 8 mL of 47% Na-HPO, were added to precipitate excess Pb^{2+} ion. This was mixed and completed to volume with water. The mixture was filtered twice through same filter paper to remove excess lead phosphate, 10ml of purified filtrate was transferred into clean Ertyn-Meyer flask and treated with 10 mL Baljet reagent.

A blank titration was carried out using 10 mL distilled water and 10 mL Baljet reagent. This was allowed to stand for 1 hr for complete color development. The color intensity was measured calorimetrically at 495nm. Calculation was done as follows-

$$\% \text{ of glycoside} = Ax100/77 \text{ g}\%$$

OBSERVATION AND RESULTS

Macroscopic

Root is elongated, thick with the dark brown surface. Less branched with brownish bark having small longitudinal fissures. Cut root surface shows nearly circular or oval shaped with dark brown outer cork region, cortex region appears greenish brown. Centre occupied by large woody part with a dilated center point having unevenly distributed small sized pores of varying size.

Microscopic

Transverse section of root of Karavira shows following characteristics. Outermost part showing cork contains much lignified layers and its linear. Inner cortex shows presence of 20-25 cells forming layered parenchymatous zone of compact cells. Small secretory canals are present in numerous. Xylem is in the middle surrounded by bundles of phloem. Major fibrillar zone vessels scattered with small lumen size and linear arrangement are xylem vessels. Phloem surrounds xylem forming distinct compact zone. Pith is very small present in the Centre. Between xylem vessels narrow or thin medullary rays are present which connects pith with cortex (Figures 1 and 2).

Table 2: Results of Phytochemical analysis.

Phytochemicals	Tests	Ashodhita sample	Shodhita sample
Carbohydrate	Fehling's test Benedict's test	Present	Present
Tannin	Ferric chloride test	Absent	Absent
Alkaloids	Mayer's test Wagner's test	Present	Present
Starch	Iodine solution test	Absent	Absent
Flavonoids	Shinoda test	Present	Present
Steroids	Salkowski test	Absent	Absent
Saponins	Froth test	Present	Present

Table 3: Results of quantitative phytochemical estimation.

	Ashodhita extract	Shodhita extract
Total polyphenolic content	4.14% w/w	2.59% w/w
Total cardiac glycoside content	3.27% w/w	3.05% w/w

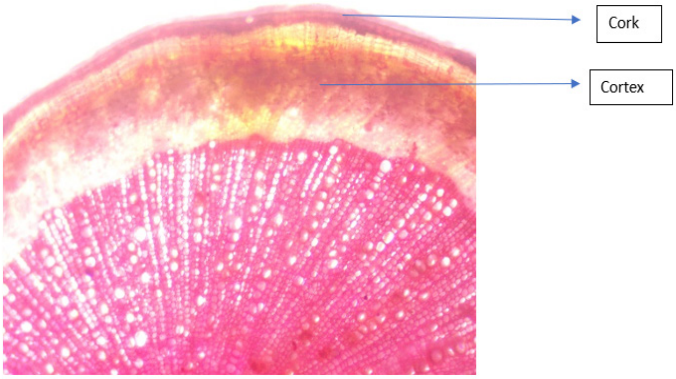

Figure 1: Showing transverse section of root of *N. indicum*.

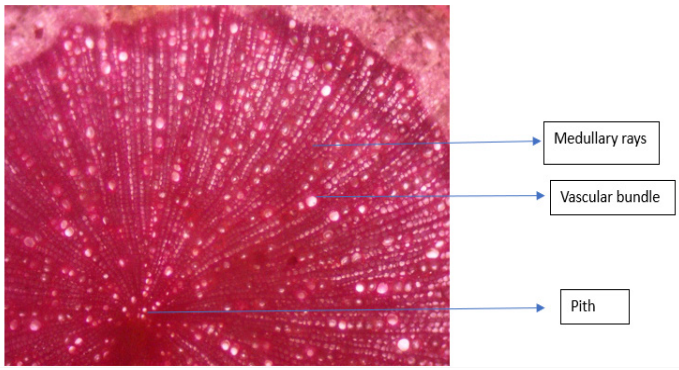

Figure 2: Showing transverse section of root of *N. indicum*.

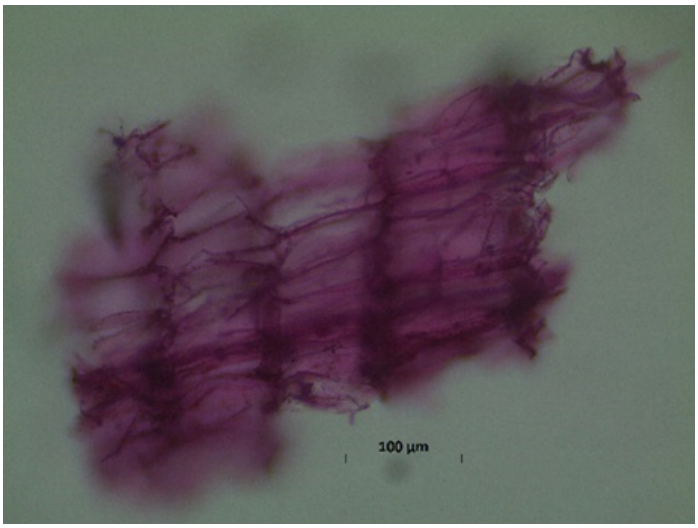

Figure 3: Cork cells in sectional view.

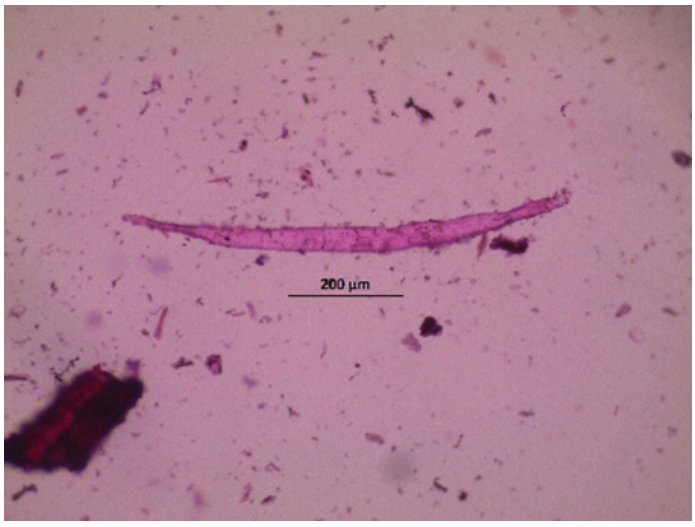

Figure 4: Fibers with tapering ends.

Powder microscopy

Powdered root of Karavira is light brown in color having faint characteristic odor and bitter taste. After treating with Saffranin and Glycerol powdered root shows presence of cork cells, calcium oxalate crystals, fiber fragments with tapering ends (200 µm) crossing medullary ray cells, pitted vessels and fragments of vessels, reticulate vessel fragments, sclereids and stone cells. (Figures 3-8).

Physico-chemical evaluation: Table 1 exhibit results of physico-chemical evaluation.

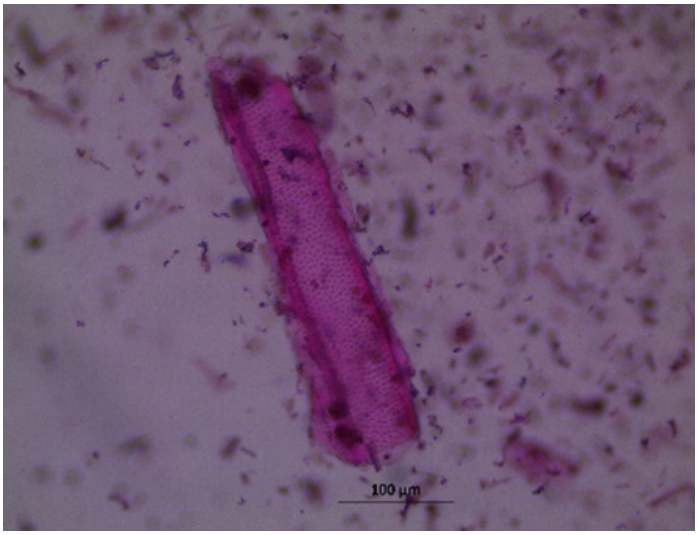

Figure 5: Fragments of vessels.

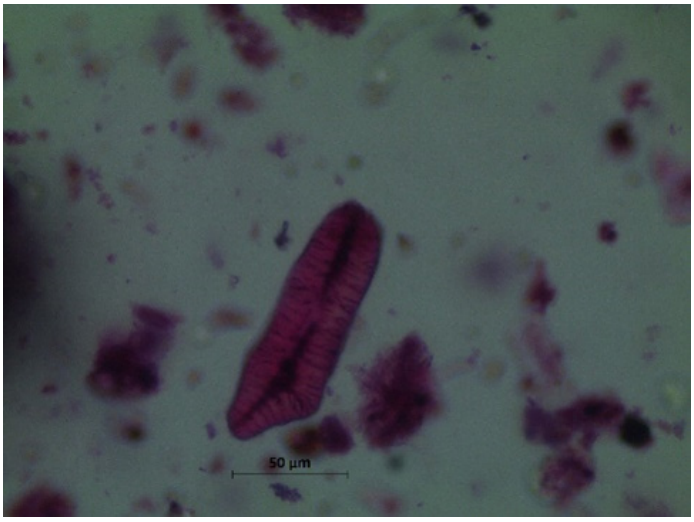

Figure 7: Stone cells.

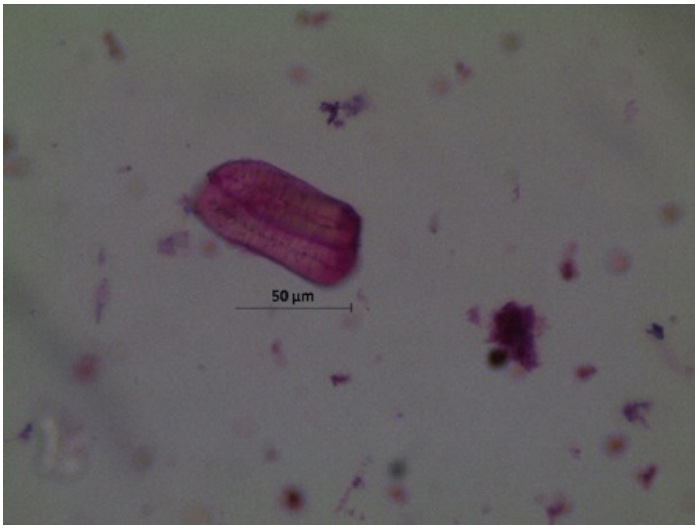

Figure 6: Sclereids.

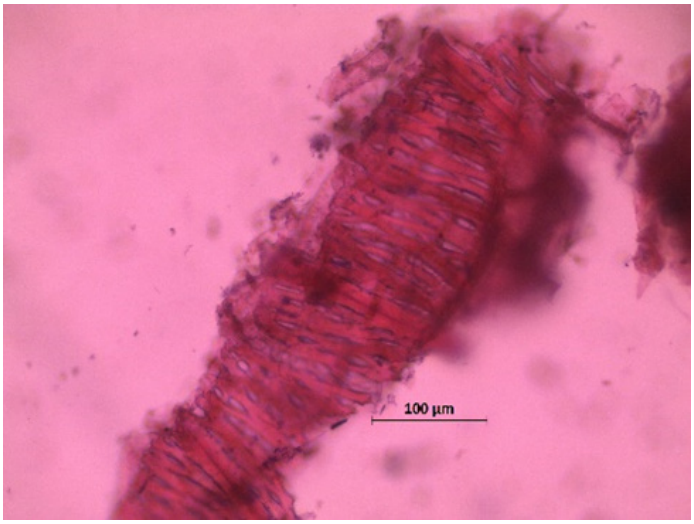

Figure 8: Reticulate vessel fragments.

Phytochemical Screening of pre and post shodhana extracts of *N. indicum* root.

Table 2 exhibit results of qualitative phytochemical analysis.

Table 3 shows results of quantitative estimation of phytochemicals.

DISCUSSION

For standard quality of herbal formulations, use of genuine crude drugs is of utmost important. Thus, there has been a focus on standardizing medicinal plants with therapeutic potential in recent years. Before conducting any tests, a macroscopic and microscopic description of a medicinal plant is recommended by the World Health Organization (WHO) as the first step in determining its identification and purity.[9] Sectioning of fresh sample shows presence of broad undifferentiated cortex, exarch xylem i.e., metaxylem is towards center and protoxylem is towards periphery and a non-cutinized epidermal cell wall confirms it as a root sample. Powder microscopy features of root identifies presence of cork cells, calcium oxalate crystals, fiber fragments with tapering ends (200 µm) crossing medullary ray cells, pitted vessels and fragments of vessels, reticulate vessel fragments, sclereids and stone cells. The physico-chemical evaluation of drugs is a key parameter in identifying adulteration or improper handling of drugs.[10] As per WHO recommendations, a number of physico-chemical properties were assessed for the root. The ash value is one of these metrics, which provides information on the inorganic composition and other impurities in plant drugs. The detection of metal, salts, and silica depends greatly on the total ash value.[11] The total ash value of root was 3.3% and water-soluble ash value was found to be 2.3% indicating presence of cellulosic substances. Acid insoluble ash value was found to be 1.0% designating silicacious substance existence. The stability of a crude medicine when there is a probability for microbial growth is closely correlated with its moisture content. The stability of that medicine will be better and there will be less likelihood of microbial development as the lower the moisture

level is. Lowering the drug's moisture content also increases its shelf life.[12] Loss on drying is the widely used method to check for moisture content. Percentage loss of drug after drying in oven is 4% i.e., the drug imbibes very less moisture when it is properly dried and it can be stored for longer duration. The percentage extractive value indicates the quantity and nature of constituents in the extracts. Hydroalcoholic extract of Ashodhita (not purified) and Shodhita (purified) sample were 13.47 g (14.96%) and 13.20 g (14.66%) respectively for 90 g of crude drug. As already known the extractive value is more with polar solvents and less as polarity of solvent decreases.

The phytochemical screening reveals the presence of carbohydrate, alkaloids, flavonoids, and saponins. The presence of these secondary metabolites suggests that the plant might be medicinal importance. An essential characteristic that indicates the presence of pharmacologically active metabolites in a plant is the identification of its many classes of phytochemical constituents.[13] Qualitative phytochemical analysis of *N. indicum* Shodhita and Ashodhita extract shows presence of similar metabolites such as Carbohydrate, Alkaloids, Flavonoids and Saponins and negative test for Tannins, Starch and Steroids. This result indicates that there is no qualitative phytochemical change after shodhana of drug in milk. Ayurvedic classics claims reduction of toxicity after shodhana process hence there may be changes in quantitative metabolites which are responsible for toxicity. Oleandrin, digitoxigenin, neriin, folinerin, and rosagenin are cardiac glycosides[14] that are mainly responsible for toxicity of Nerium root therefore quantitative estimation of total cardiac glycoside was carried out. It was found to be 3.27% w/w and 3.05% w/w of Ashodhita and Shodhita extract respectively. There was not much difference in cardiac glycoside content after shodhana (purification) process. Also, total polyphenolic content was assessed as plant polyphenols are large group of compounds containing flavonoids, stilbenes, lignans etc.[15] They inherit good antioxidant activity as well as helps in various biological conditions for prevention and cure of diseases.[16] Total polyphenolic content of Ashodhita and Shodhita extract is 4.14% w/w and 2.59% w/w which signifies quantitative reduction in polyphenols after heating with milk. Cardiac glycosides reduced in less quantity compared to total polyphenolic content. The results of the current study indicate that the physico-chemical and phytochemical analyses of *Nerium indicum* root, produced a set of qualitative and quantitative pharmaco-botanical parameters that can be used as a valuable source of data to establish the identity and to assess the quality and purity of the plant material in subsequent studies.

CONCLUSION

Pharmacognostic research of *N. indicum* root sample establishes standardisation standards that aid in preventing adulterations and ensures plant identity. These research will aid in plant identification and guarantee the reproducible quality of herbal products, resulting in the safety and effectiveness of natural products. Studying phytochemical difference between Ashodhita and Shodhita sample helps in validation of Ayurvedic shodhana (purification) procedure.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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