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

International Journal of Ayurvedic Medicine, Vol 13 (3), 2022; 634-639

A comparative analytical study of *Ashodhit* and *Shodhit* roots of *Thevetia neriifolia Juss*. W.S.R *Yogratnakar*

Research Article

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Abstract

Background: In *Ayurveda* whenever poisonous drugs *(Visha Dravya)* used as a part of medicine, they need purification (*Shodhana*) before therapeutic use. *Peet Karveer* (*Thevetia neriifolia Juss.)* is a cardiac poison which contains mainly cardiac glycosides like Cerberin, Nerifolin, Thevetin A and B, Peruvoside, Ruvoside. If the toxic components are removed then *Thevetia neriifolia Juss* is a good source of medicine in many Ayurvedic formulations as an ingredient. Aim and Objectives: The aim of the present study was to compare various physicochemical parameters and HPLC analysis of *Shodhit* and *Ashodhit Thevetia neriifolia Juss* root with special reference to *Yogratnakar*. Material and Method: One part of *Thevetia neriifolia Juss* roots were pounded and made fine powder as Sample A and another part of *Thevetia neriifolia Juss* roots were subjected for boiling (*Swedan*) procedure in cow's milk for 3 hours and made fine powder as Sample B. And studied the physicochemical and phytochemical analysis by HPLC. Result: After purification, different physicochemical parameters and phytochemical analysis which were performed using generally accepted laboratory technique, it was found that there was change in their values. Conclusion: The present study was reflected the importance of *Shodhana* (purification) method of the *Thevetia neriifolia Juss* roots (the toxic drug) which shows the reduction of toxic constituents.

Key Words: *Purification, Peet Karveer, Shodhana, Swedan procedure, Thevetia neriifolia Juss.*

Introduction

Ayurveda is mostly consists of eight branches, one of them is toxicology. According to *Ayurveda,* Toxicology is called as *Danstrachikitsa, Agadtantra, Vishatantra, Visha Vidya, Jaangulika, Visha Vaidyaka*. (1) *Agadtantra* is one of the branch of *Astanga Ayurved*a by Classical texts, which deals with the detailed description about the *Visha* (poison), *Upavisha*, *Mahavisha* and its classification, its examination, diseases caused by it, different treatment and preventive measures of *Visha* (poison) are described. *Visha* (poison) is classified into *Mahavisha* and *Upavisha* by *Rasatanginikar. Mahavisha* are nine in number and *Upavisha* are eleven in number. (2)

Visha dravya (poisonous drug) possess *Laghu* (lightness), *Ushna* (hot), *Tikshna* (sharp), *vyavi* (spread without transformation), *Vikashi* (breaks bodily connection), *Ashukari* (quickly spread) property (3), so that they absorb and spread quickly by showing desired effect. When we used poisonous drug alone or in combination with the other drugs, they increase the efficacy and potency of that formulation by their action. In *Ayurveda,* so many formulations are found including *Visha dravya* (poisonous drugs). But due to their poisonous effect, many physicians get hesitated to describe it in routine practice.

Shodhana is one of the concept of *Ayurveda* not only covers the detoxification or purification of chemical and physical impurities which covers the minimum side effects, also improving the potency and their therapeutic efficacy. Purification is a process of by which removal unwanted impurities or poisonous compounds are separated from the substance by various pharmaceutical methods like washing, grinding, boiling etc. with specific drugs (media) that enhances the minimal toxicity of the substance. (4) All these procedure may leads to either in the disintegration of molecule or making a finest form of particle so that it is easy to acceptable by body tissue and organs. These *Shodhana* procedures help to remove soluble volatile and washable impurities from the ingredients.

Karveer is one of the *upavisha*. There are four varieties of *Karveer. Shweta, Rakta, Peet* and *Krushna*. (5) In this study, we had taken *Peer Karveer. Peet Karveer* is included in *Sthavaraupavisha* or *mulavisha* (6) and in modern it is described as a Cardiac poison. (7) It is included in Apocynaceae family. Plant containing all the parts like leaves, flowers, fruit, seeds,

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roots is poisonous. Roots are considered for the present study. It contains mainly cardiac glycosides like Cerberin, Nerifolin, Thevetin A and B, Peruvoside, Ruvoside.(8) In *Ayurveda*, *vanaspatic* (herbal) plants are the prime source of *Ayurvedic* formulation. Some medicinal plants are toxic in nature which has been in the category of *Visha* (poisonous) and *Upavisha* (semi-poisonous). *Thevetia neriifolia Juss.* is a good source of medicine after removing the toxic components.

In this study we have taken *Shodhana* method from *Yogratnakara.* (9) *Acharya* has mentioned that if poisonous drug is used with proper dose after purification in medicinal preparations, it can act as good as *Amruta.* Purification procedures reduce the toxicity and enhance the safety and potency of the drugs.

Aims and Objectives

Aim

- Comparative study of *Ashodhit* (unpurified) and *Shodhit* (purified) *Thevetia neriifolia Juss*.roots with special reference to *Shodhana Samskara* of *Yogratnakar*.

Objectives

- To study the physicochemical and phyto-chemical changes in roots of *Thevetia neriifolia Juss* before purification.
- To study the physicochemical and phyto-chemical changes in roots of *Thevetia neriifolia Juss* after purification.
- To compare the physicochemical and phyto-chemical changes before and after purification process of the roots of *Thevetia neriifolia Juss.*

Materials and Methods

Materials

Collection of Drug

Roots of *Thevetia neriifolia Juss* were collected from the Herbal garden of MGACH & RC, Salod (H), Wardha.

Identification of Drug

The roots of *Thevetia neriifolia Juss* were identified and authenticated by the *Dravyaguna* department, MGACH & RC, Salod (H) Wardha.

Conceptual review of *Karveer*

Table no. 1: Showing the Classical Categorization of *Karveer*

Charaka (10)	*Kushthaghnagana, Tikttaskandha*
Sushruta (11)	*Lakshadigana, Sirovirechaniyagana*
Raj Nighantu (12)	*Karveeradi Varga*
Madanapal Nighantu (13)	*Abhayadi Varga*
Shodhal Nighantu (14)	*Karveeradi Varga*
Nighantu Aadarsh (15)	*Kutajadi Varga*
Kaideva Nighantu (16)	*Aushadhi Varga*
Dhanvantari Nighantu (17)	*Karveeradi Varga*
Priya Nighantu (18)	*Haritakyadi Varga*

Table no. 2: showing the Description of *Peet Karveer*: (19)

Botanical Name	*Thevetia neriifolia juss.*
Family	Apocynaeae
Classical Name	*Karavira, Hayamaraka, Haripriya, Asvamaraka, Ashwamaraka, Shatkumbha, Chandata, Laguda*
Parts used	Root
Active Principle	Cerberin, Nerifolin, Thevetin A and B, Peruvoside, Ruvoside
Common name	Yellow oleander, digoxin, lucky nut, yellow bell

Table no. 3: Showing the Vernacular Names of *Karveer*: (20)

Eng	Indian oleander, Sweet scented oleander, Roseberry spurge
Hindi	*Kaner, Karber, Karuvira*
Beng	*Karabi, Karavi*
Guj	*Kanher, Kanera*
Kan	*Konagilu, Paddale*
Mal	*Arali, Kanaviram, Karaviram*
Mar	*Kaner, Kanher, Kaneri*
Punj	*Kanher, Kanira, Ganhira*
Tam	*Arali, Irattai Sivapparali, Vellaiarali, Aralivayr*
Tel	*Ganneru, Karaviramu,Kasturipattu, Turi-patte*
Arab	*Diffi, Sum-el-himar*
Oriya	*Konero, Korobiro*
Pers	*Khar-sahrah*
Santhal	*Rajbaka*

Table no. 4: Showing the pharmacodynamics of *Peet Karveer*: (21)

Rasa	*Katu, Tikta, Kashaya*
Guna	*Laghu, Ruksha, Tikshna*
Veerya	*Ushna*
Vipaka	*Katu*
Prabhava	*Hridya*
Doshaghnata	*Kaphavatashamaka*
Rogaghnata	*Kushtha, Vrana, Shotha, Netraroga, Agnimandya, Vibandha, Udararoga, Raktavikara,, shwasakrichchhra, Hridaurbalya, Hridaroga, Vishamajwara, Mootrachchhra, Ashmari, Upadansha, Phirangajanyavrana*
Karma	*Kushthghna, Vranashodhana, Vranaropana, Shothahara, Deepana, Vidahi, Bhedana, Hridya, Raktashodhaka, Shwasahara, Mootrajanana, Swedajana, Jwarghna, Vishamajwara, Teevravisha, Kandughna, Krimighna, Netrakopavranahara.*
Pharmacological activities	Anti-inflammatory, Analegesic, Diuretic, Anti-biotic, Anti-stress, Anti- fungal, Cardio-kinetic, Spasmodic, Anti-cancer, Anti-pyretic.
Formulations	*Brhanmaricadya Taila, Karaviradya Taila, Gunjadya Taila, Kasisadi Taila, Karvira yoga*
Chemical Constituents	Glycosides – Cardiac Glycosides and Resinous Matter

Methodology

Study Design: Analytical study

Ethics Committee Approval – After approval from Institutional Ethical Committee (with reference number DMIMS (DU)/IE 2017-18/6594) on dated 23/08/2017, study was carried out at Mahatma Gandhi Ayurved College, Hospital and Research Centre Salod (H),Wardha

Method:

1. Coding of Sample:
   - Sample A (Un-purified sample of *Thevetia neriifolia juss* root)
   - Sample B (Purified sample of *Thevetia neriifolia juss* root)

Preparation Methods:

Sample A (Unpurified sample of *Thevetia neriifolia juss* root):

- Matured *Thevetia neriifolia juss* roots were collected from authentic source.
- After identification *Thevetia neriifolia juss* roots were cut into small pieces.
- These *Thevetia neriifolia juss* roots had been dried in sunlight.
- After that fine powder is prepared.
- The powder is packed in a sealed jar and labelled.

Figure No.1

Thevetia neriifolia juss.root

Small pieces of *Thevetia neriifolia juss*.root

Fine powder of *Thevetia neriifolia juss*.root

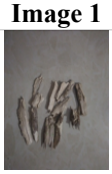
Sample B (Purified sample of *Thevetia neriifolia juss.* root):

- Matured *Thevetia neriifolia juss.* roots were collected from authentic source.
- After identification *Thevetia neriifolia juss.* roots were cut into small pieces
- Purification procedure was done according to special reference of *Yogratanakar*. (22)

Purification *(Shodhana)* Procedure: (23)

- Small pieces of *Thevetia neriifolia juss* roots were taken into a cloth bag and made a Pottali.
- A Pottali containing *Thevetia neriifolia juss* root were suspended in *Dolayantra* (heating pot) having capacity of 1-2 litre.
- Approximately 1 litre of purifying drugs *(Shodhana Dravya)* cow milk was filled in *Dolayantra* (heating pot).
- The *Pottali* was suspended to a glass rod on the mouth of pot in such a way that it did not touch the bottom of pot; it was swinging and submerged in purifying drugs.
- The pot was heated to boil gently the purifying drugs for 3 hours on low flame.
- Additional cow milk was added frequently to maintain the level of the purifying drug.
- This is turn increase in the heating by ½ an hour as it decreases the temperature of purifying drugs.
- Hence total heating time was 3½ hours.
- After that, *Thevetia neriifolia juss* roots were drawn out from *Pottali* and washed by luke warm water and then dried them and grinded to make fine powder.
- The powder is then packed in a sealed jar and labelled it.

Figure no.2

Image 1	Image 2	Image 3	Image 4
			
Small pieces of *Thevetia neriifolia juss*.root	Tied in a small *pottali*	*Swedana* (heating) in *godugdha* (cow milk)	*Swedana* (heating) upto 3 hours
Image 5	**Image 6**	**Image 7**	**Image 8**
			
Washed with luke warm water	Dried in a sunlight	Fine powder of *Shodhit Thevetia neriifolia juss.* roots	*Ashodhit* (unpurified) and *Shodhit* (purified) Sample

II. Physico-chemical Analysis: (24)

Organoleptic characters

- *Sparsha* (consistency)
- *Rupa* (Colour)
- *Rasa* (Taste)
- *Gandha* (odour)

pH

Dissolved one tablet of different pH in 100 ml of distilled water and prepared the solution of different pH 4,7,and 9 respectively and labelled. Switched the instrument and leave it for some time. Buffer solution was taken in beaker and dipped the electrode in it. Same procedure was done for every buffer solution after washing the electrode. Test sample was taken and dipped the electrode in it and noted the pH value.

Total Ash

Sample A and Sample B, each of 3 gm respectively were placed in a silica dish and kept in a muffle furnace at temperature not exceeding 600ºC. Both the dishes cooled in desiccators and after that

weighed them. Calculated the percentage of ash with reference to the air - dried drugs.

Acid insoluble Ash

Ash collected from above procedure were mixed with 100ml of hydrochloric acid in a 250ml beaker of each. Heated the beakers till the liquid boils. Filtered both the solution by 41 number whattman filter paper and collected the insoluble matter. After washing by hot water kept this filter paper into original crucible and dried on hot plate and ignited at 600ºC in a muffle furnace. After cooling weighed the residue. Repeated the procedure until constant weight obtained of both the samples. After that calculated the Acid insoluble ash value with reference to air dried drugs.

Water soluble Ash

Boiled the ash of both the samples with 25ml of water. Collected the insoluble matter on ash-less filter paper washed by hot water and ignited for 15 minutes at a temperature not exceeding 600º C. Subtracted the weight of insoluble matter from the weight of ash, the difference of weight represents the water soluble ash. Calculated the percentage of water soluble ash with reference to the air dried drugs.

Water soluble extractive value

5 gm coarse powder of both the samples macerated with 100 ml of distilled water in a closed flask for 24 hours. For 6 hours shake them frequently and kept stand for 18 hours. After that filtered. Filtrate was poured on evaporating dish for dryness at 105ºC to constant weight. Calculate the percentage of water-soluble extractive with reference to air dried drugs.

Alcohol soluble extractive value

5 gm coarse powder of both the samples macerated with 100 ml of alcohol of specified strength in a closed flask for 24 hours. For 6 hours shake them frequently and kept stand for 18 hours. After that filtered. Filtrate was poured on evaporating dish for dryness at 105ºC to constant weight. Calculate the percentage of water-soluble extractive with reference to air dried drugs.

Moisture content

Both the samples was taken in powered form accurately about 5gm and widens the dishes homogeneously. Kept the dishes in the instrument adjust according at zero and set the temperature at 105º C. When the reading becomes constant, noted down.

Phytochemical Analysis: (25)

HPLC (High Pressure Liquid Chromatography)

Instrumentation

This chromatographic technique was performed by the Shimadzu Prominence HPLC instrument. It include quaternary pump, degasser DGU-20As, LC20 -80, Autosampler SIL-20, column oven CTO -10As, Detector UVSPDM20A. Diode-Array. Required phytochemical were analysed with Prime Sil C18ncolumn (250*4.6mm. ID) It was purchased from Ultrachrom Innvatives Pvt.Ltd.

Chemicals

For HPLC analysis, HPLC grade methanol and water were used. Also 0.2 µ sample filters and 0.45 µ nylon solvent filters.

Sample extraction

Sample A and Sample B were separately dissolved in 20ml of ethanol. They are kept at room temperature to improve the release of all potential components for 24 hrs. After that they were ultra-solicited for 30 minutes, filtered through 0.2 µ nylon sample filters.

HPLC Analysis

First 20 µl of freshly prepared stock solution of sample A and Sample B were injected to the C18 column and it was eluted at the flow rate of 1.5ml /min. In HPLC analysis, binary composition of methanol and water including ammonium acetate were engaged. For achieving better peak shape and peak area, 254nm UV wavelength and temperature were considered. Sample A and Sample B were separately analysed and graphical analysis report were generated.

Observations and Results

Table no 5: Showing the result of physicochemical analysis of *Ashodhita* (un-purified) and *Shodhita* (purified) *Thevetia neriifolia juss*. sample

Sr.no.	Parameters	Sample A (*Ashodhita Thevetia neriifolia juss*)	Sample B (*Shodhita Thevetia neriifolia juss*)
1	Colour	Buff colour	Buff colour
2	Odour	Characteristic	Characteristic
3	Loss on drying at 105^0 C	1.65%	0.7%
4	Total Ash Value	10.9%	2.3%
5	Water soluble ash value	0.45%	0.15%
6	Acid insoluble ash value	0.55%	0.95%
7	Water soluble extractive	78.05%	29%
8	Alcohol soluble extractive	60%	55.4%
9	pH	6	6

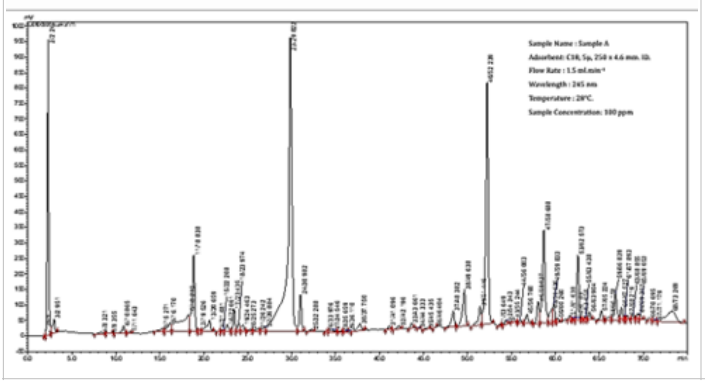
Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (3), 2022; 634-639

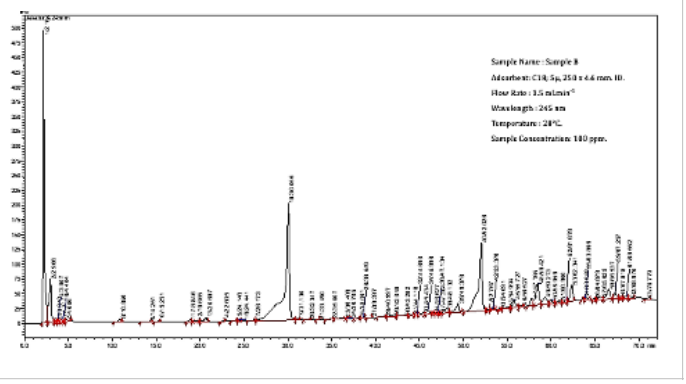
Phytochemical Analysis

HPLC graphs of Sample A and B

Graph 1: Sample A (*Ashodhit Thevetia neriifolia juss* roots)



Graph 2: Sample B (*Shodhit Thevetia neriifolia juss* roots)



Discussion

Acharya Charaka has mentioned the used of *Thevetia neriifolia juss* in the treatment of various skin diseases. By Acharya Sushruta, it is classified under the toxicity known as horse poison or *Ashwamarak.* Its cardio tonic activity was proved at 1863 and after that the whole plant parts was lighten as toxic. Whenever these types of poisonous drugs should be used in therapeutic medicine, they need purification (*Shodhana*). Without purification these drugs shows hazardous effect rather than beneficial. So, by using purification methods we reduce the higher concentration of toxins, ultimately toxicity will reduce. *Thevetia neriifolia juss* is an evergreen tropical shrub or small tree. The useful parts are toxic in nature contain various cardiac glycosides like Thevetin A, Thevetin B, peruosides, Ruvosides. Some of these are taken in low doses; it is useful in curing cardiac arrest, menstrual problem, skin diseases and seizures.

If the toxic component is removed then *PeetKarveer* (*Thevetia neriifolia*)is a good source of medicine in many Ayurvedic formulations as an ingredient. This toxic part is removed by *Shodhana*(purification)procedure which described in Ayurveda texts such as Rasatarangini, Yogratnakara, Rasa shastra, Rasa ratnasamuchaya. In this study, specifically we mentioned the methods of Acharya Yogratnakara.

The present study provides compelling evidence of Thevetianeriifolia roots for determination of different physicochemical parameters which was performed using generally accepted laboratory technique for physico-chemical analysis. After purification, it was found that there is slight change in colour of sample.

Moisture content

After loss on drying at 105˚ C, the moisture content in *Ashodhit*(un-purified)sample of *PeetKarveer*(Thevetia neriifolia) root powder was 1.65% which was decreased in *Shodhi*t (purified) sample i. e 0.7% .(Table no.5)

Total Ash Value

Total Ash value of *Ashodhit* (un-purified) sample was found 10.9% which was decrease in *Shodhit*(purified) sample that is 2.3%. (Table no.5)

Water soluble Extractive Value

Water soluble extractive value of *Ashodhit* (un-purified) sample was 0.45% after purification it was decreased in 0.15%. (Table no.5)

Acid insoluble Ash Value

Acid insoluble Ash Valueof *Ashodhit*(un-purified) sample was 0.55% after purification it was increased in 0.95%. (Table no.5)

Water soluble extractive value

Water soluble extractive valueof *Ashodhit* (un-purified) sample was 78.05% after purification it was decreased in 29%. (Table no.5)

Alcohol soluble extractive value

Alcohol soluble extractive valueof *Ashodhit* (un-purified) sample was 60% after purification it was decreased in 55.4 %. (Table no.5)

pH value

pH value of both the *Ashodhit* (un-purified) and *Shodhit*(purified)sample was 6. It is being observed that both the samples are having acidic pH. According to some experts, the acidic pH indicates *Ushnavirya* (hot potency).

With reference to above observed results of physico-chemical parameters it can be hypothized that the changes in the parameters were found due to the proper Shodhana (purification) processes.

Phytochemical Analysis

After performing the chromatographic technique HPLC (High Performance Liquid Chromatography) in Sample A/ *Ashodhit* (un-purified) sample, several acid – base strengthen compounds like phytoamines and polyphenolic acids detected in 1-25 mins run time Few polar flavonoids were detected at 28 -29 min run time. Besides the presence of ionic components, there were number of moderately polar and neutral polyphenols were detected at 53-60 min run time and sugar conjugated sterols like glycosides were detected at 62-66 min run time seen in Graph 1.

Importantly after evaluating the Sample B / *Shodhit* (purified) sample with Sample A / *Ashodhit*

(un-purified) sample, phytoamines, might some extent polyphenolic acids, neutral moderately aqueous soluble polyphenols also detected as flavonoids and sugar conjugated sterols like glycosides, they were either completely missing or their concentration was reduced to some extent in Sample B / *Shodhit* (purified) sample in Graph 2. It was assumed that the poisonous activity of this plant would associate with these phytoamines.

However, after comparing the result of Sample B *Shodhit* (purified) with Sample A *Ashodhit* (un-purified) of *Thevetia neriifolia juss* roots, many of these chemical constituents were either missing or their concentration were sharply decreased.

Conclusion

Purification process showed the changes in physicochemical and phyto-chemical analysis by reducing the chemical constituents of *Thevetia neriifolia juss.*

Acknowledgement

I would express my sincere gratitude to Dr. Sonali Chalakh, Dr. Akshay Pargaonkar, and Dr. Priyanka Bangare and specially Dr. Pankaj Kharabe for their generous support for performing Analytical study.

Funding support- nil.
Conflict of interest- nil.

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