



Research Article

COMPARATIVE PHYSICO-CHEMICAL ANALYSIS OF ROOTS OF YELLOW KARWEER (*THEVETIA NERIIFOLIA* JUSS) BEFORE AND AFTER PURIFICATION PROCESS

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ABSTRACT

Yellow *Karweer*/ oleander (*Thevetia neriifolia* synonym *Thevetia peruviana*) belongs to the family Apocynaceae. It is a poisonous plant, with various pharmacological activities. It is used by Ayurvedic physician in various diseases, especially in the skin disease. *Karweer* are potentially lethal plants after ingestion. All parts of the plants are toxic and contain a variety of cardiac glycosides. Excess use of *Karweer* results in nausea, vomiting, abdominal pain, diarrhoea, dysrhythmia and hypocalcaemia in most cases. Administration of *Karweer* is recommended only after the *Shodhana* (purification procedures) in *Godugdha* (cow's milk).

Method

After purification with cow's milk by boiling method, Yellow *Karweer* root was evaluated for their pharmacognostical and pharmaceutical analysis. The obtained roots were investigated for their powder microscopically characters, phytochemical characters like preliminary phytochemical screening and Thin layer chromatography as per the API guidelines.

Results

Organoleptic study shows variations in colour, odour, taste and appearance in all samples after purification. Microscopically study of powder highlights some changes like more number of oil globules in *Godugdha* (cow's milk) purified sample. Preliminary phytochemical screening reveals the presence of alkaloids, saponin glycoside, flavonoids, reducing sugar, etc.

Conclusion

The observed data can be helpful to delineate the impact of purification process on yellow *Karweer* roots. Media has an important role to reduce the toxic effects and increase the potency.

KEYWORDS: Yellow *Karweer*, Purification, Cow's milk, *Thevetia neriifolia*, Chemical analysis, TLC.

INTRODUCTION

Ayurvedic toxicology is the special branch of Ashtang Ayurved that is one of the eight classical disciplines of Ayurved, having its own importance. According to *Ayurveda Visha* (poisons) is the substance which after entering in to the body damages the *Rasadisaptadhatu* (all tissues) to certain extent which ultimately results in to death.^[1] It creates sorrowfulness (*Vishada*) to all living organisms by making them grievously ill & leading to death.

India has a very long tradition of using herbal medicines in various disorders as a single drug or as an ingredient of compound formulations. Among herbal origin certain plants are categorized under *Visha* (poisonous) and *Upavisha* (semi-poisonous) drugs.

Purification or detoxification (*Shodhana*) is unique process described in Ayurved as it not only vanish the poisonous qualities but it also increases the therapeutic properties of a particular drug. *Shodhana* reduces all the *Bhautic* (physical), *Rasaynik* (chemical) & *Sahaja* (natural) impurities.^[2] The media used in the process of purification has very important role in either breaking down or destroying the chemical constituent which are not required.^[3]

Yellow *Karweer* (*Thevetia neriifolia* synonym *Thevetia peruviana*) belongs to the family Apocynaceae & commonly known as yellow oleander & Lucky nut. This plant contains different glycosides especially cardiac glycosides in every parts of the plant. ^[4] It comes under *Moolavisha*^[5] (root poisons) and *Sthavarvisha* group. ^[6-7]

Different parts of *Thevetia nerifolia* are known to possess various medicinal properties. The plant is commonly used in domestic medicine in tropical America and in tropical Asia. *Thevetia nerifolia* is used traditionally in treatment of amenorrhoea, malarial fever, jaundice, haemorrhoids, constipation, headaches, skin disorders etc.^[8]

Analytical and microscopic evaluation of various parts of yellow *Karweer* has been already well established but the difference between the roots of yellow *Karweer* before and after purification and it's by phytochemical analysis has not yet been demonstrated scientifically.

In the present study, an attempt has been made to evaluate microscopical characters, qualitative tests and quantitative estimation of root after proper processing through *Godugdha* (cow's milk).^[9,10] Study was aimed to prove the impact of the Ayurvedic purification method and to analyse the raw and purified samples analytically.

MATERIALS AND METHODS

Collection of drug

The root of *Karweer* was collected in the month of November- December from the surroundings of Bharati Vidyapeeth, Dhankawadi campus, Pune, in their natural habitat. Roots were identified and authenticated by an expert plant taxonomist. The plant specimen was submitted to the departmental museum of the institute for future reference.

Procedure

Purification of roots was carried out by the classically approved method by the process of *Swedana* (boiling) in *Godugdha*.^[11] Earthen pot of 7 litter capacity was used for the *Swedana* (boiling) procedure. The cleaned roots were cut in 3 inch pieces and were placed in a muslin cloth and made into a *Potali* (linen bundle or bag). The *Potali* was suspended from a centre of the rod fixed in the neck holes of earthen pot and the vessel was filled with freshly *Godugdha* (cow's milk), *Potali* was kept as per *Swedana* (boiling) procedure for three hours. After boiling for three hours, the roots were taken out from *Potali* and washed with lukewarm water.

Yellow *Karweer*

Roots were kept for the shade drying. After proper drying, the roots were collected and stored in air tight zip locked bags.

Preparation of samples

The raw roots of *Karweer* purified were powdered with grinder and passed through 100 mesh sieves. These powdered materials used for analytical study. Cow's milk used for purification was also analysed.

Organoleptic Study^[12]

In the Organoleptic evaluation various sensory parameters such as color, odor, taste and texture were investigated. *Dwivedi et al* has laid down certain parameters and perform to identify drugs on the basis of *Panchendriya Pariksha* (Organoleptic examination).

Physico-chemical parameters^[13]

Total ash content, Acid insoluble and water soluble ash, fluorescence analysis, alcoholic water and petroleum ether extractive values were analyzed as per WHO guidelines and Ayurvedic pharmacopeia of India.

Preliminary qualitative tests^[14]

The extracts were analyzed for the presence of sugars, carbohydrates, tannins, steroids, flavonoids and saponins using standard protocol.

Chromatographic study

Thin layer chromatography (TLC) of extract was carried out for the normal phase separation of components.

RESULTS AND DISCUSSION

Macroscopic study

Thevetia nerifolia juss is a large, evergreen, glabrous shrub or small tree growing wild. The root is highly branched and browning or greyish in colour with long irregular streaks caused by the rupture of the bark.

Organoleptic analysis of yellow *Karweer* and Cow's milk

Before and after purification process all samples such as yellow *Karweer* root and cow's milk were observed by organoleptic parameters (Table 1).

Table 1: Organoleptic examination of all collected samples

Observations	Before purification	After purification
Shape	Cylindrical	Cylindrical
Colour	Gray color	Grayish brown
Odour	Herbal smell	Slightly milky
Taste	Bitter	Bitter
Surface characters	Irregular streaks caused by the rupture of the bark	Irregular streaks caused by the rupture of the bark
Fracture	Short in the bark and wood	Short in the bark and wood

Table 2: PH value of Cow Milk Before and After purification

Observations	Before purification	After purification
Colour	White	Creamy
Odour	No smell	Burnt smell
PH	6.05	5.5

Microscopic study

Comparative microscopic study on the powdered roots before and after purification process was carried out. Both the root powders were taken in a test tube and treated with 25 % choral hydrate solution. This treatment was done repeatedly until decolouration of the powder. Treated powders was mounted on the slides and observed under the microscope.

Similar microscopic characteristics were seen in both the roots before and after purification process as follows-

- Fragments of vessel with reticulate thicken
- Starch granules either single or grouped of 3-6 in to clusters
- Xylem fibers- lignified fibres with pitted wall and tapering at both the ends.

- Xylem parenchyma thin walled parenchymatous cells, rectangular in shape
- Calcium oxalate crystals
- Group of tracheids and tracheidal vessels

Before and after purification process both milks were observed under microscope. Fat globules and Brownian motion were observed.

Phytochemical analysis

Physical analysis of root powder showed particle Size moderately coarse 80% passes through 710 micrometer and 20% passes through 250 micrometer. The value of total ash shows the presence of residue after ignition, representing the plant residue, and external materials.

Table 3: Physicochemical analysis of all samples of yellow Karweer

Parameters	Roots	
	Before purification	After purification
Loss on drying	1.8931%	3.3598%
Total Ash value	4.6124%	4.7356%
Water soluble ash	0.761	0.790
Acid insoluble ash	1.7571	2.0493
Water extractive value	7.92	6.8362
Alcohol extractive value	8.9320	9.3728
Petroleum ether extractive value	1.1809	1.5293
Fluorescence analysis	Blue	Blue

Chemical studies

In the preliminary phytochemical screening for commonly occurring plant constituents was carried using the maceration procedure. The testing was carried out on residue obtained by evaporation of water, alcohol and chloroform extracts of drug. Results of phytochemical screening are listed in following table.

Table 4: Chemical tests of root extracts of yellow Karweer before and after purification

Tests	Roots Before purification			Roots After purification		
	Aqueous extract	Alcohol extract	Chloroform extract	Aqueous extract	Alcohol extract	Chloroform extract
Alkaloids	++	++	++	+	+	+
Amino acids	+	+	+	+	+	+
Anthraquene glycosides	-	-	-	-	-	-
Cardiac glycosides	++	++	++	+	+	+
Caumarins	-	-	-	-	-	-
Cynogenic glycosides	-	-	-	-	-	-

Flavonoides	+	+	+	+	+	+
Mucilage	+	+	+	+	+	+
Proteins	-	-	-	-	-	-
Saponins	+	+	+	-	-	-
Steroids	+	+	+	+	+	+
Sugars	+	+	+	+	+	+
Tannins	-	-	-	-	-	-

Table 5: Chemical analysis of milk before and after purification

Tests	Milk	
	Before purification	After purification
PH	6.05	5.5
Color	White	Creamy
Odor	No smell	Burning smell
Test for Fats	+ve	+ve
Test for Casein	+ve	+ve
Test for phosphorous	+ve	+ve
Test for lactoalbumin and lactoglobulin	+ve	+ve
Test for calcium	+ve	+ve
Test for carbohydrates	+ve	+ve

Thin Layer Chromatographic study of ethanol extract

Thin-layer chromatography was executed for the normal phase separation of components of ethanol extracts of root of *Thevetia neriiifolia*.

Solvent system: Ethyl acetate: Methanol: Water (81:11:8)

Stationary phase: silica gel G60F254

Reagent: Con H₂SO₄ in 5 to 10 % C₅H₅OH

U/V light: wavelength 254 and 366 nm

R_f = distance travelled by solute/distance travelled by solvent.

Table showing the results of TLC

Table 6: TLC profile

Spots	Before Purification	After Purification
1	0.554	0.543
2	0.724	0.724
3	0.810	0.8188
4	0.934	0.905

Colour of all the four spots was light brown to dark brown.

PHOTOGRAPHS



Yellow Karweer (*Thevetia neriiifolia*)



Purification by Dolayantra



Roots before purification



Roots after purification

DISCUSSION

Organoleptic analysis of cow's milk before & after purification shows significant difference in terms of colour, odour and pH. Before *Shodhana* it has white colour & not having specific smell but after *Shodhana* it has creamy colour & burnt smell. PH was also changed which means that during *Shodhana* there is absorption of some of the chemical constituents of *Karweer* which may be responsible to produce toxic effects.

Physical analysis of *Karweer* shows significant difference in terms of acid insoluble, loss on drying, soluble extractives. Results showed change in physical properties because of purification of *Karweer* with cow's milk.

TLC study of *Karweer* shows 4 spots each in presence of day light, 365 nm light before and after *Shodhana*. It indicates there is loss of some chemical constituents during *Shodhana* which may reduce its toxic effects.

From Ayurvedic point of view, cow's milk consists of ten qualities - *Madhura* (sweet) *Sheeta* (cold) *Mrudu* (soft) *Snigdha* (unctuous) *Bahala* (density, thick) *Shlakshna* (smoothness) *Picchila* (slimness, stickiness) *Guru* (heavy) *Manda* (slowness) and *Prasanna* (calming, clear).^[13] Yellow *Karweer* is *Katu* (pungent) *Tikta* (bitter) *Kashaya* (astringent) in taste; *Laghu* (light) *Rooksha* (dry) *Teekshna* (sharp) in physical qualities, pungent in post digestive effect and hot in potency, these qualities are opposite to cow's milk. Cow's milk is *Madhura* (sweet) *Guru* (heavy) *Snigdha* (unctuous) *Manda* (slow); sweet in post digestive effect and cold in potency. This may be the cause of reduction in the compounds after purification of yellow *Karweer* with cow's milk. Heat treatment or constant boiling for specific time in specific media resulted in modifies the chemical constituents, enhancing pharmacological actions of a substance, change in the state and nature of a substance, mode of action becomes safe, smooth.

CONCLUSION

Shodhana (purification) process brought significant changes in physical & chemical constituents of yellow *Karweer* which nullifies its toxic effects & enhances its therapeutic properties. Cow's milk has definitely an important role in making a drug act without causing side-effects/adverse effects. These findings strongly confirm the claims of the traditional practitioners of Ayurveda that the *Shodhana* (purification) process of *Karweer* by cow's milk successfully reduces the toxic elements of the drug and this process may be practiced routinely in future.

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