

<https://doi.org/10.33472/AFJBS.6.Si2.2024.3069-3075>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

Phytochemical Analysis of Strychnine content of Processed Nux-vomica seeds in different media.

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Volume 6, Issue Si2, 2024

Received: 09 March 2024

Accepted: 10 April 2024

Published: 20 May 2024

[doi:10.33472/AFJBS.6.Si2.2024.3069-3075](https://doi.org/10.33472/AFJBS.6.Si2.2024.3069-3075)

Abstract:

Background: Strychnos Nux vomica is a poisonous plant having more therapeutic potential. Before application of this poisonous herb in therapeutics Ayurveda mentioned certain processing, known as *Shodhana Sanskar*. (Purification) This brings poisonous medicinal plants into the mainstream and holds them more accountable. For *Shodhana*, *Ayurveda* uses a wide variety of media. *Godugdha*, *Gomutra*, and *Ghoghrit* are common media used for *Shodhana* purpose. In the current study strychnine content of Nux Vomica seeds was screened by processing seeds in common media along with special media.. **Material & Methodology:** Six different media including *Godugdha* (cow's milk), *Goghrita* (cow's ghee), *Gomutra* (cow's urine), *Kanji* (sour gruel), *Erand Taila* and one combined media (*Gomutra* ,*Godugdha*, *Goghrit*) was used for *Shodhana* procedure. After shodhana phytochemical analysis of strychnine was done by HPTLC method. **Observation:** Study revealed that concentration of strychnine gets reduced in samples processed in the *Godugdha* (81.2) and *Erand Taila*(83.4). **Conclusion:** These findings strongly confirmed the claims of ancient classics of Ayurveda that *Shodhna* successfully reduces the toxic effects of poisonous drugs and simplify it for therapeutic purpose. **Keywords:** Strychnine, Nux-Vomica, Shodhana, Phytochemical, HPTLC

Background:

A medicine is a poison in a large dosage, and a poison is a medicine in a small dose.[1] In Ayurveda, numerous toxic drugs are used either as single medications or in compound formulations. *Kupeelu* (*Strychnos nuxvomica* Linn), a well-known poisonous herb in Indian system of medicine. In large dosages, it is a potent poison that causes tetanic convulsions and ultimately death. [2]Strychnine and brucine, two deadly alkaloids found in *S. nux-vomica*, are present in the roots, wood, leaves, bark, fruit pulp, and seeds [3]. It is included in Schedule E of the 1940 Drug and Cosmetic Act[4].Despite the fact that the plant is classified under the "Upavisa Vargas" (sub toxic group[5], its seeds have been successfully employed in various formulations to treat a variety of ailments after undergoing the required process known as *Shodhana* (Detoxification) [6]. In Ayurveda, the concept of *shodhana* (Processing or Purification) refers to both a Purification/Detoxification procedure as well as a method to increase the drug's strength and effectiveness [7].Purified *Nux vomica* seeds are mostly used as a stimulant, aphrodisiac, appetiser, digestive & purgative. In animal experiment it proved to possess hepatoprotective [8] analgesic and anti-inflammatory [9], antioxidant [10], anti-tumor [11] anti-snake venom [12], anti-diarrheal [13] effects.. For *Shodhana*,of *Nux vomica* *Ayurveda* uses a wide variety of media. *Godugdha* (Cow's Milk) *Gomutra* (cows Urine), and *Ghoghrit* (Cows Ghee), *Kanji* (Sour Gruel) and *Erand Tail*(Castor oil) are the different media recommended for the *shodhana* of *Nux-vomica* . Hence the purpose of the current study was to determine effect of various *shodhana* media on the Strychnine content in *Nux vomica* seeds using the HPTLC method.

Material & Method:

Collection of seeds: Fully matured *Kupeelu* seeds were collected from Shri Shail Pharma, Nagpur, Maharashtra and were authenticated by Dravyaguna dept of Mahatma Gandhi Ayurved College, Wardha.

Collection of Media: *Gomutra* and *Godugdha* were collected from the local cow shed in the morning at 6 am .*Goghrit* and *Erand Tail* was purchased from local market. *Kanji* was prepared in Dattatraya Ayurved Rasashala, Mahatma Gandhi Ayurved College, Wardha.

Chemicals: Pure strychnine was purchased from Sigma Aldrich USA and pre-coated silica gel 60 F₂₅₄ TLC aluminum plates (10 × 10 cm, 0.2 mm thick), AR grade toluene, ethyl acetate, diethyl amine, methanol and chloroform were obtained from M/ S Merck Ltd. (Mumbai, India).

Equipment for HPTLC : HPTLC analysis was performed on CAMAG TLC Scanner "Scanner_181112" S/N 181112 (2.01.02) equipped with win CATS Planar Chromatography Manager. CAMAG Linomat 5 "Linomat5_180945" S/N 180945 (1.00.13) Twin Trough Chamber 10x10cm with HPTLC plates silica gel 60 F 25. Including these, analytical balance (ME-205, Mettler-Toledo), pH meter (FiveEasy-A211, Mettler-Toledo), and ultra-sonicator (Labman[®]) were used throughout the HPTLC analysis. Linomat 5 application parameters Spray gas was used Inert gas .Sample solvent type with methanol

Method of Shodhana of Strychnos seeds in Different Media:

1.Shodhana in *Gomutra* ,*Godugdha* followed by frying in *Goghrit* :

Nux vomica seeds were submerged in *Gomutra* for 7 days and then on 8th day seeds were wrapped in muslin cloth and tied into a bundle and then boiled in *Godugdha* for three hours. Then the seeds were fried in *Goghrit* and then pulverised immediately. [14]

2. Shodhana in *Gomutra*:

In a stainless steel tray, 100 g of clean, dried raw seeds were submerged in 1 litre *Gomutra* for 7 days. The *Gomutra* was changed every day at nine in the morning. The seeds were removed on the eighth day and cleaned with lukewarm water (40-45⁰C). The seeds were then ground into a powder and stored in an airtight glass container. [15]

3.Shodhana in *Godugdha*:

100 g of *Kupeelu* raw seeds were placed in a muslin cloth, and a bundle was made. *Godugdha* was then added to the stainless steel pot in which the bundle was hanging, fully submerging it. Then, it was boiled for three hours at 100⁰C. The seeds were removed from the bundle and rinsed with lukewarm water after three hours of cooking. The embryo and seed coat were cut off with a knife, and the cotyledons were dried and stored in an airtight container [16]

4. Shodhana in Goghrit : Seeds were fried in *Goghrita* on low flame until the outer skin becomes reddish yellow. Remove the skin and pulverised thereafter.[17].

5. Shodhana in Erand Tail :

100gm of seeds were kept in a pan and then fried in 30 ml of Erand tail at a low temperature (60⁰C) until the seeds swelled and turned reddish-yellow in colour. The seeds were then withdrawn from the pan; their coats were removed completely by rubbing them over the fingers and immediately made into powder form. Powder was then kept in air tight container. [18].

6. Shodhana in Kanji: *Kanji* was freshly prepared following the method mentioned in Ayurvedic formulary of India [19]. *Nuxvomica* seeds were soaked in *Kanji* for three days. Then their skin were separated and dried in sunlight. Dried seeds were powdered and kept in airtight container. [20]

Estimation of Strychnine content by HPTLC:

Methanolic extract of all individual samples were spotted with standard solution of Strychnine. The plates were developed in mobile phase used was Toluene: Ethyl acetate (7:3).

The instrument used in studies was CAMAG Automatic TLC sampler (Linomat 5) with sample solvent as methanol, dosage speed of 150 nl/s 0.2 µl as pre-dosage volume and 100 µl as syringe size. There were a total of seven numbers of tracks of all samples including standard. Application position was 8.0mm and band length was 6.0mm. The plates were scanned at 254 wavelengths.

Observation & Result:

Methanolic extract of all samples showed well resolved spot on HPTLC plate.

[Table 1, Fig 1]

Table 1: HPTLC analysis of processed *Nuxvomica* seeds in different media

Track no.	Details of Sample	Rf	Maximum Height	Area
1	Std. Strychnine	0.02	86.6	2087
2	Mixed Media (Gomutra+ Godugdha+ Goghrit)	0.02	127.8	2639.6
3	Gomutra	0.03	128	3289.9
4	Godugdha	0.03	81.2	1696
5	Goghrit	0.03	157.2	2646.5
6	Erand Tail	0.03	83.4	2435.6
7	Kanji	0.04	135.5	4205.6

In HPTLC study strychnine was considered as a standard and it was observed that Rf value of *Gomutra*, *Godugdha*, *Goghrit* and *Erand tail* Processed seeds is same - 0.03 while Rf value of *Kanji* processed seeds is 0.04. But Rf value of mixed media (*Gomutra*, *Godugdha* and *Goghrit*) is same with standard.

Fig 1 : HPTLC of standard and processed *Nuxvomica* seeds in different media

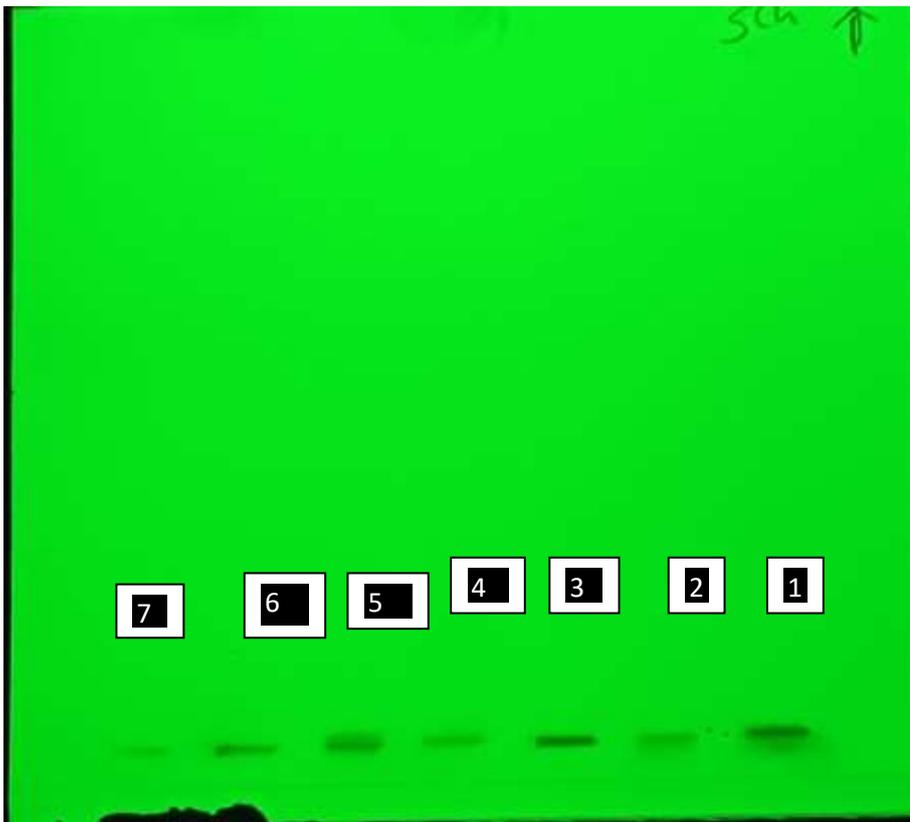


Fig 2: 3 D overlay of HPTLC chromatogram of all tracks

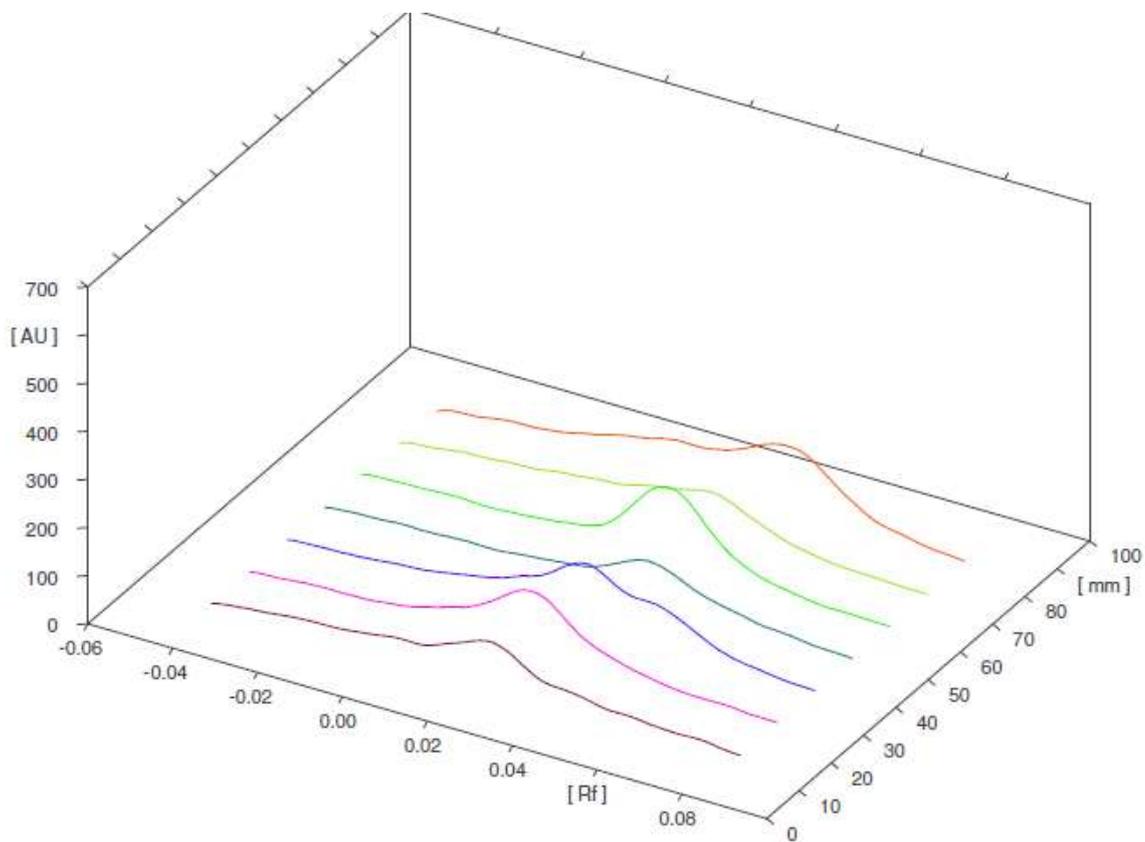


Fig 4: Track of Standard Strychnine

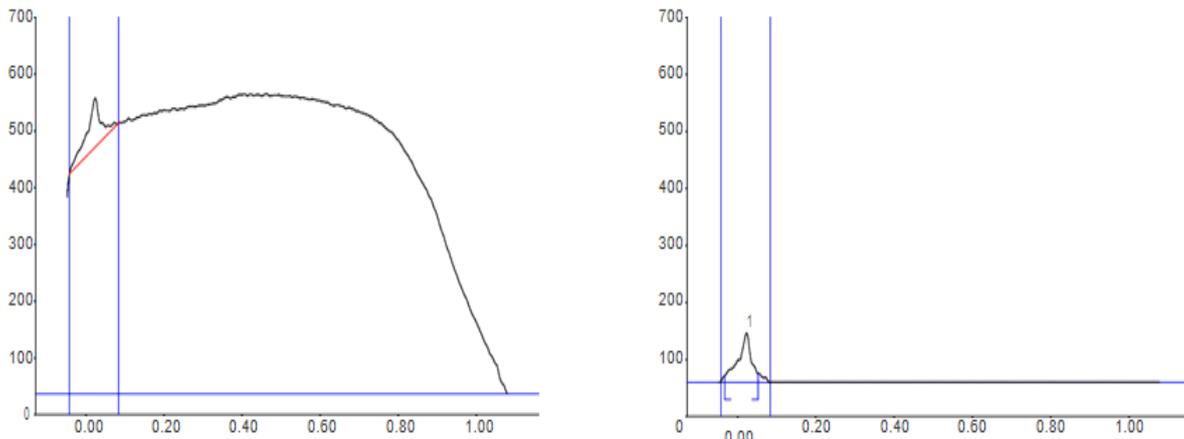


Fig 5: Track of Nux-vomica seeds processed in *Godughda* followed by immersion in *Gomutra* followed by frying in *Goghrit* :

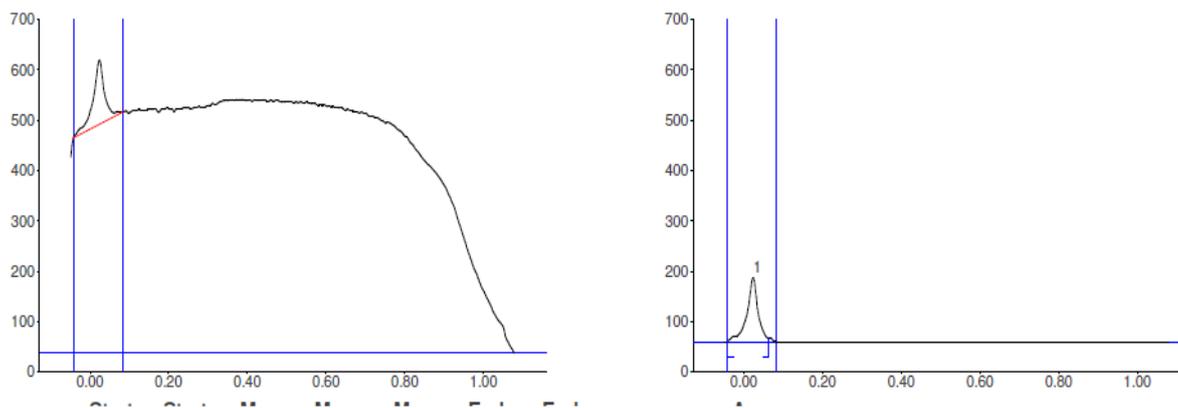


Fig 6: Track of Nux vomica seeds processed in *Gomutra*

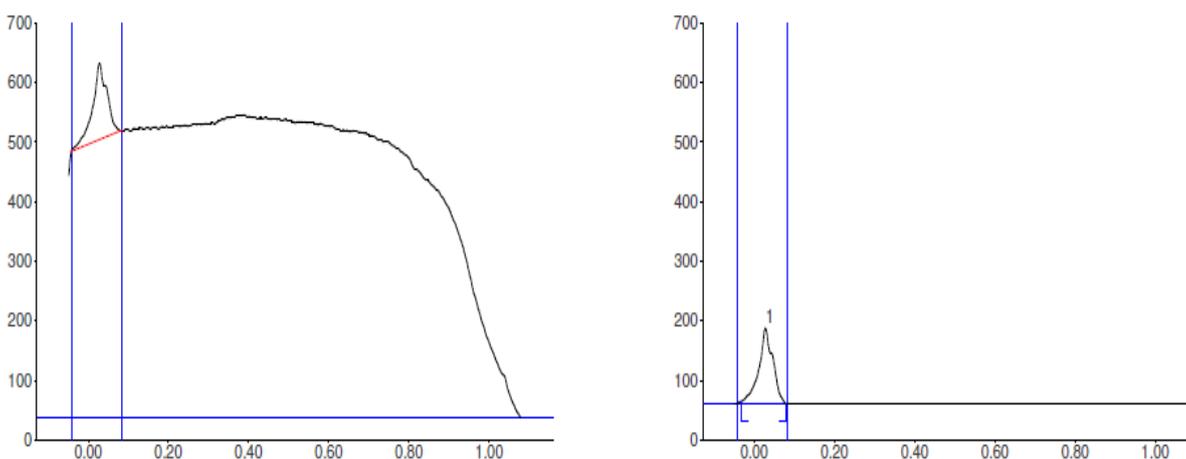


Fig 7: Track of Nux vomica seeds processed in *Godughda*

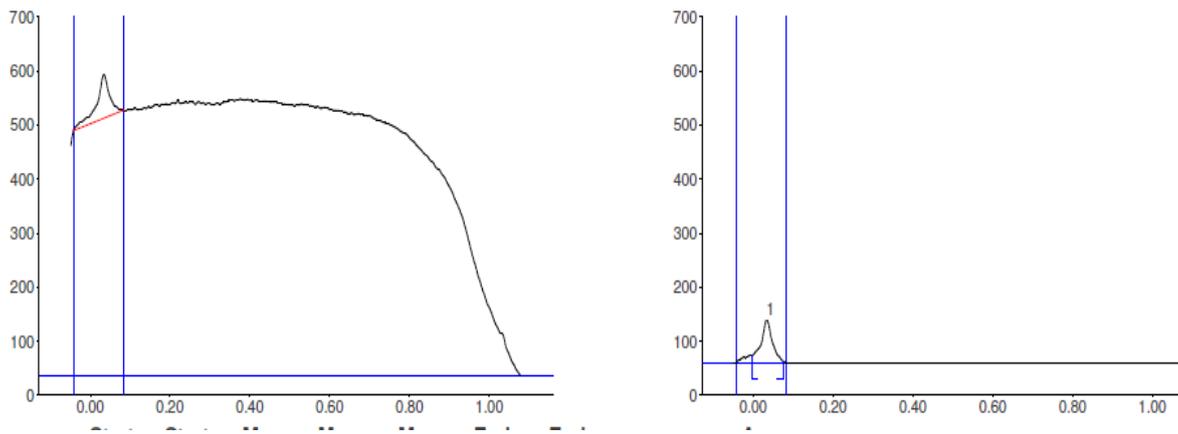


Fig 8: Track of Nux vomica seeds processed in Goghrit

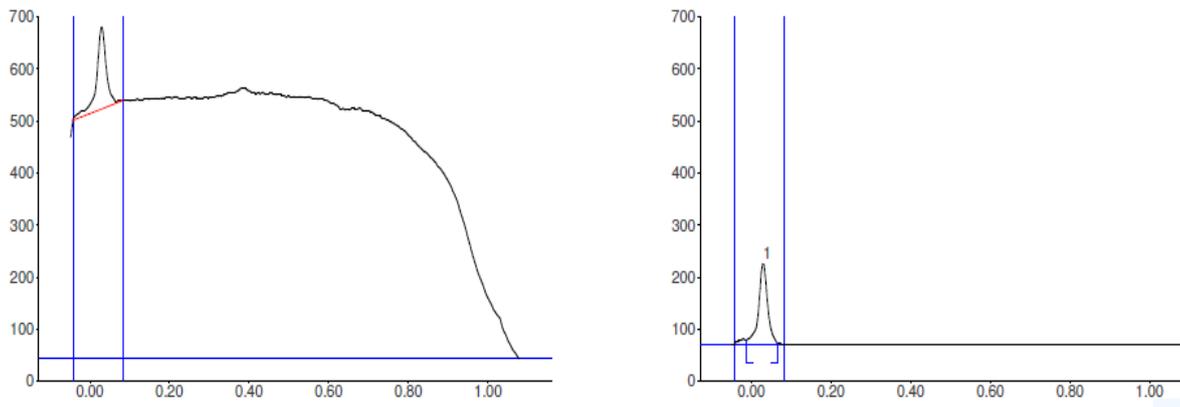


Fig 8: Track of Nux vomica seeds processed in Erand Tail

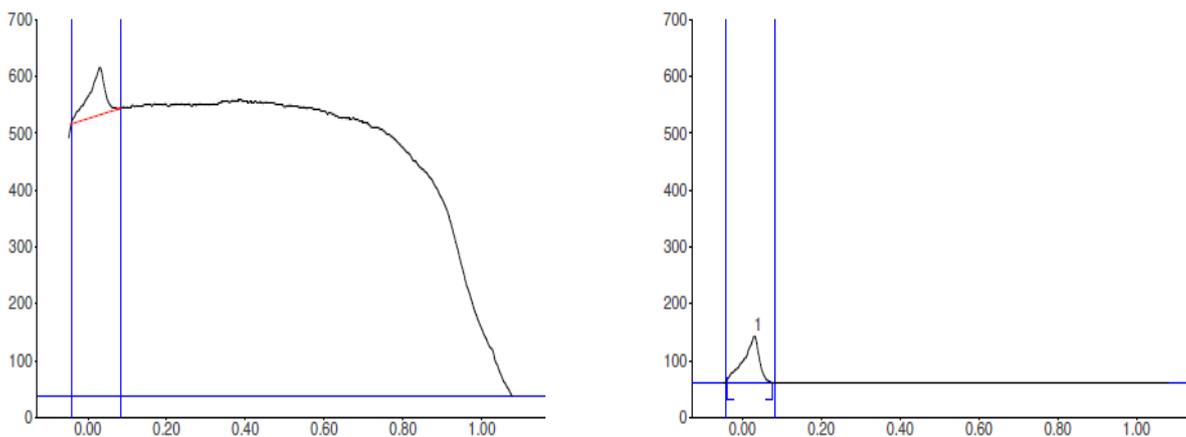
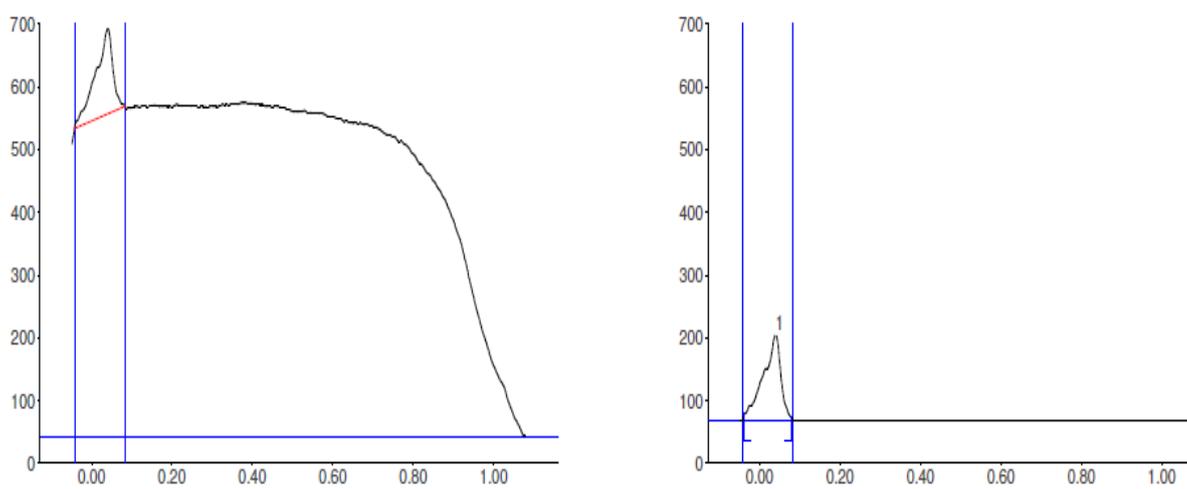


Fig 8: Track of Nux vomica seeds processed in Kanji



Discussion: Nux-vomica is poisonous herbs. It has the poisonous alkaloids strychnine (1.23% in seeds) and brucine (1.55% in seeds). It has iridoids like glycoside, loganin, loganic acid, chlorogenic acid, and minor related alkaloids like -colubrine, proto strychnine, vomicine, novacine, N strychnine, pseudo- strychnine, and iso strychnine. [21] Numerous Ayurvedic and contemporary textbooks indicate that different parts of this plant have broad spectrum of therapeutic activities on a variety of ailments. Significant therapeutic indications include hemiplegia, facial palsy, infertility, weight loss, skin conditions, ulcers, diarrhoea, and sciatica.[22] Although the whole plant is poisonous, as the seeds are extensively used for therapeutic purpose and mainly indicated in chronic rheumatic diseases, neurogenic diseases, anaemia, asthma, bronchitis, constipation, malarial fever, insomnia [23]

Even in ancient times, drug safety was a top priority hence numerous detoxifying/ purification process are described under the '*Shodhana Karma*' to increase the drug's safety and effectiveness [24].

Media used for processing has very important role either in breaking down or reducing the component that is not required. The chemical components of drugs get changed may be because of heating or prolonged continual boiling or contact of particular medium.[25] .HPTLC performed on different processed sample of Nux- vomica seeds showed different concentration of strychnine as illustrated in **figure1 and table 1**. Fig 1 represents strychnine bands of standard and processed Nux- Vomica seeds. From the Table no. 1 it was observed that maximum height of strychnine get reduced in samples processed in the *Godugdha* and *Errand Taila*. This is due to the possibility that Strychnine may have been transformed into less harmful derivatives, such as Strychnine Noxide, and isostrychnine [26]. Additionally, in Ayurveda different media are specifically recommended for certain disorders aimed to be not to reduced poisonous content of drug but also enhance the particular therapeutic effect. The seed purified with cow's milk is specifically mentioned in Vata roga [27]. Hence different media is to be used for shodhana as per the diseases condition.

Conclusion:

From this study it may be conclude that comparing to other media *Godugdha & Errand taila*, is an efficient processing medium for reducing strychnine content which strongly confirmed the claims of ancient classics of Ayurveda that Shodhna successfully reduces the toxic effects of poisonous drugs and simplify it for therapeutic purpose with enhancing its pharmacological activity.

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