

Comparative Estimation of Strychnine and Brucine in *Ashodhit* (Impure) and *Shodhit* (Pure) *Kuchala* (*Strychnos Nux Vomica*) By Hptlc Study.

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Abstract: *Kuchala* (*Strychnos nux vomica*) is the most important *Upavisha* which have wide therapeutic potential. Its main alkaloids Strychnine and Brucine are Neurotic Poison. So, it needs proper purification in order to pacify the toxic effect of these alkaloids. In present study, *Shodhana* (Detoxification) of *Kuchala* is done by 3 different methods. It is purified by using Kanji, Godugdha and Gomutra. After proper *Shodhana* got 62.5%, 70% and 65% yield of *Shuddha kuchala*. In present study, estimation of alkaloids in raw *Kuchala* Done by HPTLC study with respect to two markers i.e Strychnine and Brucine. HPTLC study shows that Strychnine % gets lower in Gomutra and Brucine % gets lower in Kanji. HPTLC profile of Raw and *Shodhita Kuchala* indicate that some peaks disappeared and some new peaks appeared after *shodhana* process. This disappearance and appearance of new peaks suggest the extraction of some components like Strychnine, Brucine and formation of some new compound during *Shodhana* process. In HPTLC study, highest reduction in the toxic alkaloids strychnine and Brucine contents in the Gomutra and Kanji respectively. This study shows that alkaloids present in the *Kuchala* are soluble in alkaline (Gomutra) and acidic (Kanji) media.

Keywords: *Kuchala*, *Shodhana*, Strychnine, HPTLC of *Kuchala*

INTRODUCTION:

Rasa shastra and *Bhaishajyakalpana* (pharmaceutical Science) is specialized branch of *Ayurveda* which deals with such poisonous drugs along with metals, minerals and animal-oriented drugs which convert it into therapeutic dosage form. Judicial use of these dosage forms (*Rasaushadhies*) gives therapeutic success to justify the need of this precious science. Although *Visha* and *Upvisha* (*Poisons*) are plant originated drugs, but it is categorised under *Rasaushadhies* because of its low dose, quick action, easy palatability and long expiry of its formulations. This poison is detoxified by scientific processing techniques known as *Shodhana* and used as main component in formulations.

The poisonous drugs are broadly classified into two subgroups that is *Visha* group and *Upvisha* group on the basis of their severity and virulence^[1]. In *Rasatarangini* there are eleven drugs in *Visha* and *Upvisha* group, *Kuchala* (*Strychnos nux vomica*) is categorized under *Upvisha* group^[2]. It has *Katu*, *Tikta*, *Kashaya Ras* (Taste) and *Ushna*, *Tikshna guna* (properties). *Shothahara*, *Vedanasthapan*, *Akshepajanan*, *Shoolaprashaman*, *Kaphaghna*, *Ardita*, *Pakshaghat* are the therapeutic uses of *Kuchala*. *Kuchala* is a poisonous drug mainly producing Tetanus like convulsions and eventually death in large doses and mental instability in lesser doses^[3], due to presence of toxic alkaloids, but shows potent therapeutic effects after *shodhana* as per *Ayurveda*. Alkaloids are the main bioactive principles in *Strychnos nux vomica*, responsible for its pharmacologic and toxic effects. Total alkaloid count for 2.6-3.0% out of which 1.25-1.5 % Strychnine and 1.7% Brucine are the main alkaloids apart from others viz. Vamicine, Colubrine, Pseudo-strychnine, Navacine, Icajine etc^[4]. Among all alkaloids, Strychnine & Brucine are the potent toxins.

All the *vishas* are purified before internal use and for this *visha dravyas* should be cut into small pieces or the seeds should be dipped or kept in vessel filled with *Swarasa*, *Kwatha*, *Gomutra* or *Godugdha* or fried in *Goghruta*^[5]. The heat treatment – constant boiling of the drug in a particular media for particular duration has a role in the modification of the chemical constituents. Specific media has definitely an important role in making the drug to act without causing any side effect or adverse effect. So the aim of traditional method is to minimise the toxic effect of the drug^[6].

After detoxification, it can be used for medication or internal use and does not produce harmful toxic effects in the body.

There are different methods of *Kuchala shodhana* such as *Nimmajjana* (soaking) in Gomutra and Kanji, *Swedana* (Boiling) in Godugdha and *Bharjana* (Frying) in Goghrita and Eranda taila. The media used in the process of *Shodhana* has very important role in either breaking down or destroying the chemical constituent which is not required. This can be assessed by various Qualitative and quantitative analysis.

Chromatographic and sophisticated modern techniques of standardization such as HPTLC provide Quantitative and semi-quantitative information about the main active constituents or marker compounds present in the crude drug or herbal products. Markers plays an important role in fingerprinting of herbs. Quality of drug can also be assessed by chromatographic fingerprint.

So in present study three *Shodhana* media (*Kanji*, *Godugdha* and *Gomutra*) have been used to carry out *Kuchala shodhana* and % of Main markers of *Kuchala* (before and after *Shodhana*) have been evaluated by using HPTLC study

AIM:

To evaluate the % of alkaloids i.e. Strychnine and Brucine in *Shodhita Kuchala* by 3 different *Shodhan* media.

OBJECTIVES:

- 1) To carry out *Shodhana* of *Kuchala* seeds by 3 methods in 3 different media (*Kanji*, *Gomutra* & *Godugdha*) mentioned in the classics.
- 2) To compare % markers of *Ashuddha* and *shuddha* samples of *Kuchala* seeds by HPTLC study.

MATERIAL & METHODS:**MATERIALS:**

Kuchala seeds were procured from reliable sources and authenticated by *Dravyaguna* experts of the institution.

Material – There are various instruments (*yantra*) are used in the *Shodhana* procedure.

1. **Gas burner** – Used to giving required amount of heat at different temperature LPG cylinder is used to provide required amount of heat.
2. **Dolayantra** – A thick mud pot having height 25 cm, upper diameter 15 cm and middle diameter is 30 cm used for *Kuchala shodhana*.
3. **Sieve** – Sieves are commonly employed for particle size separation of a powder. Sieves are usually prepared using a metal or plastic frame and wire or a fabric mesh. 80 no. mesh used in present study.
4. **Cotton cloth** – Cotton cloth used for Pottali, which is used for *Kuchala shodhana*.
Dimension – 30 × 30 cm

METHODS:**1) Preparation of Kanji :-**

a) Reference :- AFI Part 1-B (*Parada vidnyana*)

b) Type of procedure :- *Sandhana* (fermentation)

c) Equipments :- Gas stove, Lighter, Stainless steel, Spatula, Measuring jar, Porcelain jar / China pot, mud smeared cloth.

d) Ingredients :- Rice (*Tandula*) – 1.5 kg

Potable water – 12 litres (7.5 litres for cooking rice , 4.5 litres for adding to cooked rice).

e) Procedure :-

- Rice was washed properly by tap water and according to '*Annaparibhasha*' from *Bhavaprakasha*, 5 times (7.5 litres) of water was added to it and boiled until cooked.
The slurry formed at the top of water while cooking is discarded.
- The china pot was cleaned thoroughly and dried in sunlight.
- The 1.5 kg of cooked rice is added in clean and dried china pot , followed by 4.5 litres of boiled water. One third of space of the pot was left vacant for gases formed during fermentation.
- After proper cooking rice and water both were mixed properly by continuous and vigorous stirring.
- Mouth of the pot was properly closed by the lid and sealed by mud smeared cloth to resist entry of air.
- The labelling was done on pot according to date, time and quantity.
- The pot was placed in dark place for further fermentation to occur.
- It was kept as it is for 21 days. On 22nd days completion, tests were performed and Kanji was collected by straining and used for *shodhan*.

f) Completion tests –

- Match stick test : Negative
- Lime water test : Negative
- Taste : Sour
- Effervescence : Absent
- Ph : 3.2

<i>Kanji</i> yield	Colour	Taste	Smell	pH
5 litres	Whitish	Sour	Strongly acidic	3.2

Table no. 1 : Physico-chemical properties of Kanji –

2) *Shodhana in Kanji* ^[7]:

विषतिन्दुकबीजानि विन्यस्येद् गृहवारिणि ।
 दिनत्रयं प्रयत्नेन त्वपनीय बहिस्त्वचम् ॥ १७२ ॥
 निदाधे चाथ संशोष्य चूर्णयेद्विषजां वरः ।
 एवं विशुद्धिमायाति सर्वथा विषतिन्दुकम् ॥ १७३ ॥

र.तं. २४ /१७२ -१७३

(Nimajjana process)

Seeds are soaked in kanji for 3 days. Then outer seed coat and embryo are removed, cotyledons are dried in sunlight and pulverised.

3) *Shodhana in Godugdha* ^[8]:

विषतिन्दुकबीजानी पोदृल्यां विन्यसेदभिषक् ।
 स्वेदयेद् गव्यपयसः दालिकायन्त्रमागतः ॥१७६॥
 एवं यामैकमात्रेण शुद्धिमायान्त्यनुत्तमाम् ।
 शोधितं तिन्दुकं त्वेवं वीतशंकः प्रयोजयेत् ॥१७७॥

र.तं. २४/ १७६-१७७

(Swedana process)

Swedana is done in *Godugdha* by *Dolayantra* method for three hours. Then outer covering is scrapped with knife, seed coat and embryo are removed, cotyledons are dried in sunlight and pulverised.

4) *Shodhana in Gomutra* ^[9]:*(Nimajjana process)*

Seeds are soaked in *Gomutra* for seven days with daily *Gomutra* changing. Then outer covering is scrapped with knife, seed coat and embryo are removed, cotyledons are dried in sunlight and pulverised.

5) **Preparation of Churna of Shuddha Kuchala:-**a) **Total time taken** – 2 daysb) **Equipment** – Grinder and mesh (80 N) and pot for collection.c) **Procedure** – *Shuddha Kuchala* was powdered with the help of grinder and sieved in a pot through 80 No. mesh. After that powder was kept in an airtight container.d) **Precaution** –1) *Kuchala* should be dried completely before the procedure of powdering.

2) During the procedure of powdering, care must be taken, Hence a disposable hand gloves and mask should be used.

6) **HPTLC estimation of Strychnine and Brucine:**

A CAMAG HPTLC system equipped with a sample applicator Linomat 5 sample applicator was used. Pure strychnine and Brucine were obtained. Mobile phase was Toluene : Ethyleacetate : Triethylamine (7:2:2 v/v). Peaks areas were noted and their quantities were calculated.

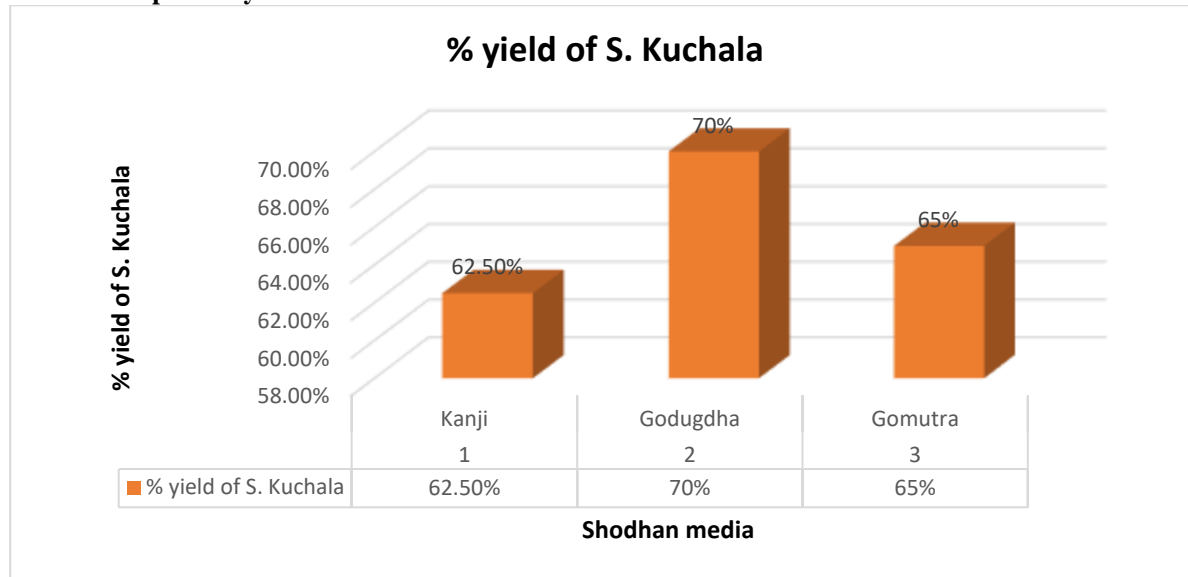
OBSERVATION:

- Total yield of *Shuddha Kuchala* Obtained By 3 different methods:

Table no 2: Showing Total yield of *Shuddha Kuchala* Obtained By 3 different methods:

Sr. no.	Shodhan Dravya	Quantity of A. Kuchala	Wt. loss After Shodhan	% yield of S. Kuchala	% of Loss
1.	Kanji	400 gm	250 gm	62.5 %	37.5 %
2.	Godugdha	400 gm	280 gm	70 %	30 %
3.	Gomutra	400 gm	260 gm	65 %	35 %

Graph - % yield of *S. Kuchala* :-



1) Ayurvedic parameters :-

Table no 2- Showing ayurvedic parameters for *Kuchala churna shodhita by Kanji, Godugdha and Gomutra.*

	<i>Kanji shodhita Kuchala churna</i>	<i>Godugdha shodhita Kuchala churna</i>	<i>Gomutra shodhita Kuchala churna</i>
Sparsha	<i>Mrudu</i>	<i>Mrudu</i>	<i>Mrudu</i>
Rupa	<i>Greyish white</i>	<i>Greyish white</i>	<i>Greyish white</i>
Rasa	<i>Katu, Tikta</i>	<i>Katu, Tikta</i>	<i>Katu, Tikta</i>
Gandha	<i>Amla Gandhi</i>	<i>Katu</i>	<i>Gomutra Gandhi</i>

2) Modern parameters :-

Table no 3: The quantification of Strychnine & Brucine by HPTLC

Parameters	Standard Strychnine	Kuchala (Impure)	Purified Kuchala (Gomutra)	Purified Kuchala (Godudha)	Purified Kuchala (Kanji)
Weight	10.0 mg	1.007 gm	1.018 gm	1.056 gm	1.056 gm
Rf Value	0.24	0.24	0.24	0.25	0.24
AUC	13935.5	14650.8	11735.7	12223.1	14208.1
% Strychnine	---	0.827 %	0.656 %	0.658 %	0.765 %
Parameters	Standard Brucine	Kuchala (Impure)	Purified Kuchala (Gomutra)	Purified Kuchala (Godudha)	Purified Kuchala (Kanji)
Weight	10.0 mg	1.007 gm	1.018 gm	1.056 gm	1.056 gm
Rf Value	0.09	0.10	0.10	0.11	0.10
AUC	11136.2	8740.4	8296.0	8316.5	7428.2
% Brucine	---	0.749 %	0.700 %	0.680 %	0.607 %

Table no 4: Showing R_f value of Impure *Kuchala churna* and *shodhita Kuchala Churna*:

Sample	No. of spot	R _f value
Strychnine	2	0.25, 0.50
Brucine	1	0.10

Impure <i>Kuchala</i>	5	0.10, 0.17, 0.25, 0.34, 0.50
Purified <i>Kuchala</i> (SK)	8	0.10, 0.25, 0.35, 0.50, 0.63, 0.69, 0.78, 0.89
Purified <i>Kuchala</i> (SGd)	6	0.10, 0.17, 0.25, 0.35, 0.50, 0.78
Purified <i>Kuchala</i> (SGm)	5	0.10, 0.17, 0.25, 0.35, 0.50

(SK- Shodhan in Kanji, SGd- Shodhan in Godughda, SGm- Shodhan in Gomutra)

Shodhana of *Kuchala* done by three different media and processes i.e *Kanji*, *Godughda* and *Gomutra*; Colour of *Kanji* changed after *Shodhana*, whitish *kanji* became blakish white in colour, Colour of *Godughda* changed to yellowish after *Shodhana* and Colour of *Gomutra* changed after *shodhana*, yellowish *Gomutra* became brown in colour.

Shodhana of *Kuchala* by three different media *Kanji*, *Godughda* and *Gomutra* is compared by evaluating percentage loss in Strychnine and Brucine before and after procedure as shown in table no. 3 and 4:

HPTLC profile of Raw and *Shodhita Kuchala* indicate that some peaks disappeared and some new peaks appeared after *shodhana* process. In raw sample, Total peaks were found, where as 5-8 peaks were observed in purified samples under 254nm. This disappearance and appearance of new peaks suggest the extraction of some components like Strychnine, Brucine and formation of some new compound during *Shodhana* process.

The percentage of Brucine in crude *Kuchala* (impure) is 0.749%, Brucine % in (SKK) is 0.607%, Brucine % in (SKGd) is 0.680% and Brucine % in (SKGm) is 0.700%. The Brucine % was marked lower in *Kanji Shodhita Kuchala Churna*.

Strychnine % in crude *Kuchala* (impure) is 0.827%, Strychnine % in (SKK) is 0.765 %, Strychnine % in (SKGd) is 0.658 % and Strychnine % in (SKGm) is 0.656 %. The Strychnine % was marked lower in *Gomutra Shodhita Kuchala churna*.

DISCUSSION:

Kuchala is a poisonous drug, modern toxicology has classified *Strychnos nux vomica* as a Neurotic Spinal excitant poison [10]. It shows incredible therapeutic effects after detoxification. According to *Ayurvedic* textbooks, there are classical methods of detoxification; makes it suitable to use on Human body for the treatment of various Diseases.

The *Shodhana* processes which are mentioned i.e, heating process or boiling of Raw *Kuchala* in particular media for specific time has a role in modification of the chemical constituents [11].

Kuchala is purified in *Kanji* and *Gomutra* by *Nimajjana* process. In this procedure Impurities or Toxic substances from the drug gets dissolved in the solvent or media used for its extraction [12].

Kuchala is purified in *Godughda* by *Swedana* process. It is the method of extraction where toxic principles gets dissolved in media used for boiling [13].

However, in present study, HPTLC study was done; two peaks of strychnine were observed. which may be due impurity of strychnine markers. So purity of marker needs to be considered before HPTLC study.

In the present Study it was observed the percentage loss of strychnine and Brucine, Raw *Kuchala* is purified by using 3 different media. The Brucine % was marked lower in *Kanji Shodhita Kuchala Churna* which suggests Brucine is more extracted in Acidic media. The Strychnine % was marked lower in *Gomutra Shodhita Kuchala churna* which suggest Strychnine is more extracted in alkaline media. Media has special role in either breaking down or destroying the chemical constituents, which are not required [14].

All the steps mentioned in the purification process of *Kuchala* (*Strychnos nux vomica*) should be performed carefully with respect to time, temperature and purity of media.

CONCLUSION:

Ayurvedic formulations which containing poisonous drugs like *Kuchala* in impure form can be deadly poisonous to human. proper *Shodhan* process is necessary in order to reduce its toxicity and increase therapeutic efficacy. It can be evident by analytical study. In HPTLC study, highest reduction in the toxic alkaloids strychnine and Brucine contents are seen in the *Gomutra* and *Kanji* respectively. This study shows that alkaloids present in the *Kuchala* are soluble in alkaline (*Gomutra*) and acidic (*Kanji*) media.

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