Original Research Article

Identification, Validation and Standardization of Bioactive Molecules of *kupeelu* Using flash chromatography

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Submission: 06.05.2024 Acceptance: 27.05.2024 Publication: 28.06.2024

Abstract

Introduction: Ayurveda literature mentions several poisonous plants that can be used in medicine after thorough detoxification. Proper purification processes can transform poison into nectar, One such plant is *Kupeelu*(strychnos nux vomica). Strychnine and brucine present in *Kupeelu* can be highly harmful or even lethal when used in unpurified form. To purify *Kupeelu* seeds, various *Shodhana* (purification) procedures have been used. Standardization of the purification process is essential to avoid harmful side effects of such toxic herbs. Therefore, the objective of the current study is to perform the *Kupeelu* seed *Shodhana* as described in various Ayurvedic books and literature, and to examine the pharmacognosy traits and phytochemical evaluation of the *Kupeelu* seeds before and after purification.

Materials, Methods and Result: *Shodhana* of *Kupeelu* seeds were carried out by different methods, using *Goghruta, Godugdha, Gomutra* and *Kanji.* Organoleptic and physiochemical assessment of the *Kupeelu* was done pre and post shodhana. Quantitative analysis of strychanine and brucine content was done by flash chromatography. Flash chromatography showed reduction in contents of stychaninne and brucine, active alkaloid after purification process.

Discussion: The current study aimed to investigate the effects of various media, including *Gomutra, Godugdha, Goghrita,* and *Kanji,* on the *Shodhana* (purification) of *Kupeelu* seeds through *Nimajjana* (dipping), Swedana (boiling), and Bharjana (frying) methods. The media used in the Shodhana process play a crucial role in eliminating toxic chemical components or transforming them into non-toxic substances. The study also suggests that the media used may provide new organic or inorganic principles to the drug, enhancing its therapeutic efficacy. The different *Shodhana* procedures had varying degrees of reduction in toxicity, with the *Nimajjana* method in *Gomutra* and the *Bharjana* method in *Goghrita* showing the highest reduction in toxicity.

Conclusion: The *Shodhana* treatments utilising different medium rendered *Kupeelu* seeds less toxic and maybe more effective for medical purposes. The appreciated media for *shodhana* are *Gomutra* and *Godugdha* since they have the highest toxicity reduction; the study also discovered that the various *Shodhana* methods had varying degrees of toxicity reduction. In conventional medicine, proper purification processes are required to ensure the safe and efficient use of potentially harmful therapeutic herbs.

Keywords : Kupeelu, Shodhana, Godugdha, Goghruta, Gomutra, Kanji, Bioactive Molecules, Flash Chromatography

Introduction

The use of medicinal plants, as described in *Ayurveda*, has a rich history deeply rooted in traditional healing practices. Among the plethora of botanical remedies mentioned in Ayurvedic literature, some include plants with potential toxic properties. However, Ayurveda, with its meticulous approach to traditional medicine, has developed processes for detoxifying and harnessing the therapeutic potential of these poisonous plants. When conducted correctly, these traditional *Shodhana* (purification) methods can transform poison into nectar, unlocking their healing properties while minimizing risks. On the flip side, if purification is inadequate, the consequences can be severe, even fatal.[1,2,3]

One such plant frequently mentioned in Ayurvedic classical texts is *Kupeelu* (*Strychnos nux-vomica Linn*), renowned for its potent medicinal properties and toxicity.[3] Interestingly, while it is widely cited in *Ayurveda*, it was not found in the *'Brihat Trayee'* texts. Instead, it appears as *Vishamustika* in *Susruta's Surasadi gana*, which is botanically identified as *S. nux-vomica.* This intriguing plant, also referred to as *Raja Nimba* by *Dalhana*, has been utilized in Ayurveda since the era of "*Brinda Madhava"* (9th A.D.). Its diverse applications range from treating chronic rheumatism, dyspepsia, and limb paralysis to addressing conditions like *Vrana, Jwara, Kushta, Arsha,* and many others.[4]

However, it's crucial to note that the seeds of Strychnos nux-vomica contain dangerous chemical compounds, including strychnine and brucine, which can be highly toxic when used in their crude and unpurified form. Ayurveda prescribes specific purification methods involving various media, such as cow's urine, cow's milk, cow's ghee, and sour gruel *(Kanji),* to render *Kupeelu* safe for therapeutic use.[5,6]

Various purification procedures have been employed over time, some derived from classical Ayurvedic texts and others from traditional practices. These *Shodhana* methods utilize media to either partially or completely neutralize the hazardous chemical components, rendering them harmless. Occasionally, a medium may serve as a solvent to dissolve these substances, effectively separating them from insoluble contaminants. Despite the long history of these purification processes, their impact on the primary chemical components of Kupeelu seeds remains inadequately studied.[6]

The objective of the current study is to rigorously follow the Kupeelu seed Shodhana processes outlined in various Ayurvedic texts and literature. Furthermore, we aim to comprehensively examine the pharmacognosy traits and phytochemical composition of Kupeelu seeds both before and during the purification process. By shedding light on the scientific aspects of this traditional practice, we hope to contribute to the understanding of Ayurvedic medicine's safety and efficacy, bridging the gap between ancient wisdom and evidence-based healthcare.

Aims and Objectives :

1. To perform *Kupeelu* seed *shodhana by four different methods* as described in many *Ayurveda* books and literature.

2. To research the biological attributes and conduct a pre- and post-purification analysis of biologically active compounds of kupeelu.

Materials and Methods-

Kupeelu fruits were collected when fully matured, and the seeds were removed. The fruits were then cleaned with tap water and dried. First, dry seeds were put into a water-filled beaker. The seeds that sank to the bottom of the container were picked for proper processing whereas the seeds that floated on the surface of the water or were discovered cracked and black in color were rejected. *Shodhana* of *Kupeelu* seeds were carried out by different methods.

A.*Shodhana of* Kupeelu seeds with *Godugdha* (Cow Milk)[7]

Principle: *Swedana* (Boiling) Ingredients: *Ashuddha Kupeelu* seeds -100 g. *Godugdha*- 6 lit. Cow milk (*Godugdha)* was collected from the local cowshed daily. Equipment: *Dolayantra* Stainless steel vessel, Stainless steel rod, Cotton threads, Muslin cloth, Measuring mug, Stainless steel knife, cooker, weighing machine

Procedure: 100 g of raw seeds of *Kupeelu* were kept in a muslin cloth and was made into a *Pottali*. Cow milk was added to the stainless steel jar in which the *Pottali* was suspended until it was completely submerged. Next, during the experiment, it was boiled on a cooker for three hours. A total of 6 litres of cow milk was used in the procedure. The seeds were removed from *Pottali* after three hours of cooking and cleaned with lukewarm water. With a knife, the cotyledons were cut into tiny pieces and dried in the shade after being removed of their seed coat and embryo. Seeds were dried and made into powder.

Everyday fresh *Godugdha* was collected, Volume of *Godugdha* was sufficient to immerse the *Pottali* completely. *Pottali* was hanged in such a manner that it did not touch the bottom or sides of the vessel. Observations- the *Pottali* floated on top of the milk at the beginning of the process, but it fully sank after the boiling process began. Temperature of milk remained constant at 100^0C throughout the boiling. As *Swedana* was finished, the *Pottali* grew larger and once more floated on the milk's surface. Following *Shodhana,* milk thickened more than fresh milk did. During the *Shodhana* method, the cow milk acquired a creamy-white hue, was thick, and had a swedish-bitter flavour. It was discovered that following *Shodhana,* 70% of purified *Kupeelu* was obtained. During processing, the severely bitter raw *Kupeelu* became sweetish bitter.

B. *Shodhana of* Kupeelu seeds with *Goghrita* (Cow Ghee)[7]

Principle: Bharjana (Frying)

Ingredients: *Ashuddha Kupeelu* seeds - 100 g, *Goghrita*- 25 ml

Cow ghee (*Goghrita)* was procured from the local market. Equipment- Measuring cylinder, Stainless steel knife, cooker, weighing machine, Stainless steel spatula, Frying pan, etc.

Procedure: 100g of seeds were fried in a mildly heated pan with 25 ml of cow ghee until the seeds swelled and the color of the outer covering turned reddish yellow. Stirring was continuously done during frying to prevent the seeds from burning. *Mandagni* was maintained throughout the process The seeds were then removed from the frying pan, the seed coatings were peeled off with the aid of a knife, and as much of the coat as possible was removed by rubbing the seeds over the fingers. Seeds were dried and made into powder.

Observations- Immediately after being fried, seeds became easily breakable when they were still hot. However, the seeds cooled down and turned quite hard after a short while, making it challenging to grind them into powder. Inner endosperm of the seeds turned golden yellow in color after *shodhana.* The cow ghee became dark brown in color and sweetish-bitter in taste after completion of *Shodhana* procedure. An average of 45 min. time was required for frying the seeds properly. It was discovered that following *Shodhana* with cow ghee, 52.33 percent of pure *Kupeelu* was obtained. After processing, the very bitter raw *Kupeelu* became sweetish-bitter, and the raw seeds' grayish color changed to a golden brown color.

C. .*Shodhana of Kupeelu seeds* with *Gomutra (*Cow Urine)[7]

Principle: *Nimajjana* (Dipping) Ingredients: *Ashuddha Kupeelu* seeds (100 g.*Gomutra:* 7 lit. (1 liter for each day) Cow urine *(Gomutra*) was collected from the local cowshed daily in the morning at 6 A.M.

Equipment- Stainless steel vessel, Stainless steel knife, Muslin cloth , Digital weighing machine , Measuring mug.

Procedure: 100 g of raw *Kupeelu* seeds that had been cleaned, dried, and placed in a stainless steel tray. It was then added with a liter of cow urine and stored for seven days. The cow urine (1 litre.) was replaced each day at 7 a.m. with new urine. The seeds were removed from *Gomutra* on the eighth day and cleaned with lukewarm water. Care was taken to completely submerge the seeds in *Gomutra. Gomutra* was changed at a specific time each day. With the use of a knife, the cotyledons were chopped into small pieces and the seed coats and embryo were removed. Seeds were dried and made into powder.

Observations - During the first two days of dipping, frothing was noted on *Gomutra*; however, no frothing was visible on subsequent days. *Gomutra* started to turn turbid after the first day. After *Shodhana*, the uncooked *Kupeelu* seeds swelled and softened. Seed coat removal became simpler than previously. Cow urine was needed for the *Shodhana* process in total of 7000 ml, of which an average of 6210 ml was recovered. It shows that, on average, 12 % of cow urine was used in the procedure. After *Shodhana,* the cow's urine turned brown, was turbid, had a strong odour, and tasted salty-bitter. It was discovered that 66.43% of the purified *Kupeelu* was derived from cow urine after *Shodhana*. Raw *Kupeelu*, which was bitter, turned salty.

D. *Shodhana* Of *Kupeelu* seeds With *Kanji* (Sour Gruel)[8,9]

Principle*:* *Nimajjana* (Dipping) Ingredients: *Ashuddha Kupeelu* seeds -100 g. *Kanji*-3 lit. (1lit for each day). Equipments for *Shodhana*- china clay vessel, Muslin cloth, Measuring mug , Glass rod, Digital weighing machine.

Procedure: 100 g of carefully chosen raw *Kupeelu* seeds were placed in a china clay container. It was mixed with one liter of *Kanji* (pH: 3.2-3.4; 1L.) from previously made stock, and stored for three days. Every 24 hours, the media would be replaced. *Kupeelu* seeds were adequately coated with *kanji*. Every day a new *Kanji* was used in place of the old one. The seeds were removed and given a lukewarm water wash on the fourth day. Cotyledons were divided into small pieces and dried in the shade after the outer seed coat and embryo were removed. After the *Shodhana* procedure was finished, the outer seed coat was carefully peeled off and seeds were dried, made into powder.

Observations - The color of the *Asuddha Kupeelu* seeds changed from gray to greenish. Seeds swelled after being dipped in the *Kanji,* which also turned thick. The *Shodhana* process required a total of 3000 ml (on average) of *Kanji*, of which an average of 2730 ml was recovered. It shows that, on average, 12.26% of *Kanji* were used in the procedure. After the *Shodhana*, the Kanji acquired an extremely sour taste and an offensive odor. Every day of sinking resulted in at least a small shift in pH value, which ranged from 3.5 to 3.7. It was discovered that following *Shodhana* with Kanji, 54.20% purified *Kupeelu* were obtained. After processing, the extremely bitter raw *Kupeelu* seeds turned into a grayish powder that was just somewhat bitter.

Results

The organoleptic characters of different samples of Kupeelu seeds are as mentioned in Table 1.

Table 1- Comparative organoleptic characters of raw and *Shodhita Kupeelu*

Sr no.	Sample	Colour	Odour	Taste	Touch
Before *shodhana*	Raw *kupeelu*	Pale brown to yellowish gray	odourless	Intensely bitter	Rough
After *shodhana* 1	*Godugdha Shodhit*	Creamy-white	Not characteristic	Sweetish-bitter	Slightly soft
2	*Goghrita shodhit*	Dark brown	Not characteristic	Sweetish-bitter	Slightly soft
3	*Gomutra Shodhit*	Whitish gray	Characteristic of urine(pungent)	Salty bitter	Slightly soft
4	*Kanji Shodhit*	white	Characteristic of *Kanji*	Moderately bitter	Slightly soft

The physiochemical analysis of *ashodhit* and *shodhit Kupeelu* is as mentioned in Table no. 2

Table no.2-Physiochemical Parameters of Raw and *Shodhit Kupeelu*-

Parameter	**Raw *kupeelu***	***Godugdha shodhit***	***Goghrita shodhit***	***Gomutra shodhit***	***Kanji shodhit***
Loss on drying@110 c	3.37%	5.74%	5.74%	3.64%	5.17%
Ash value (%)	1.32%	1.13%	1.23%	1.49%	1.05%
Water soluble extractives (%)	36.49%	35.17%	28.49%	22.13%	23.65%
Alcohol (methanol) soluble extractives (%)	4.02%	1.08%	3.75%	1.02 %	1.72 %
PH	4.37	4.74	4.53	6.30	4.86

Table 3 shows analysis of Quantification of strychnine and Brucine by Flash Chromatography in *Ashuddha* and *Shodhit* samples of *Kupeelu* seeds.

Table no.3-Quantification of strychnine and Brucine by Flash Chromatography-

Sr no.		**Strychnine %**	**Brucine %**
Before *shodhana*	*Raw kupeelu*	1.439	0.629
After *shodhana* 1	*GodugdhaShodhit*	0.237	0.207
2	*Goghrita shodhit*	0.298	0.238
3	*GomutraShodhit*	0.437	0.263
4	*KanjiShodhit*	0.769	0.571

Discussion

Kupeelu is utilized successfully in treating various ailments, after proper purification, known as *Shodhana.*[10,11] As a result of the *Shodhana* processes physical and chemical changes are observed in *Kupeelu* seeds. These observations are pivotal in understanding how traditional purification methods can transform a potentially toxic substance into a safe therapeutic agent.

Nimajjana and *Swedana* methods follow common stages of extraction processes, where the solvent enters through the pores into the cells, causing swelling of the tissues and solution of the soluble constituents, followed by the escape of dissolved material through the solvent boundary layer by diffusion, leading to the separation of the solution from the drug. The rate of extraction and solubility is increased by the elevation of temperature, which increases the concentration gradient across the cell membrane, resulting in increased mass transfer of active principles from solid material to the solvent.[12]

After *shodhana* in *Godugdha,* the *Kupeelu* seeds underwent a transformation in texture, becoming more easily breakable. This change indicates the alteration in the seeds' internal structure, which is a crucial outcome of purification. The shift from the initial bitterness to a "sweetish-bitter" flavor suggests a reduction in toxic compounds. The recovery of 70% of purified *Kupeelu* after this process highlights its effectiveness.

The *Shodhana* process employing *Goghrita* resulted in the seeds changing color from grayish to golden brown. The seeds swelled during this method, suggesting a modification in their physical properties. The ghee itself turned dark brown and sweetish-bitter in taste. The recovery of 52.33% of purified *Kupeelu* indicates a substantial reduction in the initial bitterness and toxicity.

Using *Gomutra* for purification led to significant changes in the seeds. The seeds swelled and softened, making the removal of the seed coat easier. The color of the cow's urine turned brown, became turbid, and acquired a salty-bitter taste. The recovery of 66.43% of purified *Kupeelu* suggests that cow's urine was an effective medium for *Shodhana.* This transformation from the initial bitterness to a salty taste demonstrates the alteration of toxic compounds.

The use of *Kanji* for purification caused the color of the *Kupeelu* seeds to change from gray to greenish. The seeds swelled and softened during this process. The sour gruel turned thick, and a slight shift in pH was observed during each day of the process. The recovery of 54.20% of purified *Kupeelu* suggests that this method was effective in reducing bitterness and toxicity.

The observations and results presented in the study underscore the efficacy of traditional *Shodhana* processes in reducing the toxicity of *Kupeelu* seeds. These transformations in color, texture, and taste are indicative of changes in the chemical composition of the seeds. The recovery percentages of purified Kupeelu after each *Shodhana* method substantiate their effectiveness in making this botanical resource safer for medicinal use in Ayurveda.

The observed reduction in toxicity and the transformation of Kupeelu seeds have profound implications for Ayurvedic medicine. It reaffirms the safety and efficacy of traditional purification methods, which have been employed for centuries.[13]

While the study provides valuable insights into the purification processes, it also highlights the need for further research. Future investigations could delve deeper into the specific changes in chemical compounds and toxicity levels. Clinical trials are warranted to validate the safety and efficacy of purified *Kupeelu* seeds in treating various health conditions mentioned in *Ayurvedic* texts.

Conclusion

The study's detailed observations and results emphasize the effectiveness of traditional *Shodhana* methods in transforming *Kupeelu* seeds, reducing their toxicity, and making them potentially safe for Ayurvedic medicinal use. These findings support the continued utilization of *Kupeelu* in Ayurvedic formulations, reaffirming the relevance of traditional practices in modern healthcare. However, further research, including in-depth chemical analysis and clinical trials, is warranted to validate the safety and efficacy of purified *Kupeelu* seeds for specific health conditions. This study represents a valuable bridge between traditional wisdom and contemporary scientific inquiry in Ayurvedic medicine.

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