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Effect of *Shodhana* (processing) on *Kupeelu* (*Strychnos nux-vomica* Linn.) with special reference to strychnine and brucine content

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Abstract

Kupeelu (*Strychnos nux-vomica* Linn.) commonly known as nux vomica is a poisonous plant used extensively in various ayurvedic formulations, with great therapeutic significance. Ayurveda recommended the administration of *Kupeelu* only after purification in different media like cow's urine (*Go mutra*), cow's milk (*Go dugdha*), cow's ghee (*Go ghrita*), *Kanji* (sour gruel), and so on. Apart from the classical methods some other methods are also adopted by the traditional practitioners using castor oil (*Eranda taila*), ginger juice (*Ardraka swarasa*), in the purification of *Kupeelu* seeds. In the present study an attempt has been made to purify the seeds by performing two different methods (one classical and another traditional) using *Kanji* and *Ardraka swarasa* as *Shodhana* media. This study reveals that both the methods studied reduce the strychnine and brucine contents in comparison to the raw seeds as determined by *high performance thin layer chromatography* (HPTLC). After purification in *Kanji* and *Ardraka swarasa,* the strychnine content was reduced by 39.25% and 67.82%, respectively, and the brucine content in the purified seeds was also found to have decreased by 17.60% and 40.06%, in comparison to the raw seeds.

Key words: *Ardraka swarasa, brucine, kanji, kupeelu, shodhana, strychnine*

Introduction

There are many poisonous plants reported in the ancient scriptures of Ayurveda, which are still in practice widely, to combat a number of diseases after proper *Shodhana* (purification/detoxification).[1] *Kupeelu* (*Strychnos nux-vomica* Linn.) is such a plant described under the '*Upavisa Vargas*' (semi poisonous group)[2] and its seeds have been used successfully in cure of many diseases after proper *Shodhana*.[3] Nux vomica was introduced in Europe in the sixteenth century, but was not used much in medicine, being chiefly employed to poison dogs, cats, crows, and etc.[4] It is cited in the treatises of Ayurveda that the '*Visha*' (poison) becomes '*Amrita*' (nectar) after logical administration[5] and the ancient physicians of Ayurveda successfully used this drug in a number of diseases after proper purification in some specific media. Either *S. nux-vomica* or its alkaloids have been reported for their analgesic, anti-inflammatory,[6] anti-oxidant,[7] anti-tumor[8], anti-snake venom,[9] anti-diarrheal,[10] and hepatoprotective[11] activities in different modern literature. Although 16 alkaloids have been separated and identified from crude nux vomica, 80% of them are strychnine and brucine and their derivatives such as isostrychnine and brucine N-oxide.[12] The major chemical constituents of nux vomica, (strychnine and brucine) have been reported for their adverse effects.[13]

Specific *Shodhana* (purification) procedures have been adopted for the purification of nux vomica seeds and these methods are either mentioned in the classics of Ayurveda or practiced traditionally.[14-17] The concept of *Shodhana* (purification) in Ayurveda is not only a process of purification / detoxification, but also a process to enhance the potency and efficacy of the drug.[18] For instance it is reported that aconite (*Vatsanabha*) purified by cow's urine is converted to a cardiac stimulant, whereas, raw aconite is a cardiac depressant.[19] Purified *Kupeelu* is also claimed to be a potent drug in countering old age problems and specially recommended during senility as a *Rasayana* (antioxidant).[20] Different techniques[21-25] have been used for the analysis and quantification of strychnine and brucine in raw and processed seeds. Few reports are also present, which explore various methods of purification of nux vomica seeds as per Chinese,[26] Unani,[27] and Ayurveda[28] systems of medicine. However, the methods of purification

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and analytical techniques are different from those considered under the present study. The purpose of the study is to evaluate the role of purification in the quantitative reduction of toxic alkaloids of *Kupeelu* seeds by the high performance thin layer chromatography (HPTLC) technique.

Materials and Methods

Collection of drugs

Fully matured *Kupeelu* (*Strychnos nux-vomica* Linn.) fruits were collected from Bankura district, West Bengal, in India, during the month of December, and were botanically authenticated by pharmacognosists. The sample specimen were kept in the museum for future reference. The seeds were taken out from the fruit pulp, thoroughly washed with tap water, and shade dried.

Preparation of media

Kanji was freshly prepared following the method mentioned in the Ayurvedic Formulary of India[29] and fresh *Ardraka* (ginger) was procured from the local market. Fresh juice from the *Ardraka* was extracted in the early morning and used as the media for purification.

Selection of seeds

The dried seeds were first dropped in a beaker containing water. The seeds that floated on the surface of water or found broken, black in color, were rejected and the seeds that settled at the bottom of the beaker were selected for purification after drying in air[11] and were considered as raw drug (KR).

Equipments for *Shodhana* (Purification)

A China clay jar having a capacity of 10 L, China clay vessel (16 cm radius of mouth × 12 cm depth) having capacity of 2 L, glass rod (length 28 cm), cotton thread 30 cm, measuring mug (capacity of 1 L), muslin cloth (45 cm × 45 cm), stainless steel spatula (length 30 cm), digital weighing machine, and induction heater.

Equipments for high performance thin layer chromatography

A CAMAG (Switzerland) high performance thin layer chromatography (HPTLC) system equipped with a sample applicator Linomat V sample applicator was used for the application of samples. CAMAG TLC Scanner 3, Reprostar, and Wincats 4.02 were used for scanning the plates. A CAMAG twin through a glass chamber was used for developing the plates.

Chemicals

Pure strychnine and brucine were obtained from Sigma Aldrich, USA, and precoated silica gel 60 F_{254} 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.

Methods of purification of *Kupeelu* (*Strychnos nux-vomica* Linn.) in different media

Each purification method was carried out in three batches, by using two different media as per classical and traditional processes, as mentioned herewith.

I. Purification with Kanji

Seeds (100 g) were processed by dipping in 1L *Kanji* (pH: 3.4) for three consecutive days in a china clay vessel.[14] The media was changed every 24 hours. On the fourth day, the seeds were taken out and washed with lukewarm water. The outer seed coat and embryo were removed and cotyledons were dried and pulverized. The pulverized material was kept in an air tight glass container and marked as 'KKJ powder' for further use.

II. Purification using *Ardraka swarasa* (freshly extracted ginger juice) as media

Seeds (100 g), were soaked in 1L of *Ardraka swarasa* for 20 days in a china clay vessel.[16] Every day the seeds were stirred well with a glass rod. On the twenty-first day, the seeds were taken out, washed with lukewarm water, the outer seed coat and embryo were removed, cotyledons were dried and pulverized. The pulverized materials were kept in an air tight glass container and marked as 'KAS powder' for further use.

High performance thin layer chromatography method for estimation of strychnine and brucine

Preparation of standard strychnine and brucine solution

Strychnine standard (10 mg) and brucine standard (10 mg) were accurately weighed and dissolved in methanol in two standard flasks and the final volumes were adjusted to 10 ml with methanol. (1 μg / μl)

Calibration curve for strychnine and brucine

The standard solutions corresponding to 2 μg to 6 μg of standard strychnine and brucine were applied on TLC plates (10 cm× 10 cm), precoated with silica gel as 6 mm bands by using CAMAG Linomat IV sample applicator. The plate was developed in a solvent system of Toluene : Ethyl acetate : Diethyl amine (7 : 2 : 1, v / v) in a CAMAG twin through a chamber up to a distance of 7.5 cm at a temperature of 30 ± 2°C. The plates were air dried and scanned at a wavelength of 254 nm using the CAMAG TLC scanner and CATS V 4.06 software. The peak area of strychnine and brucine were recorded for each concentration. The calibration curves of strychnine and brucine were obtained by plotting the graphs of the peak areas versus the concentrations of strychnine and brucine.

Preparation of sample solutions for estimation of strychnine and brucine

Both the purified samples (2 g each) were defatted individually with petroleum ether. The defatted samples were then mixed with 10% ammonia and finally extracted with 25 ml methanol for one hour, under reflux. The methanol extracts were filtered and concentrated to 5 ml and used as test solutions. Each test solution of 5 μl was spotted along with 2 to 6 μl standard solutions of strychnine and brucine. The plates were developed in a mobile phase of Toluene : Ethyl acetate : Diethyl amine (7 : 2 : 1, v / v) and scanned at 254 nm for strychnine and brucine. Peak areas were noted and the quantities of strychnine and brucine were calculated by comparing the areas of the standard solutions from calibration curve.

Results and Discussion

The preliminary phytochemical investigation showed the presence of alkaloids, tannins, carbohydrates, proteins, fixed oils

in methanolic extracts of raw and purified seeds. The glycoside loganin, which was present in raw seeds was found to be absent in the purified seeds. The presence of strychnine and brucine was confirmed by comparing the Rf values with those of the standard markers by HPTLC.

It was also observed that 54.20 and 66.30% of purified *Kupeelu* was obtained after purification in *Kanji* and *Ardraka swarasa*, respectively [Tables 1 and 2]. The moisture content in *Kupeelu* was increased after purification with *Kanji*, but decreased when the seeds were processed in *Ardraka swarasa* [Table 3]. The water-soluble and alcohol-soluble extractive values were decreased in both the samples after purification [Table 3]. Water and alcohol-soluble extractive values in *Kanji* purified seeds were comparatively less than those in the *Ardraka swarasa* purified samples. Therefore, better extraction of alkaloids, along with other chemical constituents, took place when the raw samples were kept in *Kanji*. However, reduction in the alkaloid content like strychnine and brucine was only observed when the samples were processed in *Ardraka swarasa*. Therefore, a hypothesis can be drawn that as far as reduction in concentrations of toxic alkaloids are concerned either by extraction or by transformation into another form *Ardraka swarasa* may be a better media than *Kanji*.

Kanji (pH 3.4) being acidic in nature may facilitate the extraction of alkaloids like strychnine and brucine, along with other chemical constituents from nux vomica seeds. Thus, it may be assumed that *Kanji* is a better extraction media than *Ardraka swarasa*.

In the HPTLC chromatogram, the UV spectrum of standard strychnine (R_f 0.54) at 254 nm and standard brucine (R_f 0.34) [Figures 1 and 2], and the peak areas of strychnine and brucine in all the samples are exposed [Figures 3-5]. Calibration curves of strychnine and brucine were prepared by plotting concentrations of strychnine and brucine in the range of 2–6 μg / spot versus average area of the peak. The responses for concentrations of standard strychnine and brucine were found to be linear [Figures 6 and 7]. The amount of strychnine and brucine in the raw and purified samples were computed from the calibration curves, which suggested that the highest reduction of strychnine and brucine was found in the *Ardraka swarasa* purified samples [Table 4]. It might be due to the fact that prolonged contact of the seeds with *Ardraka swarasa* not only helped to diffuse out some quantity of the alkaloids from the seeds, but also some

Table 1: Effect of *Shodhana* (purification) by *Kanji* on the yield of the final products and organoleptic characters

Batch	Weight (g) of the KKJ seeds				Organoleptic characters of KKJ powder		
	Initial	After soaking	After removing seed coat and embryo	After drying	Color	Odor	Taste
KKJ-1	100	162.10	98.60	56.60	White	Characteristic of *Kanji*	Bitter
KKJ-2	100	160.70	97.10	54.70	White	Characteristic of *Kanji*	Bitter
KKJ-3	100	159.70	96.80	51.30	White	Characteristic of *Kanji*	Bitter
Average	100	160.83	97.50	54.20			

Table 2: Effect of *Shodhana* (purification) by *Ardraka swarasa* on the yield of the final products and organoleptic characters

Batch	Weight (g) of the KAS seeds				Organoleptic characters of KAS powder		
	Initial	After soaking	After removing seed coat and embryo	After drying	Color	Odor	Taste
KAS-1	100	163.20	96.80	66.80	Whitish gray	Characteristic of *Ardraka*	Bitter
KAS-2	100	162.70	97.10	65.10	Whitish gray	Characteristic of *Ardraka*	Bitter
KAS-3	100	161.00	98.50	67.00	Whitish gray	Characteristic of *Ardraka*	Bitter
Average	100	162.30	97.46	66.30			

Table 3: Physicochemical parameters of raw and purified seeds

Parameters	Samples		
	Raw Kupeelu	Purified by *Kanji*	Purified by *Ardraka swarasa*
Loss on drying	3.39% w / w	5.28% w / w	2.78% w / w
Ash value	1.11% w / w	1.06% w / w	1.42% w / w
Water-soluble extractive	37.83% w / w	22.55% w / w	25.79% w / w
Methanol-soluble extractive	3.89% w / w	1.83% w / w	2.20% w / w

Table 4: Results of estimation of strychnine and brucine in raw and purified samples of *Kupeelu* by HPTLC

Samples	Amount of strychnine found (% w / w)	Amount of brucine found (% w / w)
Raw *Kupeelu* (KR)	1.44	0.66
Kupeelu purified by *Kanji* (KKJ)	0.87	0.54
Kupeelu purified by *Ardraka swarasa* (KAS)	0.46	0.34

HPTLC: High performance thin layer chromatography

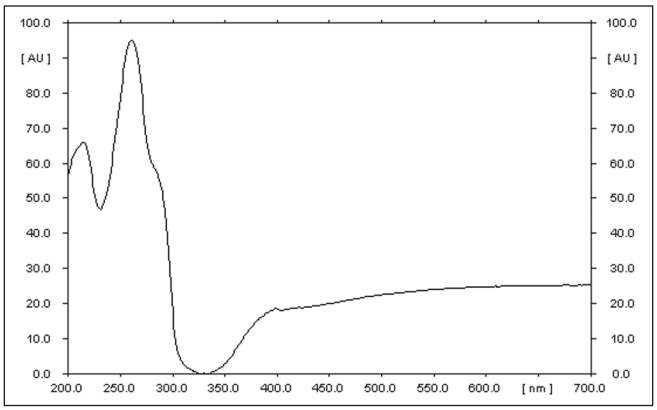

Figure 1: HPTLC profile of standard strychnine

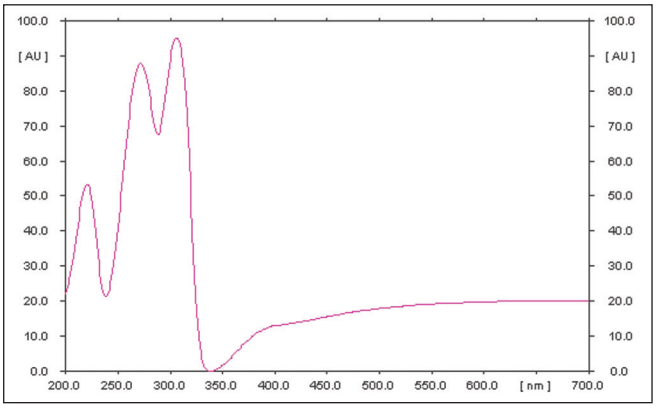

Figure 2: HPTLC profile of standard brucine

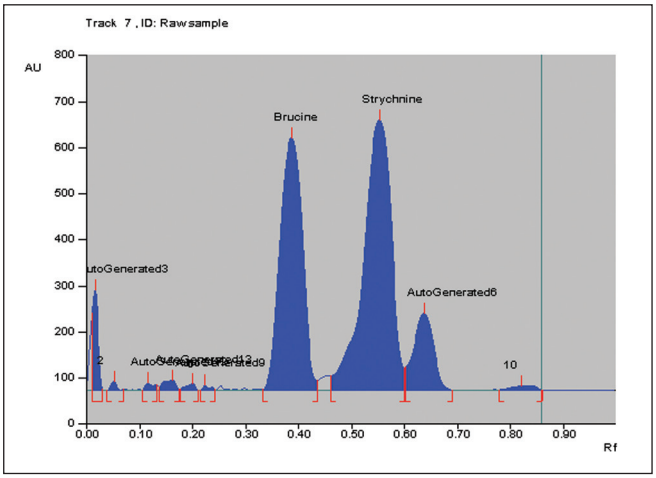

Figure 3: HPTLC of raw *Kupeelu* showing peak areas of strychnine and brucine

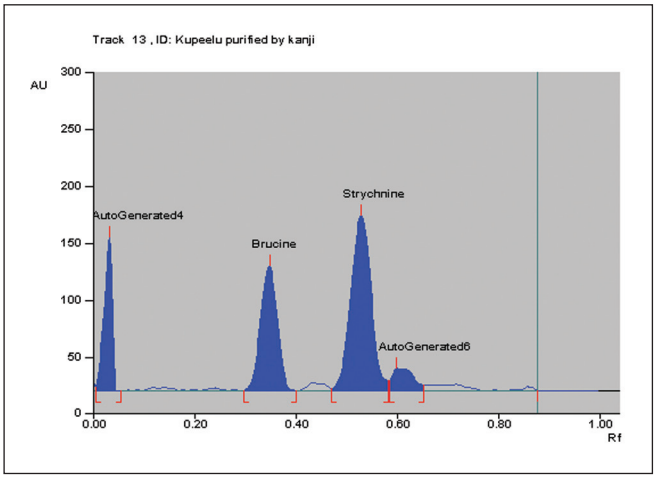

Figure 4: HPTLC of *Kupeelu* purified by *Kanji* showing peak areas of strychnine and brucine

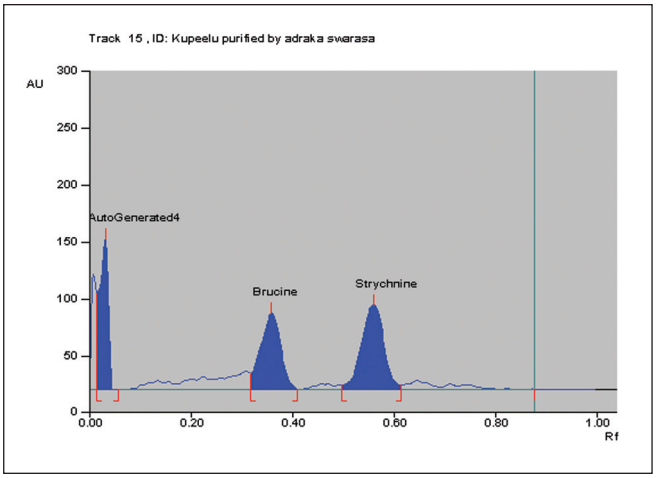

Figure 5: PHPTLC of *Kupeelu* purified by *Ardraka swarasa* showing peak areas of strychnine and brucine

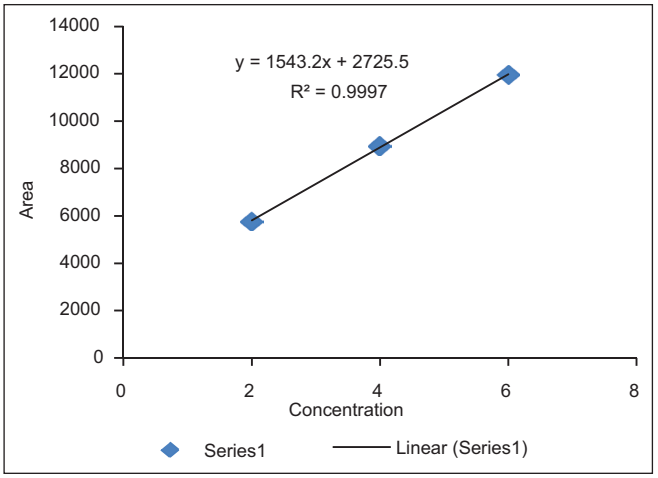

Figure 6: Calibration curve of strychnine

amount of strychnine and brucine might have been converted into less toxic derivatives like isostrychnine, isobrucine, strychnine N-oxide, brucine N-oxide, and so on.

Conclusion

From this study it may be concluded that for purification of *Kupeelu*, *Kanji* is a better extraction media than *Ardraka swarasa*.

Seeds of *Kupeelu* purified by *Ardraka swarasa* may be regarded as better method of purification as far as toxic alkaloids are concerned. These findings strongly confirm the claims of the traditional practitioners of Ayurveda that the *Shodhana* (purification) process of *Kupeelu* by *Ardraka swarasa* successfully

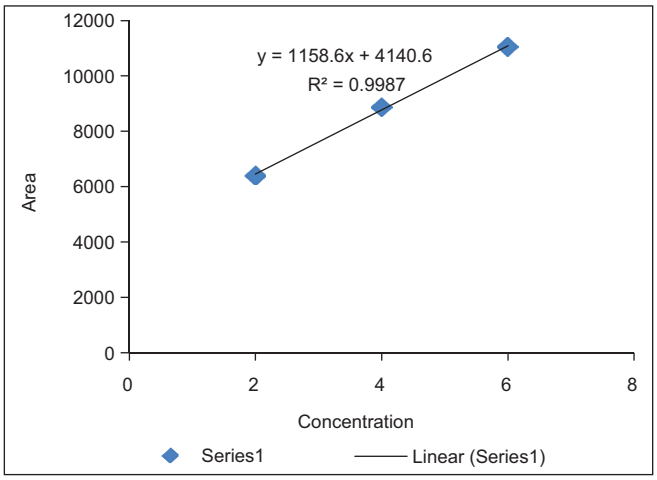

Figure 7: Calibration curve of brucine

reduces the toxic elements of the drug and this process may be practiced routinely in future.

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हिन्दी सारांश

स्ट्रिकनिन एवं ब्रुसिन के संदर्भ में कुपिलु बीज का कान्जी एवं आर्द्रक स्वरस के माध्यम से शोधन

स्वर्णेन्दु मित्रा, वी. जे. शुक्ला, रबिनारायण आचार्य

कुपिलु अर्थात नाक्सवोमिका एक विषाक्त वनस्पति है। इसका प्रयोग आयुर्वेद औषधि में वर्षों से हो रहा है। आयुर्वेद में कुपिलु बीज का प्रयोग केवलमात्र शोधन के पश्चात ही बताया गया है। शोधन के लिये आयुर्वेद शास्त्र में गोमूत्र, गोदुग्ध, गोघृत, कान्जी इत्यादि का वर्णन तो है ही, उपरान्त अनेक वैद्य शोधन के लिये एरन्ड तैल आर्द्रक स्वरस आदि का प्रयोग सफलता से करते चलेआ रहे हैं। प्रस्तुत शोध पत्र में कुपिलु बीज का शोधन कान्जी एवं आर्द्रक स्वरस के माध्यम से किया गयाहै। रासायनिक परीक्षण करने से ये प्रमाणित होता है कि कान्जी कि अपेक्षा आर्द्रक स्वरस शोधनार्थ कुपिलु में विषाक्त स्ट्रिकनिन एवं ब्रुसिन की मात्रा अशुद्ध कुपिलु कि तुलना में सबसे कम मिलती है।