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Comparative Pharmaceutical Study of White & Black *Gunja* (*Abrus prectorius* L.) Kernels

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ABSTRACT
A.precatorius (*Gunja*) is belonging to family fabaceae was usually known as crabs' eye, Indian liquorice. It is a woody twining found almost in all parts of India, ascending to an altitude of 3500 ft. In the classical texts of *Rasa Shastra*, *A. precatorius* is mentioned under *Upavisha Gana* (semi-poisonous). Several methods of purifications were mentioned in the classical text of *Ayurveda*. After proper purification, *Gunja* has been used as a treatment for various diseases like alopecia, itching, edema, urinary disorders, etc. Three seed varieties of *Gunja* and their different *Shodhana* methods were mentioned in the ancient classical literature. But there is no any clue who described that which method is superior to the other and in what respect. Therefore the current study was carried out to evaluate the comparative effect of *Shodhana* of white and black kernels of *A. prectorius* through *Godugdha* (cow's milk) and *Kanji* (sour gruel) by using phytochemical study. White and black varieties of kernels of *A. prectorius* were subjected to *Swedana* process (*Shodhana*) in cow milk and *Kanji* (sour gruel) separately for six and three hours respectively. After purification both samples were found decreased in weight, change in color and decrease the number of phytoconstituents was observed. In *Ashodhit* kernels of white *Gunja* (Wk), 3 spots, *Shodhana* with cow's milk 4 spots and *Kanji Shodhita Gunja* 7 spots were observed at different Rf values. While in *Ashodhit*a kernels of black *Gunja* (Bk), 7 spots, *Shodhana* with milk 5 spots and *Kanji Shodhita Gunja* 6 spots were observed at different Rf values. White varieties of *Gunja* seed was found more prominent as compared with black seed.

KEYWORDS: *A. prectorius, Gunja, Shodhana, Upavisha*

INTRODUCTION
Ayurveda is the most popular prehistoric traditional system of medicine about 5000 years BC, practiced in India and other nation state (Sharma *et al.,* 2016). According to *Ayurveda*, it is believed that after proper *Shodhana* (purification) any metal, mineral or plant drug which contains *Visha* (poison) can change into *Amrita* (nectar) (Manisha, 2016). In the classical texts of *Rasa Shastra*, *A. precatorius* belongs to *Upavishagana* (semi-poisonous) (Sharma, 1998). *Abrus precatorius* Linn., (Fabaceae) usually known as crabs' eye, Indian liquorice is a woody twining found almost in all parts of India, ascending to an altitude of 3500 ft. Leaves of the plant are characterized by pinnate and glabrous oblong, obtuse, truncate at both ends, apprised hairy, with many leaflets arranged in pairs. Stems are cylindrical, wrinkled, bark smooth-textured, brown. Flowers are pink or pinkish-white numerous, auxiliary racemes. Calyx is long and hispid with 9-12 mm long petals. Stamens are monadelphous, subsessile ovary and many ovules, with style incurved. Pods are oblong, a little inflated, fulvo-puberulent. Glossy seed, bright scarlet, with the area around the hilum (point of attachment) being black (Kritikar and Basu, 1999). Three varieties of *A. precatorious* (black, white and red) commonly found throughout the tropics. *A. precatorious* seeds enclose with toxic chemical

constituent like abrin, abrussic acid, choline, urease, hypaphorine and much more alkaloids and steroidal oils (Gautam *et al.*, 1998). The fatal dose of abrin is found to be 0.1-1 µg/kg in adults (Dickers *et al.*, 2003). In classical texts of *Ayurveda*, several methods of purifications for *A. precatorious* were mentioned. After being processed through purification, seeds, roots and leaves of *A. precatorious* have been used to treat alopecia, itching, edema, urinary disorders, etc. Three seed varieties of *Gunja* and their different *Shodhana* methods were mentioned in the *Ayurvedic* classical literature. However there is no any evidence who described that which method is superior to the other and in what respect. The present study was carried out to evaluate the comparative effect of *Shodhana* of white and black kernels of *A. prectorius* through cow's milk and *Kanji* (sour gruel) by using phytochemical study.

MATERIAL AND METHODS

Shodhana in cow's milk and *Kanji*

The *Shodhana* process was performed of both (white and black) seed kernels (250 gm each) of *A.precatorious* by *Swedana* method (*Dola-Yantra*) in cow's milk and *Kanji* for 6 and 3h respectively. After that, the shodhita kernels of both varieties were washed with warm water and kept for drying in the shade. (API, 2008)

Table 1: Ingredients of *Kanji*

Drug	Part used	Quantity
Oryze sativa L.	Seeds	5 kg.
Dolichos uniflorus Lamk	Seeds	5 kg.
Sodium Chloride		5 kg.
Vigna radiate L.	Dough	1.25 kg
Curcuma longa L.	Rhizome	1.25 kg
Brassica nigra L.	Seed	1.25 kg
Ferula assafoetida L.	Resin	1.25 kg
Mustard	Seed Oil	1.25 mL.
Bambusa arundinaceae Retz.	Leaves	1.25 kg
Cuminum cyminum L.	Seeds	650 gm.
Zingiber officinale Rosc.	Rhizome	650 gm.
Oryze sativa L.		160 Lt.

Preparation of *Kanji* (Sour gruel)

Rice (*O. sativa*) and *Kulthi* (*D. biflorus*) were taken in stainless steel pots. 40 liters of distilled water were added separately. The pots were placed over a gentle heat, boiled and reduced to one-fourth and filtered through cotton cloth. Both the prepared *Kwath* (decoction) of rice and *Kulthi* were mixed and kept in an earthen pot layered inside with mustard oil. The Hingu (*F. assafoetida*) was roasted in oil, and all other ingredients (3-8) were also kept in the pot. The mouth of the pot was tied by cloth and kept for 15 days and filtered before use. Thus 20 liters of *Kanji* was prepared (Anonymus, 2001).

Estimation of different physicochemical values

Extraction with polar solvents (ethanol) was made with the help of soxhlet apparatus, and the percentage of the extract was calculated after evaporating the alcoholic solvents. For Thin layer chromatography (TLC) analysis both varieties of *Gunja* were analyzed on pre-coated aluminum silica gel plates (60 F-254) as the stationary phase. The varying polarity of different mixtures of solvents (Mobile phases, Toluene: Ethyl formate: Formic acid: Water (50:40:10:1) was used for developing the chromatogram. Spraying of different

reagents confirmed the presence of various phytochemical and their respective Rf values were calculated and recorded (Sharma, 2015).

Estimation of heavy metals in Kernels of *Gunja*

2 gm of kernel powder of both varieties of *Gunja* were taken in 100 mL of the beaker. 25 mL of conc. nitric acid was measured accurately and added it in kernel powder. The beaker was covered with a watch glass and heated on a hot plate until all kernel powder was dissolved. After that, the beaker was kept out for cooling for 5 minutes and then added one mL aliquots of 10 mL of hydrogen peroxide. The mixture was heating continue until the volume was reduced up to 5 mL. After cooling for 5 minutes, all the mixture was transferred to 100 mL volumetric flask and makeup to the mark with distilled water and filtered it with the help of filter paper to remove the organic plastic precipitate (Anonymous, 2001).

Results:

Details of *Ashodhita* (crude) samples

Weight of white and black kernels of *A. precatorious*- 250gms

Table 2: Details of *Shodhita* samples of white kernel of *Gunja*

Parameters	Milk	*Kanji*
Consumed volume of vehicle	3.5 liters	4 liters.
Time consumed in the entire process of *Shodhana*	7 hrs	4.2 hrs.
Net volume of the total cow's milk and *Kanji* remained in the vessel of *Dola-Yantra* after the *Shodhana* process was over	1.5 liters (semisolid)	1.6 liters (semisolid)
The weight of white and black kernels of *Gunja* obtained after the *Shodhana* process and dried in the shade.	220 gm.	225gm.
Color of Vehicle	Color of the milk changes from the milky to light brown to dark brown color	Color of the *Kanji* changes from the bright yellow to dark yellow color
Color of Kernel	The color of white kernels changed light cream to brown.	The color of white kernels changed light cream to yellow color.

Table 3: Details of *Shodhita* samples with Black kernel of *Gunja*

Parameters	Milk	*Kanji*
Volume of cow's milk consumed	3 liters	3 liters.
Time consumed in the entire process of *Shodhana*	6.8 hrs	6 hrs.
Net volume of the total cow's milk remained in the vessel of *Dola-Yantra* after the *Shodhana* process was over	1.5 liters (semisolid)	1.7 liters (semisolid)
The weight of white and black kernels of *Gunja* obtained after the *Shodhana* process and dried in the shade.	215gm.	225 gm.
Color of vehicle	Color of the milk changes from the milky to light brown to dark brown color	Color of the *Kanji* changes from the bright yellow to dark yellow color
Color of Kernel	The color of black kernels changed black to dark black.	The color of black kernels changed black to dark chocolaty.

Table 4: Estimation of heavy metals in white kernel of *Gunja*

Element	Wavelength	Instrument detection limit	Concentration in ppm	Limit
Cadmium	228.802	0.0029	Not detected	0.3 ppm
Lead	220.353	0.0450	Not detected	10 ppm
Mercury	253.652	0.0580	Not detected	1 ppm
Arsenic	193.696	0.0530	Not detected	3 ppm

Table 5: Estimation of heavy metals in black kernel of *Gunja*

Element	Wavelength	Instrument detection limit	Concentration in ppm	Limit
Cadmium	228.802	0.0029	Not detected	0.3 ppm
Lead	220.353	0.0450	Not detected	10 ppm
Mercury	253.652	0.0580	Not detected	1 ppm
Arsenic	193.696	0.0530	Not detected	3 ppm

Fig. No 1: TLC profile of alcohol extractive

Solvent system, Toluene: Ethyl formate: Formic Acid: Water

50 40 10 1

Table 6: Rf values of alcohol extractive (from TLC plate) of white *Gunja* kernels in solvent system Toluene: Ethyl formate: Formic acid: Water (50:40:10:1)

Rf values	Ashodhita and Shodhita kernels of *Gunja*		
	Wk	WkM	WkK
0.014	+	+	+
0.037	+	+	+
0.066	+	+	+
0.089	-	-	+
0.171	-	-	+
0.193	-	+	+
0.334	-	-	+

Spraying reagent- Anisaldehydesulphuric aci

Abbreviations: Wk- White *Gunja* kernels, WkM-White *Gunja* kernels Shodhita in cow's milk, WkK- White *Gunja* kernels Shodhita in *Kanji*.

Table 7: Rf values of alcohol extractive (from TLC plate) of black *Gunja* kernels in solvent system Toluene: Ethyl formate: Formic acid: Water (50:40:10:1)

Rf values	Ashodhita and Shodhita kernels of *Gunja*		
	Bk	BkM	BkK
0.014	+	+	+
0.037	+	+	+
0.066	+	+	+
0.133	+	-	-
0.193	+	-	-
0.207	+	+	+
0.358	-	+	+
0.408	+	-	-
0.550	-	-	+

Spraying reagent- Anisaldehydesulphuric acid

Abbreviations: Bk- black *Gunja* kernels, BkM-black *Gunja* kernels Shodhita in cow's milk, BkK- black *Gunja* kernels Shodhita in *Kanji*.

Discussion:

At present era, the importance of the standardization technique increased due to the therapeutic potential of the medicinal plant. The recent standardization techniques accessible make the detection and evaluation of plant drugs more trustworthy, precise and reasonably priced (Kunle *et al.*, 2012). Traditional medicinal plants have played a major role in the healthcare system of medicine. In the present study, I have standardized the effect of *Shodhana* on two varieties of the seed (kernels) of *A. precatorious* both studies physicochemical and phytochemical. The white and black varieties of the seed of *A. precatorious* are proven to be of potent and high-quality medicinal value by different tribal of the states of India (Gautam, 2017). Therefore there is a need to precisely build up a standard monograph for the plant which will provide an excellent source of information to the customers, researchers as well as manufacturers. In this context, two varieties (white and black) of *Gunja* were processed in cow's milk and *Kanji* and subjected to various physicochemical and phytochemical studies. The observations regarding organoleptic character showed that there was a change in the color of both varieties of *Gunja* i.e. white and black. In WkM, the color of white kernels changed light cream to brown while BkM, the color of black kernels changed light cream to yellow color. The Same color change was observed in both samples when treated with *Kanji*. In WkK, the color of white kernels changed light cream to yellow color whereas, in BkK, the color of black kernels changed black to dark chocolaty. Both varieties of *Gunja* were found soft in nature after complete *Shodhana* process (Table 2, 3). Extractive values are giving useful information about active phytoconstituents which is already present in different part of plants using different solvents (Sasidharan *et al.*, 2011). In medicinal plants, a number of metal and minerals are present. These metal and mineral were important for build up nutrition in the human body. Moreover, many other elements (cadmium, lead, mercury, arsenic) are also present in plants which are caused a harmful effect in the human body (Baker and Brooks, 1989; Lasisi et al., 2005). The presence of these elements in the soil involves their accumulation in medicinal plants (Khan et al., 2007). The ethanolic extract of both varieties of *Gunja* was performed for detection of heavy metal analysis with the help of Atomic absorption spectroscopy (AAS). The elements analyzed were cadmium, lead, mercury and arsenic and their amounts were recorded in parts per million (ppm). The results (Table 4, 5) show that after *Shodhana*, all the elements were not detected in our permicible limits. The aim of the present research work is to carry out the effect of *Shodhana* on the different seed variety of *Gunja*. In our Ayurvedic classical text different *Shodhana* process is mentioned for the same drug. For that reason, in a current

scenario, many questions were raised like which of the *Shodhana* process is more effective or which variety of plant drugs is more potent, etc. (Gautam, 2016). In this context, two varieties of *Gunja* (white and black) were processed with two different vehicles with same *Shodhana* process. After purification process, both varieties of *Gunja* were subjected to extraction with ethanol. Both ethanolic extracts were performed for TLC (thin layer chromatography) in solvent system Toluene: Ethyl formate: Formic acid: Water (50:40:10:1) (figure 1). The retardation factor (Rf) value was calculated and recorded. In *Ashodhit* kernels of white *Gunja*, three spots at Rf values (0.014, 0.037, 0.066) were present. After *Shodhana* with milk 4 spots at Rf values (0.014, 0.037, 0.066, 0.193) were observed but in *Kanji Shodhita Gunja* 7 spots were observed at Rf values (0.014, 0.037, 0.066, 0.089, 0.171, 0.193, 0.334) (Table 6). In Ashodhit kernels of black *Gunja*, 7 spots at Rf values (0.014, 0.037, 0.066, 0.133, 0.193, 0.207, 0.408) were present. After *Shodhana* with milk 5 spots at Rf values (0.014, 0.037, 0.066, 0.207, 0.408) were observed but in *Kanji Shodhita Gunja* 6 spots were observed at Rf values (0.014, 0.037, 0.066, 0.207, 0.358, 0.550) (Table 7). The variations of Rf value may be explained due to *Shodhana*. During *Shodhana* process, some constituents were either soluble in milk or *Kanji*. It might be due to the fact that extended contact of the kernel with milk and *Kanji* not only reduce the phytoconstituents but also converts the highly toxic phytoconstituents into less toxic (Gautam, 2016). Another hypothesis can be drawn that during *Shodhana* some highly toxic phytoconstituents were leached out and some therapeutically active phytoconstituents were absorbed with vehicle (either milk, *Kanji*). Which has been increased the therapeutic value of seeds?

Conclusion:

It was found that the phytochemicals decreased in *Shodhita* samples of black *Gunja* while increased in *Shodhita* sample of white *Gunja* due to leaching and absorption. Some compounds were absent in *Shodhit* samples of black *Gunja* while some new compounds were present in *Kanji Shodhit* sample of white *Gunja*. The study showed that white varieties and *Shodhana* in *Kanji* is more effective. Therefore, all the analytical tools used in this study can be used as a marker for *Shodhana* for the both samples.

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