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Comparative Study of Processed (Shodhit) and Unprocessed Seeds of 'Gunja'- *Abrus precatorius* L.

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Abstract – 'Gunja' is attributable to the plant *Abrus precatorius* L. (Leguminosae). Three forms with red, brown and another with white seeds are known to occur in this species and are employed for different therapeutic uses *viz.* as purgative, emetic, aphrodisiac, tonic and also as an abortifacient. According to some Ayurvedic literature the seeds are poisonous and should be given to the patients after proper processing ('Shodhan'). A comparative study of various phytochemical parameters, namely, percentage of successive extractives, total proteins, tannins, total ash and acid insoluble ash of these three forms of the processed (with cow's milk and Kanji) and unprocessed seeds was done. TLC and densitometric scanning of successive extractives was also carried out to serve as markers for processed and unprocessed seeds. The percentage of proteins, tannins, alcohol and water soluble extractives decreased in the processed material. Besides, their acute toxicity, CNS activity were also studied in albino mice and it was found that white seeds are more toxic as compared to the red and brown. The toxic effect was reduced with the processing. Further, the 'Kanji' processed seeds are less toxic than the milk processed one.

Key words – 'Gunja' *Abrus precatorius*, processing, milk 'Kanji'.

Introduction

'Gunja' attributable to *A. precatorius* L. (Leguminosae) is a very important member of the 'Upavishagana' (having potent and toxic chemical constituents), and used as therapeutic agent with high therapeutic and pharmacological attributes (Anonymous, 1982; Bhaisaya Ratnawali, 1981; Bhavprakash Nighantu, 1974; Hari Har Prasad, 1927; Pandey, 1949). Three forms of plants with white, red and brown seeds with black spot around the hilum occur in this species (Singh and Chunekar, 1972). Each part of this plant is used medicinally. The roots and leaves contain glycyrrhizin, the principal constituent of liquorice (*Glycyrrhiza glabra*) and are used as substitute for liquorice in cough and catarrhal affections; hence *A. precatorius* is known as Indian Liquorice. The seeds are described as poisonous and useful in affections of the nervous system and externally in skin diseases, ulcers, piles, etc. (Charakha Samhita, 1949; Chunekar, 1969; Atridev, 1975; Sharma, 1979; Sharma, 1982).

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The seeds, reduced to a paste are administered internally in the affections of the nervous system and applied locally in sciatica, stiffness of the shoulder joints, paralysis (Anonymous, 1948; Asolkar, *et al.*, 1992). The seeds are said to be useful in diarrhoea and dysentery and possess anthelmintic activity. Ethanolic extracts of the seeds inhibited the growth of *Micrococcus pyogenes*- enteric and dysenteric group of microorganisms (Kirtikatr and Basu, 1933).

A. precatorius is not only used in indigenous systems of medicine but has a great significance in folklore, too. Several tribes of our country use this plant differently. The root is considered emetic, alexiteric and used in nervous disorders, sore throat, dry cough, rheumatism and leucoderma (Nautial, 1981; Bhalla, *et al.*, 1982; Saxena and Vyas, 1983). The seeds are used as abortifacient, purgative and in nervous disorders, stomach troubles, pneumonia, skin diseases and antifertility and as antimicrobial agents by various tribes of India (Malhotra and Murthy, 1973; Saxena and Datta, 1975; Billore and Audichya, 1978; Maheshwari, *et al.*, 1981; Bhalla, *et al.*, 1982; Saxena and Vyas,1983; Tarafder, 1983; Bennet, 1985). In view of the seeds being described as poisonous, it has

been mentioned in various Ayurvedic literature that these should be given to patients after processing ('Shodhan') (Chunekar, 1969; Singh, 1969). Several methods of processing ('Shodhan') have also been suggested by different Ayurvedacharyas. The present study has been undertaken with a view to observe (1) which type of seeds are more toxic than the other; (2) what is the effect of processing ('Shodhan sanskar') on the percentage of various physicochemical values of seeds; (3) which is the most appropriate processing method in reducing or eliminating the toxicity of seeds of *Abrus precatorius* by calculating the percentage of total proteins, tannins, successive extractives, total ash and acid insoluble ash of white, red and brown kernels processed with cow's milk and Kanji (Sour gruel) and unprocessed kernels. Together with this TLC and their densitometric scans were also carried out. Besides, their acute toxicity, CNS activity were also studied in albino mice.

Materials and Methods

Three forms of *Abrus precatorius* seeds, white (Vouch. No. NBRI 200480), red (Vouch. No. NBRI 200455) and brown (Vouch. No. NBRI 200479), were collected and identity confirmed by matching with the seeds deposited in **NBRI** museum.

Shodhan Sanskar or Processing – Seeds 4 kg of each variety were coarsely ground and subjected for 'Shodhan' according to the following procedures.

Shodhan in Cow's Milk – Coarsely ground materials 1 kg of all the three types were tied separately in coarse cotton cloth (cloth pouch) and immersed in 'Dola Yantra' (an earthen vessel or hanging apparatus which is generally used for processing or 'Shodhan') (Sen Gupta, 1906) containing 10 liters of cow's milk in such a way that its lower portion should not touch the bottom of the 'Yantra' and boiled for 6 hours in order to keep the volume of the milk constant, an additional quantity of milk was added from time to time. After six hours of boiling the pouch was removed from the 'Yantra' and ground seed materials were thoroughly washed with warm water. Then the seeds were placed for shade drying. After drying the pericarp was removed from the seeds.

Shodhan in 'Kanji' (Sour gruel) – The process of 'Shodhan' in 'Kanji' was similar to that of milk except that the boiling time was 3 hours instead of 6 hours. The 'Kanji' was prepared with the following ingredients.

1. 'Tandul' (Rice) *Oryza sativa* L. (Poaceae) Endosperm – 5 kg. (Vouch. No. NBRI 200478).
2. 'Kulthi' *Dolichos uniflorus* Lamk. (Leguminosae) seeds – 5 kg. (Vouch. No. NBRI 200468).
3. 'Jeerak' *Cuminum cyminum* L. (Apiaceae) seeds – 750 gm. (Vouch No. NBRI 200481)
4. 'Saindhav Lavan' Sodium Chloride – 5 kg.
5. 'Shunthi' *Zingiber officinale* Rosc. (Zingiberaceae) dried rhizome – 750 gm (Vouch. No. NBRI 2000476).
6. 'Urad' (Bara) *Vigna radiata* (L.) Wilczek var. radiata (Leguminosae) seeds – 1.25 kg. (Vouch. No. NBRI 200469).
7. 'Haldi' (Turmeric) *Curcuma longa* L. (Zingiberaceae) Rhizome – 1.25 kg. (Vouch. No. NBRI 200470)
8. 'Rai' *Brassica nigra* (L.) Koch. (Brassicaceae) seeds – 1.25 kg. (Vouch. No. NBRI 200472)
9. 'Hingu' *Ferula assa-foetida* L. (Apiaceae) Resin – 1.25 kg. (This plant does not occur in India therefore we procured resin from the local market and confirmed the purity of the sample according to the method described by Handa and Kapoor, 1993)
10. Mustard oil (purchased from the local market Dhara) – 1.25 kg.
11. Vansha patra *Bambusa arundinaceae* (Retz.) Roxb. (Poaceae) Leaves 1.25 kg. (Collected from the labelled plants of NBRI Garden)
12. 'Distilled water' 160 Lit.

Preparation of 'Kanji' (Sour gruel) – *Oryza sativa* and *Dolichos uniflorus* (5 kg. of each) were boiled separately in 80 litres of distilled water till water was reduced to one fourth and filtered through fine cotton cloth and mixed. All the ingredients (3-8) mentioned above kept in dried earthen pot coated inside with mustard oil. After that 1.25 kg. fried *Ferula assa-foetida* in oil was put into the earthen pot after which small pieces of leaves of *Bambusa arundinacea* (1.25 kg.) were also placed in the pot. The mouth of the earthen pot was tied by cloth and kept for 15 days and filtered before use. Thus 40 liters of 'Kanji' was prepared.

Estimation of different physicochemical values – For quantitative estimation of total ash and acid insoluble ash methods mentioned by Anonymous (1966) were followed. The tannin percentage was calculated by spectrophotometric method described by Anonymous (1984-9.110-9.112) using Folin and Ciocalteus Phneol reagent, and tannic acid as a standard solution, readings were taken at 760 nm absorbance. The

protein percentage was also calculated by spectrophotometric method described by Lowery *et al.* (1951), a standard curve at 700 nm absorbance was prepared with bovine serum albumin (B.S.A.) and 2% potassium sodium tartrate, 1% $CuSO_4 \cdot 5H_2O$, 2% sodium carbonate in 0.1N NaOH were used as test solutions (see detailes in Lowery, *et al.*, 1951). Extraction with non-polar to polar solvents (hexane, chloroform, ethyl alcohol and water) was made with the help of Soxhlet apparatus and the percentage of each extractive was calculated after evaporating the respective solvents. The thin layer chromatography was performed on pre-coated silica gel G254F plates using different solvent systems according to the nature of the constituents present in the extractives. These plates were further screened through the Hitachi fluorescence spectrophotometer 650-60 at different excitation and emission wave lengths. In addition to these the elucidation of different compounds on silica gel G plates was also made after spraying with different developing reagents (Wagner, *et al.*, 1984).

Toxicological study – Experiments were conducted on healthy swiss strain of Albino mice of both sexes weighing between 20-30 gms. All animals were kept in colony cages under identical housing conditions in the animal house of the Department of Pharmaceutics, I. T. B. H. U. Varanasi. Animals were acclimatized for one week and fasted overnight (18 hours) before experimentation. The experiments were conducted in the months of March to May. The mean minimum and maximum environmental temperature during these months was 24°C to 38°C. All the experiments were conducted during the day time from 9 a.m. to 5 p.m., when the ambient temperature was nearer the maximum. For pharmacological study, methods mentioned by Robert (1971) were adopted.

Preparation of test drug samples – The unprocessed and processed ('Shodhit') seeds from the white, red and brown 'Gunja' were taken. Weighed quantities of powder was dissolved in 100 ml of distilled water and macerated in the cold and kept overnight for extraction. the material was then centrifuged at 500×g for 15 min. in a REMI centrifuge and the supernatant obtained was diluted with water for 3 different doses 0.25 (D1), 0.5 (D2) and 1.0 (D3) mg per kg body weight of the mice and the drug was administered intraperitoneally (i.p.) to the animals for LD_{50} and LD_{100}. The acute toxicity of processed and unprocessed seeds was studied in terms of mortality rate (Table 2).

Table 1. Physicochemical values of processed and unprocessed seeds of *Abrus precatorius*

MATERIAL		PARAMETERS (In percentage)							
		Total ash *Mean±SD	Acid insol. Ash *Mean±SD	Protein *Mean±SD	Tannins *Mean±SD	Hexane extractive *Mean±SD	Chloroform extractive *Mean±SD	Alcohol extractive *Mean±SD	Water extractive *Mean±SD
WHITE	Unprocessed	2.8±0.356	0.565±0.060	18±0.638	0.753±0.009	1.98±0.070	0.405±0.020	7.66±0.133	40.18±0.448
	Processed with milk	1.53±0.115	0.606±0.027	14.00±0.146	0.153±0.004	5.84±0.1100	0.76±0.014	5.53±0.117	33.66±0.266
	Processed with Kanji	1.11±0.456	0.568±0.031	6.40±0.480	0.351±0.004	2.46±0.086	0.51±0.025	4.50±0.290	14.40±0.092
RED	Unprocessed	3.7±0.137	0.151±0.005	11.01±0.581	0.650± 0.025	2.74±0.102	0.421±0.019	6.64±0.152	26.42±0.302
	Processed with milk	2.50±0.584	0.595±0.013	8.50±0.195	0.250±0.008	4.04±0.079	0.65±0.014	5.16±0.163	20.02±0.093
	Processed with Kanji	1.72 ±0.063	0.566±0.029	2.41±0.045	0.450±0.006	2.61±0.046	0.269±0.012	4.50±0.268	10.35±0.037
BROWN	Unprocessed	3.91±0.145	0.211±0.017	14.1±0.216	0.645±0.026	2.79±0.023	0.41±0.031	6.51±0.172	24.77±0.475
	Processed with milk	1.68±0.068	0.518±0.025	11.13±0.247	0.25±0.006	4.46±0.326	0.53±0.021	5.43±0.301	20.18±0.156
	Processed with Kanji	1.198±0.350	0.517±0.015	3.24±0.043	0.351±0.005	1.63±0.028	0.29±0.009	4.58±0.172	8.80±0.107

*Mean is the average of 6 readings.
Differences between the mean values of total ash, protein, water soluable extractives of unprocessed and processed (with milk and kanji) seeds of *Abrus precatorius* are highly significant and the values have been calculated by the student 't' test.

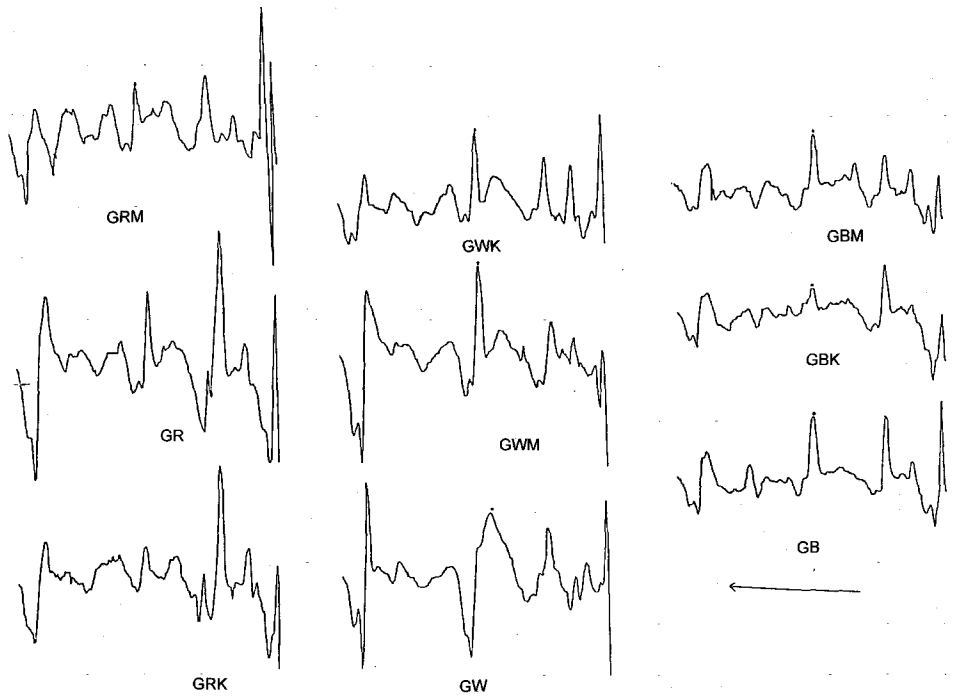

Fig. 1. Densitometric scans of chloroform extractive of processed and unprocessed seeds of 'Gunja'. Solevent used *n*-Heptane: ethyl-methylketone:methanol (58:34:8), Excitation wave length 470 nm, emission wave length 470 nm, Energy range 0.2V, excitation and emission slit 8 nm, Chart and plate speed 30 nm/min.

Table 2. Some significant R_f values of chloroform extractive

Rf Values	GW	GWM	GWK	GR	GRM	GRK	GB	GBM	GBK
0.06	+	+	+	+	+	+	+	+	+
0.11	+	+	+	+	+	+	+	+	+
0.20	+	+	+	+	+	+	+	+	+
0.28	+	+	+	+	+	+	+	+	+
0.32	+	–	–	+	–	–	+	–	–
0.39	+	–	–	+	–	–	+	–	–
0.43	+	–	–	+	–	–	+	–	–
0.50	+	+	+	+	+	+	+	+	+
0.65	+	+	+	+	+	+	+	+	+
0.76	–	+	+	–	+	+	–	+	+
0.80	+	+	–	+	–	–	+	+	–

Spray reagent 10% methanolic H_2SO_4.
GW, White seeds; GWM, White seeds processed with milk; GWK, White seeds processed with 'Kanji'; GR, Red seeds; GRM, Red seeds processed with milk; GRK, Red seeds processed with 'Kanji'; GB, Brown seeds; GBM, Brown seeds processed with milk; GBK, Brown seeds processed with 'Kanji'.

Observations

The observations regarding various physicochemical values are very well depicted in Table 1. The percentage of total ash was significantly reduced in processed ('Shodhit') material as compared to the unprocessed ones. For instance it was 1.5, 2.5, 1.7% in milk Shodhit and 1.1, 1.7 and 1.2% in 'Kanji shodhit' material while it was 2.8, 3.7 and 3.9% in unprocessed seeds, respectively. Likewise the percentage of protein in unprocessed and processed material was 18, 11, 14% unprocessed) 14.0, 8.5, 11.0 (milk Shodhit); 6.4, 2.4, 3.2% ('Kanji shodhit') in white red and brown kernels, respectively. Simultaneously the decrease was more prominent in 'Kanji' as compared to milk. As most of the tannins are water soluble, the reduction in tannin percentage was also observed. (Table 1).

The percentage of hexane and chloroform extractives in milk processed kernels increased in all the samples. On the contrary, the percentage of ethyl alcohol and water soluble extractives decreased. On systematic evaluation and comparison of thin layer chromatograms and their finger prints (Fig. 1) from different extractives of processed and unprocessed seeds it was revealed that these parameters can be utilized as markers for quality evaluation of processed and unprocessed seeds of all the three types of

'Gunja'. However, some significant changes with regard to some spots were also observed; for example the three spots at Rf values 0.32, 0.39 and 0.43 were present in chloroform extractive in solvent systems *n*-heptane, ethyl-methylketone and methanol (58:34:8) in all of the unprocessed materials and were absent in processed ones (Table 2). Similarly the concentration of the spot at Rf value 0.50 in white variety is slightly higher in comparison to the brown and red varieties (Fig. 1). These variations may be explained that due to processing ('Shodhan') there is some decline in the concentration of some of the constituents which are either soluble in milk and 'Kanji' or may be due to the reduction and degeneration.

The aforesaid changes in the percentage of proteins, water and alcohol extractives were reflected in the pharmacological behavior of the processed seeds with regard to their pharmacological action and toxicity. For instance animals fed with different doses of seed powder show significant pharmacological action *viz.* depression on CNS activity; marked decrease in heart and respiratory rates; sluggish spontaneous activity and reactivity; elongation of the body; aggressive behavior and coloration of skin from white to black. On the contrary no effect on pupil size, body temperature, tremor and convulsions was observed in the seed powder-fed animals except animals fed with white seed at D3 dose level (1 mg/kg body weight) show tremors and convulsions. It is quite interesting to note that aforesaid activities were quite prominent in white seeds fed animals as compared to the red and brown seeds fed animals. However, animals treated with milk processed material also showed lesser aforesaid changes, but almost no change was observed in 'Kanji Shodhit' material. Further it was also observed that all the activities were dose related. Acute toxicity of processed ('Shodhit') and unprocessed seeds was also studied (Table 3). The ratio between the white, red and brown seeds producing toxic effect was 1:2:2.

The LD_{50} and LD_{100} value of all the samples of processed and unprocessed seeds of *Abrus precatorius* were calculated and it was found that LD_{50} was 0.5 to 1.0 mg and LD_{100} was 1.0 to 2.0 mg per kg. body weight in white and red variety of unprocessed material respectively. However, these values got increased in the processed material with both milk and 'Kanji' *viz.* LD_{50} was 1.0 to 2.0 mg in milk and 15.0-20.0 mg in Kanji shodhit seeds while LD_{100} was 2.5 to 5.0 in milk and 20.0 to 30.0 mg/kg body weight in 'Kanji shodhit' seeds of white and red forms (Table 3). It is interesting to note that animal injected with red and brown seeds behaved in a similar fashion with regard to their toxicity and pharmacological action in spite of the changes recorded in the percentage of protein.

Table 3. Acute toxicity of processed and unprocessed seeds

Mortality (mg/kg body weight)						
	LD_{50}			LD_{100}		
Parameters	White seeds	Red seeds	Brown seeds	White seeds	Red seeds	Brown seeds
Unprocessed	0.5	1.0	1.0	1.0	2.0	2.0
Milk Shodhit	1.0	2.0	2.0	2.5	5.0	5.0
Kanji Shodhit	15.0	20.0	20.0	20.0	30.0	30.0

Discussion and Conclusion

The objective of the present paper is to study the effect of processing ('Shodhan') on the (1) concentration of various phytoconstituents resulting in acute toxicity of 'Gunja' which is attributable to *Abrus precatorius* seeds of white, red and brown varieties (2) which of the Shodhan process is more effective and (3) which is the most toxic variety among three colored seeds. In this context brown, red and white seeds of 'Gunja' were processed in cow's milk and 'Kanji' and subjected to various phytochemical tests and pharmacological/ toxicological action.

As brought out by the present studies, the percentage of total ash decreased in processed material suggesting that leaching out of some amount of inorganic salts may have occurred during the processing. Similarly the decrease in protein percentage from 18, 11, 14 to 14, 8.5, 11.00 in milk and 6.4, 2.4 and 3.2 percent in 'Kanji' was also due to the dissolution of water soluble protein in processed material and denaturalization of toxic proteins during heating. Simultaneously the decrease was more prominent in 'Kanji' as compared to milk, suggesting that 'Kanji shodhan' process is much better. Further, the percentage of protein in white seeds is higher as compared to red and brown seeds. This finding validates the saying of some Ayurvedacharyas Chunekar (1969) that white seeds are more toxic than the other two. On the other hand the amount of hexane and chloroform soluble portions increased in the processed material which may be due to the absorption of fats present in the milk by the seeds during processing. However,

the percentage of alcohol and water soluble extractive decreases, which is possibly due to the extraction of water and alcohol soluble substances like sugar, glycosides, in milk and 'Kanji'. The reduction of protein, water and alcohol extractives was further confirmed by the pharmacological behavior of the processed and unprocessed seeds. For instance animals fed with unprocessed seeds show noteworthy changes on the CNS activities such as marked decrease in heart and respiratory rates; aggressive behavior and elongation of the body with coloration of skin from white to black. However, animals treated with milk-processed material show lesser aforesaid changes. But no significant changes were observed in animals fed with 'Kanji' treated seeds.

Further, the aforesaid activities are more prominent in white seed-fed animals, which also show tremors and convulsions at D3 dose level (1 mg/kg body weight) confirming that the white seeds are more toxic as compared to the other two i.e., red and brown. The above differences were further confirmed from the toxicological studies. The LD_{50} and LD_{100} values of all the processed material increased both in milk and Kanji (Table 3).

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