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Comparative chemical profiling of purified and unpurified *Strychnos nux-vomica* Linn seeds: An attempt to reduce toxic Brucine content

Research Article

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Abstract

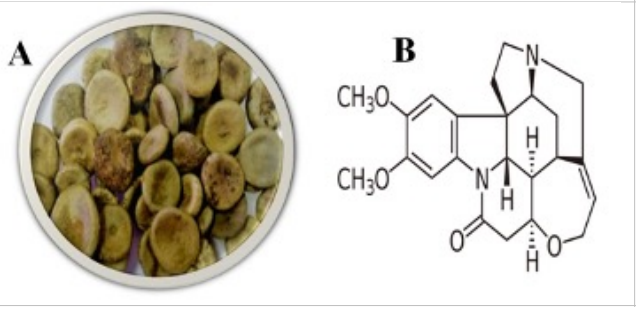
Vishamusthi (*Strychnos nux-vomica* Linn.), a medicinal plant described as Upavisha (semi-poisonous) group of Ayurvedic Pharmacopoeia of India. *Vishamusthi* has widely been used and being practiced in several illness namely nervous debility, paralysis, weakness of limbs, sexual weakness, dyspepsia and etc. Ayurveda practices strictly recommend the use of *Vishamusthi* in therapeutics only after proper *shodhana* (purificatory procedure) through specific medias such as *Gomutra* (cow's urine), *Godugdha* (cow's milk), *Goghrita* (cow's ghee), and etc. Although various *shodhana* procedures are recommended in Ayurvedic treatise, but updated scientific researches regarding the *shodhana* methods are lacking. The present study was undertaken to investigate the physicochemical and phytochemical parameters, quantitative estimation of brucine using cutting edge research tools such as high-performance thin layer chromatography (HPTLC), liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) analyses of *Vishamusthi* seeds before and after purification. The remarkable changes have been observed in different physicochemical parameters, HPTLC, HPLC, GC-MS and LC-MS chromatographic profiling before and after *shodhana* process of *Vishamusthi* seeds. Quantitative HPLC studies revealed that the process of *shodhana* resulted in depletion of toxic brucine (chief poisonous constituent of *Vishamusthi* seeds) reduced to 79.66% in chloroform extract and 64.54% in ethanol extract after *shodhana* process.

Key Words: Ayurveda, *Shodhana*, Brucine, HPLC, Strychnos nux-vomica Linn. Chemical profiling.

Introduction

Vishamusthi consists of dried seeds of *Strychnos nux-vomica* Linn. (Family: Fabaceae), a medicinal plant used in Ayurvedic system of medicine for treating various ailments such as nervous debility, paralysis, weakness of limbs, sexual weakness, dyspepsia, dysentery and chronic rheumatism [1]. It is used as a potent rasayana drug for old age problems [2]. Additionally, it is employed in the treatment of anaemia, asthma, bronchitis, colic, intermittent fever, hysteria, etc., in a specific therapeutic dose. V*ishamusthi* and its alkaloids have been reported to possess anti-oxidant, analgesic, anti-diarrheal, anti-inflammatory, anti-tumor, hepatoprotective and anti-snake venom properties in different experimental models [1]. Although 16 different alkaloids have been isolated and identified from crude nux vomica, major chemical constituents are strychnine, brucine and their derivatives such as isostrychnine and brucine N-oxide [1]. Strychnine and brucine are the most important and strongly toxic alkaloids not only in the seed but also in the roots, bark, leaves, fruit-pulp, and the hard fruit-shells [3]. Nux-vomica is a highly poisonous at large doses, producing tetanic convulsions and eventually death and in lesser doses it may manifest mental derangement [4]. Illustrations of raw seeds of *Vishamusthi* and chemical structure of brucine has been provided in Figure 1.

Figure 1. (A) Raw seeds of *Vishamusthi*, and (B) Chemical structure of brucine



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As per the Ayurvedic treatise, *Vishamusthi* seeds are to be administered in therapeutics only after going through certain purificatory measures (*shodhana*). In the Ayurvedic literature, it is well established that the '*Visha*' (poison) becomes '*Amrita*' (nectar) after logical proper purificatory measures in a number of specific media and the ancient physicians of Ayurveda successfully used purified drugs in several diseases [5]. Different methods for specific *shodhana* procedures are reported for nux vomica seeds [1] [6-9]. In Ayurveda, The *shodhana* is not only a process of detoxifying toxic substances but also it is a process of enhancing the potency as well as efficacy of the drug [10].Akbar et al. reported the reduction in strychnine content by following the detoxification of nux vomica seeds as mentioned in Unani system of medicine [11]. Mitra et al reported that raw as well as purified *vishamusthi* exhibited anti-inflammatory activity in formaldehyde induced hind paw oedema in albino rats. An attempt was made by Mitra et al to purify the *vishamusthi* seeds using cow's urine, cow's milk and cow's urine and cow's milk as media and fund that highest reduction in toxic strychnine and brucine contents after purification in cow's urine for one week followed by boiling in cow's milk for three hours [8]. However, the present study used this method to purify *vishamusthi* seeds .The present study was undertaken to investigate the physicochemical and phytochemical parameters, quantitative estimation of brucine using cutting edge research tools such as high-performance thin layer chromatography (HPTLC), liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) analyses of *Vishamusthi* seeds before and after purification.

Materials and methods

Collection of plant material

The *vishamusthi (Strychnos nux-vomica* Linn.*)* seeds were procured from local crude drug market Chennai, Tamilnadu, India and authenticated at Botany Department of Captain Srinivasa Murthy Central Ayurveda Research Institute, Chennai with the help of flora. Voucher specimen was deposited in the Botany department of the Institute. Copy of authentication certificate is available, if needed.

Shodhana (Purificatory measures) procedure

The procedure for purification was followed as reported by [8] The seeds of *Vishamusth*i was cleaned and separated from foreign matter. Seeds of *vishamusthi* were soaked in Gomutra (Cow's urine) for 7 days and the urine was replaced by fresh Cow's urine every day. Then after, the seeds were cleaned with warm water followed by boiling in Godugdha (Cow's milk) for 3 hours with Dola-yantra method. Then the outer seed coat and embryo were removed from the cotyledons. The seeds were dried in sunlight properly and fried in Cow's ghee on low flame till it gets kapish (slightly brownish) colour, then seeds were pulverized for further studies [12].

HPTLC finger print profile of *Visamusthi seeds*

The dried powdered seeds of *vishamusthi* both materials each approximately 10g before and after *shodhana* process were extracted with 200 ml of absolute ethanol by using soxhlet extraction for 24 hrs. The extracts were evaporated to dryness under reduced pressure. The same procedure was followed for chloroform extraction. In order to prepare test solutions, the residues obtained from ethanol and chloroform extracts were weighed and dissolved in methanol using 10 ml volumetric flask, filtered through 0.22 µ membrane filters and used for HPTLC fingerprint profiling and identification of reference standard Brucine biomarker compound. In order to prepare standard curve, 4.1mg of Brucine was accurately weighed and added to a 10 ml volumetric flask, dissolved in HPLC grade methanol and the volume was made up to 10 ml to obtain 0.41mg/ml Brucine stock solution. The solvent system Chloroform: Methanol: Formic acid (8.5:1.5:0.4) was used. In order to run the test, accurately, 15l of the test solution of chloroform and ethanol extracts and 10 l of brucine standard solution was applied on different tracks on a precoated silica gel 60 F_{254} TLC plate (E. Merck) of 0.2 mm thickness. The plate was developed in the suitable solvent system till the solvent rises to a distance of 8cm. The plate was observed through TLC Visualizer under UV at 254 nm and 366 nm and photos were documented. Finally, the plate was dipped in vanillin-sulphuric acid reagent and heated in hot air oven at 105°C until the colour of the spots were appeared and photo was documentation under white light and calculate the R_f value. Before derivatization the plate was scanned under UV at 254nm and 366 nm using deuterium and mercury lamps respectively. After derivatization plate was scanned at 540 nm using tungsten lamp. The R_f values and fingerprint data were recorded by WIN CATS software [13-14].

HPLC chromatographic profiling of *Vishamusthi seeds*

In order to prepare test solutions, the residues obtained from chloroform and ethanol extracts of before (unprocessed) and after (processed) *shodhana* process samples were accurately weighed same amount and dissolved in methanol using 5 ml volumetric flask, filtered through 0.22 µ membrane filters and used for HPLC analysis chromatographic profiling. Chloroform and ethanol extracts of the *vishamusthi* seeds before and after s*hodhana* process, the processed and unprocessed samples are compared under the same chromatographic conditions. HPLC column of ZORBAX Eclipse XBD-C18 (4.6 mm x 150 mm) (particle size 5µm) was used. Phosphate Buffer and Acetonitrile in the ratio of 70:30 was used as mobile phase. For detection DAD detector at 230 nm was used. The injection volume of 10µl from processed and purified samples was used. The flow Rate was kept at 1.0 ml/min [15-16].

Quantitative Estimation of Brucine in *Vishamusthi seeds* by HPLC

The test and standard solutions were prepared as described above. HPLC column of ZORBAX Eclipse XBD- C18 (4.6 mm x 150 mm) (particle size 5µm) was used. Phosphate Buffer and methanol in the ratio of 95:5 was used as starting mobile phase. The HPLC operated at gradient elution. For detection VWD Detector at 230 nm was used. The injection volume of 10µl from processed and purified samples was used. The flow Rate was kept at 1.0 ml/min. In order to prepare calibration curve, 0.41 mg/ml Brucine stock solution was appropriately diluted further to get a concentration of 0.200, 0.100, 0.050 and 0.025 mg/ml of brucine standards. Each of the standard solution was run through the HPLC and recorded the respective peak areas. In order to estimation of brucine, accurately10 µl of each test solution injected to HPLC system. Record the chromatogram and determine the area of the peak of the test solution corresponding to that of brucine as described above from the calibration curve [16-17].

GC-MS Chromatographic profiling of *vishamusthi seeds*

The test solution was prepared by dissolving the dried extract of chloroform in chloroform and methanol solvents of desired volume. Test solutions were filtered and GC-MS analysis was performed as per the standard protocol reported [18].

LC-MS Chromatographic profiling of *vishamusthi seeds*

The test solution was prepared by dissolving of ethanol extracts of *Visamusthi* seeds before and after s*hodhana* process in HPLC grade methanol upto 1.0ml volume. Test solutions were filtered and LC-MS analysis was performed as per the standard protocol reported [17].

Results

Preliminary phytochemical screening results showed the presence or absence of certain phytochemicals in the *vishamusthi* seeds. The comparative preliminary phytochemicals screening results of *vishamusthi* seeds before and after s*hodhana* a process are tabulated in Table 1.

Table 1. Preliminary phytochemicals screening of *Strychnos nux- vomica* seeds before and after shodhana process

S. No.	Parameters	*Strychnos nux- vomica*	
		Before shodhana	After shodhana
1	Acids	-	-
2	Alkaloids	+++	+
3	Coumarins	++	+
4	Flavonoids	+	+
5	Glycosides	+	++
6	Phenols	-	-
7	Proteins	+	+
8	Saponins	+	-
9	Steroids	++	+
10	Sugar	-	-
11	Tannins	+++	+

Physico-chemical analysis was done to ascertain the quality of the raw material used in the study. The comparative analysis results of physicochemical parameters for *vishamusthi* samples before and after s*hodhana* processes are tabulated in table 2. The results of all the parameters complying with the Ayurvedic Pharmacopeia of India (API) standards. The percentage of alcohol soluble extractive, ash content, acid-insoluble ash and pH were found reduced and water-soluble extractive, loss on drying at 105°C increased in the *shodhit* (processed) *vishamusthi* seeds as compare to *ashodhit* (unprocessed) sample.

Table 2. Physicochemical parameters of *Strychnos nux-vomica* seeds before and after shodhana process

S. No.	Parameters	*Strychnos nux- vomica*	
		Before shodhana	After shodhana
1	pH (10% w/v aqueous solution)	6.31	5.26
2	Loss on drying at 105°C (% w/w)	7.63	8.18
3	Water soluble extractive (% w/w)	12.24	29.59
4	Alcohol soluble extractive (% w/w)	5.43	4.66
5	Ash content (% w/w)	1.42	1.36
6	Acid-insoluble ash (% w/w)	0.19	0.06

HPTLC finger print analysis of vishamusthi seeds

The obtained residue weights for the ethanol and chloroform extractions given in the table 3.

Table: 3. Extractive values of *Strychnos nux-vomica* seeds before and after shodhana process

S. No.	Name of extracts	Weight of sample (g)		Weight of extract (g)		Extractive value % (w/w)	
		Before	After	Before	After	Before	After
1	Chloroform extract	10.1555	10.0362	0.3219	0.4379	3.1698	3.9750
2	Ethanol extract	10.0337	10.1131	0.3418	0.4020	3.4065	4.3632

A band 254 nm (Green, R_f 0.59) corresponding to brucine is visible in both the reference standard and test solution tracks of chloroform and ethanol extracts of *visamusthi* seeds before and after s*hodhana* process. HPTLC fingerprint profile of *vishamusthi* seeds chloroform & ethanol extracts before and after s*hodhana* process at UV 254 nm, 366 nm and 540 nm showed in Figure 2-5. Rf values of *vishamusthi* seeds chloroform & ethanol extracts before and after s*hodhana* process and brucine reference standard at UV 254 nm, 366 nm and 540 nm showed in Table 4-6.

Table 4. R_f values of *Strychnos nux-vomica* seeds chloroform & ethanol extracts before and after shodhana process and brucine reference standard at UV 254 nm

Tracks	Before shodhana			After shodhana		
	Peak	R_f	Color	Peak	Max R_f	Color
Track: 1 & 5 Chloroform extract	1	0.22	Green	-	-	-
	2	0.25	Green	1	0.24	Green
	3	0.28	Green	2	0.27	Green
	-	-	-	3	0.55	Green
	4	**0.59**	**Dark green**	**4**	**0.59**	**Dark green**
	5	0.62	Dark green	5	0.67	Green
	6	0.69	Green	6	0.84	Green
	7	0.93	Green			
Track: 2 & 4 Ethanol extract	1	0.01	Green	1	0.01	Green
	2	0.08	Dark green	2	0.08	Dark green
	-	-	-	3	0.12	Green
	3	0.20	Green	4	0.17	Green
	4	0.23	Green	-	-	-
	5	0.29	Green	5	0.28	Green
	6	0.39	Green	6	0.37	Green
	7	0.45	Green	-	-	-
	8	0.55	Dark green	7	0.56	Dark green
	9	**0.59**	**Dark green**	**8**	**0.59**	**Dark green**
	10	0.64	Green	-	-	-
	11	0.67	Green	9	0.67	Green
	12	0.74	Green	10	0.72	Green
	-	-	-	11	0.78	Green
	13	0.86	Green	12	0.84	Green
	14	0.91	Green	13	0.91	Green
	15	0.93	Green	-	-	-
Track:3 Brucine Standard	**1**	**0.59**	**Dark green**	**1**	**0.59**	**Dark green**

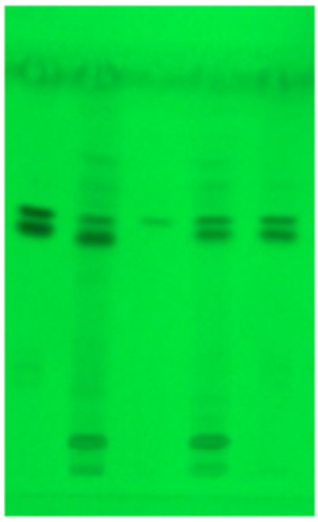
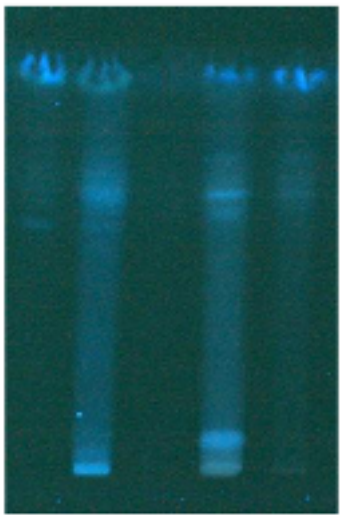
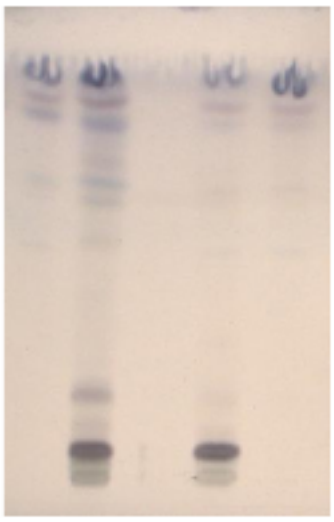
Table 5. R_f values of *Strychnos nux-vomica* seeds chloroform & ethanol extracts before and after shodhana process at UV 366 nm

Tracks	Before shodhana			After shodhana		
	Peak	R_f	Color	Peak	R_f	Color
Track: 1 & 5 Chloroform extract	1	0.59	-	1	0.55	Blue
	2	0.93	Dark blue	2	0.91	Dark blue
	3	0.95	Dark blue	-	-	-
Track: 2 & 4 Ethanol extract	1	0.01	Blue	1	0.01	Blue
	-	-	-	2	0.08	Dark blue
	2	0.56	Blue	3	0.60	Blue
	-	-	-	4	0.65	Blue
	-	-	-	5	0.71	Blue
	3	0.91	Blue	6	0.90	Blue

Table 6. R_f values of *Strychnos nux-vomica* seeds chloroform & ethanol extracts before and after shodhana process at 540 nm

Tracks	Before shodhana			After shodhana		
	Peak	R_f	Color	Peak	R_f	Color
Track: 1 & 5 Chloroform extract	1	0.53	Grey	1	0.53	Grey
	-	-	-	2	0.57	Grey
	2	0.68	Blue	3	0.68	Grey
	3	0.85	Blue	4	0.86	Violet
	4	0.88	Violet	-	-	-
Track: 2 & 4 Ethanol extract	-	-	-	1	0.01	Grey
	1	0.06	Dark grey	2	0.07	Grey
	2	0.12	Grey	-	-	-
	3	0.19	Grey	3	0.17	Grey
	4	0.26	Grey	4	0.31	Grey
	5	0.42	Grey	-	-	-
	6	0.55	Grey	5	0.53	Grey
	7	0.64	Grey	-	-	-
	8	0.68	Blue	6	0.67	Grey
	9	0.73	Grey	-	-	-
	10	0.82	Blue	7	0.83	Blue
	11	0.87	Violet	8	0.86	Violet

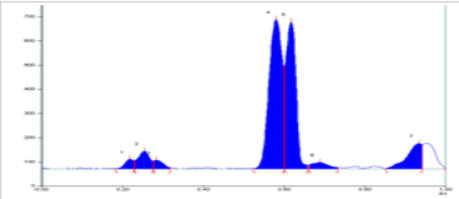
Figure 2. HPTLC fingerprint Profiling of *visamusthi* seeds chloroform & ethanol extracts before and after s*hodhana* process and brucine reference standard

At UV 254 nm	At UV 366 nm	Derivatised with vanillin H_2SO_4
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
		

Chloroform extract: Track-1: Before shodhana; **Track-5**: After shodhana;
Reference standard: Track-3: Brucine
Ethanol extract: Track-2: Before shodhana; **Track-4**: After shodhana

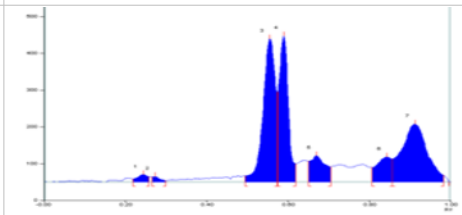
Figure 3. HPTLC fingerprint profile of *visamusthi* seeds chloroform & ethanol extracts before and after s*hodhana* process and brucine standard at UV 254 nm

Chloroform extract before shodhana



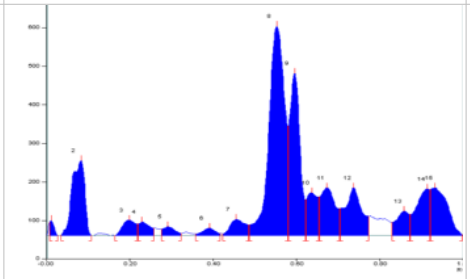
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.18 Rf	1.5 AU	0.22 Rf	40.0 AU	2.93 %	0.23 Rf	34.5 AU	817.8 AU	1.91 %
2	0.23 Rf	34.7 AU	0.25 Rf	73.8 AU	4.62 %	0.27 Rf	32.9 AU	1953.8 AU	4.56 %
3	0.28 Rf	33.3 AU	0.28 Rf	35.8 AU	2.24 %	0.32 Rf	5.5 AU	802.6 AU	1.87 %
4	0.52 Rf	0.7 AU	0.58 Rf	617.2 AU	38.61 %	0.60 Rf	22.3 AU	18554.2 AU	43.28 %
5	0.60 Rf	424.5 AU	0.62 Rf	607.8 AU	38.02 %	0.66 Rf	14.9 AU	14114.6 AU	32.92 %
6	0.66 Rf	16.5 AU	0.69 Rf	25.4 AU	1.59 %	0.73 Rf	4.4 AU	953.2 AU	2.22 %
7	0.88 Rf	19.2 AU	0.93 Rf	99.5 AU	6.22 %	0.94 Rf	34.8 AU	3000.5 AU	7.00 %
8	0.94 Rf	94.7 AU	0.95 Rf	99.3 AU	6.21 %	0.99 Rf	6.2 AU	2674.9 AU	6.24 %

Chloroform extract after shodhana



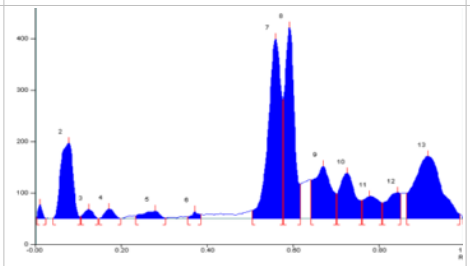
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.22 Rf	8.3 AU	0.24 Rf	20.2 AU	1.81 %	0.26 Rf	14.4 AU	498.8 AU	1.47 %
2	0.26 Rf	14.0 AU	0.27 Rf	14.8 AU	1.32 %	0.30 Rf	3.3 AU	264.7 AU	0.78 %
3	0.49 Rf	16.5 AU	0.55 Rf	389.6 AU	34.82 %	0.57 Rf	44.3 AU	10788.5 AU	31.90 %
4	0.57 Rf	245.1 AU	0.59 Rf	397.3 AU	35.50 %	0.62 Rf	49.2 AU	8356.8 AU	24.71 %
5	0.65 Rf	56.4 AU	0.67 Rf	71.8 AU	6.42 %	0.70 Rf	41.2 AU	2492.4 AU	7.37 %
6	0.81 Rf	39.4 AU	0.84 Rf	67.9 AU	6.07 %	0.85 Rf	52.7 AU	2277.4 AU	6.73 %
7	0.86 Rf	62.7 AU	0.91 Rf	157.4 AU	14.06 %	0.96 Rf	18.3 AU	9145.8 AU	27.04 %

Ethanol extract before shodhana



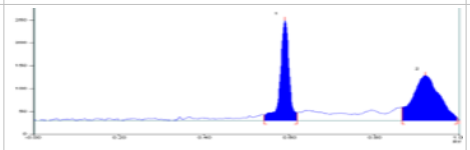
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	33.7 AU	0.01 Rf	39.2 AU	1.96 %	0.03 Rf	0.4 AU	366.2 AU	0.62 %
2	0.05 Rf	0.9 AU	0.08 Rf	194.9 AU	9.72 %	0.11 Rf	0.3 AU	5463.7 AU	9.27 %
3	0.16 Rf	0.2 AU	0.20 Rf	40.2 AU	2.01 %	0.22 Rf	29.7 AU	1155.9 AU	1.96 %
4	0.22 Rf	29.7 AU	0.23 Rf	35.4 AU	1.76 %	0.26 Rf	18.7 AU	885.9 AU	1.50 %
5	0.27 Rf	16.3 AU	0.29 Rf	22.8 AU	1.14 %	0.32 Rf	4.8 AU	593.1 AU	1.01 %
6	0.36 Rf	2.9 AU	0.39 Rf	18.2 AU	0.91 %	0.42 Rf	4.4 AU	509.7 AU	0.86 %
7	0.42 Rf	3.4 AU	0.45 Rf	40.9 AU	2.04 %	0.48 Rf	26.1 AU	1385.1 AU	2.35 %
8	0.48 Rf	25.3 AU	0.55 Rf	541.0 AU	27.02 %	0.58 Rf	32.3 AU	10604.6 AU	31.55 %
9	0.58 Rf	282.5 AU	0.59 Rf	419.5 AU	20.92 %	0.62 Rf	31.0 AU	9530.4 AU	16.16 %
10	0.62 Rf	31.5 AU	0.64 Rf	108.8 AU	5.43 %	0.65 Rf	48.1 AU	2561.3 AU	4.34 %
11	0.65 Rf	98.3 AU	0.67 Rf	122.1 AU	6.09 %	0.70 Rf	37.3 AU	3969.6 AU	6.73 %
12	0.70 Rf	87.6 AU	0.74 Rf	121.5 AU	6.06 %	0.77 Rf	47.7 AU	4423.6 AU	7.50 %
13	0.83 Rf	31.5 AU	0.86 Rf	60.8 AU	3.03 %	0.87 Rf	31.0 AU	1099.1 AU	2.86 %
14	0.87 Rf	52.0 AU	0.91 Rf	117.8 AU	5.88 %	0.92 Rf	16.4 AU	3407.9 AU	5.78 %
15	0.92 Rf	118.5 AU	0.93 Rf	121.1 AU	6.04 %	0.98 Rf	0.0 AU	4425.6 AU	7.51 %

Ethanol extract after shodhana



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	2.8 AU	0.01 Rf	27.0 AU	1.97 %	0.02 Rf	0.4 AU	233.0 AU	0.60 %
2	0.04 Rf	0.7 AU	0.08 Rf	147.5 AU	10.78 %	0.10 Rf	3.8 AU	3807.1 AU	9.73 %
3	0.10 Rf	3.9 AU	0.12 Rf	17.9 AU	1.31 %	0.14 Rf	0.0 AU	346.0 AU	0.88 %
4	0.15 Rf	0.2 AU	0.17 Rf	18.7 AU	1.37 %	0.20 Rf	0.1 AU	375.9 AU	0.96 %
5	0.23 Rf	5.2 AU	0.28 Rf	15.1 AU	1.10 %	0.30 Rf	0.8 AU	530.1 AU	1.36 %
6	0.35 Rf	3.1 AU	0.37 Rf	13.7 AU	1.00 %	0.38 Rf	8.1 AU	198.3 AU	0.51 %
7	0.50 Rf	15.8 AU	0.56 Rf	349.3 AU	25.52 %	0.58 Rf	29.7 AU	9630.4 AU	24.62 %
8	0.58 Rf	238.1 AU	0.59 Rf	372.0 AU	27.19 %	0.62 Rf	56.8 AU	7406.4 AU	18.93 %
9	0.64 Rf	74.8 AU	0.67 Rf	102.4 AU	7.48 %	0.70 Rf	18.4 AU	3718.5 AU	9.51 %
10	0.70 Rf	48.7 AU	0.72 Rf	88.4 AU	6.46 %	0.76 Rf	34.8 AU	2924.4 AU	7.48 %
11	0.76 Rf	35.1 AU	0.79 Rf	43.8 AU	3.20 %	0.81 Rf	30.1 AU	1463.9 AU	3.74 %
12	0.81 Rf	30.1 AU	0.84 Rf	50.9 AU	3.72 %	0.85 Rf	48.5 AU	1442.3 AU	3.69 %
13	0.86 Rf	49.4 AU	0.91 Rf	121.7 AU	8.89 %	0.99 Rf	8.4 AU	7038.3 AU	17.99 %

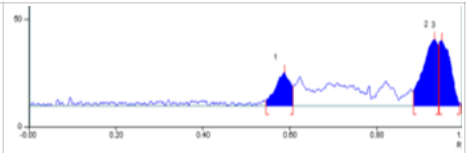
Brucine reference standard



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.54 Rf	10.8 AU	0.59 Rf	217.6 AU	69.76 %	0.62 Rf	15.1 AU	4115.0 AU	44.44 %
2	0.86 Rf	24.9 AU	0.92 Rf	94.3 AU	30.24 %	0.99 Rf	1.1 AU	5144.0 AU	55.56 %

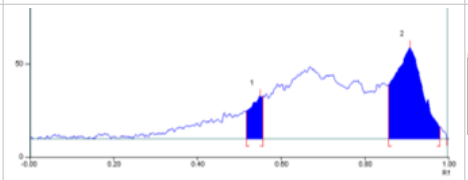
Figure 4. HPTLC fingerprint profile of *visamusthi* seeds chloroform & ethanol extracts before and after s*hodhana* process at UV 366nm

Chloroform extract before shodhana



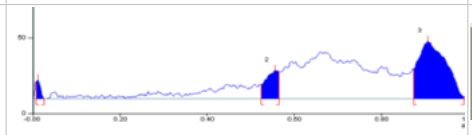
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.54 Rf	2.4 AU	0.59 Rf	15.4 AU	20.13 %	0.61 Rf	9.1 AU	482.6 AU	22.06 %
2	0.86 Rf	6.9 AU	0.93 Rf	30.7 AU	40.26 %	0.94 Rf	28.2 AU	962.7 AU	44.00 %
3	0.94 Rf	28.6 AU	0.95 Rf	30.2 AU	39.61 %	0.99 Rf	1.5 AU	742.8 AU	33.95 %

Chloroform extract after shodhana



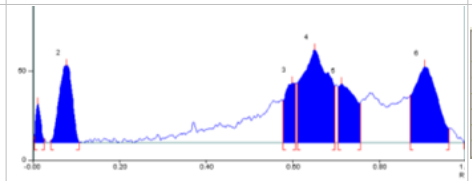
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.52 Rf	14.9 AU	0.55 Rf	22.9 AU	31.85 %	0.56 Rf	22.9 AU	629.4 AU	17.26 %
2	0.85 Rf	28.9 AU	0.91 Rf	49.0 AU	68.15 %	0.98 Rf	6.2 AU	3018.1 AU	82.74 %

Ethanol extract before shodhana



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	11.2 AU	0.01 Rf	12.4 AU	18.14 %	0.02 Rf	0.0 AU	107.6 AU	3.80 %
2	0.52 Rf	9.5 AU	0.56 Rf	18.5 AU	26.99 %	0.56 Rf	17.8 AU	498.3 AU	17.62 %
3	0.87 Rf	19.7 AU	0.91 Rf	37.6 AU	54.86 %	0.99 Rf	1.4 AU	2222.5 AU	78.58 %

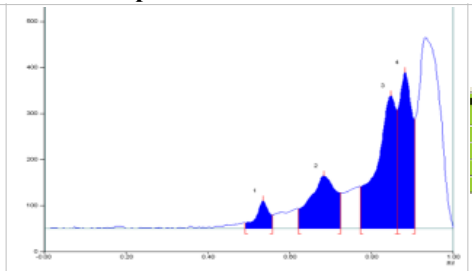
Ethanol extract after shodhana



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	3.5 AU	0.01 Rf	21.7 AU	9.60 %	0.02 Rf	2.3 AU	225.6 AU	2.72 %
2	0.04 Rf	1.4 AU	0.08 Rf	43.3 AU	19.15 %	0.11 Rf	0.2 AU	1197.3 AU	14.45 %
3	0.58 Rf	23.4 AU	0.60 Rf	33.5 AU	14.82 %	0.61 Rf	33.3 AU	732.3 AU	8.84 %
4	0.61 Rf	33.1 AU	0.65 Rf	51.9 AU	22.98 %	0.70 Rf	31.9 AU	2866.7 AU	34.84 %
5	0.70 Rf	30.9 AU	0.71 Rf	33.0 AU	14.60 %	0.75 Rf	22.1 AU	1165.6 AU	14.07 %
6	0.87 Rf	26.6 AU	0.90 Rf	42.6 AU	18.85 %	0.96 Rf	8.0 AU	2079.2 AU	25.09 %

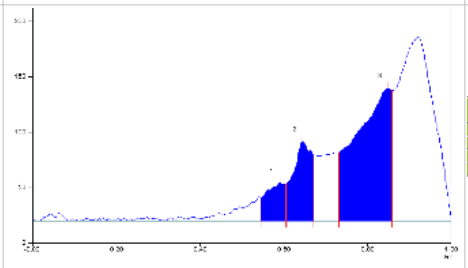
Figure 5. HPTLC fingerprint profile of *visamusthi* seeds chloroform & ethanol extracts before and after *shodhana* process at 540 nm

Chloroform extract before shodhana



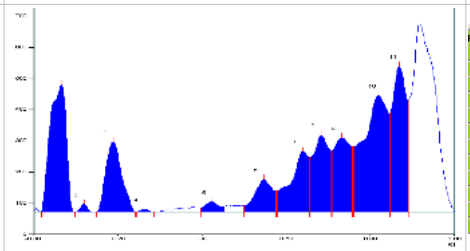
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.49 Rf	12.3 AU	0.53 Rf	60.0 AU	7.49 %	0.56 Rf	28.1 AU	1719.0 AU	5.52 %
2	0.62 Rf	42.9 AU	0.68 Rf	114.4 AU	14.27 %	0.72 Rf	77.6 AU	6732.2 AU	21.60 %
3	0.77 Rf	91.1 AU	0.85 Rf	288.2 AU	35.97 %	0.88 Rf	56.8 AU	12842.0 AU	41.21 %
4	0.88 Rf	256.0 AU	0.89 Rf	338.6 AU	42.27 %	0.90 Rf	37.5 AU	9869.4 AU	31.67 %

Chloroform extract after shodhana



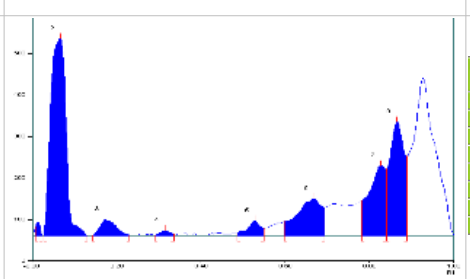
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.48 Rf	11.2 AU	0.53 Rf	41.4 AU	6.81 %	0.55 Rf	20.9 AU	1194.0 AU	4.85 %
2	0.55 Rf	21.0 AU	0.57 Rf	31.5 AU	6.70 %	0.58 Rf	29.8 AU	557.9 AU	2.27 %
3	0.61 Rf	29.0 AU	0.66 Rf	97.9 AU	20.84 %	0.70 Rf	23.3 AU	4800.0 AU	20.15 %
4	0.75 Rf	82.2 AU	0.88 Rf	200.1 AU	69.85 %	0.89 Rf	24.6 AU	17850.7 AU	72.73 %

Ethanol extract before shodhana



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	1.5 AU	0.06 Rf	408.3 AU	17.60 %	0.10 Rf	0.0 AU	14430.8 AU	16.48 %
2	0.10 Rf	0.1 AU	0.12 Rf	26.0 AU	1.12 %	0.14 Rf	0.3 AU	389.8 AU	0.45 %
3	0.15 Rf	0.1 AU	0.18 Rf	225.7 AU	9.73 %	0.24 Rf	0.2 AU	7871.3 AU	8.99 %
4	0.24 Rf	0.1 AU	0.26 Rf	10.0 AU	0.43 %	0.28 Rf	0.1 AU	172.0 AU	0.20 %
5	0.40 Rf	12.4 AU	0.42 Rf	32.7 AU	1.41 %	0.45 Rf	16.3 AU	1057.7 AU	1.21 %
6	0.50 Rf	19.5 AU	0.55 Rf	104.4 AU	4.50 %	0.58 Rf	57.1 AU	4099.7 AU	4.68 %
7	0.58 Rf	66.8 AU	0.64 Rf	168.3 AU	8.33 %	0.66 Rf	74.4 AU	7830.7 AU	8.94 %
8	0.66 Rf	174.6 AU	0.68 Rf	249.1 AU	10.57 %	0.71 Rf	94.6 AU	8699.0 AU	9.93 %
9	0.71 Rf	194.7 AU	0.73 Rf	236.1 AU	10.18 %	0.76 Rf	36.4 AU	8778.5 AU	10.02 %
10	0.76 Rf	208.4 AU	0.82 Rf	371.5 AU	16.02 %	0.84 Rf	12.8 AU	19921.1 AU	22.75 %
11	0.85 Rf	313.3 AU	0.87 Rf	466.2 AU	20.10 %	0.89 Rf	55.4 AU	14361.0 AU	16.40 %

Ethanol extract after shodhana



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	21.8 AU	0.01 Rf	32.6 AU	2.90 %	0.02 Rf	0.2 AU	298.2 AU	0.77 %
2	0.03 Rf	2.6 AU	0.07 Rf	474.8 AU	42.25 %	0.13 Rf	0.2 AU	16028.2 AU	41.37 %
3	0.14 Rf	0.6 AU	0.17 Rf	39.5 AU	3.52 %	0.23 Rf	2.0 AU	1372.5 AU	3.54 %
4	0.29 Rf	1.8 AU	0.31 Rf	12.1 AU	1.07 %	0.34 Rf	4.4 AU	282.3 AU	0.73 %
5	0.48 Rf	5.7 AU	0.53 Rf	35.5 AU	3.16 %	0.55 Rf	16.9 AU	1042.9 AU	2.69 %
6	0.60 Rf	33.0 AU	0.67 Rf	88.8 AU	7.90 %	0.69 Rf	57.1 AU	4887.4 AU	12.61 %
7	0.78 Rf	83.2 AU	0.83 Rf	167.6 AU	14.91 %	0.84 Rf	58.8 AU	6009.8 AU	15.51 %
8	0.84 Rf	158.9 AU	0.86 Rf	273.0 AU	24.29 %	0.89 Rf	30.4 AU	8824.6 AU	22.78 %

HPLC chromatographic analysis of *vishamusthi* seeds

In comparison of HPLC Chromatographic profiling of chloroform extracts, 10 peaks in unprocessed and 08 peaks in processed samples were detected, presented in Figure 6. HPLC Profiling Chromatogram of *vishamusthi* seeds showed 20 peaks in unprocessed and 6 peaks in processed samples of each ethanol extracts were detected as revealed by Figure 7. It is observed that the peak area of all peaks of processed samples was reduced as compared to unprocessed samples. The results of detailed peak identification and peak area of *vishamusthi* seeds in chloroform and ethanol extracts before and after s*hodhana* process, are presented in Table 7 and 8. The remarkable changes have been observed in the chloroform and ethanol extracts HPLC profiling chromatograms of the *visamusthi* seeds before and after *shodhana* process.

Table 7. HPLC peak details of *Strychnos nux-vomica* seeds chloroform extracts before and after shodhana process

Unprocessed sample				Processed sample			
Peak No.	**Ret. Time [min]**	**Area [mAU*s]**	**Area %**	**Peak No.**	**Ret. Time [min]**	**Area [mAU*s]**	**Area %**
1	1.502	30.06450	0.2583	1	1.456	32.65804	0.6177
2	1.622	18.43875	0.1584	2	1.626	8.16754	0.1545
3	1.693	13.35016	0.1147	3	1.699	8.50321	0.1608
4	1.887	9.62238	0.0827	4	1.959	14.31698	0.2708
5	2.001	13.45921	0.1156	5	3.939	105.70403	1.9993
6	2.356	13.76966	0.1183	6	11.439	597.53699	11.3018
7	3.944	199.26936	1.7122	7	15.165	876.25647	16.5734
8	9.736	14.43178	0.1240	8	23.248	3643.96851	68.9217
9	10.281	4085.67187	35.1056	-	-	-	-
10	13.886	7240.17236	62.2101	-	-	-	-
Total	1.16383E+04	**100.0000**	**Total**	5287.11175	**100.0000**		

Table 8. HPLC peak of *Strychnos nux-vomica* seeds ethanol extracts before and after shodhana process

Unprocessed sample				Processed sample			
Peak No.	**Ret. Time [min]**	**Area [mAU*s]**	**Area %**	**Peak No.**	**Ret. Time [min]**	**Area [mAU*s]**	**Area %**
1	1.495	117.95510	0.6796	1	1.489	22.73930	4.4118
2	1.620	1704.65710	9.8213	2	1.591	103.70794	20.1213
3	1.847	101.61682	0.5855	3	1.870	12.80527	2.4845
4	2.032	320.67776	1.8476	4	2.029	20.26344	3.9315
5	2.602	79.79147	0.4597	5	2.760	29.12658	5.6511
6	2.888	449.43628	2.5894	6	4.061	326.77200	63.3998
7	3.345	6267.98975	36.1128	-	-	-	-
8	3.701	52.62699	0.3032	-	-	-	-
9	3.879	67.14089	0.3868	-	-	-	-
10	3.984	72.32104	0.4167	-	-	-	-
11	4.126	85.54170	0.4928	-	-	-	-
12	4.368	1400.76794	8.0705	-	-	-	-
13	4.578	946.41052	5.4527	-	-	-	-
14	4.731	1320.47620	7.6079	-	-	-	-
15	4.836	1958.20703	11.2821	-	-	-	-
16	5.269	1091.63794	6.2894	-	-	-	-
17	5.519	191.54636	1.1036	-	-	-	-
18	5.638	199.44115	1.1491	-	-	-	-
19	5.775	116.65503	0.6721	-	-	-	-
20	5.898	811.80676	4.6772	-	-	-	-
Total	**1.73567E+04**	**100.0000**	**Total**	**515.41453**	**100.0000**		

Figure 6. HPLC Profiling Chromatogram of *visamusthi* seeds chloroform extracts before and after *shodhana* process

Unprocessed sample | **Processed sample**

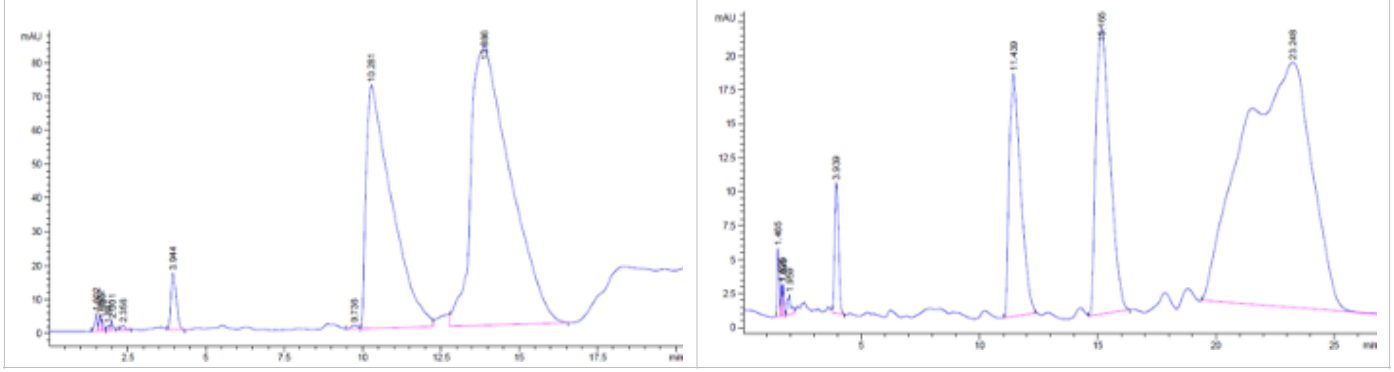
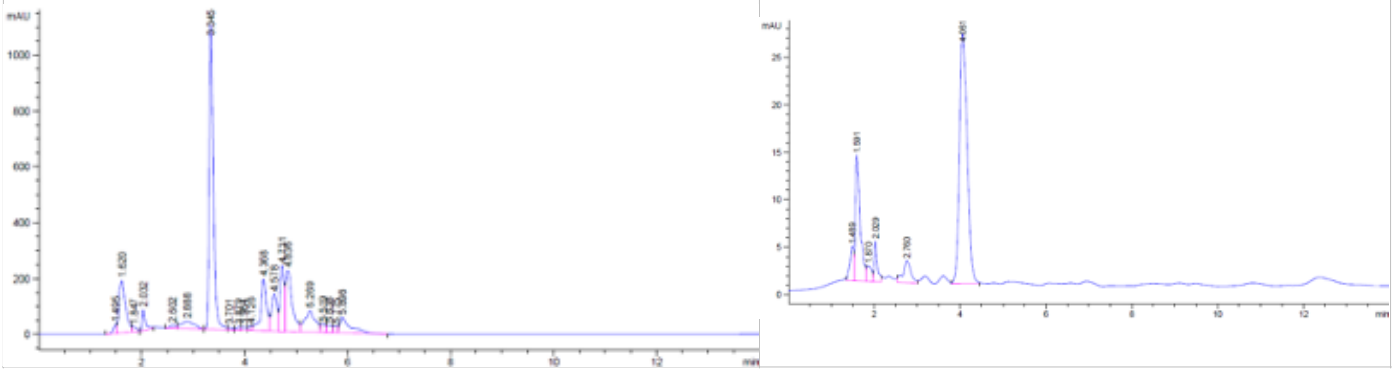


Figure 7. HPLC Profiling Chromatogram of *visamusthi* seeds ethanol extracts before and after *shodhana* process

Unprocessed sample | **Processed sample**



Quantitative analysis of brucine in vishamusthi seeds by HPLC

Calibration curve was established for peak area Vs concentration of brucine applied, has been shown in figure 8. The amount of brucine present in the residues extracted in ethanol and chloroform for each test sample obtained before and after *shodhana* samples of *vishamusthi* seeds, has been provided in table 9 while respected chromatograms has been showing in figure 9. The results obtained from HPLC analysis revealed that the remarkable depletion in the level of brucine after *shodhana* process of *vishamusthi* seeds as compared to unprocessed seeds. HPLC analysis showed that the percentage of Brucine was reduced 79.66% in chloroform extract and 64.54% in ethanol extract after *shodhana* process respectively. The HPLC chromatogram of *vishamusthi* seeds corresponding to standard brucine was showed at a retention time of 6.787 min, at 210 nm wavelengths.

Figure 8. HPLC Chromatogram of brucine Standard and Calibration curve

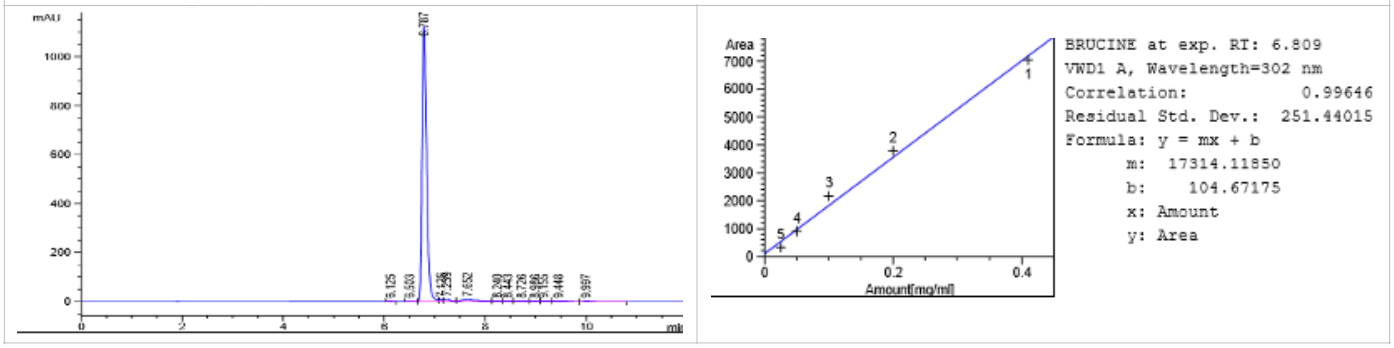
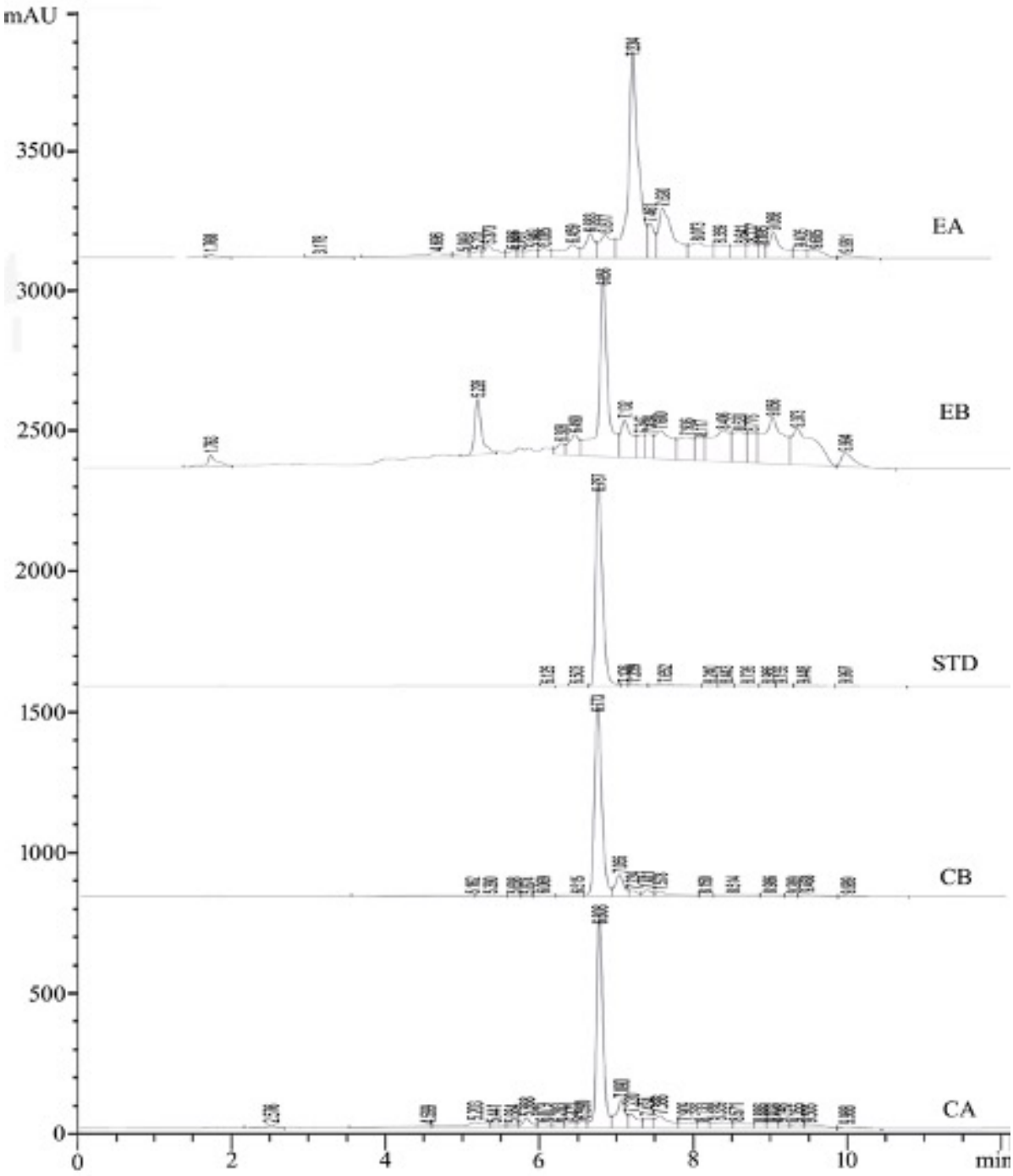


Table 9. Estimation of Brucine in the *Strychnos nux-vomica* of chloroform and ethanol extracts of before and after shodhana process

S. No.	Name of extracts	Brucine (% w/w)				
		Before shodhana		After shodhana		Percentage reduced
		Results	Mean	Results	Mean	
1	**Chloroform extract**	0.1582	0.1567	0.0317	0.0319	79.66%
		0.1571		0.0315		
		0.1549		0.0324		
2	**Ethanol extract**	0.0084	0.0085	0.0030	0.0030	64.54%
		0.0085		0.0030		
		0.0086		0.0030		

*Percentage of results was given from the means of triplicates for both before and after shodhana samples of chloroform and ethanol extracts.

Figure 9. Estimation of brucine in *visamusthi* seeds chloroform and ethanol extracts before and after *shodhana* process



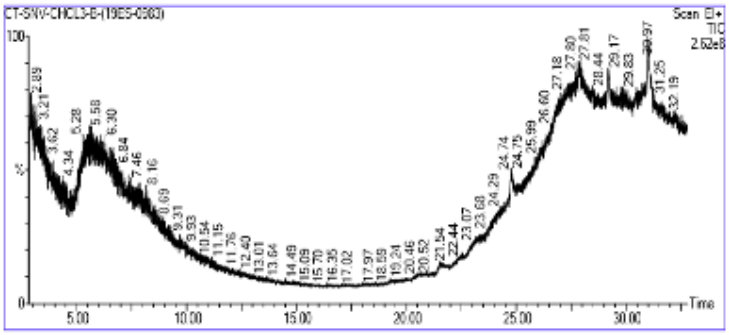
EB:Ethanol extract before shodhana; **EA:** Ethanol extract after shodhana; **STD:** Brucine Standard; **CB:** Chloroform extract before shodhana; **CA:** Chloroform extract after shodhana

GC-MS Chromatographic analysis of vishamusthi seeds

The chloroform extracts of the *visamusthi* seeds before and after *shodhana* process, the processed and unprocessed samples are compared under the same chromatographic conditions. GC-MS analysis of chloroform extracts of *visamusthi* seeds shows the presence of 18 peaks in unprocessed sample and 10 peaks in processed sample. The detailed peak identification showed in Figure10 and retention time, peak area, area percentage, compound name and molecular weight are presented in Table 10. The remarkable changes have been observed in the chloroform extracts, GC-MS profiling chromatograms of the *Visamusthi* seeds before and after *shodhana* process.

Figure 10. GC-MS Chromatogram of *visamusthi* chloroform extracts before and after *shodhana* process

Unprocessed sample



Processed sample

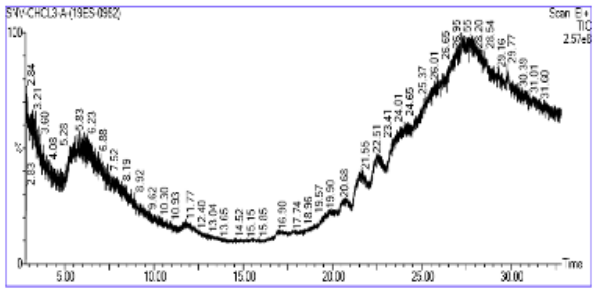


Table 10. GC-MS Peak of *Strychnos nux-vomica seeds* chloroform extracts before and after shodhana process

Unprocessed Sample

Peak	RT	Area	Area %	Name of the compound	Molecular weight
1	5.194	12,92,763.1	1.689	Trichloromethane	118
2	5.499	11,80,258.8	1.542		118
3	6.395	10,94,078.6	1.429		118
4	26.818	28,64,305.8	3.742	2-Methyl-6-methylene-octa-1,7-dien-3-ol	152
5	26.873	23,38,793.0	3.055	Oleic acid	282
6	27.033	34,07,232.8	4.451	4-Tetradecanol	214
7	27.123	21,27,486.2	2.779	Estran-3-one, 17- (acetyloxy) -2-methyl-, (2.alpha. 5. alpha., 17.beta.)-	332
8	27.323	79,17,713.5	10.343	Pseduosarsasapogenin-5, 20-dien methyl ether	428
9	27.459	19,63,992.4	2.566	Hexadecane, 1,16-dichloro	294
10	27.699	1,07,40,507.0	14.030	Pentanoic acid, 2-(aminooxy)-	133
11	27.899	1,07,29,815.0	14.016	3-Methyl-4-(phenylthio)- 2-prop- 2-enyl-2,5-dihydrothiophene 1,1-dioxid	280
12	28.059	1,01,48,400.0	13.257	Pentadecanoic acid	242
13	28.439	14,52,697.0	1.898	D-Mannitol, 1-O-(22-hydroxydocosyl)-	506
14	28.534	37,58,858.5	4.910	Pseduosarsasapogenin-5,20-dien	414
15	29.064	10,52,095.8	1.374	D-Mannitol, 1-O-(22-hydroxydocosyl)-	506
16	29.169	45,72,997.5	5.974	D-Mannitol, 1-decyl sulfonyl	370
17	30.975	87,84,969.0	11.476	2,7-Octadiene-1,6-diol, 2,6-dimethyl-, (Z)-	170
18	31.600	11,24,873.8	1.469	T-Butyl cyclopentaneperoxy carboxylate	186

Processed sample

Peak	RT	Area	Area %	Name of the compound	Molecular weight
1	25.508	26,44,144.0	2.315	Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy -1-methoxy-7-(7-methoxy-1,3-	388
2	25.713	21,82,052.8	1.911	Z-2-Acetoxy-12- etradecenitrile	279
3	25.873	22,49,412.8	1.970	Undecanoic acid, 10-bromo	264
4	26.148	34,02,081.0	2.979	2,7-Octadiene-1,6-diol, 2,6-dimethyl -, (Z)-	170
5	27.218	4,81,04,500.0	42.122	Pentanoic acid, 2-(aminooxY)-	133
6	27.829	3,62,07,884.0	31.705	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta. 4.alpha.)-	398
7	28.539	91,18,143.0	7.984	1-Naphthalene propanol, alpha -ethyldecahydro-5-(hydroxymethyl)-.	308
8	29.059	45,00,372.0	3.941	Oleic acid	282
9	29.504	18,60,675.4	1.629	2,6-Lutidine 3,5-dichloro-4-dodecylthion	375
10	29.770	39,33,129.0	3.444	Pregnan-3,11-diol- 20-one	334

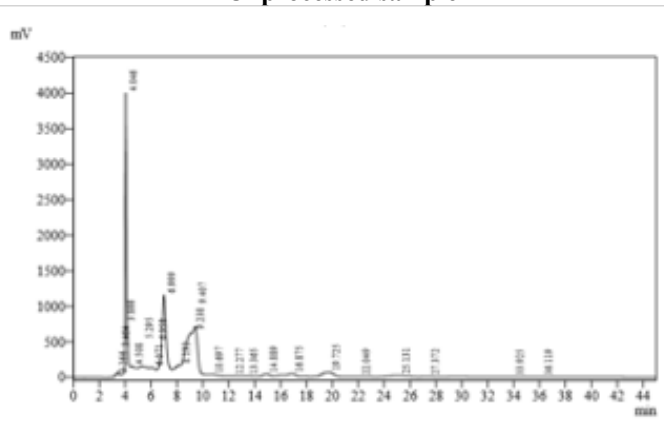
LC-MS Chromatographic analysis of vishamusthi seeds

The ethanol extracts of the *visamusthi* seeds before and after *shodhana* process, the processed and unprocessed samples are compared under the same chromatographic conditions. LC-MS Chromatographic profiling of ethanol extracts of *visamusthi* seedsshows the presence of 23 peaks in unprocessed sample and 20 peaks in processed sample. The detailed peak identification shown in Figure 11 and retention time, peak area and area percentage are

given in Table 11. The remarkable changes have been observed in the ethanol extracts LC-MS profiling chromatograms of the *visamusthi* seeds before and after *shodhana* process.

Figure 11. LC-MS Chromatogram of *visamusthi* ethanolic extracts before and after *shodhana* process

Unprocessed sample



Processed sample

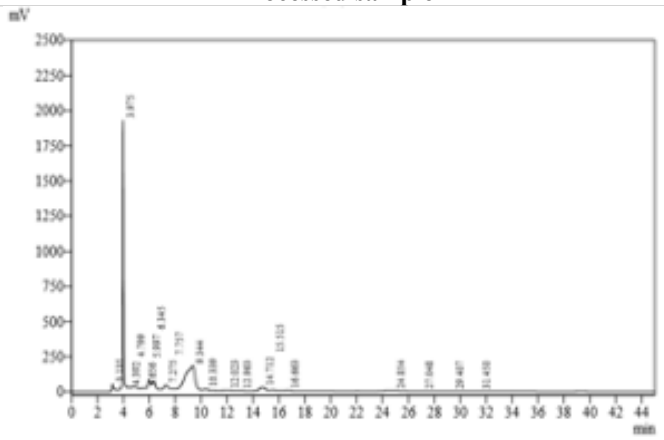


Table 11. LC-MS Peaks details of *Strychnos nux-vomica* seeds ethanol extracts before and after shodhana process

Unprocessed sample				Processed sample			
Peak	**Ret. Time**	**Area**	**Area %**	**Peak**	**Ret. Time**	**Area**	**Area %**
1	3.266	385112	0.371	1	3.135	474782	2.040
2	3.464	720513	0.695	2	3.975	8737746	37.542
3	3.866	417639	0.403	3	4.392	23029	0.099
4	4.046	28046821	27.055	4	4.799	256553	1.102
5	4.508	3152079	3.041	5	5.656	100787	0.433
6	5.295	5187641	5.004	6	5.997	982434	4.221
7	6.071	1393362	1.344	7	6.345	892718	3.836
8	6.358	278831	0.269	8	7.275	710395	3.052
9	6.999	22335767	21.546	9	7.757	122688	0.527
10	8.132	254842	0.246	10	9.344	8812150	37.862
11	9.238	18812919	18.148	11	10.339	181320	0.779
12	9.407	13118424	12.655	12	12.023	79821	0.343
13	10.697	261677	0.252	13	12.963	16394	0.070
14	12.277	62142	0.060	14	14.712	856245	3.679
15	13.365	103001	0.099	15	15.515	259091	1.113
16	14.889	1082368	1.044	16	16.663	175862	0.756
17	16.875	2242967	2.164	17	24.854	453449	1.948
18	19.725	3623200	3.495	18	27.048	27101	0.116
19	22.049	131007	0.126	19	29.407	92291	0.397
20	25.131	1746849	1.685	20	31.450	19523	0.084
21	27.372	172334	0.166	-	-	-	-
22	33.925	32968	0.032	-	-	-	-
23	36.119	102793	0.099	-	-	-	-
Total	**103665258**	**100.000**	**Total**	**23274378**	**100.000**		

Discussion

The preliminary phytochemical investigation revealed the presence alkaloids, coumarins, flavonoids, glycosides, proteins, saponins, steroids and tannins. However, there was a decrease in alkaloids, coumarins, steroids and tannins content in processed samples as compared to unprocessed samples. Moreover, there was an increase glycoside content in processed samples as compared to unprocessed samples. The decrease in alkaloidal content, might be an indication of decrease in toxic alkaloidal constituent strychnine and brucine. The water-soluble extractive value in *vishamusthi* seeds was increased approximately 2.5 folds after in processed samples as compared to unprocessed samples. Whereas, the alcohol soluble extractive value and ash content (also acid insoluble ash) was decreased in processed samples as compared to unprocessed samples. Chloroform as well as ethanolic extractive values (% w/ w) and weight of extract (g) were increased in processed *vishamusthi* as compared to unprocessed *vishamusthi*. Mitra et al reported that, owing to the alkaline nature of cow's urine [pH 8.10 (ranging from pH7.27-8.71)], it facilitates the extraction of alkaloidal content like strychnine as well as brucine from nux-vomica. In HPTLC chromatographic analysis, brucine content was observed at UV spectrum at 254, 366 and 540 nm with respect to standard brucine (R_f 0.59). Cai et al reported that, alkalinity of cow's urine initiated the extraction process and further it was potentiated by milk as boiling in milk converted the strychnine into less toxic isostrychnine [19].

HPLC chromatographic analysis revealed a significant decrease in the number of peaks in processed samples as compared to unprocessed samples of both chloroform and ethanolic extracts. There was more than two fold decrease in peaks were observed in the processed samples of ethanolic extract. Quantitative analysis of brucine by HPLC confirmed a significant decrease in brucine content to 79.66% and 64.54% in processed samples of chloroform and ethanolic extract, respectively. It has been reported that brucine content in raw/crude (Unpurified/unprocessed) *vishamusthi* seed is 0.77%. Whereas, brucine content after purification in cow's milk and is reduced to 0.68%. It is worthy to mention that brucine content after sequential purification in cow's urine, milk, ghee is 0.57% [20].

GC-MS analysis of chloroform extracts of *vishamusthi* seeds shows the presence of 18 peaks in unprocessed sample and 10 peaks in processed sample. Whereas, LC-MS Chromatographic profiling of ethanol extracts of *vishamusthi* seeds shows the presence of 23 peaks in unprocessed sample and 20 peaks in processed sample. V. G. S. Sharma & Reddy reported that acute toxic dose of *vishamusthi* unprocessed seed ethanolic extract was ranging from 15-50 mg/kg, whereas seeds purified in media (Cow's urine, milk and ghee) showed acute toxic dose ranging from 300-600 mg/kg [21].

Many Ayurveda formulations containing *vishamusthi* seeds are used vatadosha (mainly responsible for pain and neurological disorders). Some of them formulations are *Karaskara ghritham*, *Agnitundi rasa*, *Navajivana rasa*, *Lakshmivilasa rasa*, *Sulnirmulana rasa*, *Supti vatari rasa*, *Sarameya vishahara yoga*, *Vishatinduka taila*, *Krimimudgara rasa*, *Mahavishagarbha taila*, *Ekangavira rasa* and *Vishatinduka vati.*

Conclusions

The percentage of alcohol soluble extractive, ash content, acid-insoluble ash and pH were found reduced and water-soluble extractive, loss on drying at 105°C increased in the shodhit (processed) *vishamusthi* seeds as compare to ashodhit (unprocessed) sample. The remarkable changes have been observed in different physicochemical parameters, HPTLC, HPLC, GC-MS and LC-MS chromatographic profiling before and after *shodhana* process of *Vishamusthi* seeds. In the HPLC chromatographic profiling it is observed that the peak area of all peaks of processed samples was reduced as compared to unprocessed samples.

HPLC studies revealed that the process of s*hodhana* resulted in depletion of more toxic brucine, which is the chief poisonous constituent of *vishamusthi* seeds. Study showed that the percentage of brucine was reduced 79.66% in chloroform extract and 64.54% in ethanol extract after s*hodhana* process respectively.

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