Research Article

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COMPARATIVE PHYSICO-CHEMICAL PROFILE OF 'VATSANABHA' (*Aconitum ferox*, Ranunculaceae) MULA PROCESSED THROUGH COW'S URINE AND COW'S MILK

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Received on: 30/07/17 Accepted on: 02/09/17

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DOI: 10.7897/2277-4343.085278

ABSTRACT

Toxic effects of Vatsanabha (*Aconitum ferox* Wall.) Mula (root), a poisonous plant drug, are well noted in Ayurvedic texts. This may even be fatal and so is recommended to be used only in proper dose and after proper processing (Shodhana) with some specific media. The concept of Shodhana (purificatory measures) in Ayurveda is not only a process of detoxification but also a process to enhance the potency and efficacy of the drug. In Ayurvedic literature, media like Gomutra (Cow's urine) and Godugdha (Cow's milk) has been reported for processing of Vatsanabha. This study focuses on evaluation of these two Shodhana (purification) processes. Vatsanabha Mula was processed by using Gomutra (cow's urine) and Godugdha (cow's milk) and the raw roots were taken as control. Changes in every physicochemical parameter confirm the effect of purification on Vatsanabha. No change was observed in the pH value of two samples. In HPTLC analysis, spots detected under both 254nm and 366nm were less in number after purification with cow's urine compared to purification with cow's milk, indicating denaturation of some component after purification. Hence, Vatsanabha Mula purified by Gomutra (cow's urine) may be regarded as better method of purification as far as toxic alkaloids are concerned.

Keywords: Vatsanabha Mula, Alkaloids, Shodhana, Analytical study, HPTLC.

INTRODUCTION

Aconite (Vatsanabha) found its place in Ayurvedic Pharmacopoeia for centuries back with its first possible mention in Atharvaveda.[1]The most common aconite-based medicinal plant Vatsanabha (*Aconitum ferox* Wall.) is a therapeutically potent plant in Ayurveda and its roots are being used extensively in different classical formulations.[2] The plant is reported under the poisonous group. A purification process of Aconite root which was supposed to reduce the toxic effect of the drug without compromising its pharmacological properties was thus described in many ancient Ayurvedic pharmacopoeias. It is cited in the treatises of Ayurveda, the Visha (poison) becomes Amrita (nectar) after logical administration.[3] If properly administered it helps in rejuvenation of the body.[4] In classical text books of Ayurveda, actions of Vatsanabha are explained as cardiac tonic and imparts strength. Vatsanabha is indicated in Fever, Rheumatoid Arthritis, and Sciatica.[5] It is used as an ingredient in preparations of many formulations such as Mrityunjaya rasa, Hinguleshwara rasa, Anandabhairwa Rasa.[6] The tuber of Vatsanabha contains 0.4–0.8% diterpene alkaloids and the concentration of aconite in the fresh plant is between 0.3% and 2.0% in tubers and 0.2% and 1.2% in the leaves. The highest concentration of aconite is found in the winter.[7] Major alkaloids are aconitine, pseudaconitine, bikhaconitine, diacetyl pseudaconitine, aconine, picroaconine, veratry pseudaconitine, chamaconitine, veratryl gama aconine, and di-Ac–Y-aconitine.[8] It has been reported that cow's urine converts Aconite to a compound with a cardiac stimulant property, whereas raw Aconite showed cardiac depressant properties.[9] Its major alkaloid aconitine has the chemical formula $C_{34}H_{47}NO_{11}$, and is soluble in chloroform or benzene, slightly in alcohol or ether, and only very slightly in water. It is a neurotoxin that opens TTX-sensitive Na+ channels in the heart and other tissues, and is used for creating models of cardiac arrhythmia.[10] In Ayurveda; different methods are in practice for the specific Shodhana (purificatory measures) procedures of Vatsanabha.

Therefore, in the present study, the effect of two different methods of processing mentioned in the ancient Ayurveda scriptures was compared (i.e. with cow's milk and cow's urine).

MATERIALS AND METHODS

Collection and selection of drug

Fully matured Vatsanabha (*Aconitum ferox* Wall.) roots were collected from the local market of Haridwar in India during the month of December and were botanically authenticated by pharmacognosists and sample specimens were kept in the museum for future reference.

Cow's urine (Gomutra) and Cow's milk (Godugdha) were collected from the local cow shed in the morning at 6 A.M. and were used for purificatory measures of the root.

Equipment for Shodhana (Purificatory measures)

Stainless steel tray (48 cm × 30 cm × 7 cm); capacity of 3 L, stainless steel vessel (20 cm × 30 cm) having capacity of 7 L, used as Dolayantra, stainless steel rod (28 cm.), Stainless steel vessel (48 cm x 30 cm x7 cm); capacity of 3L., cotton thread 30 cm. in length, measuring mug (capacity of 1 L), muslin cloth (45 cm × 45 cm), stainless steel spatula (length 30 cm), digital weighing machine, pyrometer, digital induction cooker.

Preparation of sample

The raw (RVM) and purified Vatsanabha (Both GDSV &

GMSV) roots were powdered with mechanical grinder and passed though mesh no. 60.

Physico chemical parameters

Assessment of the parameters such as organoleptic characters, pH with pH paper moisture content, Total ash value, water soluble extractive value alcohol soluble extractive value, acid insoluble ash was carried out following standard procedures recommended by Ayurvedic Pharmacopoeia of India(API).[11]

Determination of pH Value
Method

pH meter and Electrode System was operated according to the manual instructions. At the end of a set of measurements, reading of the solution used for standardizing the meter and electrodes was taken. This reading should not differ by more than 0.02 from the original value at which the apparatus was standardized. One gram sample was put in 5ml of water and pH of the solution was determined.[12]

Determination of Total Ash Value

Two grams of accurately weighed sample was taken and transferred to the cleaned, dried and weighed silica crucible and was subjected to ignition using electric furnace at 450ºC for an hour. Silica crucible was taken out from the furnace and was allowed to cool and weighed. After cooling the weight of the ash was obtained and the ash value of sample was calculated.[13]

Determination of Acid Insoluble Ash

2g of sample was digested with 25 ml diluted hydrochloric acid for 5 minutes, then filtered through Whatman paper and washed with water. The residue was taken in a crucible dried and ignited, allowed to cool and weighed.[13]

Determination of Water Soluble Ash

Ash was boiled for 5 minutes with 25 ml of water. Insoluble matter was collected in an ash less filter paper; washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450ºC. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in the weight represents the water soluble ash. Percentage of water soluble ash was calculated with reference to the air dried drug.

HPTLC study
Chemicals

Percolated silica gel 60 F254 TLC aluminium plates (10×10 cms, 0.2mm thick), AR grade toluene, ethyl acetate, glacial acetic acid, methanol was obtained from M/S Merck Ltd. Mumbai, India.

Samples for HPTLC

The extract of two samples (Sample 1(GDSV) & Sample 2(GMSV)) for HPTLC, were made in same process as mentioned below. 1. Methanolic extract - 2 g of sample was macerated with 20ml of methanol for 24 hrs & filtered. Filtrate was concentrated to 5ml & used for spotting. The samples were titled as Track-1, Track-2.

Track-1: Methanolic extract of Sample 1(GDSV).

Track-2: Methanolic extract of Sample 2(GMSV).

Mobile phase: Toluene: Ethyl acetate: Glacial acetic acid (6.5: 3.5: 0.2) v/v/v

Detection: Spray with Vanilline-H2SO4Application mode - Camag Linomat V
Development Chamber - Camag Twin through Chamber
Plates - Precoated Silica Gel GF254 Plates.
Chamber Saturation - 30 min.
Development Time -30 min.
Development distance -7 cm
Scanner - Camag Scanner III.
Detection - Deuterium lamp, Tungsten lamp
Data System - Win cats software

Procedure

Each purificatory measure was carried out in three different batches by using two different media (cow's milk and cow's urine) individually.

Purificatory measures of Vatsanabha using cow's urine as media[14]

100 g of clean and dried roots of Vatsanabha were made into pea sized pieces and were kept in one litre of cow's urine for three consecutive days. Each day the cow's urine was replaced with the fresh one. On the fourth day, the roots were washed with water; the outer cortical layers were peeled off and the product was again washed with warm water. The dried pieces were pulverized and kept in an air tight glass container and the final product was labelled as Gomutra (cow's urine) shodhita (purified) Vatsanabha (GMSV).

Purificatory measures of Vatsanabha using cow's milk as media[15]

100 g of Vatsanabha was tied in a muslin cloth into a poultice which was suspended in the centre of a pot with the help of a stick. Cow's milk was poured in the vessel to completely immerse the bundle. It was then heated on a stove for six hrs at 100°C. Later, the pieces of Vatsanabha were taken out and washed with water. The outer cortical layers of the roots were peeled off. After proper drying the Vatsanabha pieces were then made into powder and kept in an air tight glass container as 'GDSV powder'.

RESULT AND DISCUSSION

Net loss of 32% and 36% of Vatsanabha root was observed after processing in Cow's urine and Cow's milk respectively (Table 1).

Both urine and milk purified vatsanabha samples were evaluated for organoleptic characters such as color, odor, taste and appearance. (Table 2)

Physicochemical parameters such as Moisture Content, ash values, extractive values including the pH were determined. (Table 3)

It was observed that the moisture content of GMSV was comparatively lower than the raw and GDSV (Table 3).

Presence of Moisture content affects the physical, chemical aspects of drug which relates with the freshness and stability for the storage for a long period of time.[16]

Table 1: Effect on yield of final product after purification with Cow's Milk and Cow's Urine

S.No	Sample	Initial Quantity(g)	Final Weight (Avg.) (g)	% of weight loss
1	**GDSV**	100	64	36
2	**GMSV**	100	68	32

Table 2: Organoleptic characters of the sample

S.No	Features	Impure Vatsanabha	GDSV	GMSV
1	**Colour**	Brown	Pale yellow	Light brown
2	**Odour**	Typical	Characteristic of milk	Odourless
3	**Touch**	Hard	Soft	Slightly soft
4	**Taste**	Bitter	Sweetish Bitter	Salty Bitter
5	**Appearance**	Smooth	Dull	Dull

Table 3: Physicochemical Parameters of Raw and Purified Vatsanabha

Tests	Vatsanabha (R)	Vatsanabha (GMSV) %w/w	Vatsanabha (GDSV) %w/w
pH	6.08	6.07	6.08
Moisture Content	9.64	12.70	12.78
Acid insoluble Ash	1.24	1.38	1.38
Water Soluble extractive value	17.71	13.85	14.21
Alcohol Soluble extractive value	4.88	3.43	3.92
Total Ash	4.93	5.11%	5.11

RVM= Raw Vatsanabha Mula; GMSV = Gomutra shodhita Vatsanabha Mula; GDSV= Godugdha shodhita Vatsanabha Mula

Table 4: Rf value in Short UV 254 nm of the Methanolic extract of the samples

S.No	Sample	No. of spot	R_F Value
1	**GDSV**	13	0.02, 0.13, 0.18, 0.21, 0.27, 0.29, 0.36, 0.42, 0.46, 0.74, 0.79, 0.86, 0.93,
2	**GMSV**	12	0.02, 0.10, 0.13, 0.17, 0.22, 0.28, 0.33, 0.40, 0.63, 0.77, 0.85, 0.93,

GMSV = Gomutra shodhita Vatsanabha Mula; GDSV= Godugdha shodhita Vatsanabha Mula

Table 5: Rf value in Short UV 366 nm of the Methanolic extract of the samples

S.No	Sample	No. of spot	R_F Value
1	**GDSV**	9	0.02, 0.13, 0.16, 0.21, 0.24, 0.26, 0.41, 0.65, 0.90
2	**GMSV**	5	0.01, 0.10, 0.17, 0.23, 0.92

GMSV = Gomutra shodhita Vatsanabha Mula; GDSV= Godugdha shodhita Vatsanabha Mula

HPTLC profile

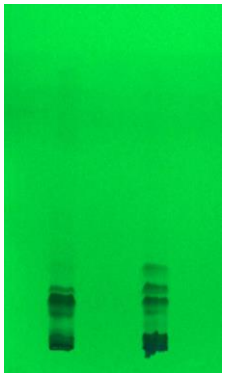


Figure 1: Short UV 254 nm

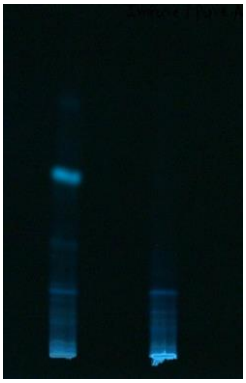
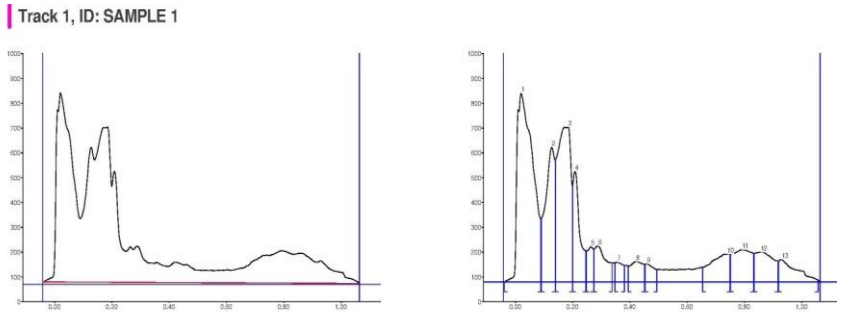


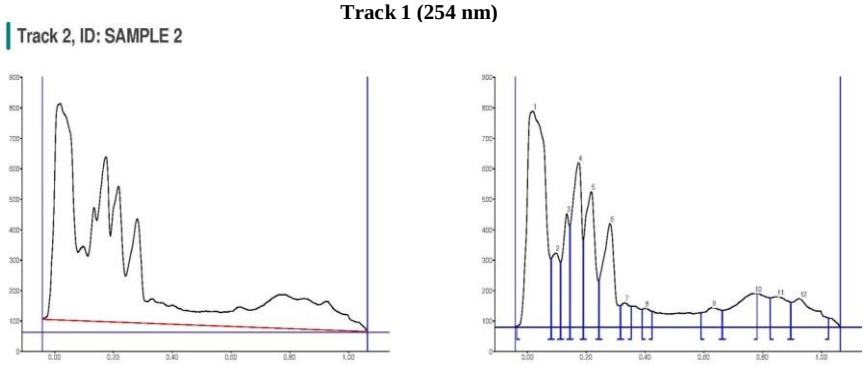
Figure 2: Long UV 366 nm

Figure 3: After spraying

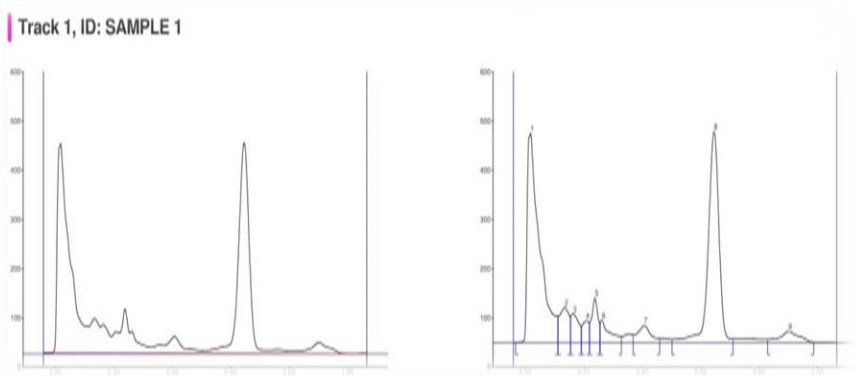
Track-1 HPTLC for Methanolic extract of Vatsanabha purified with Cow's Milk
Track-2 HPTLC for Methanolic extract of Vatsanabha purified with Cow's Urine



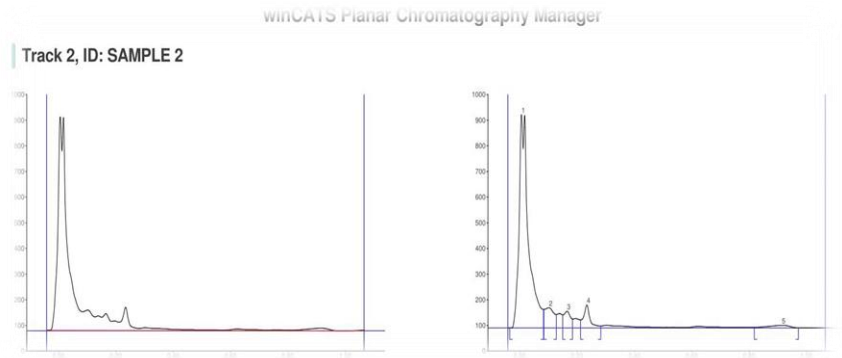
Track 1 (254 nm)



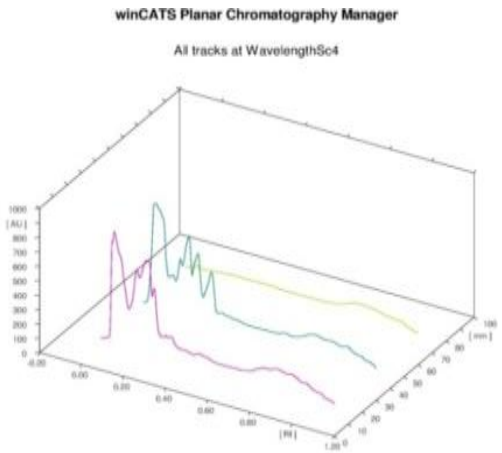
Track 2 (254 nm)



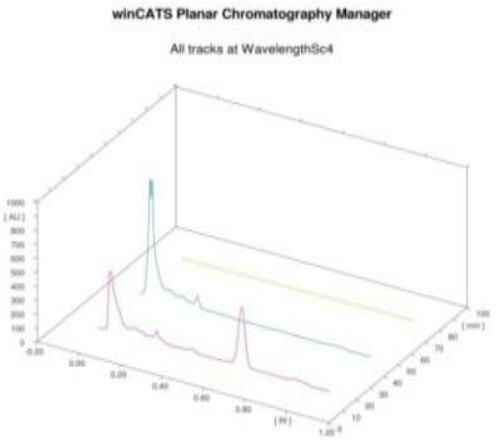
Track 1 (366 nm)



Track 2 (366 nm)



Multiple Tracks at 254 nm



Multiple Tracks at 366nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	0.2	0.02	425.7	34.91	0.11	53.7	14962.3	39.85	unknown *
2	0.11	53.8	0.13	70.3	5.77	0.15	53.1	1909.6	5.09	unknown *
3	0.16	53.3	0.16	57.9	4.75	0.19	34.1	1301.5	3.47	unknown *
4	0.19	34.3	0.21	44.1	3.62	0.22	40.8	813.9	2.17	unknown *
5	0.22	41.1	0.24	90.3	7.40	0.26	41.1	1610.0	4.29	unknown *
6	0.26	41.7	0.26	44.6	3.66	0.33	12.8	1199.7	3.20	unknown *
7	0.37	15.5	0.41	34.3	2.81	0.46	9.2	1320.7	3.52	unknown *
8	0.50	6.2	0.65	429.1	35.19	0.71	7.6	13157.9	35.04	unknown *
9	0.83	6.1	0.90	23.0	1.89	0.99	0.4	1274.7	3.39	unknown *

Showing Rf values of Vatsanabha purified with Cow's Milk in Densitometric scan at 366 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	0.1	0.01	831.5	77.16	0.08	72.7	23052.2	79.27	unknown *
2	0.08	72.7	0.10	79.1	7.34	0.13	52.7	2220.7	7.64	unknown *
3	0.15	51.3	0.17	65.8	6.11	0.18	35.1	1369.3	4.71	unknown *
4	0.21	32.2	0.23	90.7	8.41	0.28	8.0	1742.9	5.99	unknown *
5	0.82	2.7	0.92	10.5	0.97	0.97	0.4	695.3	2.39	unknown *

Showing Rf values of Vatsanabha purified with Cow's Urine in Densitometric scan at 366 nm

Figure 4: Densitogram of Standard (visible Spectrum Comparison)
Track-1- HPTLC for Methanolic extract of Vatsanabha purified with Cow's Milk
Track-2-HPTLC for Methanolic extract of Vatsanabha purified with Cow's Urine

It was observed that the percentage of water-soluble extractive values were higher than alcohol soluble extractives values in both the samples.

Ash value was increased in both cases after purification. It was observed that all samples were acidic (Table 3). No change was observed in the pH value of two samples. Lower the pH value indicates more acidic in nature, which is more capable to inhibit microbes.

In HPTLC, at short UV 254nm (Figure 1&4), different spots were found in both the samples indicating presence of different components. (Table 4) Presence of three common Rf values (0.02, 0.13 and 0.93) in both the samples (Figure 4) indicates the presence of one common component in both the samples. At long UV 366 nm (Figure 2 & 4) Godugdha shodhita Vatsanabha Mula (GDSV) and Gomutra shodhita Vatsanabha Mula (GDSV) showed 9 and 5 spots respectively. (Table 5)

CONCLUSION

From this study, it is concluded that shodhana (purification) alters the physicochemical parameters of Vatsanabha Mula and also the Rf value of the sample in HPTLC. Less Numbers of

spots were detected under both 254nm and 366nm after Shodhana with gomutra, indicating denaturation of some component after Shodhana. From this study it may be concluded that for purification of Vatsanabha Mula, Gomutra is a better extraction media than Godugdha as far as toxic alkaloids are concerned.

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Cite this article as:

Neha Arya and Hemant Kumar. Comparative physico-chemical profile of 'Vatsanabha' (*Aconitum ferox*, Ranunculaceae) mula processed through cow's urine and cow's milk. Int. J. Res. Ayurveda Pharm. 2017;8(5):217-222 http://dx.doi.org/ 10.7897/2277-4343.085278

Source of support: Nil, Conflict of interest: None Declared