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SCIENTIFIC VALIDATION ON SIDDHA PURIFICATION PROCESS OF NABHI

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ABSTRACT: Suddhi is a unique process of detoxification which is employed topotentiate as well as enhance therapeutic properties the effect of raw drugs. Nabhi (*Aconitum ferox*), a herbal poisonous rhizome commonly known as aconitum is used extensively in various Siddha formulations, with great therapeutic significance. Siddha system recommends the administration of Nabhi only after suddhi (purification) in different medias. Hence in this pretext one of the purification process of Nabhi mentioned in Theraiyar Yemaga Venba book gets validated scientifically with alleged reasoning through estimation of aconitine by HPTLC method and along with physicochemical parameters.The amount of Aconitine present is found to be reduced from 0.2107 % to 0.00 % after purification. In this present scenario may be presumed that the toxic compound aconitine might have been underwent transformation into some nontoxic compounds like Benzyl aconine and Aconine.

INTRODUCTION: According to Siddha doctrines, everything found in nature is having two qualities, nalvinai (good effect) and thee vinai (bad effect). Similarly the raw drugs also possess two extreme qualities like therapeutic effect, toxic effect [1]. Likewise Nabhi (*Aconitum ferox*), a herbal poisonous rhizome commonly known as aconitum is used extensively in various Siddha formulations, with great therapeutic significance. Nabhi Roots are traditionally used in treatment of Scorpion sting, Snake bite [2]. It has Diaphoretic, Diuretic, Anti periodic, Anodyne, Narcotic and Sedative actions. It contains major toxic alkaloids such as aconitine and pseudoaconitine [3]. Siddha system recommends the administration of Nabhi only after suddhi (purification) in different medias [4].

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The word "Suddhi" means "to get rid of impurities". Suddhi is a unique process of detoxification which is employed to purify/detoxify and to potentiate the effect of various kinds of raw drugs used in Siddha formulations with a view to reduce their toxic contents /effects as well as to enhance their therapeutic properties [5]. In Siddha system, all the drugs must be purified individually as told in the text before converting them into medicine; the process of purification of drugs are variable according to the the medicine that is to be prepared. Since Many Siddha formulations containing Nabhi roots become more popular, methods for purification of Nabhi is in demand. Besides that since now no scientific validation of changes that occurred during purification process of Nabhi is carried out.

Hence in this pretext one of the purification process of Nabhi gets validated scientifically with alleged reasoning through estimation of aconitine by HPTLC method and along with physicochemical parameters.

MATERIALS AND METHODS: Nabhi was collected from reputed shop in Assam, identified and authenticated by botanist, National institute of Siddha, Chennai. (Voucher No: NISMB 1262014)

Method of Purification: 35gm of Nabhi was taken and soaked it in 210 gm of butter milk (Media 1), placed in sunlight from sun rise to sun set, continued this process for 10 days, after that repeated the same with cow's butter (Media 2). Again the same process was repeated with cow dung solution for another 10 days (Media 3). Then take the sample out and was dried in sun light for another two more days [7].

Sample:

1. Raw Nabhi, Sample
2. Nabhi obtained after soaking in butter milk, sample
3. Nabhi obtained after soaking in butter, Sample
4. Nabhi obtained finally after soaking in cow dung solution

Organoleptic evaluation, Physiochemical analysis, Analysis of microbial load were done for Sample 1 and 4 [8-11]. The pH of aqueous extract of sample 1, sample 4 and all the medias used in different stages of purification process were estimated as per the method prescribed in the Indian standard (IS) - 6940(1982) using DIGISUN digital pH meter. Preliminary phytochemical analysis were done for Sample 1, Sample 4 and all the medias used individually [12]. Aconitine level estimated in sample 1&4 by TLC and HPTLC analysis as perthe procedures recommended for the analysis of aconitine Wagner H and Bladt S, 1996 [13-4].

RESULT:

Morphological Examination: Organoleptic evaluation of sample 1 and sample 4 described below in **Table 1.**

TABLE 1: ORGANOLEPTIC EVALUATION OF SAMPLE 1 AND SAMPLE 4

S.no.	Organoleptic Evaluation	Sample 1 (Raw Nabhi)	Sample 4 (Purified Nabhi)
1.	Colour	Blackish brown outside and white inside.	Blackish colour reduced. Sandal colour inside.
2.	Odour	Odourless	Butter smell
3.	Surface Characterstics	Rough and hard, Brittleness absent.	Hardness reduced. Brittleness absent but slightly softer than earlier.

Table 2: Colour changes in Medias used in different stages of purification process: The medias used in various stages of purification process were observed to determine the colour changes before and after soaking Nabhi. The results are noted below.

TABLE 2: COLOUR CHANGES IN MEDIAS USED IN DIFFERENT STAGES OF PURIFICATION PROCESS

Sl .no	Medias used	Intial colour of media before soaking drug	Final colour of media after soaking drug
1	Butter milk	White in colour	Brownish white
2	Butter	Yellow in colour nearing to white.	Greenish yellow solution with dispersed white colour
3	Cow dung solution	Solution was Greenish colour	Solution turned to Greenish black

Physico -Chemical Analysis: Physico- chemical analysis of sample 1 and sample 4 were done and the results are illustrated in **Table 3**. The pH of aqueous extract of sample 1, sample 4 and all the medias used in different stages of purification process were shown in **Table 4-7**.

A. Foreign matter: The sample 1 and sample 4 were tested for the presence of any foreign matter, but the given samples were free from them.

TABLE 3: PHYSICO-CHEMICAL ANALYSIS OF SAMPLE 1 AND SAMPLE 4

S. No.	Parameter	Results		Permissible limit
		Sample 1	Sample 4	
1.	Loss on drying at 105^{o}C	10.52%	11.33%	1-20%
	Ash Values			
	a. Total Ash	3.46%	1.19%	1-25%

2.	b. Acid Insoluble Ash	.82%	.53%	1-10%
	Extract Values			
3.	a. Water	17.72%	3.38%	4-85%
	b. Alcohol	4.46%	3.31%	4-85%

C. pH of Nabhi and Various Medias at Different Stages of Purification Process.

TABLE 4: pH OF ALL THE SAMPLES OF NABHI OBTAINED DURING VARIOUS STAGES OF PURIFICATION PROCESS

Sample	pH
Sample 1	6.2
Sample 2	6.7
Sample 3	6.1
Sample 4	7.9

TABLE 5: AVERAGE CHANGE IN pH OF MEDIAS BEFORE AND AFTER PURIFICATION

Medias used	Average change in pH of medias before soaking Nabhi	Average change in pH of medias after soaking Nabhi
Media 1	5.15	3.2
Media 2	5.25	3.26
Media 3	5.1	3.5

Preliminary Phytochemical Screening: Preliminary phytochemical analysis were done for aqueous extract of sample 1, 4 and the medias used in various stages of purification procedure so as to detect the Phytoconstituents present them. The result of the analysis were illustrated in **Table 6**.

TABLE 6: PRELIMINARY PHYTOCHEMICAL SCREENING OF SAMPLE 1 AND 4, VARIOUS MEDIAS

S.no	Procedures	Media 1		Media 2		Media 3		Samples	
		B/F	A/F	B/F	A/F	B/F	A/F	Sample 1	Sample 4
1	Test for Phenolic compounds	–	–	–	–	–	–	–	–
2	Test for reducing sugar	+	+	+	+	+	+	+	+
3	Test for carbohydrates	+	+	+	+	+	+	+	+
4	Test for Flavanoid	–	–	–	–	–	–	–	–
5	Test for Glycosides	–	–	–	–	–	–	–	–
6	Test for steroids	+	+	+	+	+	+	–	–
7	Test for Alkaloids	–	++	–	++	+	++	++	++
8	Test for Anthraquinones	–	–	–	–	–	–	–	–
9	Test for Quinones.	–	–	–	–	–	–	–	–
10	Test for Aminoacids	–	–	–	–	–	+	–	–
11	Test for saponins	–	–	–	–	–	–	+	+
12	Test for proteins	+	+	+	+	+	+	–	–
13	Test for Tannins	–	–	–	–	–	–	–	–

(+, ++ depends on strength and frequency of colour change, B/F before soaking Nabhi, A/F after soaking Nabhi, Media 1: Cow's butter milk, Media 2: Cow's butter, Media 3: cow dung)

Determination of Microbial Load: The determination of microbial load as described below was carried out in sample 1 and 4 as per the WHO guidelines (Anonymous 1998) and the results were indicated in **Table 7.**

TABLE 7: MICROBIAL LOAD OF SAMPLE 1 AND 4

S. No.	Parameters	Results		Permissible Limit for Internal use
		Sample 1	Sample 4	
1	Total Bacterial Count (TBC)	$8x10^3$ cfu/g	$5x10^3$ cfu/g	10^5cfu/g
2	Total Fungal Count (TFC)	Less than 10 cfu/g	Less than 10 cfu/g	10^3cfu/g
3	Enterobacteriaceae	Absent	Absent	10^3cfu/g
4	*Escherichia coli*	Absent	Absent	10 cfu/g
5	*Salmonella Spp*	Absent	Absent	Absent
6	*Staphylococcus aureus*	Absent	Absent	Absent

B. Heavy Metal Analysis: In this study heavy metal analysis was done for both the samples 1 and 4 so as to determine heavy metal contamination as per standard WHO Guidelines using AAS. The results were shown in **Table 8**.

TABLE 8: HEAVY METAL ANALYSIS OF SAMPLE 1 AND SAMPLE 4

S. No.	Name of the Element	Sample 1	Sample 4	Permissible Limit
1	Lead	0.1052 ppm	Not detected	10 ppm (WHO)
2	Cadmium	Not detected	Not detected	0.3 ppm (WHO)
3	Arsenic	Not detected	Not detected	3 ppm (API)
4	Mercury	Not detected	Not detected	1 ppm (API)

E. Estimation Aconitine by HPTLC Method: Estimation aconitine by HPTLC method done based on recommended procedures for the analysis of TLC and HPTLC analysis as per Wagner H and Bladt S, 1996 and the results obtained with reference to aconitine in Sample 1 and 4 described in tables **Fig. 1.**

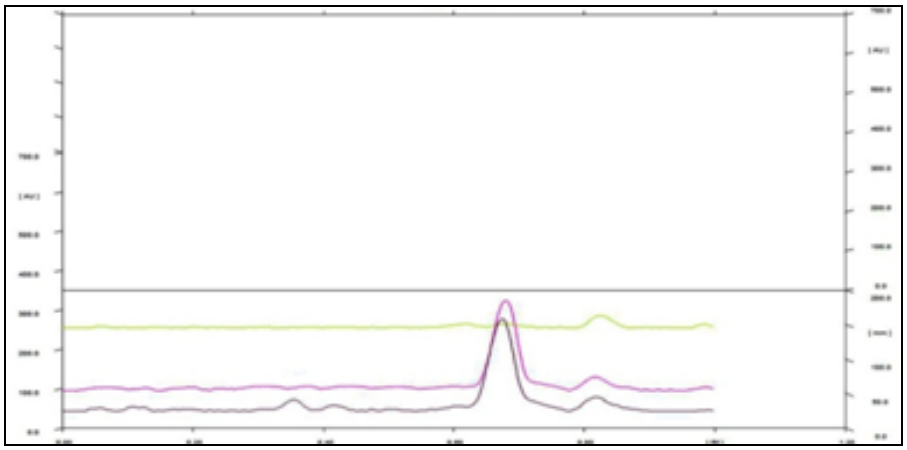

FIG. 1: DENSITOMETRIC CHROMATOGRAM OF SAMPLE - 1, 4 AND ACONITINE

Percentage of Aconitine: The amount of Aconitine present in the samples calculated from the calibration curve of Aconitine. The amount of Aconitine present in Sample 1 is: 0.2107 %; Sample 4 is: 0.00 %.

DISCUSSION: In this present study an attempt was made for deriving a monograph for it and to standardise the purification process by using some sophisticated instrumental studies as per WHO Guidelines [15]. On morphological examination it was revealed that there were considerable changes in organoleptic characters in raw sample and purified sample. Besides it was also interesting that there were some observable changes found in the media used. Physiochemical evaluation is still of important in deciding identity of a drug. Ash value determination furnishes the basis for judging the identity and cleanliness of any drug and gives information relative to its adulteration/ contamination with inorganic matter. Here there was considerable change in ash value from 3.46% to 1.19%, indicating reduction impurities during purification procedure. Loss on Drying of the Nabhi before and after purification (10.52% to 11.33 %) was found within acceptable range (1%-20%), which implies that the drug can be stored for a long period and would not easily be attacked by microbes and moulds. As acid-insoluble ash the samples decreases after purification process (0.82 to 0.53) shows that the reduction in of inorganic adulterants [16-8]. Alcohol, water soluble extractive of raw sample reduced from 4.46%, 17.72% to 3.31%, 3.38% in purified sample respectively. However the water soluble extractive values showed marked reduction after purification indicating loss of water soluble constituents from Aconitum ferox[18].

Phytochemical investigation was performed for Sample1 and sample 4, showed the presence of Carbohydrates, alkaloids, Reducing sugars, Saponins. After purification all the medias were subjected for phytochemical analysis, Showed the presence of alkaloids which was not present earlier

in it. It is also quite interesting that there were some observable changes in colour of media and pH of media after purification process. It was noted that the media become more acidic after purification procedure, Increase in pH of the drug after purification (6.2 to 7.9) encourages the drug absorption in intestine [19-20].

The microbial load was detected within normal limits in the both the samples ensured safety and efficacy of drug during ingestion. It was noted that total microbial count found to be 8×10^3 in Sample 1as compared 5×10^3 in sample 4. The physicochemical and phytochemical characteristics obtained confirmed the effectiveness and stability of the Drug after purification [21-2].

Apart from these as per WHO Quantitative analysis is also essential to ensure safety and efficacy of drug [23-5]. Based on this the drug was quantitatively analysed for heavy metals Content by AAS. Heavy metal analysis Complied with the limits prescribed.

According to WHO Chromatography is better to estabilish identity of a particular chemical constituent reported to be present in the drug [26-7]. Hence HPTLC analysis was carried to estimate the amount of aconitine present in raw Aconitum ferox and purified Aconitum feoxby using reference standard aconitine. The amount of Aconitine present in raw Aconitum ferox is found to be reduced from 0.2107 % to 0.00 % in purified one.

The reduction of aconitine can be explained by means of hydrolysis [28-9]. This may be the possible mechanism that Aconitine undergoes hydrolysis in dilute acid or basic medium. Here the used medias like butter milk and butter are acidic and the Cow dung is essentially alkaline in nature. Sunlight is essential to increase the temperature of the medium, this suggest the possibility of hydrolysis of aconitine, readily to less toxic material like aconine and benzyl aconine [30].

The Physical and chemical changes that occurs in medias also played an important role to assure the detoxification of Nabhi. It may be speculate that emulsification property of butter milk and alkaline nature of cow dung possibly mitigates the toxic alkaloid in Nabhi. From this it can be implicated that the medias like butter milk dissolves the soluble polar compounds like aconitine by its emulsificant property. The results obtained from the study could be utilized as a reference for setting limits for the reference standards for the quality control and quality assurance of Aconitum ferox.

CONCLUSION: From this study, it can be concluded that the concept of detoxification procedure as mentioned in Siddha text provides contemporary evidence with good scientific background. These explorations will definitely help to set a standard procedure for purification of Aconitum ferox. Subsequent validation for studying it's detoxification through pharmacological profile is essential.

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CONFLICT OF INTEREST: None declared.

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