Research Article

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Pharmaceutical Study of Aconitum Heterophyllum (Ativisha)Shodhana and Analysis Based on Hptlc and Hplc

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ABSTRACT

Ativisha (Aconitum heterophyllum), a prominent drug in the Ranunculaceae family, is extensively cited in the Charaka and Sushruta Samhitas for its therapeutic efficacy. However, its potent diterpene alkaloids necessitate "Shodhana" (systematic purification) to ensure clinical safety and enhance pharmacological action. This review examines classical Shodhana protocols and their impact on the plant's chemical profile, specifically evaluating how these traditional processes mitigate toxicity. This work highlights the importance of classical Shodhana process in optimizing the bioavailability of phytochemical metabolites and impact on the plant's chemical profile ensuring the safety of herbal interventions in primary healthcare.

Keywords: Ativisha; Shodhana; Nighantu; HPTLC; HPLC; Swedana

INTRODUCTION

A vital component of ayurvedic system is *Ativisha* (*Aconitum heterophyllum*), a Himalayan herb revered by practitioners as *Balanam Sarvada Pathya*[1] (always wholesome for children) for its pediatric efficacy. While classical texts like the *Charaka Samhita*[2] quote it as *Deepaniya, Pachaniya, Sangrahai* and *Sarvdoshaharananam*, explains its use for obesity and gastrointestinal disorders, its potent phytochemical profile requires cautious ***Shodhana*** (purification). *Shodhana* of *Ativisha* is a critical pharmaceutical intervention to refine *its* immune-modulatory properties while ensuring its clinical safety and to perform a **qualitative analysis for secondary metabolites, HPTLC and HPLC.**

The ***Shodhana*** process is essential to transform the raw tuber into a safe therapeutic agent. This introduction explores how systematic purification protocols optimize the herb's pharmacological benefits.

DESCRIPTION OF ATIVIHSA [3]

Latine name : *Aconitum heterophyllum* Wall.

Family : Ranunculaceae

Synonyms : *Aruna, Ghunapriya, Visha, Shishu Bhaishajya, Shuklakanda, Bhangura.*

Classification according to Bhruhatrayi, Laghutrayi and Nighantu:[4]

· ***Charka***- Lekhaniya, arsoghna, tikta skandha, sirovirecana

· ***Sushruta***- Pippalyadi, mustadi, vacadi

· ***Ashtang Sangraha***- Lekhaniya, Arsoghna, Pippalyadi, Mustadi, Vacadi

· ***Astanga Hrdaya***- Pippalyadi, Mustadi, Vacadi

· ***Dhanvantari Nighantu***- Guducyadi Varga

· ***Sodhala Nighantu***- Guducyadi Varga, Anekartha Varga

· ***Kaiyadeva Nighantu***- Osadhi Varga

· ***Bhav Prakasa Nighantu***- Haritakyadi Varga

· ***Raja Nighantu***- Pippalyadi Varga, Upavisa Gana

· ***Priya Nighantu***[5]- *Shatapushpadi Varga*

Properties of Ativisha [6]

Rasa : *Tikta* (bitter)*, Katu* (pungent)

Guna : Laghu, Ruksha

Virya : *Ushna* (Hot potency)

Vipaka : Katu

Karma : *Dipana, Pachana, Sanghrahika, Kaphapittahara*

Definition of Shodhana7

In AFI, Shodhana is explained as process of elminiating impurities and increase drug potency. *Shodhana* is a purification process in which *kshalana* (washing), *Mardana* (pounding), *Bhavana* (Levigation), *Swedana* (boiling), *Bharjana* (frying), *Nirvana* (Heating & dipping in specified liquids) are carried out on drugs to eliminate impurities and enhance their therapeutic use.

Importance of Shodhana

1. Eliminate physical & chemical impurities from raw drug.

2. detoxifies drug by Neutralizing toxins

3. Enhance therapeutic effects of drug.

4. Impart desirable organic properties.

5. Make drugs safe and suitable for administration.

METHODOLOGY

Shodhana of *Ativisha* is not described in *Bhruhatrayi*, *Laghutrayi* and *Nighantus* except *rajnighantu.*

Shodhana of Ativihsa 8

Reference - *Raj Nighantu (Pippaliadi Varga)*

Principle method - *Swedana*

Equipment - Heater, weighing machine, container, Spatula.

Ingredients - *Ativisha* (120gm)*, Gomaya* (1kg)

Pre-procedure- 1 kg of *Gomaya* was taken in stainless-steel vessel and 4 times of water was added in it. It was heated over medium flame until the liquid was reduced to 1/4th and then filtered through cotton cloth. 1 litre of *Gomaya kwatha* was prepared.

Procedure-

Ativisha was dried in sunlight to remove moisture and all the physical impurities were removed. *Ativisha* was pounded in *Ulukhala Yantra* and tied into *Pottali* using clean cotton cloth. Then it was subjected to *Swedana* using *Gomaya kwatha* in *Dolayantra* for 3 hours.

After 3 hours*, Pottali* was untied, and *Ativisha* washed with hot water and dried in sun light until dries completely. *Shuddha Ativisha* was collected and stored in air tight container.

Table 1: Observation after *Shodhana* process:

Colour	Brown
Taste	Bitter
odour	*Gomaya Sadrishya*

Precautions-

Physical impurities need to be eliminated.

Medium heat is used for *Dolayantra Swedana*

Properly dried under sunlight.

Proper gloves in hand and face mask should be used.

Table 2: Result of *Ativisha Shodhana*

Initial weight of *Ativisha* (g)	After removing impurities and pounding (g)	After *Swedana* (g)	After drying (g)	Loss (%)
120	95	124	92	23.3%

ANALYTICAL STUDY

Alkaloids, carbohydrate, proteins and amino acids, saponins, glycosides, quinones, flavonoids, terpenoids are present in the leaf, root and stem of *Aconitum heterophyllum*.

HPTLC High-Performance Thin-Layer Chromatography (HPTLC) is a widely utilized analytical technique that offers significant advantages in the qualitative and quantitative analysis of a variety of chemical substances.

HPLC (high performance liquid chromatography) is a type of liquid chromatography, meaning the mobile phase is a liquid. The information that can be obtained using HPLC includes identification, quantification, and resolution of a compound.

HPTLC and HPLC used to determine qualitative analysis of secondary metabolites in *Aconitum heterophyllum* (*Atis*) roots

RESULTS

Qualitative analysis of secondary metabolites in Aconitum heterophyllum (Atis) roots

Table 3: Showing secondary metabolites in *Aconitum heterophyllum* (*Atis*) roots

S. No	Sample name	Alkaloids	Flavonoid	Phenol	Saponin	Tannin
1	*Aconitum heterophyllum* roots	Positive	Positive	Positive	Positive	Negative

HPTLC Fingerprinting of Aconitum heterophyllum roots

Preparation of sample solution: 10 g of sample grounded and then 100 mL of methanol is added the sample is sonicated for 30 min. after that the sample solution is filtered and the clear solution was used for analysis.

Chromatographic condition

Stationary phase : TLC Silica gel 60 F_{254} Aluminium sheet

Mobile phase : Chloroform: Methanol (8:2)

Saturation time : 20 minutes

Band length : 8.0 mm

Injection volume : 10 µL

Migration distance : 70 mm

Visualization : At 254 nm and 366 nm UV

Interpretation: In HPTLC fingerprints bands of phyto-chemicals were observed under UV 254 nm and 366 nm. This is specific fingerprint for roots of Aconitum heterophyllum in the mentioned chromatographic conditions.

Peak profiling of Aconitum heterophyllum RM by HPLC-PDA

Experimental Methodology: Analysis was performed on Prominence-i HPLC system (Shimadzu, Japan). Separation was achieved using a Shodex C18-4E (5 µm, 4.6*250 mm) column subjected to binary gradient elution. The two solvents used for the analysis were water containing 0.1% Acetic acid in water (solvent A) and Acetonitrile (solvent B). Column temperature was kept 30 0C and flow was set 1.0 mL/min during the analysis. 10 µL of standard and test solution were injected. Wavelength set at 345 nm.

Gradient program:

Table 4: showing gradient program

Time (Min)	Solvent B %
0	5
5	10
15	15
25	15
30	20
50	30
60	60
65	80
70	5
75	5

Sample Preparation: 2.0 gm sample was dissolved in 25 mL of (Water: methanol:: 5:5) by sonicating for 30 min then centrifuged at 9000 rpm for 10 min and filtered using 0.45µ nylon filters then used for the analysis.

Chromatogram

Contents (Area %)

Table 5: Showing Retention time with area (%)

S. No	Retention time	Area	Area%
1	3.12	22502	1.09
2	3.95	19936	0.966
3	11.15	150966	7.316
4	12.001	169346	8.207
5	13.062	271120	13.139
6	13.605	47138	2.284
7	26.305	581195	28.165
8	31.055	421354	20.419
9	36.664	31192	1.512
10	37.217	25121	1.217
11	43.642	26982	1.308
12	44.068	29536	1.431
13	55.919	52043	2.522

Interpretation: Thirteen peaks are detected in the given sample. Out of which two major peaks are observed at RT 26.305 min and 31.055 min with area % 28.165 and 20.419 % respectively.

DISCUSSION

The analysis revealed a rich phytochemical profile that supports the therapeutic claims discussed in our previous conversations regarding *Ativisha*'s potency.

Qualitative Screening: The roots tested **positive for Alkaloids, Flavonoids, Phenols, and Saponins**. However, the sample tested **negative for Tannins**. The presence of alkaloids is particularly significant, as these often correspond to the diterpene alkaloids responsible for the herb's analgesic and anti-inflammatory properties.

HPTLC Fingerprinting: Using a mobile phase of Chloroform and Methanol (8:2), the researchers observed specific **bands of phytochemicals at 254 nm and 366 nm**. These bands serve as a specific diagnostic "fingerprint" that can be used to authenticate the roots of *Aconitum*

heterophyllum and distinguish them from substitutes like *Mustaka*.

HPLC-PDA Peak Profiling: The HPLC analysis detected **thirteen distinct peaks** in the sample. Two major peaks were identified, which dominate the chemical composition:

Peak 1: Observed at a Retention Time (RT) of **26.305 minutes**, accounting for **28.165%** of the total area.

Peak 2: Observed at a Retention Time (RT) of **31.055 minutes**, accounting for **20.419%** of the total area.

Other notable peaks appeared at RT 13.062 (13.139%) and RT 12.001 (8.207%).

CONCLUSION

This data suggests that nearly **50% of the detectable phytochemical content** is represented by just two major compounds, which likely play a central role in the plant's medicinal efficacy

Figure 1. Gomaya Kwatha preparation

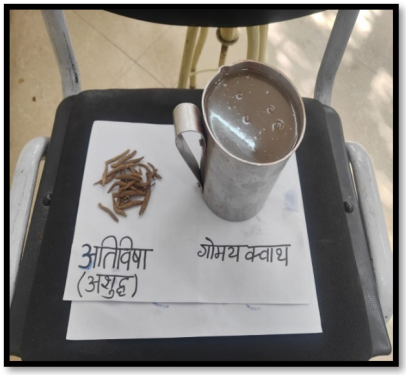

Figure 2. Ashudha Ativisha and Kwatha

Figure 3. Daulayantra Swedana

Figure 4. Shudha Ativsha in Pottali

Figure 5. Shuddha Ativisha dried

Figure 6. pounded in ulukhala for churna Nirmana

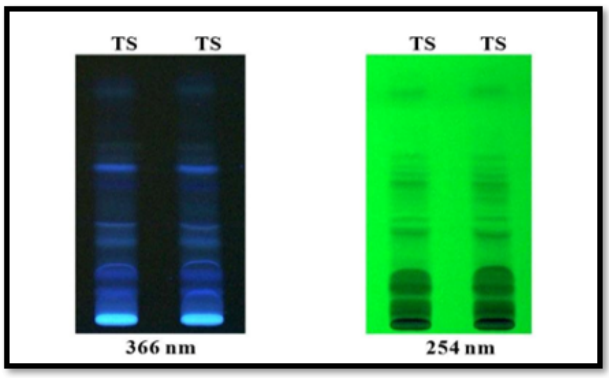

Figure 7. HPTLC fingerprint of Aconitum heterophyllum roots at 254 nm and 366 nm

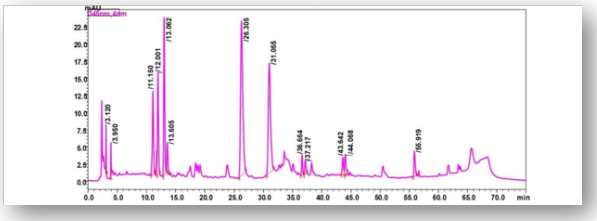

Figure 8. HPLC chromatogram of Aconitum heterophyllum

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