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PHARMACOGNOSTICAL ANALYSIS AND THIN LAYER CHROMATOGRAPHY (TLC) PROFILE OF *GLORIOSA SUPERBA (LANGALI)*

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ABSTRACT

Nature serves as the ultimate source of combinatorial chemistry, providing several remedies for numerous aliments. Studies suggest that nearly two-third of the world's herbal species possess therapeutic properties, and these plants have been the integral part of our Indian system of medicine (TSMs), promoting traditional healing since the dawn of human civilization. For the exploration of plant-derived compounds, Pharmacognosy helps in investigating its physical and chemical properties, identifying their bioactive constituents, understanding their therapeutic effects, and ensuring their safety and efficacy. This study aims to establish various organoleptic and pharmacognostical standards that will assess the purity, safety, and efficacy of *Gloriosa superba Moola* (Roots), a toxic plant, before and after undergoing the *shodhana(purification)* process. The prepared sample of both *Shodhita* and *ashodhita langali* moola were studied organoleptically for its appearance, colour, odour, and taste. The pharmacognostical investigation determined key parameters such as moisture content, total ash, acid insoluble ash, water soluble ash, water soluble extractive value and alcohol soluble extractive value of the drug. Following this, phytochemical screening was carried out to detect the presence of various phytoconstituents such as alkaloids, glycosides, tannin, saponins, Steroids, which contribute to the drug's therapeutic

properties. Thin layer chromatography (TLC) analysis further confirmed the presence of these bioactive phytoconstituents and hence, highlighted distinctive diagnostic markers in both (untreated) *ashodhita* and (treated) *shodhita* samples of *langali*. The findings provide valuable insights into the quality and stability of both the samples of Langali. Hence, in this context it can be used for future researches and quality assessment of both the sample of *langali* i.e., before and after *shodhana*.

KEYWORDS: *Gloriosa superba*, Organoleptic study, physicochemical analysis, phytochemical analysis, Thin-layer chromatography.

INTRODUCTION

A substance that might cause harm to the body when delivered, inhaled, or ingested is known as a poison. Nature has consistently served as a perpetual source of cures for countless ailments. *Langali (Gloriosa superba)*, a drug of herbal origin, is referenced in various *ayurvedic* text for its therapeutic applications. The drug *Langali (Gloriosa superb Linn.)* is being classified under *Moolavisha* (toxic roots) and is also included in the nine *Upavishas*[1] (semi poisonous drugs) in various Ayurvedic *Samhitas* and *Nighantus*. Although inherently toxic, Langali has been used for the treatment of multiple diseases as it has beneficial properties such as *Shothahara* (anti-inflammatory), *Vranahara* (wound healing), *Krimighna* (wormicidal), *Shalyaharana* (removal of foreign substances), *Garbhapaatana* (abortifacient) properties etc.[2] Despite being a poisonous plant, its roots, leaves and seeds are commonly employed in traditional and folklore medicine. While *Brihatrayi* texts acknowledge the medicinal value of *langali* but does not mention any *Shodhana* process,[3] however, other classical texts of *Ayurveda*, emphasizes that *langali* must undergo certain *Shodhana* (purificatory) procedures before its internal administration, in order to reduce its toxicity and enhance its safety for therapeutic use.[4]

In today's era of evidence-based medicine, the global acceptance of *ayurvedic* and herbal products depends largely on their scientific validation. To ensure credibility and safety of the *Ayurvedic*/ herbal products on the modern parameters, their standardization and quality control are very essential. The process of standardization can be gained by systemic approach through stepwise pharmacognostic studies.[5] These studies play a pivotal role in accurate identification and authentication of the plant material.[6]

MATERIAL AND METHOD

Collection of the plant material

The raw rhizomes of Langali *(Gloriosa superba*) were collected from Herbal Drug Vendor, Panna Lal Store, Haridwar.

Authentication

The collected raw drug was identified and authenticated by the experts of P.G department of *Dravyaguna, Rishikul* Campus, Uttarakhand Ayurved University (UAU), Haridwar. Reference no.-**DG/RC/UAU-178**

Preparation of Sample extraction

The dried sample of *langali* were initially cleaned and crushed into coarse powder with the help of iron mortar and pestle. Subsequently, 250gms of sample underwent the process of *Shodhana* (purification), in which the sample was immersed in *gomutra* (cow urine) for 24 hrs[7] and remaining 250gms *Ashodhita* sample were directly subjected for the organoleptic study. The procedure of *shodhana* was carried out in the laboratory, at the Department of chemistry, KLDAV PG College Roorkee, the *gomutra* used for the process was freshly collected in sterile bottle from *Gaushala, Chawmandi*, Roorkee. It took 24 hrs for the process of *nimajjan* (immersion), after which the sample was kept for shade dried and preserved for the further process of extraction.

Analysis of *Ashodhita* and *Shodhita* sample of *langali moola*

Organoleptic, Physico-chemical, Phytochemical and Thin layer chromatography (T.L.C) analysis were carried out at KL DAV PG College Roorkee. The Physico-chemical parameter was assessed following the standards outlined in the Ayurvedic Pharmacopeia of India (API).

- **Macroscopic Study / Organoleptic Parameters –** Various parameters like appearance, colour, odour and taste of the both the *ashodhita* and *shodhita* sample of *langali* were observed and recorded.

Soxhlet extraction method

For the purpose of extraction, distilled water and ethanol were used as a solvent, resulting in two distinct extracts of drug sample:

- Aqueous extract (using distilled water)
- Ethanolic Extract (using ethanol)

Physico-chemical Analysis

Physico-chemical parameters of both the sample *ashodhita* and *shodhita* sample of *langali* like Loss on drying, Total ash, Acid insoluble ash, Water soluble ash, Extractive values Viz., Water soluble extractive value, Alcohol soluble extractive value were estimated following the guidelines provided in the Ayurvedic Pharmacopeia of India (API). These parameters were calculated according to the standard procedures:

Loss on drying

1-gm sample of the material was used for the test, and it was equally spread after being put on a Petri dish plate. The plate was heated for one hour at 105°C in a hot air oven, cooled for one hour in the desiccator, and then weighed to determine the sample's weight. Every 30 minutes, this process was repeated until the sample's weight remained constant and no weight variation was noted.

Calculation

Weight of the empty Petri dish = W_1gm

Weight of the drug sample = X gm

Weight of the Petri dish containing the drug prior to drying (W_3) = (W_1 + X)

Weight of Petri dish after drying = W_2gm

Loss on drying in % = W_3-W_2×100/X

Determination of Total ash

2.5 gm of powdered drug sample was placed in a Silica crucible and evenly spread in a thin layer. This crucible was put in a muffle furnace and it was ignited at 800°C temperature for approximately 6 hours until the ash was completely free of carbon. After ignition, the crucible was cooled in a desiccator to stabilize its weight before being weighed. The percentage of ash was then calculated relative to the weight of the air dried.

Calculation

Wt. of Empty Silica Crucible = A_1 gm

Wt. of Sample (X) = X gm

Wt. of the Crucible with Ash = A_2 gm

Percentage of Total Ash = [A_2-A_1 /X] × 100

Percentage of Total Ash = [A_2-A_1 /X] × 100

Acid insoluble ash value

To perform this test, the total ash obtained was boiled for 5 minutes with 25 ml of 2M hydrochloric acid. The insoluble residue was filtered using an ashless filter paper, cleaned thoroughly with hot water, incinerated for 15 minutes, cooled in a desiccator, and then weighed.

The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

Calculation

Wt. of drug sample - X gm

Wt. of empty Crucible = G_1 gm

Wt. of Crucible with insoluble Ash = G_2 gm

Wt. of insoluble ash (G_3) = G_2-G_1

Percentage of acid insoluble ash = $G_3/X \times 100$.

Percentage of acid insoluble ash = $G_3/X \times 100$.

Water soluble ash value

In this test, the total ash was boiled for 5 minutes with 25 ml of distilled water. The insoluble material was filtered using either in a Gooch crucible or ashless filter paper, cleaned thoroughly with hot water, incinerated for 15 minutes, cooled in a desiccator, and then weighed. The weight of the insoluble residue was subtracted from the weight of the ash; the obtain weight represents the water-soluble ash.

The percentage of water-soluble ash was calculated in relation to the air-dried drug.

Calculation

Wt. of drug sample - X gm Wt. of total ash - A gm

Wt. of Crucible - G_1 gm

Wt. of Crucible with insoluble Ash - G_2 gm

Wt. of insoluble ash (G_3) = G_2-G_1

Soluble ash (G_4) = Wt. of total ash A gm- Wt. of insoluble(G_3)

Alcohol soluble extractive value

To determine the number of alcohol-soluble constituents, 2.5 gm fine powdered air-dried drug was macerated with 100 ml of Alcohol of the prescribed strength in a closed flask for 24 hours. The mixture was shaken frequently during the first 6 hours using a rotary shaker and

then allowed to stand undisturbed further for 18 hours. The content was filtered afterward by using filter paper. The filtrate was transferred into a pre-weighed flat-bottomed dish and evaporate to dryness on a water bath at 105° C, until a constant weight was achieved. The percentage of alcohol-soluble extractives was then calculated with reference to the air-dried drug.

Calculation

$$\textbf{Percentage of extractive value} = W_2 - W_1 \times 100 / X$$

Where,

W_2- weight of the dish with dried extract

W_I= weight of the empty dish

X= weight of the sample taken

Water soluble extractive value

The procedure followed was identical to that used for the alcohol-soluble extractive value, except distilled water was used instead of ethanol.[8]

Phytochemical screening of both *ashodhita* and *shodhita* sample of langali[9]

Test of carbohydrates

Molisch's Test (General Test): It is a chemical assay use to determine the presence of carbohydrate in a test sample.

In this process we have taken 3 ml of extracts of the sample in a different sterile test tube. A few drops of Molisch's reagent (5% solution of alpha naphthol in ethanol) were gently added, followed by the addition of concentrated H_2SO_4 along the sides of test tube to form a separate layer.

The appearance of violet ring at the junction of 2 liquids determines the presence of carbohydrate.

Test for proteins

Biuret Test (General Test): It is a chemical assay to determine the presence of protein by identification of peptide bond. In this process a small quantity 3 ml Test Solution is taken in a clean test tube. 4% NaOH is added to the solution, few drops of 1% CuSO4 solution was introduced and the mixture was gently mixed.

Any change in colour is observed. If Violet or pink colour appears represents presences of protein.

Test for Saponin

Foam Test: It is a general assay to determine the presence of saponins in test sample.

In this test, a clean test tube was taken and 2-3 ml of test solution is added. Equal volume of distilled water is added to the solution. Vigorously shake the test tube for 10-15 sec, Formation of a persistent foam or forth which lasts for several minutes determines the presences of saponins.

Tests for Glycosides

Test for Deoxysugars (Keller – Killani Test): It is a chemical assay which is used to determine the presence of deoxysugars.

2ml sample extract was taken in a clean test tube then 2 ml of glacial acetic acid is added with one drop 5% FeCl3. Gently the concentrated H_2SO_4 was poured along sides of the test tube to form a separate layer. Formation of Reddish-brown colour ring at the interface which may turn into blue or green determines the presences of glycosides.

Tests for steroids

Salkowski reaction: It is a qualitative test to determine the presence of cholesterol or another sterol in test sample.

Take 2 ml of sample extract, add 2 ml of chloroform. Gently add 2 ml concentrated H2SO4 to the test tube to form a separate layer. Allow the mixture to stand and undisturbed. The appearance of reddish violet colour of Chloroform layer and greenish yellow fluorescence colour of acid layer determines the presence of steroids.

Tests For Tannins and Phenolic Compounds

To 2 – 3 ml of sample extract in a clean test tube. Add few drops of Lead acetate solution. Formation of white precipitate indicates the presence of tannins and phenolic compounds.

Test for alkaloids

2 ml of plant extract and 2 ml of 1% HCL were mixed thoroughly and heated gently. The Mayer's and Wagner's reagents were added to the resulting mixture. The appearance of turbidity or precipitate was considered indicative of the presence of alkaloids.

Thin Layer Chromatography[10]

TLC was performed on both the *ashodhita* and *Shodhita* sample of *langali*:

- **Stationary phase-** T.L.C plates were prepared by coating them with 0.2mm layer of silica gel G.
- **Activation of pre-coated Silica gel G plates-** The silica gel G- coated plates were dried and activated by placing them in a hot oven at 105℃ for 30 minutes prior to chromatography procedure.
- **Test solution:** Alcoholic and Aqueous Extracts were used as test samples.
- **Mobile phase:** A solvent system consisting of Chloroform: methanol: ethyl acetate (60:40:1) was used for the procedure.
- **Visualization:** Iodine were used for detecting the separate spots of the extracts.

Procedure: TLC plates were prepared by coating of Silica gel G slurry (10g+6.2ml D.W) and dried. After the process of drying, the coated TLC plates were activated at 105℃ for 30 minutes and then used. Spots of both the extracts were placed approximately above 1 cm from the base of the plate by using capillary tube. Then the plates were placed in TLC chamber having different mobile phase. Once the solvent front reached an appropriate height, the plates were removed, dried and placed in iodine chamber for visualization.

Calculation of Rf Value

$$Rf = \frac{\text{Distance travelled by solute from origin line}}{\text{Distance travelled by solvent from origin line}}$$

OBSERVATION AND RESULTS

Table 1: Organoleptic Characters of *Ashodhita* and *Shodhita Langali.*

S.NO.	Organoleptic	*Ashodhita langali*	*Shodhita langali*
1.	Appearance	Coarse powder	Coarse powder
2.	Colour	Brown	Light brown
3.	Odor	Characteristics	Characteristics
4.	Taste	Acrid and bitter	Bitter

Table 2: Table showing Physicochemical Parameters of *langali.*

S.No.	Parameters	*Ashodhita langali*	*Shodhita Langali*
1.	Loss on drying	4.05	9.51
2.	Total ash	4.8	3.2
3.	Acid insoluble ash	0.64	0.5
4.	Water soluble ash	19.68	21.51
5.	Water soluble extractive value	17.35	18.49

6.	Alcoholic soluble extractive value	9.52	23.36

Table 3: Results showing the phytochemical analysis of *langali.*

S.No.	Phytochemicals	*Ashodhita* extract		*Shodhita* extract	
		Aqueous extract	Alcoholic extract	Aqueous extract	Alcoholic extract
1.	Carbohydrates	+	+	+	+
2.	Alkaloids	+	+	+	+
3.	Protein	-	-	-	-
4.	Saponin	+	+	-	-
5.	Glycosides	+	+	-	-
6.	Tannins	+	+	+	+

Thin layer chromatography

The details of the chromatogram obtained when viewed in day light has been presented in

***Ashodhita* sample**	***Shodhita* Sample**
L-Ethanolic	L-Ethanolic
M- Aqueous	M-Aqueous
R- Mixture	R-Mixture

Where L= M= Middle, Left, R=Right.

Table 4: Table showing TLC profile of extracts of *Ashodhita* and *Shodhita langali* Extract.

Sample Extract		No. of Spots	Rf Value
Ashodhita Extract	Aqueous	02	**0.894, 0.273**
	Alcohol	02	**0.947, 0.463**
Shodhita Extract	Aqueous	02	**0.813, 0.934,**
	Alcohol	04	**0.772, 0.813, 0.853, 0.975,**

DISCUSSION

The physiochemical analysis of the tested sample indicates that the parameters largely fall within the acceptable limits prescribed by the Ayurvedic Pharmacopoeia of India. The loss on drying of *Ashodhita* sample is (4.05%) and (9.51%) of *Shodhita* sample, well below the maximum limit of ensuring minimal moisture content and stability of the Formulation. The aqueous extractive value of *Ashodhita* sample is (17.35%) and 18.49% of *Shodhita* sample. Alcoholic extractive value of *Ashodhita* sample is (9.52%) and 23.36 of *Shodhita* sample both the exceed Minimum required limits of 15% and 5% respectively indicating good solubility of active constituents. Acid insoluble ash content was found (0.64%) of *Ashodhita* and 0.5% in *Shodhita*, both falling within the acceptable limit of 1% reflecting low levels of siliceous impurities. However, the total Ash value content of *Ashodhita* Sample is (4.8%) and 3.2% of *Shodhita* sample is under the general permissible limit of not more than 6% suggesting acceptable total in organic content overall the sample compiles with standard quality parameters confirming its suitability for further pharmacological or therapeutic use.

The phytochemical analysis of both aqueous and alcoholic extracts indicates the presence of various bioactive constituents. Carbohydrates, alkaloids and tannins were consistently present in both extracts indicating their stable solubility in polar solvents. Proteins were absent in both suggesting their limited extractability under the given conditions. Interestingly, saponins and glycosides were detected only in the aqueous extract highlighting water's superior efficiency in extracting these compounds compared to alcohol. These results when correlated with physical chemical parameter, support the phytochemical richness and potential therapeutic value of the plant, thereby justifying its traditional uses and paving the way for further pharmacological investigation.

After the process of *Shodhana* increase the number of detectable compounds especially in the alcohol extract (from 2 to 4 spots). This suggests the purification either removed masking agents or allowed better separation of compounds. The Rf value of *Shodhita* extract of *Langali* are generally higher especially in alcohol medium indicating better mobility of the compounds, the *Shodhita* extract shows more distinct and separated Rf values improved resolution of phytochemicals after purification. The presence of multiple distinct Rf values in the *Shodhita* Alcohol extract suggests increased chemical complexity or better extractability of different phytoconstituent after purification. The TLC results demonstrate that *shodhana* may aid in revealing or isolating additional active constituents that were not visible in the

Ashodhita sample this support the basic fundamental of ayurveda that claims *"Samskarohinaam Gunataradanam"* i.e., purification enhance the quality and efficacy of herbal extract.

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