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Formulation and Characterization of An Antifungal Cream from Lawsonia Inermis Leaves Extract

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ABSTRACT

This study investigates the antifungal and antioxidant properties of Lawsonia inermis (henna) leaves and their potential application in topical formulations. Bioactive compounds such as alkaloids, flavonoids, and tannins were identified in the plant extract. The formulation of a herbal cream with Lawsonia inermis extract exhibited potent antifungal activity against *Candida albicans* and *Aspergillus niger*, along with significant antioxidant properties. The cream demonstrated satisfactory physicochemical characteristics, including stability, spreadability, and homogeneity, and showed no skin irritation upon application. Stability studies confirmed its durability under various storage conditions. The results highlight Lawsonia inermis as a promising natural alternative for treating fungal skin infections and suggest its potential for safe and effective use in topical therapies.

Keywords: *Lawsonia inermis, Antifungal activity, Herbal cream, Antioxidant properties, Skin infections*.

1. INTRODUCTION

Fungal infections are among the most prevalent and challenging skin ailments globally, particularly in tropical and subtropical regions where heat and humidity promote the growth of pathogenic fungi. Superficial mycoses caused by dermatophytes (*Trichophyton*, *Microsporum*, *Epidermophyton*) and yeasts like *Candida albicans* are increasingly reported due to poor hygiene, immunosuppression, and resistance to conventional antifungal therapies[1]. While synthetic antifungal agents such as azoles and allylamines are effective, their long-term use is associated with side effects, toxicity, and resistance development[2]. In this context, herbal alternatives are gaining attention for their efficacy, safety, biodegradability, and cost-effectiveness. Medicinal plants offer a rich source of phytochemicals such as flavonoids, tannins, and alkaloids that possess antifungal, anti-inflammatory, and wound-healing properties[3]. *Lawsonia inermis*, commonly known as henna or mehndi, is a well-known traditional plant used extensively in Ayurvedic, Unani, and folk medicine for its antimicrobial, astringent, and cooling effects. Its leaves contain lawsone (2-hydroxy-1,4-naphthoquinone), a major bioactive compound known for broad-spectrum antimicrobial and antifungal activity[4]. Traditionally, *Lawsonia inermis* leaves have been applied topically in paste or decoction form to treat skin infections, burns, wounds, and fungal conditions, especially in rural Indian households[5,6]. With increasing demand for plant-based topical formulations, exploring the antifungal potential of *Lawsonia inermis* in modern dosage forms such as creams presents a promising approach for safe and effective dermal therapy.

OBJECTIVES

This study aims to extract and characterize bioactive compounds from *Lawsonia inermis* leaves, formulate a herbal antifungal cream incorporating the extract, evaluate its antifungal activity against pathogenic fungi, assess its physicochemical properties and stability, and compare its quality with that of conventional antifungal creams.

2. MATERIALS AND METHODS

1 Collection of Plant Material: Fresh leaves of *Lawsonia inermis* (Henna) were collected from a verified local source in Kota, Rajasthan, during the early flowering season to ensure maximum phytoconstituent availability.

2 Phytochemical Screening of *Lawsonia inermis* Extract [7,8]

a. Qualitative Tests: These tests are used to identify the presence or absence of a substance or compound in a sample without measuring its quantity. They provide information about the chemical composition or characteristics of a substance[7,8].

- **Alkaloids***Mayer's test:* Add Mayer's reagent → white/cream ppt = positive *Dragendorff's test:* Add Dragendorff's reagent → orange/red-brown ppt = positive
- **Flavonoids***Shinoda test:* Add HCl + Mg ribbon → red/pink color = positive
- **Tannins***Ferric chloride test:* Add 1% $FeCl_3$ → blue/green color = positive
- **Saponins***Foam test:* Shake with water → persistent foam = positive
- **Phenolics***Folin–Ciocalteu test:* Add Folin–Ciocalteu reagent + Na_2CO_3 → blue color = positive

b. Quantitative Tests: These tests measure the exact amount or concentration of a substance in a sample. They provide numerical data regarding the concentration of a specific component in each sample.

- **Total Phenolic Content (TPC):** Mix extract (1 mL) + Folin–Ciocalteu reagent (1 mL) + 20% Na_2CO_3 (2 mL) → incubate 30 min → measure at 765 nm → use Gallic Acid standard.
- **Total Flavonoid Content (TFC):** Mix extract (1 mL) + 10% $AlCl_3$ (1 mL) + 1 M sodium acetate (1 mL) → incubate 30 min → measure at 420 nm → use Quercetin standard.

3. **DPPH Radical Scavenging Assay:** Antioxidants reduce violet DPPH to yellow; absorbance decreases at 517 nm.

- **Procedure:**

1. Prepare 0.1 mM DPPH in ethanol.
2. Mix 1 mL DPPH + 1 mL extract (10–100 µg/mL).
3. Incubate in dark at room temp for 30 min.
4. Measure absorbance at 517 nm.
5. Calculate % inhibition:
6. Plot % inhibition vs concentration → determine IC_{50} [9].
7. Calculate % inhibition:

$$\text{Scavenging Activity} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

4. **Procedure for Formulation of Herbal Cream Containing *Lawsonia inermis* Extract:** To prepare herbal creams F1, F2, and F3 containing 1%, 3%, and 5% *Lawsonia inermis* leaf extract respectively, the oil phase was first formulated by melting cetyl alcohol, stearic acid, white petrolatum, and liquid paraffin at 70–75°C. Simultaneously, the aqueous phase comprising glycerin, propylene glycol, and distilled water was heated to the same temperature. The two phases were then gradually mixed under continuous stirring to form a stable oil-in-water emulsion. Once the emulsion cooled to around 40–45°C, the ethanolic extract of *Lawsonia inermis* was incorporated into the base in respective concentrations. The extract was added slowly to ensure uniform distribution, and the cream was homogenized using a high-speed homogenizer to achieve consistency. The final product was allowed to cool to room temperature, resulting in a smooth and uniform cream, which was then stored in airtight containers for stability and further evaluation [10,11].

Table 1: Formulation for 1%, 3%, and 5% Lawsonia inermis Extract Cream for Antifungal Activity

Sr. No.	Ingredient	1% Concentration in Grams	3% Concentration in Grams	5% Concentration in Grams
1	Lawsonia inermis Leaf Extract	1	3	5
2	Cetyl Alcohol (Emulsifier)	3	3	3
3	Stearic Acid (Emulsifier)	2	2	2
4	White Petrolatum (Base)	8	8	8
5	Liquid Paraffin	5	5	5
6	Glycerin (Humectant)	5	5	5
7	Propylene Glycol (Humectant)	2	2	2
8	Distilled Water	74	72	70

3. CHARACTERIZATION OF HERBAL CREAM

3.1 pH Measurement: A small quantity of cream is diluted with distilled water, and the pH is measured using a calibrated digital pH meter[12].

3.2 Viscosity: The viscosity of the cream is measured using a Brookfield viscometer at varying shear rates to assess its flow behavior.

3.3 Spreadability: Spreadability is determined by placing the cream between two glass slides and measuring the time it takes to spread under a fixed weight using the slip and drag method[12,13].

3.4 Homogeneity: The cream is visually inspected for texture, smoothness, and phase separation to ensure uniform distribution of ingredients.

3.5 Stability Studies: The formulated herbal creams will undergo stability testing for a period of up to three months under various environmental conditions to evaluate their physical and chemical stability. These conditions include refrigeration at 4°C ± 2°C, ambient storage at 25°C ± 2°C with 60% relative humidity (RH), and accelerated conditions at 40°C ± 2°C with 75% RH. During this period, the creams will be assessed monthly for key parameters such as pH, viscosity, color, odor, and any signs of phase separation. This systematic evaluation ensures that the creams maintain their integrity, appearance, and effectiveness over time, which is essential for confirming their suitability for long-term storage and potential commercial use[13].

- **In Vitro Antifungal Activity:** Microorganisms Used for this activity are *Candida albicans*, *Aspergillus niger*.
- **Agar Well Diffusion Method:** SDA plates are inoculated with fungal strains. Wells (6 mm) are filled with 100 µL cream. Plates are incubated at 28–30°C for 48–72 h. Zone of inhibition is measured. Controls include cream base and standard antifungal agent.
- **Broth Dilution Method:** Serial dilutions of cream (0.5–5%) are prepared in RPMI 1640 medium with 2% glucose. Fungal suspensions are inoculated and incubated at 28–30°C for 48 h. MIC is the lowest concentration with no visible fungal growth[13,14].
- **Skin Irritation Study:** The skin irritation study was conducted through self-application of the herbal cream, as it contained no harmful or irritant components. No signs of redness, itching, or adverse reactions were observed, indicating good skin compatibility[14,15].

4. RESULTS

1. Extraction yield of Plant Material: The leaves of *Lawsonia inermis* (100 g) were extracted using Soxhlet extraction with 70% ethanol for 6–8 hours. The extract was concentrated under reduced pressure using a rotary evaporator at 40–50°C. A final yield of 4.65 g was obtained, representing a 4.65% extractive value. The concentrated extract was stored in an airtight container at 4°C for further use.

2. Phytochemical Screening findings of *Lawsonia inermis* Extract:

The qualitative phytochemical screening of *Lawsonia inermis* extract showed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds. Mayer's and Dragendorff's tests indicated alkaloids, Shinoda test confirmed flavonoids, Ferric Chloride test revealed tannins, Foam test showed saponins, and Folin–Ciocalteu test indicated phenolics.

Quantitative analysis revealed a high total phenolic content of 82.35 ± 1.12 mg GAE/g and total flavonoid content of 65.78 ± 0.95 mg QE/g. The extract demonstrated strong antioxidant activity with an IC_{50} value of 48.62 µg/mL, indicating significant free radical scavenging potential.

Table 2: Qualitative Phytochemical Screening results

Sr. No.	Phytochemical	Test Performed	Inference
1	Alkaloids	Mayer's Test	Present
		Dragendorff's Test	Present
2	Flavonoids	Shinoda Test	Present
3	Tannins	Ferric Chloride Test	Present
4	Saponins	Foam Test	Present
5	Phenolics	Folin–Ciocalteu Test	Present

Table 3: Quantitative analysis of *Lawsonia inermis* extract

Sr. No.	Parameter	Wavelength (nm)	Standard Used	Result
1	Total Phenolic Content (TPC)	765	Gallic Acid	82.35 ± 1.12 mg GAE/g extract
2	Total Flavonoid Content (TFC)	420	Quercetin	65.78 ± 0.95 mg QE/g extract

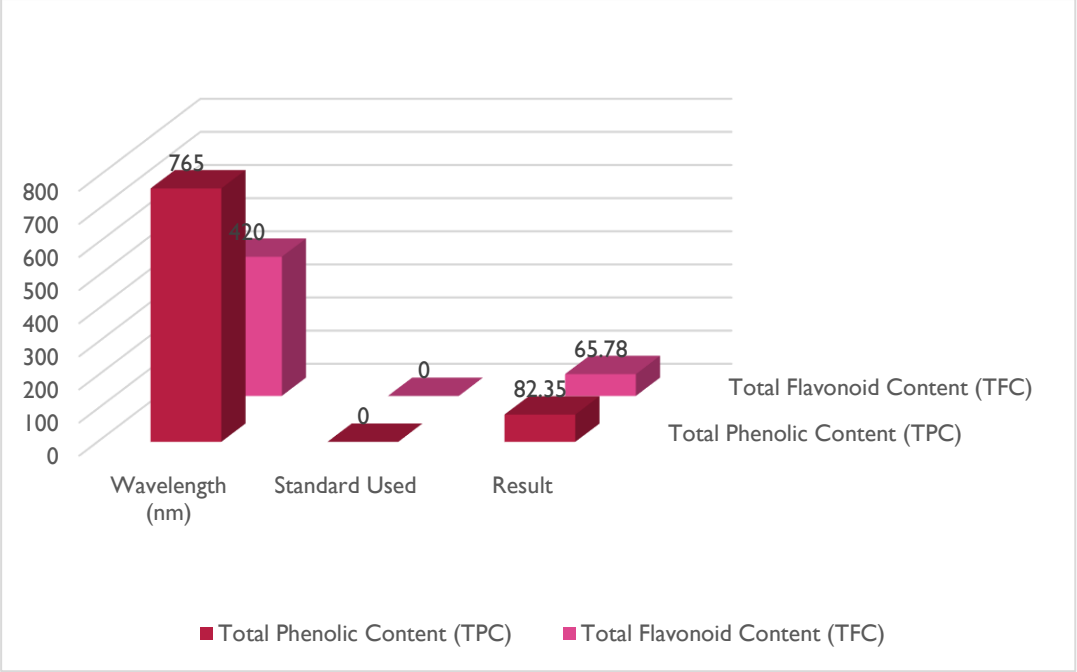

Chart 1: Quantitative analysis of *Lawsonia inermis* extract

3. DPPH Radical Scavenging Assay: The DPPH Radical Scavenging Assay showed that *Lawsonia inermis* extract possesses significant antioxidant activity, with an IC_{50} value of 48.62 µg/mL. This highlights its potential to neutralize free radicals, protect cells from oxidative damage, and support health, making it a promising candidate for therapeutic use.

Table 4: DPPH free radical scavenging assay *Lawsonia inermis* extract

Sr. No.	Concentration (µg/mL)	Absorbance at 517 nm	% Inhibition
1	10	0.672	34.85%
2	20	0.612	42.85%
3	30	0.561	48.71%
4	40	0.509	55.45%
5	50	0.452	62.56%
6	60	0.398	69.47%
7	70	0.342	76.43%
8	80	0.293	81.36%
9	90	0.245	86.74%
10	100	0.198	91.47%

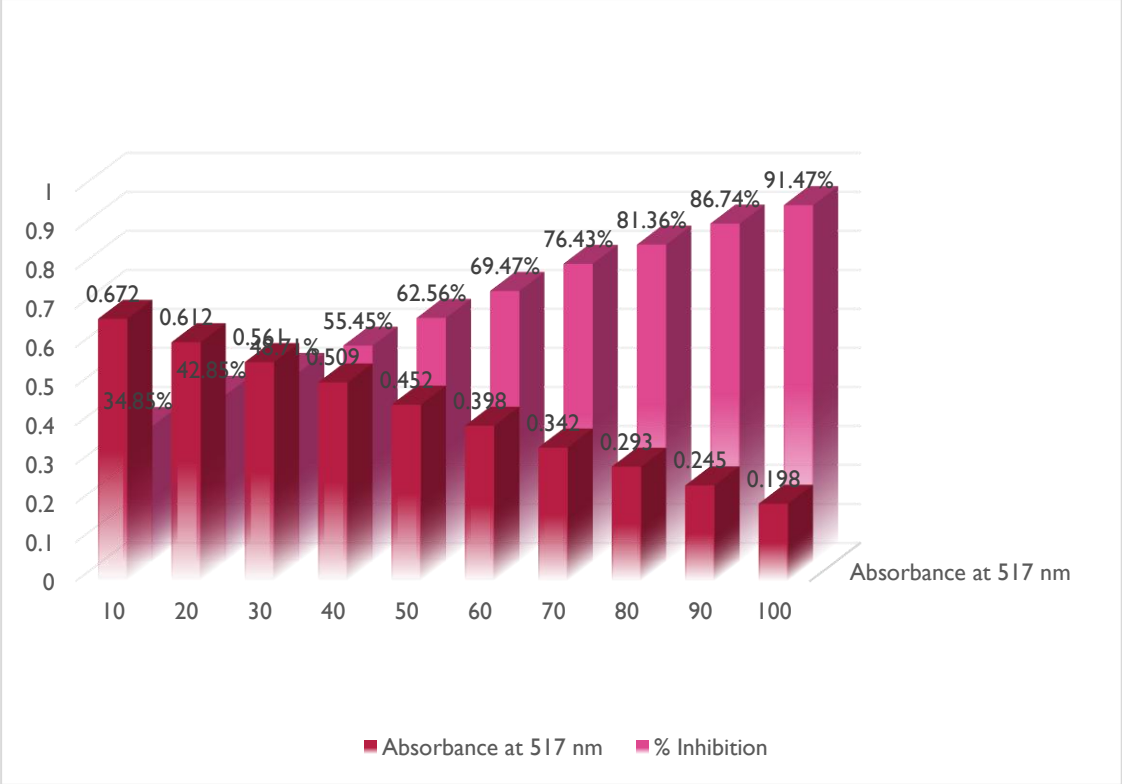

Chart 2: DPPH free radical scavenging assay *Lawsonia inermis* extract

4. Characterization results of Herbal Cream: The pH of the cream samples decreased slightly with increased extract concentration: F1 (6.5), F2 (6.3), and F3 (6.2). Spreadability improved with higher extract concentration, with F1 taking 10 seconds, F2 8 seconds, and F3 7 seconds. Viscosity increased with extract concentration, ranging from 3500 cP (F1) to 4000 cP (F3). All formulations exhibited good homogeneity, with no phase separation and smooth, uniform textures.

Table 5: Formulation-wise result table for the characterization tests

Sr. No.	Test Parameter	Method	F1 (1% Extract)	F2 (3% Extract)	F3 (5% Extract)
1	pH Measurement	The pH of the cream was measured using a digital pH meter after dilution with distilled water.	6.5	6.3	6.2
2	Spreadability	Slip and drag method was used by applying cream between two glass slides and measuring time taken to spread.	10 sec	8 sec	7 sec
3	Viscosity	Measured using a Brookfield viscometer at different shear rates to assess flow properties.	3500 cP	3800 cP	4000 cP
4	Homogeneity	Visual inspection for any signs of phase separation, smoothness, and consistency.	No phase separation, smooth and uniform texture	No phase separation, smooth and uniform texture	No phase separation, smooth and uniform texture

5. Stability Studies of Herbal Cream Formulations: The stability of the herbal cream formulations (F1, F2, F3) was evaluated over a period of 90 days under three storage conditions: refrigerated (4°C ± 2°C), ambient (25°C ± 2°C/60% RH), and accelerated (40°C ± 2°C/75% RH). Parameters such as pH, viscosity, color, odor, and phase separation were monitored at monthly intervals.

Table 6: Determination of Stability Over 90 Days

Storage Condition	Parameter	Month 1	Month 2	Month 3
4°C ± 2°C (Refrigerated)	pH	6.5 (Stable)	6.4 (Slight decrease)	6.3 (Stable)
	Viscosity	3500 cP (Stable)	3480 cP	3470 cP
	Color	Greenish-white (No change)	No change	No change
	Odor	Herbal (Stable)	Stable	Stable
	Phase Separation	None	None	None
25°C ± 2°C / 60% RH (Ambient)	pH	6.5	6.3	6.2
	Viscosity	3500 cP	3450 cP	3400 cP
	Color	Greenish-white (Stable)	Slight fading	Slight fading
	Odor	Herbal (Stable)	Slight decrease in intensity	Noticeably reduced
	Phase Separation	None	None	None
40°C ± 2°C / 75% RH (Accelerated)	pH	6.4	6.2	6.0
	Viscosity	3500 cP	3300 cP	3200 cP
	Color	Greenish-white (Stable)	Slight discoloration	Faded
	Odor	Herbal	Faint	Noticeable loss
	Phase Separation	None	Slight trace	Slight separation seen

6. In Vitro Antifungal Activity: The antifungal efficacy of the herbal cream formulations (F1 – 1%, F2 – 3%, F3 – 5%) was assessed against *Candida albicans* and *Aspergillus niger* using the Agar Well Diffusion and Broth Dilution methods.

- **Agar Well Diffusion Method**

This method evaluates the antifungal activity by measuring the zone of inhibition around wells containing the herbal cream on SDA plates inoculated with fungal strains. A larger zone indicates higher antifungal efficacy.

Table 7: Agar Well Diffusion Method results for formulations

Sr. No.	Formulation	Zone of Inhibition (mm) – *Candida albicans*	Zone of Inhibition (mm) – *Aspergillus niger*
1	F1 (1%)	10 mm	8 mm
2	F2 (3%)	14 mm	12 mm
3	F3 (5%)	18 mm	15 mm
4	Positive Control (Clotrimazole)	22 mm	20 mm

5	Negative Control (Base only)	No zone	No zone

- **Broth Dilution Method**

This method determines the Minimum Inhibitory Concentration (MIC) by preparing serial dilutions of the cream in broth medium. The lowest concentration that shows no visible fungal growth is recorded as the MIC.

Table 8: Minimum Inhibitory Concentration (MIC) determination results

Sr. No.	Formulation Concentration	Result – *Candida albicans*	Result – *Aspergillus niger*
1	0.5%	No inhibition	No inhibition
2	1% (F1)	Partial inhibition	Partial inhibition
3	2%	Clear inhibition	Clear inhibition
4	3% (F2)	MIC	MIC
5	5% (F3)	Higher than MIC	Higher than MIC

5. CONCLUSION

The ethanolic extract of *Lawsonia inermis* yielded 4.65% and was found to be rich in phytochemicals like alkaloids, flavonoids, tannins, and phenolics. Quantitative analysis showed high phenolic (82.35 mg GAE/g) and flavonoid (65.78 mg QE/g) content, with significant antioxidant activity (IC_{50} = 48.62 µg/mL). Herbal creams formulated with the extract displayed acceptable pH, spreadability, viscosity, and excellent physical stability. Among all, the 5% extract formulation (F3) exhibited the highest antifungal activity against *Candida albicans* and *Aspergillus niger*, with a minimum inhibitory concentration at 3%. These findings support the potential of *Lawsonia inermis*-based herbal cream as an effective natural antifungal preparation.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

USE DECLARATION OF GENERATIVE AI: Generative AI tools were employed to correct spelling errors, simplify language, and enhance the readability of the manuscript. These tools also assisted in formatting the document.

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