

Development and In Vitro Evaluation of *Chakramardabeejadi Pralepa* and Its Cream: Antifungal Activity and Analytical Characterization

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ABSTRACT

Background: Dermatophytosis (*Tinea*) is a common superficial fungal infection of the skin, caused predominantly by *Trichophyton rubrum* and *Microsporum canis*. In Ayurveda, it can be correlated with *Dadru Kushtha*, a form of *Kshudra Kushtha*. *Chakramardabeejadi Pralepa*, described in Chakradatta, is a classical topical formulation comprising *Chakramarda* (*Cassia tora* Linn.), *Jeerak* (*Cuminum cyminum* Linn.) and *Sudarshanmoola* (*Crinum latifolium* Linn.), traditionally recognized for *Kushthaghna* and *Kandhuaghna* properties. However, the conventional *Lepa* form has practical limitations, including limited stability, characteristic odour, staining, and inconvenience during application. **Objectives:** To prepare *Chakramardabeejadi Pralepa* and develop a cream formulation using its hydroalcoholic extract; to evaluate and compare antifungal activity against *T. rubrum* and *M. canis*; and to assess physicochemical and analytical parameters. **Methods:** The *Lepa* was prepared. Hydroalcoholic extraction was performed using the Soxhlet method (ethanol:water 1:3). The cream formulation was developed through systematic optimization of the formulation components to achieve suitable consistency and homogeneity. Antifungal activity was assessed by agar well diffusion against MTCC strains. Analytical parameters including pH, Spreadability, HPTLC fingerprinting, heavy metals, and microbial limits were evaluated per CCRAS guidelines. **Results:** Both formulations showed moderate antifungal activity compared to the reference standard (Terbinafine HCl cream): zones of inhibition of 20 mm (*Lepa*) vs. 9 mm (Cream) against *T. rubrum*, and 18 mm (*Lepa*) vs. 8 mm (Cream) against *M. canis*. The cream (pH 5.25) met all safety and quality benchmarks. **Conclusion:** *Chakramardabeejadi* Cream is an effective and patient-friendly novel dosage form with moderate antifungal activity comparable to the classical *Lepa*, supporting its potential in the management of dermatophytosis.

Keywords: *Dadru*, *Chakramardabeejadi Pralepa*, Cream, Dermatophytosis, *Cassia tora*, Antifungal Activity, Agar well diffusion, *Lepa Kalpana*, Ayurveda.

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INTRODUCTION

The skin serves as the body's primary interface with the external environment, functioning as a critical protective barrier.⁽ⁱ⁾ Disorders affecting this organ carry substantial clinical significance, imposing considerable physical, psychological, and social burdens on affected individuals, in addition to their broader socioeconomic implications.⁽ⁱⁱ⁾ Within the Ayurvedic system of medicine, cutaneous disorders are collectively classified under the umbrella term *Kushtha*, which is further subdivided into two categories: *Mahakushtha* (major skin disorders) and *Kshudrakushtha* (minor skin disorders).⁽ⁱⁱⁱ⁾ Among the *Kshudrakushtha* conditions, *Dadru* stands out as one of the most commonly encountered presentations, with its pathogenesis primarily attributed to the vitiation of *Kapha* and *Pitta doshas*.^(iv)

The cardinal features of *Dadru* include *Kandu* (pruritus), *Utsanna Mandala* (elevated circumscribed plaques), *Raga* (erythema), and *Pidika* resembling *Atasipushpa* (macular rashes similar to flax flower).^(v) Contemporary medicine correlates *Dadru* with dermatophytosis (tinea), a superficial fungal infection caused by keratin-degrading fungi of the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*.^(vi, vii) Globally, dermatophytosis is estimated to affect approximately 20–25% of the population, with a substantial burden also reported in India.^(viii)

Topical formulations (*Lepa*) occupy a central role in Ayurvedic dermatology.^(ix) *Chakramardabeejadi Pralepa*, documented in Chakradatta, is a three-ingredient formulation of *Chakramarda* (seeds), *Jeerak* (seeds) and *Sudarshanmoola* (root) in a 1:1:¼ ratio. The constituent drugs exhibit *Kushthaghna* and *Kandughna* activities.^(x)

Table no.1: Ingredients of *Chakramardabeejadi pralepa* (Database on medicinal plants vol 2 and 8, 2005 Sharma P.V., 2013)

Drugs	Botanical Name	Family	Part Used	Ratio
<i>Chakramarda</i> ^(xiii)	<i>Cassia tora</i> Linn.	Caesalpiniaceae	Seeds	1 part
<i>Jeerak</i> ^(xiv)	<i>Cuminum cyminum</i> Linn.	Umbelliferae	Seeds	1 part
<i>Sudarshana</i> ^(xv)	<i>Crinum latifolium</i> Linn.	Amaryllidiaceae	Root	1/4 part

2.4 Pharmaceutical Processing & Formulation Development

2.4.1 Preparation of *Chakramardabeejadi Pralepa Churna*

Traditional *Lepa* preparations present several practical limitations: short shelf-life, requirement for fresh preparation before use, staining, unpleasant odour, and poor patient compliance.^(xi) Semi-solid cream formulations overcome these limitations by providing ease of application, better absorption, extended shelf life, uniform drug delivery, and cosmetic acceptability.^(xii) The present study was therefore undertaken to develop a cream from the hydroalcoholic extract of *Chakramardabeejadi Pralepa* and to evaluate its pharmaceutical, analytical, and antifungal properties.

2. MATERIALS AND METHODS

2.1 Study Design & Overview

The study was conducted in three major phases: Pharmaceutical formulation development, Analytical evaluation, and Antifungal testing (In Vitro). Formulation development involved systematic optimization of the selected ingredients and excipients to obtain a stable cream with desirable consistency. The developed formulation was then subjected to Analytical characterization and Antifungal Evaluation

2.2 Procurement and Authentication of Raw Materials

Chakramarda and *Jeerak* were sourced from Authentic drug suppliers in Haridwar. *Sudarshana* was collected from the herbal garden of Rishikul Campus, Haridwar. Authentication was performed according to the Ayurvedic Pharmacopoeia of India (API) protocols and validated through pharmacognostic studies at Vasu Research Centre, Vadodara, Gujarat.

2.3 Composition Of *Chakramardabeejadi Pralepa*

Prepared following the general principles of powder preparation described in Sharngadhara Samhita (Madhyama Khanda, 6/1).

Drug Authentication → Cleaning & Shade Drying → Pulverization & Sieving (No. 80) → Weighing (1:1:¼) → Blending → *Chakramardabeejadi Lepa Churna*

2.4.2 Preparation of *Chakramardabeejadi Pralepa*

Fine powders of *Chakramarda* (1 part), *Jeerak* (1 part), and *Sudarshana* ($\frac{1}{4}$ part) were mixed in the prescribed proportion. Required quantity of water (Q.S.) was added gradually to yield a smooth, homogeneous paste.

2.4.3 Hydroalcoholic Extraction (Soxhlet Method)

Chakramarda (200 g), *Jeerak* (200 g), and *Sudarshana* (50 g) — total 450 g coarsely powdered — were subjected to Soxhlet extraction with ethanol:water (1:3, v/v). The process was continued until the solvent in the extraction chamber turned clear. The filtrate was distilled for ~3 hours to remove ethanol, followed by complete drying on a water bath. The yield of hydroalcoholic extract was 38.8% (175 g from 450 g drug). In the main study, the distillate from the hydroalcoholic solvent was utilized in place of distilled water to enhance therapeutic efficacy of the cream.

2.4.4 Formulation Development of *Chakramardabeejadi Cream*

Table No.2: Composition Of *Chakramardabeejadi Cream* (Main Study)

Ingredient	Function	Quantity (500gm)
Hydroalcoholic Extract (g)	Therapeutically active herbal extract responsible for anti fungal, anti-inflammatory, and wound-healing activity	35g
Softemul 165 (g)	Primary emulsifying agent; stabilizes oil-in-water cream emulsion	15g
Ginol 1618 (g)	Co-emulsifier and consistency enhancer; improves cream texture and stability	15g
GMS NSE (g)	Emulsion stabilizer and thickening agent; enhances viscosity and emolliency	10g
Shea Butter (g)	Emollient and moisturizer; softens skin and improves spreadability	25g
Stearic Acid (g)	Consistency enhancer and secondary emulsifier; provides body to the cream	10g
Glycerin (ml)	Humectant; retains moisture and prevents drying of the formulation	20ml
Olive Oil (ml)	Emollient and lipid phase component; nourishes skin and improves spreadability	25ml
PVP K-30 (g)	Film-forming and stabilizing agent; enhances adhesion and formulation stability	5gm
Propylene Glycol (g)	Co-solvent, humectant, and penetration enhancer; improves extract dispersion	15gm
Distilled water	Aqueous vehicle; serves as the continuous phase and facilitates uniform dispersion of the formulation components	320ml
Fragrance	Flavouring/aroma agent; masks the characteristic odour of the herbal extract and improves sensory acceptability	1ml

2.5 Analytical Study

Centre following CCRAS guidelines and ASU Pharmacopoeia limits.

Successive pilot trials were conducted at Anamika Pharmacy, Haridwar, optimizing excipient proportions and process parameters. The final formulation was prepared using the fusion (hot process) method:

The oil-phase ingredients (SOFTEMUL 165, GINOL 1618, GMS NSE, shea butter, stearic acid, and olive oil) were melted at 70 °C. In a separate vessel, the extract, glycerin, and PVP K-30 were dispersed in distilled water and heated to the same temperature. The oil phase was gradually incorporated into the aqueous phase under continuous stirring to form an emulsion. Stirring was continued for approximately 35 min, followed by gradual cooling to 40 °C. The pH was adjusted as required, and the prepared cream was transferred into a clean, sterilized container and stored at room temperature for further evaluation.

Table 3: Physico-Chemical Parameters Of *Chakramardabeejadi Lepachuran* & Its Cream

S.No.	Analytical Parameter of <i>Lepa churna</i>	Analytical Parameter of cream
Organoleptic test	Appearance Colour Odour Uniformity of content	Appearance Colour Odour Consistency
Physio-chemical parameters	pH, Alcohol soluble extractive, water soluble extractive, Loss on drying, Total ash, Acid insoluble ash.	pH Spreadability
Identification	TLC/HPTLC	TLC/HPTLC
Microbial contamination (Limits as per ASU Pharmacopoeia)	Total bacterial count, Total fungal count	Total bacterial count, Total fungal count
Test for heavy or toxic metals (Limits as per ASU Pharmacopoeia)	Lead Cadmium Mercury	Lead Cadmium Mercury
Test for specific pathogens (Limits as per ASU Pharmacopoeia)	<i>Escherichia coli</i> , <i>Salmonella spp.</i> <i>Staphylococcus aureus</i>	<i>Escherichia coli</i> , <i>Salmonella spp.</i> <i>Staphylococcus aureus</i>
	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>

2.6 Antifungal Study (In Vitro — Agar Well Diffusion Method)

Test organisms: *Trichophyton rubrum* (MTCC 8477) on Sabouraud Dextrose Agar (SDA), and *Microsporum canis* (MTCC 2820) on Potato Dextrose Agar (PDA). Media were autoclaved at 121°C / 15 lb pressure / 20 min. A 10 µL aliquot of fungal culture was inoculated into the media, plates were poured (25 mL/plate), and wells (6–8 mm) were created using a sterile borer. Test samples (100 µL each: *Chakramardabeejadi Pralepa*, *Chakramardabeejadi* Cream, and Terbinafine HCl cream as standard) were dispensed into wells. Plates were incubated at 25°C for 48 h in a BOD incubator. Zones of inhibition (ZOI) and activity indices (AI = ZOI of sample / ZOI of standard) were recorded.()

3. OBSERVATIONS AND RESULTS

3.1 Pharmaceutical Study

The percent yield of *Chakramardabeejadi Lepa Churna* was 77%. Percent yields of individual drug powders: *Chakramarda* — 78%, *Jeerak* — 90%, *Sudarshana* — 60%. Weight loss on drying: *Chakramarda* 0.6%, *Jeerak* 0.8%, *Sudarshana* 20%. The hydroalcoholic extract yield was 38.8%. The cream was successfully prepared, with a final yield of 98% with minimal transfer loss.

3.2 Analytical Study

3.2.1 Organoleptic Characteristics

Table 4: Organoleptic Characteristics Of *Chakramardabeejadi Lepa Churna* And Cream

Parameter	<i>Lepa Churna</i>	Cream
Colour	Light brown powder	Light brown cream
Odour	Characteristic	Characteristic
Consistency	Rough fibrous powder	Uniform & Smooth

3.2.2 Physicochemical Parameters

Table 5 Physicochemical Parameters Of *Lepa Churna* And Cream

Parameter	<i>Lepa Churna</i>	Cream
pH	4.72	5.25
Loss on Drying	9.91%	—
Total Ash	5.46%	—
Acid Insoluble Ash	0%	—
Water Soluble Extractive	25.21%	—
Alcohol Soluble Extractive	13.93%	—
Viscosity	—	17,500 cP
Firmness (Spreadability)	—	250.85 g
Work of Shear (Spreadability)	—	299.34 g/cm ²

3.2.3 Heavy Metal Analysis

Table 6: Heavy Metal Analysis — All Values Within Asu Pharmacopoeia Permissible Limits

Metal	<i>Lepa Churna</i> (ppm)	Cream (ppm)	Permissible Limit
Lead	1.061	0.493	NMT 10 ppm
Cadmium	0.183	0.001	NMT 0.3 ppm
Arsenic	0.416	0.016	NMT 3 ppm
Mercury	0.162	0.124	NMT 1 ppm

3.2.4 Microbial Limit Tests

Table 7: Microbial Limit Test Results — Cream Within All Permissible Limits

Parameter	<i>Lepa Churna</i>	Cream	Permissible Limit
Total Microbial Plate Count	1,745 cfu/g	< 10 cfu/g	< 10 ⁵ cfu/g
Total Yeast & Mould Count	643 cfu/g	< 10 cfu/g	< 10 ³ cfu/g
<i>Staphylococcus aureus</i>	Absent	Absent	Absent
<i>Salmonella</i> sp.	Absent	Absent	Absent
<i>Pseudomonas aeruginosa</i>	Absent	Absent	Absent
<i>Escherichia coli</i>	Absent	Absent	Absent

3.2.5 HPTLC Fingerprinting

Table 8. Comparative Hptlc Fingerprint Profile Of *Chakramardabeejadi Lepa Churna* And Cream

Wavelength	<i>Lepa Churna</i>	Cream
254 nm	7 peaks; <i>Rf</i> : 0.16–0.89	6 peaks; <i>Rf</i> : 0.11–0.64
366 nm	9 peaks; <i>Rf</i> : 0.16–0.82	8 peaks; <i>Rf</i> : 0.24–0.73
540 nm	5 peaks; <i>Rf</i> : 0.29–0.75	5 peaks; <i>Rf</i> : 0.19–0.55

Mobile phase: Toluene : Ethyl acetate : Glacial acetic acid (7:2:1, v/v).

Note: The comparable HPTLC fingerprints of the *Lepa Churna* and cream indicate the presence of detectable phytochemical constituents across the three observation wavelengths, supporting the chromatographic identity of the formulated cream.

3.3 Antifungal Activity

The antifungal activity results, expressed as ZOI and AI against *T. rubrum* and *M. canis*, are presented in Table X. An AI ≥ 0.50 was considered significant. *Chakramardabeejadi Lepa* exhibited greater inhibition against both test organisms than the cream formulation. Nevertheless, both formulations showed moderate activity in comparison with the standard drug.

Table 9: Zones of Inhibition and Activity Indices of test samples against *T. rubrum* and *M. canis*

S.No.	Sample	ZOI <i>T. rubrum</i> (mm)	Activity Index (<i>T.</i> <i>rubrum</i>)	ZOI <i>M. canis</i> (mm)	Activity Index (<i>M. canis</i>)
1	<i>Chakramardabeejadi Pralepa</i>	20	0.67	18	0.56
2	<i>Chakramardabeejadi Cream</i>	9	0.30	8	0.25
3	Terbinafine HCl Cream (Standard)	30	1.00	32	1.00
4	Blank	NZI	—	NZI	—

4. DISCUSSION

4.1 Pharmaceutical Aspects

The transformation of classical *Chakramardabeejadi Lepa* into a cream formulation using its hydroalcoholic extract marks a significant development in Ayurvedic pharmaceuticals. The use of Soxhlet extraction with a hydroalcoholic solvent (ethanol:water 1:3) ensured comprehensive recovery of both polar and non-polar bioactive constituents, including anthraquinones, alkaloids, and flavonoids from all three constituent drugs. An extract yield of 38.88% is consistent with phytochemical productivity expectations for these plant materials.

The cream was optimized through successive formulation trials to achieve the ideal balance of consistency, spreadability, stability, and pH. The use of the distillate obtained during hydroalcoholic extraction, rather than plain distilled water, as the aqueous phase may facilitate the incorporation of water-soluble and moderately water-soluble volatile constituents into the final formulation, thereby potentially enhancing the phytochemical contribution of the extract.

4.2 Analytical Parameters

The analytical evaluation of *Chakramardabeejadi Lepa Churna* and cream demonstrated satisfactory quality attributes. The *Lepa Churna* showed a pH of 4.72, LOD of 9.91%, total ash of 5.46%, acid-insoluble ash of 0%, water-soluble extractive of 25.21%, and alcohol-soluble extractive of 13.93%, while the cream exhibited a skin-compatible pH of 5.25 with acceptable spreadability. Heavy-metal levels in both formulations were within the specified ASU Pharmacopoeia limits. Microbial evaluation showed 1,745 cfu/g total microbial count and 643 cfu/g yeast and mould count in the *Lepa Churna*, whereas both counts were <10 cfu/g in the cream, with specified pathogens absent in both. HPTLC fingerprinting revealed characteristic profiles for both formulations, supporting their quality and consistency.

4.3 Antifungal Activity

The invitro antifungal findings demonstrated comparatively greater activity of *Chakramardabeejadi*

Lepa than its cream formulation against both test organisms. Against *T. rubrum*, the *Lepa* produced a ZOI of 20 mm (AI 0.67), whereas the Cream showed 9 mm (AI 0.30), compared with 30 mm for terbinafine (AI 1.00). Similarly, against *M. canis*, inhibition zones of 18 mm (AI 0.56) and 8 mm (AI 0.25) were observed for the *Lepa* and Cream, respectively, while terbinafine produced a 32 mm zone. The reduced activity of the Cream may be associated with incorporation of the hydroalcoholic extract into the semisolid base, which can lower the effective concentration bioactive constituents.

The antifungal mechanism is likely multi-factorial: chrysophanol and anthraquinone derivatives from *Cassia tora* disrupt fungal cell wall synthesis; the essential oil compounds of *Cuminum cyminum* (cumaldehyde, cuminol) inhibit ergosterol biosynthesis and membrane integrity; and the alkaloids from *Crinum latifolium* (9-methoxy-crispowellin B) impair microbial enzyme activity. Taken together, these bioactive constituents create a synergistic effect that may help circumvent single-target resistance mechanisms seen with conventional antifungals,

5. CONCLUSION

The present study demonstrated the successful development of a cream dosage form from the classical Ayurvedic *Chakramardabeejadi Pralepa*. Both the traditional *Lepa* and the developed cream exhibited antifungal activity against *T. rubrum* and *M. canis*, with comparatively higher zones of inhibition observed for the *Lepa* than the cream. Hydroalcoholic extraction using the Soxhlet method yielded 38.88% extract, which was incorporated as the principal active component of the cream. Physicochemical, heavy metal, and microbiological evaluation confirmed the quality and safety of both the *Lepa Churna* and cream as per applicable ASU Pharmacopoeial standards. Conversion into a cream dosage form provided practical advantages over the conventional *Lepa*, including improved ease of application, reduced staining and odour, and greater

convenience of use. Overall, the findings provide preliminary experimental support for the traditional use of *Chakramardabeejadi Pralepa* and indicate its potential as a topical herbal formulation for dermatophytosis, warranting further clinical evaluation.

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