ejpmr, 2025, 12(8), 238-243

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN (O): 2394-3211

ISSN (P): 3051-2573

EVALUATION OF ANTIFUNGAL BIOACTIVITY AND CHEMICAL CHARACTERISTICS OF DERMOSAP: A MODERN STUDY OF A POLYHERBAL AYURVEDIC OINTMENT

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Article Received on 31/05/2025 **Article Revised on 21/06/2025** **Article Accepted on 11/07/2025**

ABSTRACT

Introduction: *Malahara* in Ayurveda is a soothing, lipid-based ointment using herbal medicines, oils, ghee, or beeswax to directly deliver powerful herbal actives and gently heal skin conditions like ulcers, eczema, infections, and inflamed joints. **Materials and methods:** The modern formulation, Dermosap Ointment, developed by Sitaram Ayurveda Pvt. Ltd., combines major classical antifungal herbs such as *Nimba*, *Haridra*, and *Karanja*. The formulation contains a total of 27 herbs, along with coconut oil and three lipid-based carriers. The finished ointment was evaluated through organoleptic and physicochemical tests (including pH, stability), phytochemical screening, and in vitro antifungal activity against *Candida albicans* using the Kirby–Bauer disk diffusion method. **Results:** Dermosap ointment is a soothing, pale-brown semisolid with a pleasant, signature herbal aroma. Laboratory analysis revealed a mildly acidic pH of 4.8. The formulation exhibited stability under storage conditions, showed no signs of rancidity, and retained essential qualities such as low moisture content, good spread ability, and thermal resilience. Phytochemical screening confirmed the presence of bioactive compounds such as carbohydrates, flavonoids, glycosides, saponins, alkaloids. The ointment exhibited notable antifungal activity, producing a 27 mm zone of inhibition against *Candida albicans*, which closely approached the 30 mm zone produced by the standard antifungal agent, Terbinafine. **Conclusion:** Dermosap Ointment upholds the Ayurvedic legacy by integrating classical herbal actives into a stable, lipid-based formulation that meets modern pharmaceutical quality standards and demonstrates strong antifungal activity producing a 27 mm inhibition zone against *Candida albicans*, using CLSI-standardized disk diffusion methodology. In addition to its antifungal efficacy, Dermosap Ointment beneficial in managing skin irritation due to its soothing herbal base and absence of synthetic additives, making it a safe and skin-friendly alternative for prolonged topical use.

KEYWORDS: *Malahara*, Dermosap ointment, Antifungal activity, *Candida albicans*, *Dadru kushta*.

1. INTRODUCTION

In Ayurveda, the concept of *Kalpana* means formulations encompasses a rich spectrum of dosage forms, each thoughtfully designed to enhance efficacy, shelf-life, and patient suitability. In Ayurveda *Panchavidha Kashaya Kalpanas* are *swarasa* (fresh herbal juices), *kalka* (medicated pastes), *kwatha* (decoctions), *hima* (cold infusions) and *phanta* (hot infusions).[1] Along with these fundamental dosage forms Ayurveda advocates the administration of various formulations to manage different pathological conditions through various routes including internal and external. *Malahara* is an Ayurvedic formulation for external application, used to provide local effects, and also to promote ulcer healing.[2]

Malahara, the Ayurvedic medicated ointment, offers a time-tested solution for localized healing and skin nourishment. One of its greatest strengths lies in its ability to deliver herbal actives directly to the affected area, whether it's inflamed joints, chronic wounds, burns, or skin infections without burdening the digestive system or causing systemic side effects. The lipid base like oil, ghee, beeswax, or fat) used in *Malahara* enhances the penetration of herbs through the skin and supports deep tissue action, especially in conditions like skin infections, fungal infections and eczema. Because of its soothing, emollient nature, *Malahara* also helps maintain skin hydration, reduce itching, and form a protective barrier against environmental irritants. *Malaharas* represent a gentle, sustainable, and effective topical therapy in

Ayurvedic practice with minimal processing and no need for preservatives.

Sarangadhara Acharya, in his classical Ayurvedic text *Sarangadhara Samhita*, has elaborated on *Malahara* as a specialized external dosage form used for treating various skin and musculoskeletal conditions. He emphasizes the use of *churna*, oils or *snehadravya*, and wax or resins as the base to ensure stability, consistency, and effective delivery of active ingredients. The preparation process involves careful heating and blending of ingredients to ensure uniformity and therapeutic potency.

Dadrukuṣṭha is a type of *kṛmijanyatwakvikara* (fungal skin disease of parasitic origin), has become increasingly common in the present era due to *mithyaaharavihara*, *doṣaprakopa*, and changing environmental factors. Classical texts describe numerous krimighna, kāṇḍughna, and kuṣṭhaghna herbs like *nimba* (*Azadirachta indica*), *haridrā* (*Curcuma longa*), and *karanja* (*Pongamia pinnata*), known for their potent antifungal and skin-purifying actions.[3]

Dermosap Ointment, developed by Sitaram Ayurveda Pvt. Ltd., is a contemporary *Malahara* formulations is rooted in these Ayurvedic principles while embracing modern standards of formulation development and quality control. The *saṃskaras* and *samyak siddhi lakṣaṇas* are carefully observed to ensure therapeutic potency. In alignment with current formulation development and quality control protocols, the product undergoes standardization through parameters including pH, spreadability, microbial load testing etc. This formulation upholds the values of both classical wisdom and pharmaceutical rigor.

Microorganisms can be broadly divided into four main groups bacteria, fungi, viruses, and actinomycetes all of which are responsible for a wide variety of infectious diseases. Consequently, it is vital to investigate these pathogens in detail, including how they cause disease in humans, and to explore effective methods for their diagnosis and treatment. In vitro studies serve as essential preliminary screening tools for novel antimicrobial agents and are also used to test the susceptibility of isolates. This helps identify which drugs may be therapeutically effective. Any compound that halts the growth or eradicates microorganisms is classified as an antimicrobial agent; specifically, a compound that inhibits the growth of fungi is termed fungistatic, meaning it prevents fungal proliferation without necessarily killing the organism. This article discusses the standardisation and Antifungal activity of Dermosap Ointment as an Ayurvedic intervention for fungal infections, and the importance of consistent therapeutic attributes to ensure the clinical efficacy.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The raw materials required for the preparation of Dermosap Ointment were collected from the Raw Material Store of Sitaram Ayurveda Pvt. Ltd., Thrissur. All herbal ingredients were authenticated and approved by the Quality Control division based on classical Ayurvedic texts and standard references such as the Ayurvedic Pharmacopoeia of India and IS and IP guidelines. Authenticated specimens were documented and stored appropriately in the QC laboratory for further use.

2.2 Method of Preparation

Ingredients numbered 1 to 27 were used in the preparation of the medicated oil base for Dermosap Ointment. After oil preparation, ingredients numbered 28 to 31 were added into the oil to complete the final ointment formulation. The complete list of ingredients is tabulated below (Table -1).

Table 1: Ingredients of Dermosap Ointment.

Sl.	Sanskrit Name	Botanical /Common Name	Part used	Quantity
1	Sapthachada	*Alstonia scholaris*	Stem Bark	0.017 g
2	Padmaka	*Prunus cerasoides*	Heart Wood	0.017 g
3	Shyamalatha	*Ichnocarpus frutescens*	Root	0.017 g
4	Nimba	*Azadirachta indica*	Stem Bark	0.017 g
5	Gajapippali	*Scindapsus officinalis*	Fruit	0.017 g
6	Agaru	*Aquilaria agallocha*	Heart Wood	0.017 g
7	Vishala	*Citrullus colocynthis*	Whole Plant	0.017 g
8	Guduchi	*Tinospora cordifolia*	Stem	0.017 g
9	Bruhati	*Solanum anguivi*	Root	0.017 g
10	Murva	*Chonemorpha fragrans*	Root	0.017 g
11	Sathavari	*Asparagus racemosus*	Root Tuber	0.017 g
12	Patola	*Trichosanthes lobata*	Whole Plant	0.017 g
13	Mustha	*Cyperus rotundus*	Rhizome	0.017 g
14	Brahmi	*Bacopa monnieri*	Whole Plant	0.017 g
15	Dusparshaka	*Tragia involucrata*	Root	0.017 g
16	Parpataka	*Hedyotis corymbosa*	Whole Plant	0.017 g
17	Katuka	*Picrorhiza kurroa*	Root	0.017 g

18	Vacha	*Acorus calamus*	Rhizome	0.017 g
19	Harithaki	*Terminalia chebula*	Fruit Rind	0.017 g
20	Amalaki	*Emblica officinalis*	Fruit Rind	0.017 g
21	Vibhithaki	*Terminalia bellirica*	Fruit Rind	0.017 g
22	Pippali	*Piper longum*	Fruit	0.017 g
23	Yashtimadhu	*Glycyrrhiza glabra*	Root	0.017 g
24	Kutajabija	*Holarrhena antidysenterica*	Seed	0.017 g
25	Usheera	*Vetiveria zizanioides*	Root	0.017 g
26	Pata	*Cyclea peltata*	Rhizome	0.017 g
27	Tankanam	Borax	As Such	0.017 g
28	Kerathaila	Coconut oil	As Such	0.880 g
29	Psora herb oil	-	As Such	0.088 g
30	-	White Petroleum Jelly	As Such	0.058 g
31	-	Hard Paraffin	As Such	0.058 g

2.3 Organoleptic Analysis
The organoleptic characteristics of Dermosap Ointment such as colour, texture and odour were observed and tabulated.[4]

2.4 Physiochemical Analysis
Physiochemical analysis was carried out as per the standard procedures outlined in the Ayurvedic Pharmacopoeia of India for Malahara formulations. The parameters evaluated such as, pH (10% aq. solution), rancidity, refractive index, spreadability, loss on drying, thermal stability (at 5°C, 25°C, and 45°C for 5 days), acid value, saponification value and solubility.[4]

2.5 Preliminary Phytochemical Analysis
The preliminary phytochemical screening of Dermosap oil was carried out to detect the presence of various bioactive phytochemical components, including sugar, reducing sugar, ketose, amino acids, proteins, starch, quinones, glycosides, flavonoids, phenols, saponins, alkaloids, tannins, and coumarins.[5]

2.6 Antimicrobial Activity
Antimicrobial testing is essential to evaluate the ability of a formulation to inhibit or kill microbial pathogens, particularly for herbal products claiming antibacterial or antifungal efficacy. The present study was designed to assess the comparative antifungal activity of Dermosap Ointment. *Candida albicans* was selected as the test microorganism, considering its clinical relevance in fungal skin infections. The assay was performed using the disk diffusion method, following standardized protocols developed initially in the 1960s and subsequently refined by the World Health Organization (WHO) and the Clinical and Laboratory Standards Institute (CLSI).[4]

2.6.1 Place of Study
The preparation of Dermosap Ointment was carried out in the Production Department of Sitaram Ayurveda Pvt. Ltd., Thrissur, Kerala. Physicochemical and phytochemical analyses were conducted in Sitaram's Quality Control Laboratory. The in vitro antifungal study was performed at the Tropical Institute of Ecological Sciences (TIES), Kottayam, Kerala.

2.6.2 Material Requirements
- Muller Hinton agar (MHA)
- Sterile swab
- 2-15 hours (overnight) young culture of Candida albicans
- Standard antibiotic disc (Terbinafine)
- Sterile disc impregnated in sample
- Isopropyl alcohol (IPA)

2.6.3 Antifungal Sensitivity Test
The Kirby–Bauer disk diffusion method is a standardized, reliable assay widely used in clinical microbiology to assess bacterial susceptibility to antibiotics. Mueller–Hinton agar - a non-selective, starch-containing medium poured to a precise 4 mm depth and buffered to pH 7.2 -7.4 to ensure consistent diffusion of antimicrobial agents. A standardized bacterial suspension, is evenly swabbed onto the agar surface in three directions to create a confluent lawn. After allowing the plate to absorb excess moisture, the Dermosap is first dissolved in isopropyl alcohol to form a uniform solution. This solution is then used to impregnate plain discs, which are saturated with the prepared sample, gently pressed to ensure contact, Terbinafine is used as positive control and IPA impregnated disc as negative control. Incubate the plate inverted at 35°C for 16 -18 hours, clear zones of inhibition are measured in millimetres.[6]

2.6.4 Data Collection and Analysis
Diameter of the inhibitory zones was reported in Millimetres (mm) by using foot ruler.

3. RESULTS
3.1 Organoleptic Analysis
Primary evaluation of Dermosap ointment was done based on its colour, odour and texture and tabulated in the Table. 2.

Table 2: Organoleptic characteristics of Dermosap Ointment.

Sl. No.	Characteristics	Observations
1	Colour	Pale brown
2	Odour	Characteristic
3	Texture	Semisolid

3.2 Physicochemical Analysis

The results of the physicochemical analysis of Dermosap ointment is presented in Table 3.

Table 3: Physicochemical Analysis of Dermosap ointment.

Sl. No	Parameters	Results
1	pH (10% aqueous solution)	4.80
2	Rancidity	Not rancid (-ve)
3	Loss on Drying @105^{0}C	0.17 %
4	Refractive index @ 27^{o}C	1.460
5	Saponification value	175.27 mg KOH/g
6	Acid value	0.357 mg KOH/g
7	Solubility	Partially soluble in water
8	Spreadability	Uniform
9	Thermal stability (5 °C–45 °C for 5 days)	Stable

3.3 Preliminary Phytochemical Analysis.

Table 4 presents the results of the preliminary phytochemical screening of the chloroform extract of Dermosap base oil. The analysis was conducted to detect the presence or absence of key phytoconstituents such as carbohydrates, sugars, proteins, glycosides, steroids, flavonoids, phenols, and other bioactive compounds commonly associated with herbal medicinal efficacy.

Table 4: The phytochemical analysis of Dermosap Oil.

Sl. No.	Phytochemical constituents	Name of the test conducted	Dermosap Oil (Chloroform Extract)
1.	Carbohydrate	Molisch's test	+
2.	Sugar	Benedict's test	+
3.	Reducing Sugar	Fehling's test	+
4.	Ketose	Seliwanoff's test	+
5.	Protein	Biuret test	-
6.	Starch	K I test	-
7.	Glycoside	Keller killiani test	+
8.	Steroid	Salkowski test	+
9.	Terpenoid	Salkowski test	-
10.	Flavonoid	Alkaline reagent	+
11.	Phenol	Phenol reagent test	-
12.	Saponin	Foam test	+
13.	Alkaloid	Wagner's reagent	+
14.	Tannin	Ferric chloride test	-
15.	Coumarin	NaOH test	-
16.	Amino acids	Ninhydrin test	-

Remarks: +(present), - (absent)

3.5 Antifungal Analysis

Table 5: Antifungal analysis of *Candida albicans.*

Sl.No.	Pathogen	Zone of inhibition of Dermosap sample 20 µm (1mg/ml) in mm	Zone of inhibition of positive control (Terbinafine 15 mcg in mm)	Zone of inhibition of Negative control (Isopropyl alcohol)
1	***Candida albicans***	**27mm**	**30mm**	**0**

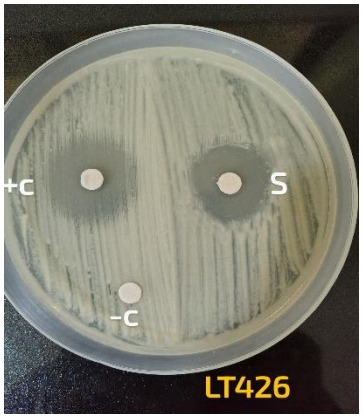

Figure 1: Zone of inhibition of Dermosap, positive control and Negative control.

4. DISCUSSION

Standardization of a finished herbal formulation begins with organoleptic evaluation, which serves as the initial qualitative measure of product identity, quality, and consumer acceptability. Organoleptic parameters such as colour, texture, and odour provide immediate sensory feedback and play a crucial role in routine quality control procedures. In Ayurvedic formulations, sensory attributes are essential as they reflect the authenticity, consistency, and effectiveness of the ingredients used. Pale brown colour of Dermosap ointment indicates the presence of herbal drugs while the characteristic odour provides insights into the natural composition. Additionally, the semisolid texture ensures ease of application and absorption. Evaluating these parameters helps maintain product integrity and enhances user experience.

Physicochemical testing is essential for quality control and standardization of Ayurveda ointments. The pH of 4.80 indicates that the ointment falls within a suitable range for topical application, ensuring compatibility with the skin's natural pH and minimizing the risk of irritation.[7] The absence of rancidity confirms that the formulation remains chemically stable and free from oxidative deterioration. The loss on drying (LOD) of 0.17% suggests minimal moisture content, reducing the risk of microbial contamination and degradation of active ingredients. The refractive index of 1.460 reflects the purity and consistency of the formulation. The saponification value of 175.27 indicates the presence of esterified fatty compounds, which contribute to the emollient properties of the ointment, enhancing skin absorption.[8] A low acid value 0.357 suggests minimal free fatty acid content, which is beneficial in preventing skin irritation.[9] Dermosap ointment shows less solubility in water. Additionally, the formulation demonstrated thermal stability, ensuring that temperature variations do not compromise its consistency and effectiveness.

The phytochemical analysis of the chloroform extract of Dermosap oil, revealed the presence of several bioactive compounds that are known to possess antifungal and skin-protective properties. Positive results for carbohydrates, sugars, reducing sugars, and ketoses suggest that the formulation contains essential energy sources and metabolic intermediates that may support skin regeneration and healing.[10] Notably, the presence of glycosides, flavonoids, saponins, and alkaloids is of particular interest due to their well-documented antifungal and antimicrobial activities. Glycosides are known to disrupt fungal cell membranes,[11] while flavonoids possess antioxidant and antifungal properties by inhibiting fungal growth and neutralizing oxidative stress in infected tissues.[12] Saponins contribute by forming complexes with sterols in fungal cell membranes, leading to increased permeability and cell lysis.[12] Alkaloids, on the other hand, exhibit fungistatic or fungicidal effects through interference with fungal enzyme systems and cellular metabolism.[13] The detection of steroids may aid in reducing inflammation and soothing the affected skin areas.[14] Although some compounds such as proteins, starch, tannins, coumarins, terpenoids, phenols, and amino acids were absent, the presence of the aforementioned bioactive supports the therapeutic rationale of Dermosap ointment as a natural and effective remedy for fungal infections.

The antifungal potential of Dermosap was evaluated against *Candida albicans* using a 20 µm (1 mg/mL) concentration, yielding a zone of inhibition of 27 mm, compared with a 30 mm zone for the positive control (15 µg terbinafine), and 0 mm for the negative control (isopropyl alcohol). This indicates that Dermosap exhibits strong fungistatic activity, nearly comparable to terbinafine - the standard antifungal agent. In the context of agar diffusion assays, an inhibition zone of ≥19 mm typically signifies sensitivity in *Candida*spp. Therefore, with a 27 mm zone, Dermosap demonstrates robust antifungal efficacy. The absence of inhibition by the negative control confirms that growth suppression is due to the active compounds in the extract.

5. CONCLUSION

Dermosap ointment demonstrates strong potential as a scientifically standardized Ayurvedic formulation for fungal infections. Its organoleptic properties confirm the presence of botanical ingredients while ensuring user-friendly application and absorption. Physicochemical assessments indicate it is compatible with skin physiology and ideal for reducing skin irritation. Phytochemical analysis recognized for their antifungal, antioxidant, and anti-inflammatory effects. These findings support the ointment's rationale as a natural therapeutic agent for skin infections. In antifungal testing against *Candida albicans* confirms strong fungistatic activity directly attributable to the formulation's active components. Together, organoleptic, physicochemical, phytochemical, and microbiological evidence validates Dermosap as a stable, effective, and skin-friendly Ayurvedic ointment. By integrating traditional botanical principles with modern quality control, Dermosap formulation offers a credible, natural alternative in

managing fungal skin conditions combining authenticity, safety, therapeutic efficacy, and enhanced user experience in effective and holistic skin care.

ACKNOWLEDGEMENT

The authors express their gratitude to Dr. D. Ramanathan (Managing Director), Dr. Vignesh Devaraj (Technical Director), Smt. Janani Ramanathan (Director), Mr. Sandeep VR (Chief General Manager), and Dr. Adithya Peethambara Panicker (Assistant General Manager-Marketing & Innovations) of Sitaram Ayurveda (P) Ltd., Thrissur, Kerala, for their continuous support and encouragement. We also sincerely acknowledge Tropical Institute of Ecological Sciences (TIES), Kottayam, Kerala for their assistance in conducting antifungal studies.

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