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Mahua Oil as a Promising Antipsoriatic Agent: Insights from In Vitro Studies

Research Article

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Abstract

Psoriasis, a chronic autoimmune skin condition, aligns with the Ayurvedic classification of *Kitibha*, which results from vitiated Vata and Kapha doshas and the accumulation of *Ama*. This study investigates the therapeutic potential of *Madhuca longifolia* (Mahua) oil in the management of psoriasis. Organoleptic Evaluation, Physicochemical analysis (Acid Value, Saponification Value, Iodine Value) and Gas Chromatography-Mass Spectrometry (GC-MS) were performed To check authenticity and purity of the oil. In vitro evaluations on HaCaT keratinocyte cell lines were conducted to assess antiproliferative and anti-inflammatory activities. Results revealed that Mahua oil significantly reduced pro-inflammatory cytokines (IL-1β, TNF-α) and nuclear transcription factor NF-κB ($p < 0.05$). Furthermore, Mahua oil demonstrated inhibitory effects on COX-2 pathway, confirming its efficacy in modulating inflammatory responses. These findings support its traditional use and propose Mahua oil as a viable natural treatment for psoriasis.

Keywords: Psoriasis, Mahua oil, GCMS, HaCaT cells, IL-1β, TNF-α, NF-κB, COX-II.

Introduction

Psoriasis is a chronic, immune-mediated dermatological condition characterized by hyperproliferation and abnormal differentiation of keratinocytes, accompanied by inflammation and immune dysregulation. It affects approximately 2–3% of the global population and significantly impairs quality of life, often leading to psychological distress, including anxiety and depression [1]. Conventional treatments primarily involve immunosuppressants, corticosteroids, and biologic agents; however, these are often associated with adverse effects, long-term dependency, and economic burden, especially in resource-limited settings [2].

In Ayurvedic literature, psoriasis is closely associated with *Kitibha*, a type of *Kshudra Kushtha*, which arises from the vitiation of *Vata* and *Kapha* doshas, along with the accumulation of *Ama* (toxins) and impairment of *Rakta dhatu* (blood tissue) [3]. Vata contributes to dryness and scaling, Kapha to thickening and plaque formation, while Pitta may exacerbate inflammation. The traditional Ayurvedic approach to treating *Kitibha* includes dosha pacification, detoxification (*shodhana*), and topical or systemic application of herbal oils known for their emollient, anti-inflammatory, and detoxifying properties.

Madhuca longifolia (Mahua) commonly known as the "Tree of Life" among tribal populations of India due to its diversified uses as nutrient, medicine and as fodder, Mahua oil has been used traditionally for managing a wide range of conditions including skin disorders, rheumatism, and inflammation [4]. Mahua oil contains an abundance of fatty acids such as linoleic, palmitic, and stearic acids, along with bioactive phytosterols that contribute to its skin conditioning, moisturizing, and anti-inflammatory effects [5]. Despite its extensive traditional usage, scientific validation of Mahua oil's efficacy in treating psoriasis is limited.

Recent advances in dermatological research emphasize the role of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and nuclear factor kappa B (NF-κB) in the pathogenesis of psoriasis [6–8]. These molecular mediators contribute to the hyperproliferation of keratinocytes and perpetuate chronic inflammation. The HaCaT keratinocyte cell line has emerged as a reliable in vitro model for investigating antipsoriatic activity due to its preserved differentiation characteristics and responsiveness to inflammatory stimuli [9].

In this study, we evaluate the physicochemical and phytochemical characteristics of cold-pressed Mahua seed oil and investigate its antiproliferative and anti-inflammatory effects on HaCaT cells. Through MTT cytotoxicity assay, cytokine quantification via ELISA, and cyclooxygenase (COX) enzyme inhibition assay, we aim to scientifically substantiate the traditional claims regarding the antipsoriatic potential of Mahua oil.[10-12]

Materials and Methods

Materials

Culture Media and Reagents: Dulbecco's Modified Eagle Medium (DMEM, AT149-1L, HiMedia), Fetal Bovine Serum (FBS, RM10432, HiMedia), Penicillin-

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Streptomycin (Sigma-Aldrich, P0781), Dimethyl Sulfoxide (DMSO, Cat. No. 67685, SRL), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich), Bovine Hemin Chloride (SRL-78372), Phenol (Fisher Scientific-35953), Arachidonic acid (SRL-20975), TMPD solution (17 mM, HiMedia, GRM445), LOX Enzyme (Sisco Research Laboratories Pvt. Ltd, Cat. No. 9029-60-1).

ELISA Kits: Human IL-1β ELISA Kit (GENLISA™, Cat. No. KB1063), Human TNF-α ELISA Kit (GENLISA™, Cat. No. KB1145), Human NF-κB ELISA Kit (GENLISA™, Cat. No. KBH14016).

Others: Tris-Cl buffer (pH 8.0, 100 mM), iron sulphate (Merck), ammonium thiocyanate (SRL), Celecoxib (positive control, TCI-C2816), sterile double-distilled water.

Procurement and Preparation of Mahua Oil

Cold-pressed seed oil of *Madhuca longifolia* was obtained from Cit Spray Aroma Science, Nagpur, Maharashtra, India.

Organoleptic Evaluation, Physicochemical analysis (Acid Value, Saponification Value, Iodine Value) and Gas Chromatography-Mass Spectrometry (GC-MS) were performed To check authenticity and purity of the oil.

Organoleptic Evaluation

The oil was examined for its characteristic color, odor, and consistency.

Physicochemical Characterization of Oil

The acid value, saponification value, and iodine value of Mahua oil were determined as per the methods outlined by the Food Safety and Standards Authority of India (FSSAI). All analyses were performed in triplicate and the results were expressed as mean ± standard deviation. [13]

GC-MS Analysis of Fatty Acid Composition

Gas Chromatography–Mass Spectrometry (GC-MS) was conducted using a SHIMADZU GCMS-QP-2010 Plus instrument equipped with an Rtx-5MS capillary column (30.0 m × 0.32 mm × 0.50 µm). Helium was used as the carrier gas with a flow rate of 41.0 mL/min. The oven temperature was programmed from 45°C to 270°C in controlled ramps. The injection port temperature was 270°C and ion source temperature was 200°C. Mass spectra were interpreted using integrated software.

In Vitro Cytotoxicity (MTT Assay)

Cell Line Culture

HaCaT human keratinocyte cell lines were procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in DMEM supplemented with 10% FBS and 1% Penicillin-Streptomycin, maintained at 37°C in a humidified incubator with 5% CO_2.

MTT Assay

Cells (10,000 cells/well) were seeded in 96-well plates and incubated for 24 h. Subsequently, cells were treated with Mahua oil at concentrations ranging from 0.078 µl to 5 µl (diluted in incomplete medium). After 24 h, MTT solution (5 mg/mL) was added and incubated for 2 h. The formazan crystals were solubilized with 100 µl DMSO, and absorbance was measured at 540 nm using a microplate reader (iMark™, Bio-Rad, USA). Percentage viability was calculated as:

$$\% \text{ Viable cells} = (A_{test} / A_{Control}) * 100$$

(A_{test} = Absorbance of test sample)
($A_{Control}$ = Absorbance of Control)

Protein Expression Analysis via ELISA

Protein expressions of IL-1β, TNF-α, and NF-κB were quantified using specific sandwich ELISA kits as per manufacturer protocols. Briefly, treated and untreated HaCaT cells (control and IC_{50} dose of Mahua oil) were used for analysis. Samples were incubated with capture and detection antibodies, followed by streptavidin-HRP and TMB substrate. The reaction was stopped and absorbance was recorded at 450 nm using an ELISA plate reader.

COX Enzyme Inhibition Assay

Cyclooxygenase (COX) Inhibition

Reaction mixtures containing Tris-Cl buffer, enzyme reagents (Bovine Hemin, Phenol), and Mahua oil dilutions were prepared in 96-well plates. The reaction was initiated by adding arachidonic acid and TMPD, incubated for 10 minutes, and read at 595 nm. Celecoxib (25 µM) was used as the positive control.

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard error of the mean (SEM). Statistical significance was assessed using one-way analysis of variance (ANOVA).

Results and Discussion

Organoleptic Evaluation and Physicochemical Properties of Mahua Oil

Table 1: Organoleptic Evaluation of Mahu oil.

Parameter	Observation
Color	pale to dark yellow liquid
Odor	Pleasant, sweet fragrance
Consistency	Viscous, smooth oily texture

Physicochemical Properties of Mahua Oil

The physicochemical parameters of Mahua oil were analyzed to confirm its purity and suitability for topical application. As shown in *Table 2*, the observed values were within acceptable ranges: acid value (24.10), saponification value (188.3), and iodine value (68.01). These values correlate well with literature findings and indicate the oils unsaturation and emollient potential and purity of oil. [5].

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Table 2. Physicochemical properties of Mahua oil.

Parameters	Reported	Observed
Acid value (mg of KOH/g)	20-25	24.10
Saponification value (mg of	187-197	188.3
Iodine value (I_2/100g)	55-70	68.01

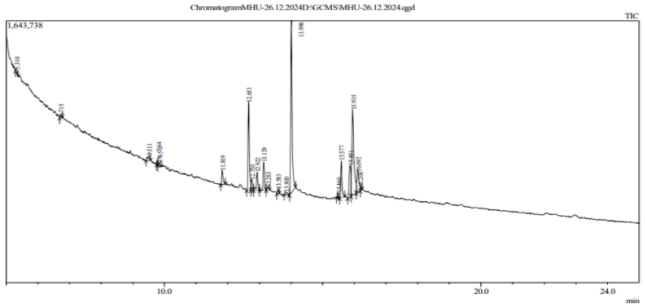
Fatty Acid Profile

GC-MS analysis identified major fatty acid components in Mahua oil. Notable constituents included palmitic acid (28.98%), methyl linolenate (21.35%), behenic acid (5.61%), and phytol (5.88%), each known for their skin conditioning and anti-inflammatory properties [4]. These constituents are crucial in barrier repair and inflammation control. (*Table 2*)

Table 3: Fatty acid composition of Mahua oil

Sr. no.	Chemical Name	Common Name	Area %	Role
1	Hexadecanoic acid	Palmitic Acid	28.98%	Skin conditioning Agent, emollient
2	Docosanoic acid	Behenic Acid	5.61%	Emollient, Moisturizing agent
3	2 heptadecanone	Stearic acid	1.83%	Moisturizing agent, Emollient
4	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	Phytol	5.88%	Antioxidant, Precursor for Vitamin E
5	9,12,15-Octadecatrienoic acid, methyl ester	Methyl Linolenate	21.35%	Anti-inflammatory property, antioxidant
6	11,14-Eicosadienoic acid, methyl ester	Methyl Eicosadienoate	6.69%	Reduces inflammation and supports skin barrier, Anti-inflammatory

Figure 1. GC-MS chromatogram of Mahua oil showing major constituents.



Antiproliferative Effects on HaCaT Cells

The skin is a continuously self-renewing organ that actively participates in the host defenses. Under homeostatic conditions, keratinocyte differentiate and mature from proliferating nucleated basal cells to the highly differentiated, nucleus-free corneocytes.

In Psoriais keratinocyte proliferation is induced by the cytokines that contributes to thickened skin, a scaly surface appearance, epidermal hyperplasia, hyperkeratosis, and parakeratosis. Altered homeostasis of proliferation and differentiation of cell act on the immune cells to continue the inflammatory response. An ideal agent for treating psoriasis should have the role in antiproliferation, anti-inflammation, and immunomodulation. [14,15]

Figure 2 A. Antiproliferative Effects of Mahua oil on HaCaT cells at various concentrations measured using MTT assay

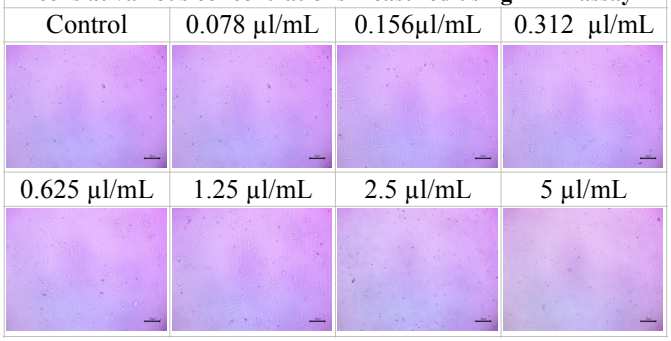
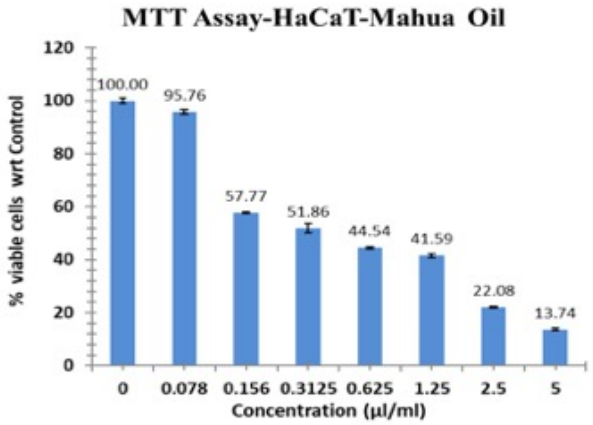


Figure 2 B: Antiproliferative Effects of Mahua oil on HaCaT cells at various concentrations measured using MTT assay



The cytotoxic effect of Mahua oil on HaCaT human keratinocyte cells was evaluated using the MTT assay. Cells were exposed to various concentrations of Mahua oil (0.078–5 µl/mL) for 24 hours, and cell viability was assessed by measuring absorbance at 540 nm. The results demonstrated a concentration-dependent reduction in cell viability (Figure 2A and 2B).

At the lowest concentration (0.078 µl/mL), cell viability was maintained at 95.76%, which was comparable to the control (100%). A significant decline in viability was observed at higher concentrations: 57.77% at 0.3125 µl/mL, 51.86% at 0.625 µl/mL, 44.54% at 1.25 µl/mL, and 22.08% at 2.5 µl/mL. The highest dose (5 µl/mL) resulted in only 13.74% cell viability. These findings indicate a dose-dependent cytotoxic effect of Mahua oil on keratinocytes.

On microscopic observations (Figure 2A) untreated control cells showed a typical characteristic appearance of epithelial cells. They are well-differentiated and growing in a healthy state and

confluency, whereas cells treated with higher concentrations of Mahua oil exhibited rounding, shrinkage, and detachment, suggestive of cytotoxicity.

All results are presented as mean ± SEM of triplicate experiments. Statistical analysis using one-way ANOVA revealed that reductions in cell viability at concentrations ≥0.3125 µl/mL were statistically significant ($p < 0.05$) when compared to the control group.

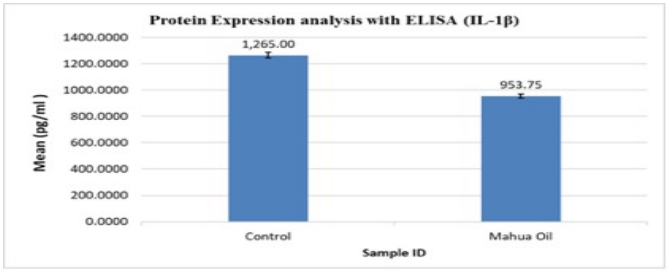
These findings suggest that Mahua oil effectively inhibits HaCaT cell proliferation, indicating its potential antipsoriatic activity via suppression of keratinocyte overgrowth.

The MTT assay demonstrated a dose-dependent reduction in keratinocyte viability. The IC_{50} value was calculated as 0.4816 ± 0.1002 µl/mL, indicating effective inhibition of cell proliferation (*Figure 2 A and B*). Morphological changes, such as cytoplasmic shrinkage and cell rounding, were observed at higher concentrations, aligning with keratinocyte cytotoxicity profiles of known antipsoriatic agents [16].

Inhibition of Inflammatory Cytokines

Mahua oil significantly downregulated the expression of pro-inflammatory cytokines:

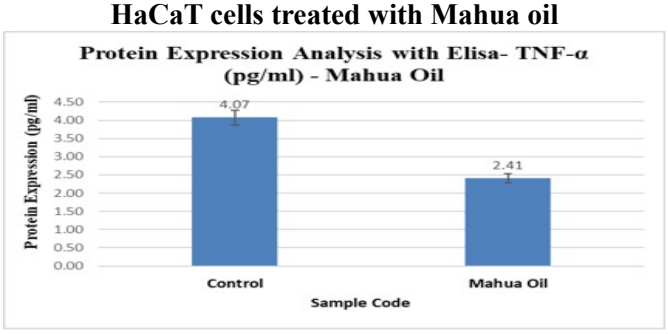
Figure 3: Downregulation of IL-1β expression in HaCaT cells treated with Mahua oil



IL-1β, a crucial pro-inflammatory cytokine, plays a central role in the pathogenesis of psoriasis by promoting the activation of keratinocytes, recruitment of inflammatory cells, and the amplification of the immune response within the skin. [17]

In this study, the mean IL-1β concentration in the untreated control group was 1265.00 ± 45.08 pg/ml, while treatment with Mahua oil significantly reduced it to 953.75 ± 29.17 pg/ml (**$p < 0.01$**). This statistically significant reduction underscores the efficacy of Mahua oil in downregulating IL-1β inflammatory cytokine expression.[6,18].

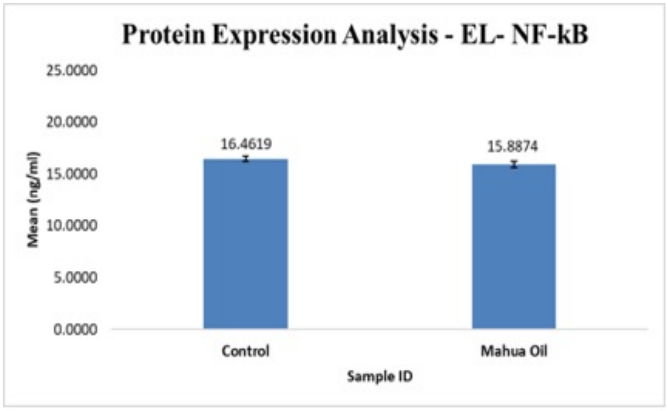
Figure 4: Downregulation of TNF expression in HaCaT cells treated with Mahua oil



TNF-α is a pivotal cytokine in the inflammatory cascade of psoriasis, playing a key role in activating keratinocytes, dendritic cells, and endothelial cells, and in promoting leukocyte infiltration into psoriatic plaques [19, 20].

The ELISA results showed that TNF-α levels dropped from 4.07 pg/ml in the control group to 2.41 pg/ml in the Mahua oil-treated group, indicating a statistically significant suppression **($p < 0.05$).** Given that TNF-α is a therapeutic target in conventional psoriasis treatments (e.g., biologics like etanercept and infliximab), Mahua oil could represent a cost-effective, natural alternative or adjunctive therapy with fewer side effects. [7]

Figures 5: Down regulation of NF-κB expression in HaCaT cells treated with Mahua oil



NF-κB plays a central role in regulating genes associated with inflammation, cell proliferation, and apoptosis. Its dysregulation has been implicated in chronic inflammatory diseases. [21]

As illustrated in Figure 5, NF-κB expression was reduced in the Mahua Oil-treated group (15.8874 ng/ml) compared to the control group (16.4619 ng/ml). Mahua Oil may exert a modest modulatory effect on NF-κB activity.

COX Inhibition

Figure 6: COX-2 enzyme inhibition by Celecoxib (positive control)

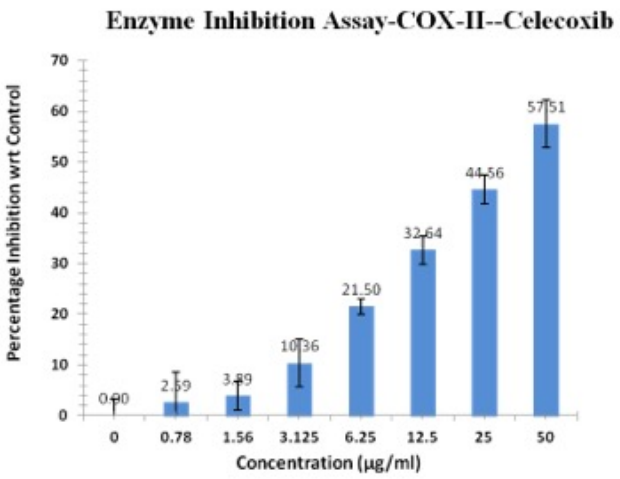
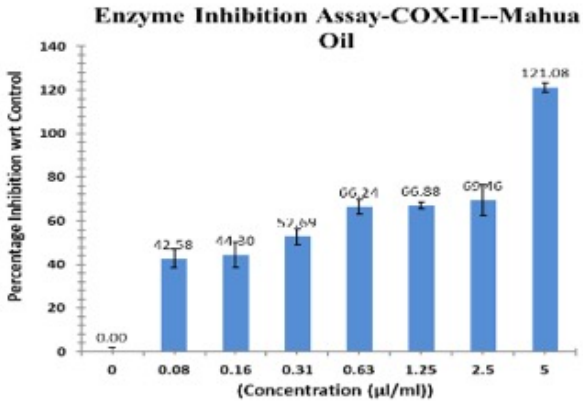


Figure 7: COX-2 enzyme inhibition by Mahua oil compared to Celecoxib (positive control)



The current study investigated the COX-II inhibitory potential of Mahua oil in comparison with the standard anti-inflammatory drug Celecoxib, using an in vitro enzyme inhibition assay. The results demonstrate a dose-dependent inhibition of COX-II by both agents, with Mahua oil exhibiting notably high inhibitory activity at higher concentrations.

Celecoxib, a selective COX-II inhibitor widely used in the management of inflammatory conditions, exhibited up to 57.51% inhibition at 50 µg/ml. In contrast, Mahua oil showed remarkable COX-II inhibition, reaching 121.08% inhibition at 5 µl/ml, suggesting potent activity potentially exceeding that of the standard drug under the conditions tested.

The observed effects of Mahua oil are particularly significant in the context of psoriasis, a chronic inflammatory skin disorder characterized by overexpression of COX-II, increased prostaglandin production, and aberrant keratinocyte proliferation. Inhibition of COX-II can attenuate the inflammatory cascade by reducing prostaglandin E2 (PGE2) levels, thus offering symptomatic relief and slowing disease progression.[22] The data were statistically analyzed using one-way ANOVA, confirming that the differences in percentage inhibition between treatment groups were statistically significant ($p < 0.05$).

Plant-derived oils are increasingly recognized in dermatology for their role in hydration, barrier restoration, and anti-inflammatory activity [23]. The results of our study align with traditional Ayurvedic principles where *Madhuca longifolia* is applied topically for skin disorders. The oil's emollient properties are enhanced by its unsaturated fatty acid content, and phytosterol likely contribute to its immunomodulatory activity.

In comparison to conventional therapies, Mahua oil offers a natural, cost-effective alternative with multifaceted actions—cytokine inhibition, enzyme blockade, and keratinocyte regulation—making it a compelling candidate for further clinical evaluation.

Conclusion

This study support the antipsoriatic potential of Mahua oil, an Ayurvedic remedy traditionally used for skin ailments. The oil exhibited significant anti-proliferative effects on HaCaT keratinocyte cell lines, with an IC_{50} of 0.4816 ± 0.1002 µl/mL, indicating strong efficacy in inhibiting abnormal skin cell growth —a hallmark of psoriasis.

Mahua oil also demonstrated down regulation of key inflammatory mediators implicated in psoriasis pathogenesis, including IL-1β, TNF-α, and NF-κB. In addition, the oil effectively suppressed COX-2 enzyme activities, further confirming its anti-inflammatory properties. The phytochemical constituents of Mahua oil, including unsaturated fatty acids and phytosterols, likely contribute to these effects through moisturization, skin barrier restoration, and immune modulation.

These findings support the traditional use of Mahua oil in Ayurvedic medicine for treating skin diseases and further advocate for its potential as a complementary therapeutic agent in managing psoriasis. However, further in vivo and clinical studies are warranted to validate its therapeutic utility and standardize dosage forms for dermatological applications.

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