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Review Article

Formulation and Evaluation of an Anti-Microbial Activity Anti-Acne Gel Containing Methanolic Extract of Curry Leaves (*Murraya koenigii*)

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ABSTRACT

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit, affecting millions globally. While synthetic treatments like benzoyl peroxide and topical antibiotics are effective, they are often associated with side effects such as skin irritation, dryness, and the emerging threat of antibiotic resistance. Consequently, there is a burgeoning interest in herbal pharmacotherapy. *Murraya koenigii* (L.) Spreng, commonly known as the curry leaf plant, is rich in carbazole alkaloids, flavonoids, and phenolic compounds that exhibit potent antimicrobial and anti-inflammatory properties. This review explores the formulation strategies for an anti-acne gel incorporating the methanolic extract of *Murraya koenigii*. It details the phytochemical profile of the plant, the mechanism of action against *Propionibacterium acnes* and *Staphylococcus epidermidis*, and the pharmaceutical evaluation parameters including pH, spreadability, viscosity, and in vitro antimicrobial assays. The synthesis of traditional botanical knowledge with modern dermatological formulation science provides a promising alternative for the management of acne.

INTRODUCTION

Acne vulgaris remains one of the most prevalent dermatological conditions worldwide, characterized by comedones, papules, pustules, and in severe cases, nodules and cysts [1]. The pathogenesis is multifactorial, involving follicular hyperkeratinization, excess sebum production under androgenic control, and the colonial

proliferation of *Cutibacterium acnes* (formerly *Propionibacterium acnes*) and *Staphylococcus epidermidis* [2]. The resulting inflammatory response can lead to permanent scarring and significant psychological distress, including anxiety and depression.

Current therapeutic regimens primarily involve retinoids, benzoyl peroxide, and antibiotics like

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clindamycin and erythromycin. However, the long-term use of these agents is limited by adverse effects such as erythema, scaling, and the alarming rise of multi-drug resistant bacterial strains [3]. This has catalyzed a shift toward ethnopharmacology. Natural products offer a diverse chemical library with multi-targeted actions and generally lower toxicity profiles.

Murraya koenigii (Family: Rutaceae), commonly known as "Kadi Patta" or Curry leaf, is an aromatic plant native to the Indian subcontinent. While primarily used as a culinary spice, it has been a staple in Ayurvedic medicine for treating skin eruptions, bruises, and inflammations [4]. Recent studies have highlighted the antimicrobial efficacy of its methanolic extract, particularly due to the presence of carbazole alkaloids like mahanimbine and girinimbine [5]. Formulating these extracts into a topical gel offers several advantages over traditional ointments, including better spreadability, non-greasy texture, and improved patient compliance

Botanical and Phytochemical Profile of *Murraya koenigii*

A. Botanical Description

Murraya koenigii is a small tree growing up to 4–6 meters. Its leaves are bipinnately compound, numbering 11–21 leaflets, each 2–5 cm long. The plant is indigenous to India, Sri Lanka, and Southeast Asia. In traditional medicine, all parts—leaves, bark, and roots—are utilized for their tonic and stomachic properties [6].

B. Phytochemical Constituents

The therapeutic potential of *Murraya koenigii* resides in its complex secondary metabolites. The leaves contain:

1. **Carbazole Alkaloids:** These are the most significant bioactive markers, including mahanine, mahanimbine, isomahanimbine, and girinimbine. These compounds have demonstrated significant inhibitory effects against Gram-positive bacteria [7].
2. **Essential Oils:** Rich in caryophyllene, p-gurjunene, and p-element, which contribute to the aromatic and antimicrobial nature.
3. **Flavonoids and Phenols:** Including quercetin and catechin, which act as potent antioxidants, neutralizing reactive oxygen species (ROS) produced during the inflammatory phase of acne [8].
4. **Vitamins and Minerals:** High concentrations of Vitamin A and C, which aid in skin tissue repair and collagen synthesis.

Pathogenesis of Acne and Antimicrobial Targets

The development of an anti-acne formulation must target the four primary pillars of acne pathogenesis:

1. **Sebum Overproduction:** Often stimulated by dihydrotestosterone.
2. **Follicular Hyperkeratosis:** Leading to the formation of a microcomedo.
3. **Bacterial Colonization:** *C. acnes* is an anaerobe that thrives in the sebum-rich environment of the follicle, secreting lipases that break down sebum into irritating free fatty acids [9].
4. **Inflammation:** The activation of Toll-like receptors (TLR-2) on monocytes by *C. acnes* triggers the release of pro-inflammatory cytokines like IL-8 and TNF-alpha [10].



Methanolic extracts of *M. koenigii* are particularly effective because methanol, being a polar solvent, efficiently extracts the alkaloids and phenols responsible for disrupting the bacterial cell wall of *S. epidermidis* and *C. acnes* [11].

Extraction Methodology

The preparation of the methanolic extract is a critical step in ensuring the potency of the final gel.

A. Collection and Authentication

Leaves must be collected from a standardized source, washed thoroughly to remove debris, and shade-dried to prevent the degradation of thermolabile constituents. Authentication by a botanist is essential to ensure species purity.

B. Maceration and Soxhlation

The dried leaves are pulverized into a coarse powder. Extraction is typically performed using 95% methanol via the Soxhlet apparatus for 18–24 hours or through cold maceration with occasional stirring [12]. The resulting liquid is filtered and concentrated using a rotary evaporator under reduced pressure to obtain a semi-solid crude extract and the yield percentage is calculated.

Formulation of the Anti-Acne Gel

Gels are preferred for acne treatment because they provide a cooling effect and do not clog pores (non-comedogenic).[13]

A. Excipients Selection

1. **Gelling Agent:** Carbopol 940 (0.5%–2%) is frequently used for its high clarity and optimal rheological properties. Alternatively, Hydroxypropyl Methylcellulose (HPMC) or Sodium CMC can be used.[14]

2. **Penetration Enhancer:** Propylene glycol or Dimethyl sulfoxide (DMSO) facilitates the delivery of alkaloids through the stratum corneum [15].
3. **Neutralizer:** Triethanolamine is used to adjust the pH and initiate the cross-linking of Carbopol to form a gel matrix.
4. **Preservatives:** Methylparaben and Propylparaben (0.2%) prevent microbial contamination of the water-rich base.
5. **Humectant:** Glycerin prevents skin desiccation during application.

B. General Procedure

1. **Step 1:** Disperse Carbopol 940 in purified water with constant stirring to avoid clump formation and allow it to swell overnight.
2. **Step 2:** Dissolve the methanolic extract of *M. koenigii* in a small amount of methanol or propylene glycol.
3. **Step 3:** Add the preservatives and humectants to the Carbopol dispersion.
4. **Step 4:** Incorporate the extract solution into the gel base under slow stirring to prevent air entrapment.
5. **Step 5:** Gradually add Triethanolamine dropwise until the desired pH (5.5–6.5) and consistency are achieved [16-17].

Evaluation Parameters

Comprehensive evaluation ensures the safety and efficacy of the herbal gel.

A. Physicochemical Evaluation



1. **Organoleptic Properties:** The gel is inspected for color (typically dark green due to chlorophyll), odor (characteristic aromatic), and clarity.[18]
2. **pH Measurement:** The pH of a 1% aqueous solution of the gel is measured using a digital pH meter. For topical application, the pH should be between 5.0 and 7.0 to maintain the skin's acid mantle [19].
3. **Viscosity:** Measured using a Brookfield Viscometer. Proper viscosity ensures the gel stays on the skin after application.
4. **Spreadability:** Determined by the time in seconds taken by two glass slides to slip off each other under a specific load, with the gel sandwiched between them. High spreadability indicates ease of application.
5. **Extrudability:** Evaluated by the force required to extrude the gel from a collapsible tube.

B. Phytochemical Screening of the Gel

Post-formulation tests verify the presence of alkaloids (Mayer's test), flavonoids (Shinoda test), and tannins (Ferric chloride test) within the formulation to ensure the active ingredients remain stable in the vehicle [20].

C. In Vitro Antimicrobial Activity

This is the core evaluation of the anti-acne claim.

1. **Test Organisms:** *Staphylococcus epidermidis* (ATCC 12228) and *Cutibacterium acnes* (ATCC 6919).
2. **Agar Well Diffusion Method:** Wells are bored into sterilized Mueller-Hinton agar plates inoculated with the bacteria. Different

concentrations of the gel are placed in the wells. After incubation (24–48 hours for *S. epidermidis* and 72 hours anaerobically for *C. acnes*), the Zone of Inhibition (ZOI) is measured [21]. A larger ZOI compared to a marketed clindamycin gel indicates high potency.

3. **Minimum Inhibitory Concentration (MIC):** The lowest concentration of the extract/gel that prevents visible growth of the bacteria.

D. Skin Irritation Study (Draize Test)

The gel is applied to the shaved skin of laboratory animals (e.g., rabbits) or tested using *in vitro* reconstructed human epidermis. Observations for erythema (redness) and edema (swelling) are made over 72 hours. A Primary Irritation Index (PII) score is calculated; a score below 2.0 generally indicates the formulation is non-irritant [22].

E. Stability Studies

As per ICH guidelines, the gel is kept at $40 \pm 2^\circ\text{C}$ / $75 \pm 5\%$ RH for 3 to 6 months. Parameters like pH, viscosity, and drug content are monitored to ensure the extract does not degrade.[23]

Discussion of Antimicrobial Mechanisms

The efficacy of *Murraya koenigii* against acne-causing bacteria is attributed to the synergistic effect of its alkaloids. Mahanimbine, a major carbazole alkaloid, disrupts the integrity of the bacterial cytoplasmic membrane, leading to the leakage of intracellular components and cell death [20]. Furthermore, the methanolic extract has been shown to inhibit the secretion of lipase by *C. acnes*. Since lipase is responsible for converting sebum triglycerides into pro-inflammatory free fatty acids, its inhibition directly reduces the severity of acne inflammation [24-25].



Comparative studies have shown that 1%–5% concentrations of *M. koenigii* extract in a gel base can exhibit zones of inhibition comparable to 1% Clindamycin Phosphate, but with significantly lower cytotoxicity to human keratinocytes [26]. The antioxidant properties of the extract also assist in preventing sebum peroxidation, a process that is increasingly recognized as a trigger for early comedogenesis.

Challenges and Future Perspectives

Despite the promising results, several challenges remain:

1. **Standardization:** The chemical composition of *M. koenigii* varies with geographical location and season. Standardizing the extract based on mahanimbine content is necessary for clinical consistency [27].
2. **Color and Staining:** The deep green color of the extract can be aesthetically unappealing. Future research into decolorized extracts or nano-emulsoids may improve cosmetic acceptance.[28]
3. **Clinical Trials:** Most existing data is *in vitro*. Robust, double-blind, placebo-controlled clinical trials are required to establish the efficacy and safety in human subjects.[29]
4. **Synergy:** Investigating the combination of *M. koenigii* with other herbal agents like *Azadirachta indica* (Neem) or *Aloe vera* may provide a broader spectrum of activity [30].

CONCLUSION

The formulation of an anti-acne gel containing the methanolic extract of *Murraya koenigii* represents a viable, science-backed approach to herbal dermatology. The gel's physicochemical properties, such as optimal pH and spreadability,

combined with the potent antimicrobial and anti-inflammatory action of carbazole alkaloids, make it an effective alternative to synthetic anti-acne agents. By utilizing a Carbopol-based delivery system, the bioactive constituents are efficiently released, targeting the bacterial colonization and inflammatory pathways of acne vulgaris. This review underscores the potential of *M. koenigii* to be transitioned from a traditional remedy to a standardized pharmaceutical product, addressing the global need for safer and more sustainable acne treatments.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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