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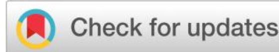

Research Article

Formulation and evaluation of Niosomal cream from moringa leaf extract for enhanced Antifungal Activity

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Abstract

Niosomal drug delivery systems have gained attention for enhancing the bioavailability and efficacy of pharmaceutical formulations. This study explores the formulation and evaluation of a niosomal antifungal cream incorporating Moringa oleifera leaf extract. Moringa leaves contain bioactive compounds with antifungal properties, making them a potential alternative to synthetic antifungal agents. Niosomes, as vesicular carriers, improve drug stability, penetration, and controlled release, enhancing therapeutic effects. The formulated cream was characterized for particle size, entrapment efficiency, pH, spreadability, and in vitro antifungal activity against common fungal strains. The results demonstrated that the niosomal formulation improved drug retention and prolonged antifungal activity compared to conventional creams. The study suggests that a niosomal antifungal cream containing Moringa leaf extract could be an effective natural treatment for fungal infections, offering a promising alternative to conventional antifungal therapies.

Keywords: Moringa oleifera, niosomal cream, antifungal activity, drug delivery, dermatophytosis, Candida albicans, vesicular drug

INTRODUCTION:

The increasing prevalence of fungal infections represents a critical challenge to global health systems, affecting populations through both superficial skin conditions and severe systemic diseases[1]. Cutaneous infections such as ringworm and yeast-related candidiasis impact millions annually, while immunocompromised groups—including HIV/AIDS patients, organ transplant recipients, and individuals undergoing chemotherapy—face elevated risks of invasive infections like aspergillosis and cryptococcal meningitis [2]. Epidemiological studies reveal that superficial fungal infections affect nearly 1.7 billion people worldwide, with dermatophyte infections alone impacting 20-25% of the global population[3].

Diagnostic complexities and escalating antifungal resistance, fueled by genetic adaptations and biofilm production, hinder effective management of these infections[4]. Conventional therapies face limitations due to adverse effects; azole antifungals, for example, carry risks of liver damage and drug interactions through cytochrome P450 enzyme inhibition[6]. Nephrotoxic effects plague polyene drugs like amphotericin B, while allylamines such as terbinafine often trigger gastrointestinal or cutaneous side effects[7]. Rising resistance patterns further diminish treatment efficacy, with fluconazole-resistant Candida strains now reported in 20-30% of cases in some regions[8]. Topical drug delivery remains suboptimal due to the skin's lipid-rich stratum corneum barrier, which restricts penetration of antifungal agents into deeper tissue layers[9].

Fungal pathogens pose distinct therapeutic challenges compared to bacterial or viral infections. Their eukaryotic biology shares cellular processes with human hosts, narrowing the scope for selective drug targets and increasing treatment-related toxicity risks[10]. Chronic infection courses demand extended therapies, amplifying concerns about medication adherence and cumulative side effects[11].

Moringa oleifera, a plant with extensive traditional medicinal applications, shows promise in addressing these challenges. Its leaves contain bioactive compounds—including isothiocyanates (e.g., sulforaphane), flavonoids (quercetin, kaempferol), saponins, and alkaloids—that demonstrate antifungal properties through multiple mechanisms, such as cell membrane disruption and inhibition of critical fungal enzymes[13,14]. Laboratory studies confirm its activity

against Candida, Aspergillus, and dermatophytes, with animal models supporting its therapeutic potential[15].

This plant's selection for antifungal research is supported by its global availability, eco-friendly cultivation, favorable safety profile, and broad-spectrum activity[16]. Its suitability for topical formulations addresses the need for localized treatment of superficial infections while minimizing systemic exposure, offering a potential solution to current therapeutic limitations[17].

Niosomal Drug Delivery Systems

Niosomes, vesicular systems formed from non-ionic surfactants, have emerged as an innovative approach for topical drug delivery due to their biocompatibility and versatility [18]. These biodegradable structures can encapsulate both water-soluble and lipid-soluble compounds, making them effective carriers for plant-based therapeutics like Moringa extracts [19]. Compared to liposomes, niosomes demonstrate superior stability and scalability, with surfactants such as Span and Tween enabling customization of vesicle characteristics [20,21]. By enhancing drug solubility and promoting fusion with skin lipids, niosomes overcome the stratum corneum barrier, improving localized delivery while protecting encapsulated agents from degradation [22,23].

MATERIALS:

Table 1: Ingredients and its roles

Ingredients	Role
Moringa leaf extract	API
Span 60	Surfactant
Cholestrol	Membrane stabilizer
Ethanol	Solvent
Beeswax	Emulsifier
Borax	Stabilizer
Triethanolamine	pH adjuster
Methyl paraben	Preservative
Rose oil	Fragnance
Distilled water	Vehicle

Table 2: ingredients with optimized batch

Ingredient	F1	F2	F3	Optimized
Moringa leaf extract	0.5	0.6	0.7	0.6
Span 60	1	1.2	1.4	1.2
Cholestrol	0.4	0.5	0.6	0.5
Beeswax	1	0.9	1	0.9
Borax	0.2	0.2	0.2	0.2
Triethanolamine	0.3	0.2	0.1	0.2
Methyl paraben	0.2	0.2	0.2	0.2
Liquid paraffin	0.8	0.9	1	0.9
Rose oil	1	1	1	1
Distilled water	q.s.	q.s.	q.s.	q.s.

Methodology:

1. Preparation of Moringa Leaf Extract

Fresh Moringa oleifera leaves were authenticated, air-dried in shaded conditions (25 ± 2°C) for one week to preserve thermolabile constituents [24], and pulverized into a fine powder using an electric grinder. The powder was sieved through a 60-mesh screen to ensure uniformity [25]. For extraction, 100 g of powder underwent cold maceration in 500 mL of 95% ethanol for 72 hours with periodic agitation [26]. The mixture was filtered through Whatman No. 1 filter paper, and the residue was re-extracted twice with fresh solvent to maximize phytochemical recovery [27]. The combined filtrates were concentrated using a vacuum rotary evaporator (Büchi Rotavapor R-210) at 40°C under reduced pressure, followed by desiccation in a vacuum chamber to yield a solvent-free extract [28]. The dried extract (yield calculated gravimetrically) was stored in sealed containers at 4°C [29].

2. Niosome Synthesis (Thin-Film Hydration)

1. Span 60, cholesterol, and Moringa extract were dissolved in 10 mL ethanol within a round-bottom flask [30].

2. Ethanol was evaporated using a rotary evaporator (60 ± 2°C, 25 mmHg, 120 rpm) to form a thin lipid film [31].

3. The film was hydrated with 10 mL phosphate-buffered saline (pH 7.4) under gentle agitation (room temperature, 1 hour) to form niosomes [32].

4. The dispersion was sonicated (Sonics Vibra Cell probe sonicator) for 5 minutes (40% amplitude, 2s on/1s off pulses) to homogenize vesicle size [33].

5. The final niosomal suspension was refrigerated (4°C) for stability [34].

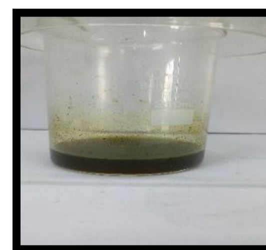

Figure 1: Moringa leaf extract

Figure 2: Niosome preparation by rotary evaporator

3. Cream Development

Oil Phase: Beeswax and liquid paraffin were melted (70 ± 2°C water bath) [35].

Aqueous Phase: Borax and methyl paraben were dissolved in heated distilled water (70 ± 2°C) [36].

Emulsification: The aqueous phase was gradually incorporated into the oil phase under high-shear mixing (Remi Motors stirrer, 1500 rpm) [37].

Cooling: Triethanolamine was added during cooling (room temperature) with continuous stirring to form a stable emulsion [38].

Niosome Integration: Niosomal dispersion was blended into the cream base using gentle spatulation to preserve vesicle integrity [39].

Finalization: Rose oil (fragrance) was incorporated, followed by homogenization (IKA T25 homogenizer, 3000 rpm, 5 minutes) [40]. The cream was stored in airtight containers at ambient conditions [41].

Phytochemical Analysis of Extract

Test for Alkaloids

-Dragendroff's Test: A volume of 2 mL of the extract solution was mixed with several drops of Dragendroff's reagent (potassium bismuth iodide solution). The appearance of an orange-red precipitate was interpreted as evidence of alkaloid presence [42].

- Hager's Test: To 2 mL of the extract solution, a few drops of Hager's reagent (saturated picric acid solution) were added. The formation of a yellow precipitate was indicative of alkaloids [43].

Test for Flavonoids

A 3 mL aliquot of the extract solution was combined with 10 mL of distilled water and shaken. Subsequently, 1 mL of 10% sodium hydroxide was added. The emergence of a yellow hue confirmed the presence of flavonoids [44].

Test for Saponins

Frothing Test: The extract solution (3 mL) was diluted with 2 mL of distilled water in a test tube and vigorously shaken for 5 minutes. A stable foam layer at the surface signaled the presence of saponins [45].

Test for Steroids

Chloroform (2 mL) was added to 2–3 mL of the extract solution, followed by careful layering of 1–2 mL of concentrated sulfuric acid. A reddish-brown interface between the layers indicated steroids. Further addition of 1–2 mL of acetic anhydride produced a reddish-pink hue, confirming steroid presence [46].

Test for Tannins

The extract solution (2 mL) was gently heated for 2 minutes, cooled, and treated with three drops of 5% ferric chloride. A greenish-black or bluish-black hue confirmed tannin content [47].

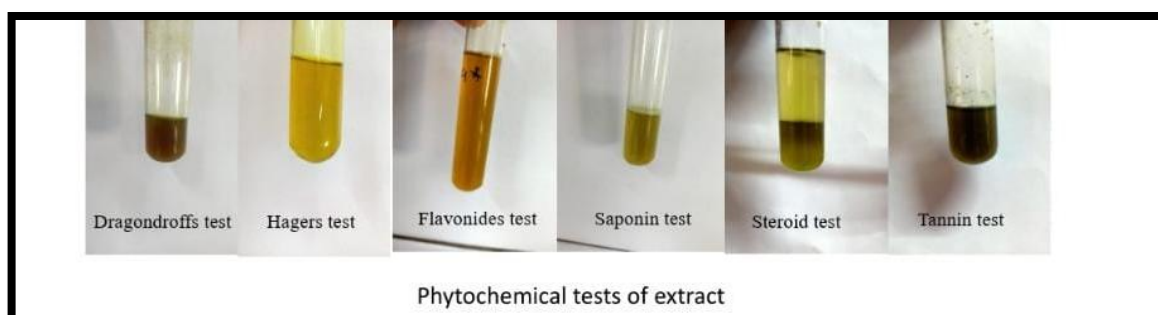

Figure 3: Phytochemical test of extract

Characterization of Niosomal Cream

Particle Size

The niosomal dispersion was diluted 1:100 with filtered deionized water to prevent multiple scattering effects. Measurements were conducted at 25°C with a 90° detection angle, with triplicate analyses performed for each sample [48].

Entrapment Efficiency (EE)

EE was assessed via ultracentrifugation to separate unentrapped extract from niosomes. The supernatant was analyzed spectrophotometrically to calculate the percentage of entrapped extract [49,50].

Zeta Potential (ZP)

ZP was measured using a Zetasizer Nano ZS (Malvern Instruments, UK). Samples were diluted 1:100 with filtered deionized water and analyzed at 25°C under 20 V/cm field strength. ZP values exceeding ±30 mV was considered indicative of stable formulations [51].

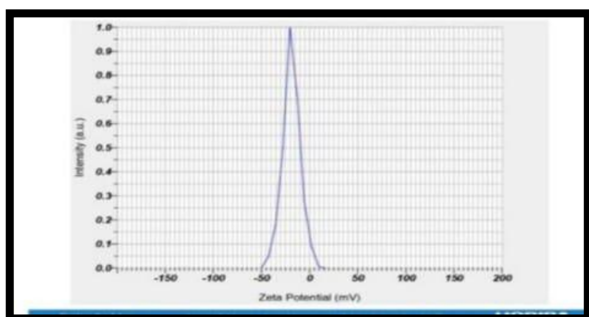

Figure 4: Graph of zeta potential

pH

The pH of a 5% aqueous cream solution was measured using a digital pH meter (Eutech pH 700, Singapore) to ensure alignment with the skin's natural pH range (5.0–6.5) [52].

Viscosity

Viscosity was determined using a Brookfield viscometer (LVDV-II+Pro, USA) with spindle #64 at 25 ± 1°C. Rheological behavior was evaluated across varying shear rates [53].

Spreadability

A fixed quantity of cream was placed between two glass plates, and the spread diameter was measured under standardized weight to assess spreadability [54].

Washability

A small cream sample applied to the skin was rinsed with water to evaluate ease of removal[55].

In Vitro Drug Diffusion Study

Figure 5: Invitro cell diffusion study

An egg membrane model was prepared by acid treatment and equilibration in phosphate buffer. Drug release kinetics were analyzed using a diffusion cell setup, with samples collected at intervals and quantified spectrophotometrically [56,57]

Stability Studies

Optimized formulations were stored at 25 ± 2°C (room temperature) and 4 ± 2°C (refrigeration) for three months to assess physical and chemical stability [58].

Antifungal Activity

Antifungal effects against Candida albicans were tested via agar diffusion on Sabouraud dextrose agar, with inhibition zones measured to gauge efficacy [59].

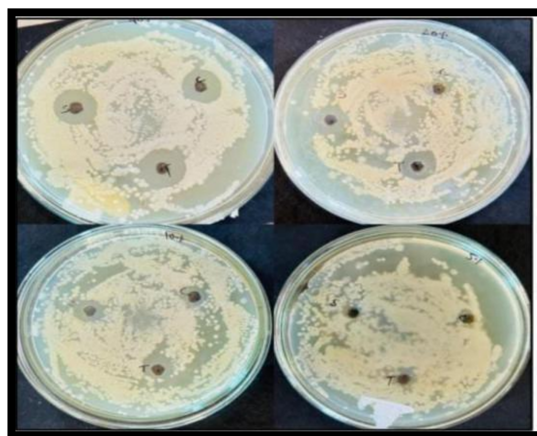

Figure 6: Antifungal study of formulation

RESULT AND DISCUSSION:

Calibration Curve:

Table 3: Calibration curve

Concentration	Absorbance
2	0.4234
4	0.5281
6	0.6202
8	0.7497
10	0.8397

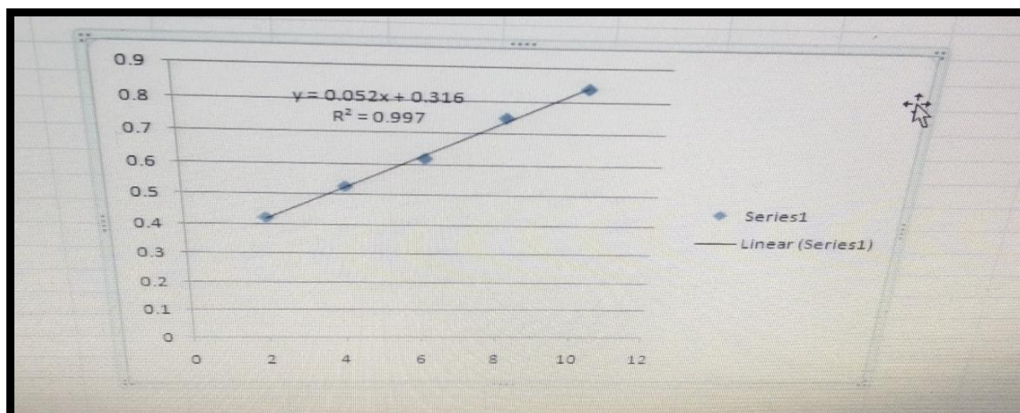

Figure 7: Graph of calibration curve

Comparative Study:

Table 4: Comparative study of formulations

Parameter	Normal cream	Standard	Niosomal Cream
Appearance	Smooth	smooth	Smooth
pH	5.5	5.4	5.5
Viscosity(cpa)	1820	980	570
Particle Size	180nm	200nm	155nm
Entrapment efficacy	NA	70	74
Zeta potential	+18	+20	+25
Spreadability	Good	Good	Good
Irritancy	None	None	None
Washability	Easily washable	Easily washable	Easily washable
Stability	Stable	Stable	Stable
Invitro drug release	65.94	75	79
Antifungal activity (Zone of inhibition)	1.53	1.9	2.5

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