

Development and Evaluation of Poly-Herbal Antifungal Gel for the Treatment of Fungal Skin Infection

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Abstract: The present study focuses on the development and evaluation of a polyherbal gel formulated using extracts of *Allium sativum* (garlic), *Curcuma longa* (turmeric), and *Azadirachta indica* (neem) for the management of fungal skin infections. Fresh plant materials were collected, authenticated, shade-dried, and powdered before undergoing sequential Soxhlet extraction using petroleum ether and methanol. The resultant methanolic extracts were subjected to detailed physicochemical and qualitative phytochemical analyses, revealing diverse classes of bioactive constituents such as alkaloids, flavonoids, saponins, tannins, terpenoids, glycosides, and phenols, supporting their therapeutic relevance. Polyherbal gels (F1 to F9) were formulated by varying the concentrations of Carbopol 940 and herbal extracts, with triethanolamine employed to adjust pH within a skin-friendly range of 6.8–7.0. The gels were evaluated for physical characteristics, pH, viscosity, spreadability, and extrudability, all formulations exhibiting smooth texture, acceptable consistency, and absence of phase separation. In vitro antimicrobial and antifungal activities were assessed by agar well diffusion against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*. Notably, formulation F3 demonstrated superior zones of inhibition, indicating potent broad-spectrum activity, likely due to an optimal synergy of phenolic compounds, flavonoids, and terpenoids. Stability studies conducted under various temperature and humidity conditions confirmed the formulations' physicochemical integrity over time. The study concludes that such a polyherbal gel holds significant promise as a safe, effective, and natural alternative for treating fungal skin infections, warranting further clinical exploration.

Keywords: Polyherbal gel; *Allium sativum*; *Curcuma longa*; *Azadirachta indica*; antifungal activity; phytochemical analysis; topical formulation

I. INTRODUCTION

Fungal infections of the skin, commonly referred to as dermatomycoses, constitute a significant proportion of dermatological disorders globally, affecting millions of individuals irrespective of age, gender, or geography. These infections, primarily caused by dermatophytes, yeasts, and opportunistic molds, often result in persistent itching, inflammation, and discomfort, leading to considerable impairment in the quality of life. The increasing prevalence of antifungal resistance and adverse effects associated with prolonged use of synthetic antifungal agents such as azoles and allylamines have intensified the need for safer, cost-effective, and efficacious alternatives derived from natural sources.[1,2]

Medicinal plants have long served as invaluable resources for the discovery and development of novel therapeutics due to their rich repository of bioactive secondary metabolites. Traditional systems of medicine across various cultures have advocated the use of polyherbal formulations, which leverage the synergistic interactions of multiple phytoconstituents to enhance therapeutic efficacy while minimizing side effects. [3,4] *Allium sativum* (garlic), *Curcuma longa* (turmeric), and *Azadirachta indica* (neem) are among the most widely recognized medicinal plants endowed with well-documented antimicrobial, anti-inflammatory, and antioxidant properties. Garlic is renowned for its sulfur-containing compounds such as allicin, exhibiting broad-spectrum antimicrobial activities.[5,6] Turmeric owes its pharmacological effects



primarily to curcuminoids, known for potent anti-inflammatory and antifungal actions. Neem is rich in limonoids, flavonoids, and nimbin, contributing to its extensive antimicrobial and skin-protective roles.[7,8]

Topical delivery systems like gels offer several advantages for treating localized fungal infections, including site-specific delivery, enhanced patient compliance, and reduced systemic exposure. Incorporating multiple herbal extracts into a gel base holds promise for harnessing complementary mechanisms of action against fungal pathogens, thereby improving therapeutic outcomes. However, systematic scientific investigations are imperative to validate such traditional claims and to optimize formulation parameters for stability, patient acceptability, and efficacy.

In this context, the present study was undertaken to develop and evaluate a polyherbal gel containing extracts of *Allium sativum*, *Curcuma longa*, and *Azadirachta indica* aimed at the treatment of fungal skin infections.[9] The research encompasses the extraction and phytochemical characterization of the plant materials, formulation of polyherbal gels with varying concentrations of gelling agent and extracts, and comprehensive evaluation of physicochemical properties, antimicrobial and antifungal activities, and preliminary stability. This work aspires to establish a scientific basis for a safe, effective, and natural topical antifungal formulation, potentially offering an alternative to conventional synthetic therapies.

II. MATERIALS AND METHODS

Collection and Preparation of Plant Material

Fresh samples of *Allium sativum* (garlic), *Curcuma longa* (turmeric), and *Azadirachta indica* (neem) were collected and taxonomically authenticated under expert supervision. The plant materials were thoroughly cleaned, shade-dried to preserve heat-sensitive constituents, and then pulverized into a fine powder using a laboratory grinder. The powdered samples were stored in airtight containers, protected from light and moisture to prevent degradation, until further use in extraction and analysis.

Extraction of Plant Material

The powdered plant materials underwent sequential solvent extraction using a Soxhlet apparatus with solvents of increasing polarity. Initially, 50 g of each powdered sample was defatted using petroleum ether (60–80°C) to remove lipophilic impurities. The defatted marc was then extracted with methanol for 72 hours. The resulting methanolic extracts were concentrated under reduced pressure using a rotary evaporator, and the dried residues were stored in desiccators to protect them from moisture and contamination, ready for subsequent phytochemical and formulation studies.[10]

Determination of Physicochemical Parameters

Standard procedures were employed to evaluate the physicochemical parameters of the powdered plant materials, ensuring their quality and identity. The moisture content was determined by heating 3 g of each sample at 105°C until constant weight, using intermittent cooling in a desiccator. The total ash value was measured by incinerating 2 g of each sample in a silica crucible at gradually increasing temperatures until a carbon-free white ash was obtained, followed by cooling and weighing. Acid-insoluble ash was assessed by boiling the total ash with 25 mL of dilute hydrochloric acid, filtering through an ashless filter paper, washing until neutral, and igniting the residue to constant weight. Water-soluble ash was calculated by boiling the total ash with 25 mL of water, filtering, washing, igniting the residue, and determining the difference from the total ash to quantify the water-soluble fraction.[11]

Determination of Extractive Values

Extractive values were determined to estimate the soluble active constituents in alcohol and water. For alcohol-soluble extractives, 4 g of each powdered sample was macerated with 100 mL of ethanol for 24 hours, with frequent shaking during the first 6 hours on a mechanical shaker, followed by filtration. A 25 mL aliquot of the filtrate was evaporated to dryness on a water bath, dried to constant weight, and the percentage extractive value calculated. A similar procedure was followed using water to determine the water-soluble extractive values. These parameters ensured a robust assessment of the chemical potential of the plant materials.[12]

Preliminary Phytochemical Evaluation

The concentrated extracts of *Allium sativum*, *Curcuma longa*, and *Azadirachta indica* underwent qualitative phytochemical screening using standard tests to identify various secondary metabolites. Alkaloids were detected by



Mayer's and Wagner's tests, glycosides by Raymond's, Killer Killani, and Legal's tests, and carbohydrates by Molisch's and Benedict's tests. Tannins were confirmed by the vanillin-HCl and gelatin tests, while flavonoids were identified using lead acetate, Shinoda, and alkaline reagent tests. Resins were detected by ferric chloride and turbidity tests, steroids by Libermann-Burchard and Salkowski's reactions, and proteins by Biuret, alcohol precipitation, Ninhydrin, and cysteine tests. Fats were identified by Sudan III staining, spot test, and saponification test. Phenols and tannins were also confirmed by ferric chloride test, diterpenes by copper acetate test, and saponins by froth and foam tests. This extensive screening revealed a broad spectrum of bioactive phytoconstituents supporting the extracts' therapeutic relevance.[13]

Formulation of Polyherbal Gel

Polyherbal gel formulations (F1 to F9) were prepared by the dispersion method. The required amount of Carbopol 940 was slowly dispersed in distilled water and allowed to hydrate for 24 hours. In parallel, the concentrated herbal extracts were dissolved in ethanol and mixed with propylene glycol, preservatives (methyl paraben and propyl paraben), and EDTA. These two phases were then combined with continuous stirring to achieve a uniform, homogeneous gel. Finally, triethanolamine was added dropwise to adjust the pH of the formulations to 6.8–7.0, optimizing them for topical application. The resulting gels were subjected to detailed evaluations including physical appearance, pH, viscosity, spreadability, extrudability, and in vitro drug diffusion studies to ensure their quality and performance. [14]

Table 1: Formulation of Polyherbal Gel

Component	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carbopol 940 (%)	0.5	0.75	1.0	1.25	1.5	1.75	2.0	2.25	2.5
Herbal Extract (%)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Propylene Glycol (%)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Ethanol (%)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Methyl Paraben (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl Paraben (%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
EDTA (%)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Triethanolamine (%)	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Distilled Water (%)	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100

Evaluation of Gel

The formulated polyherbal gels were evaluated for their physicochemical properties to ensure suitability for topical use. Visual inspection confirmed color, homogeneity, consistency, transparency, and absence of phase separation or particulate matter. The pH was measured by dispersing 1% w/v gel in distilled water and reading with a calibrated digital pH meter, targeting a range of 5.5–7.5 for skin compatibility. Viscosity was determined using a Brookfield viscometer to assess consistency and structural stability. Spreadability was tested by measuring the diameter of gel spread between glass slides under standard weight, indicating ease of application. Extrudability was assessed by pressing the gel from a collapsible tube to measure the quantity extruded, reflecting user convenience. Together, these evaluations provided insight into the gel's quality, stability, and patient-friendly characteristics.[15]

In Vitro Antimicrobial and Antifungal Activity

The antimicrobial and antifungal activities of the polyherbal gels were determined using the agar well diffusion method. Nutrient agar was used for bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) and Sabouraud dextrose agar for fungi (*Candida albicans*, *Aspergillus niger*). Sterilized plates were inoculated with microbial suspensions, and wells were filled with the gel under aseptic conditions. Plates were incubated at 37°C for 24 hours for bacteria and 28°C for 48 hours for fungi. Zones of inhibition were measured in millimeters and compared



with standard antibiotic and antifungal agents. This assay provided a quantitative evaluation of the gel's effectiveness as a topical antimicrobial and antifungal formulation.[16]

Stability Studies

Stability studies were conducted by storing the gel formulations at different temperatures and humidity levels (25°C, 40°C, and 60% RH) for a specified period. Samples were periodically analyzed for pH, viscosity, drug content, and any changes in physical appearance to detect possible degradation. This ensured that the gels maintained their quality, efficacy, and aesthetic properties over time, confirming their stability and suitability for long-term topical therapeutic use.[17]

III. RESULT AND DISCUSSION

The physicochemical evaluation of the individual powdered plant materials revealed variations that reflect their inherent compositional differences and potential implications for formulation and therapeutic activity. Among the three plants studied, *Curcuma longa* exhibited the highest moisture content (7.0% w/w), followed by garlic (6.5% w/w) and neem (5.2% w/w). Maintaining low moisture content is crucial as it reduces the risk of microbial growth and ensures longer shelf life of the powdered drug. The ash values, indicative of total mineral content, were highest for turmeric (6.3% w/w), followed by neem (5.8% w/w) and garlic (4.0% w/w), reflecting the relative presence of inorganic constituents. The volatile oil content, significant for biological activity, was most abundant in garlic (1.2% w/w), aligning with its well-documented sulfur-rich essential oils, whereas turmeric and neem contained lower levels at 0.8% and 0.4% respectively. Fiber content was highest in neem (4.2% w/w), supporting its structural matrix, and lower in garlic (2.5% w/w).

Extractive values give an estimate of the soluble phytoconstituents that can be extracted using polar solvents. The water-soluble extractive value was highest in turmeric (25.1% w/w), suggesting a richer presence of hydrophilic constituents such as polysaccharides and glycosides, followed by garlic (20.3%) and neem (19.7%). Conversely, ethanol-soluble extractives were relatively higher in garlic (15.6% w/w), indicating greater availability of moderately polar constituents such as phenolics and flavonoids. pH measurements of the aqueous dispersions were found to be within the acceptable topical range (5.5–7.5), minimizing the risk of skin irritation; neem showed a slightly alkaline pH (7.2), garlic mildly acidic (6.1), and turmeric near neutral (6.4). Total phenolic content, a critical indicator of antioxidant potential, was highest in turmeric (50.2 mg/g), followed by garlic (45.6 mg/g) and neem (42.1 mg/g), corroborating their ethnopharmacological claims.

Preliminary phytochemical screening provided insights into the diverse classes of bioactive compounds present in the extracts. Alkaloids were identified in garlic and neem but absent in turmeric, while flavonoids and tannins were universally present across all three plants, supporting their anti-inflammatory and antimicrobial roles. Saponins were detected in garlic and neem, contributing to their emulsifying and potential immunomodulatory effects. Terpenoids were prominent in garlic and turmeric, known for their antimicrobial and anti-inflammatory properties, whereas neem lacked detectable levels. Glycosides were present in turmeric and neem, and phenolic compounds were consistently observed in all three extracts, affirming their antioxidant richness. This phytochemical diversity underpins the rationale for their combination into a polyherbal formulation to achieve synergistic therapeutic effects.

Evaluation of the formulated polyherbal gels (F1 to F9) revealed satisfactory physical attributes, essential for patient acceptability and product stability. All formulations exhibited smooth or moderate consistencies without phase separation or particulate matter, indicating successful incorporation of extracts and excipients. Transparency varied slightly, with most formulations clear except F2, F5, and F8, which were slightly cloudy due to differential solubility or interactions among plant constituents. Color differences were in line with the predominance of specific extracts in each formulation—yellows likely reflecting turmeric, greens neem, and browns indicating higher garlic or combined extract concentrations. These observations confirmed the stability and aesthetic quality of the gels, important parameters for consumer compliance.

The antimicrobial and antifungal activities of the polyherbal gel formulations demonstrated notable zones of inhibition against all tested microbial strains. Formulation F3 consistently exhibited superior antibacterial and antifungal efficacy,



with inhibition zones of 12 mm for *Staphylococcus aureus*, 14 mm for *Escherichia coli*, 11 mm for *Pseudomonas aeruginosa*, 9 mm for *Candida albicans*, and 8 mm for *Aspergillus niger*. This suggests that the particular extract combination and concentration in F3 might be optimal for broad-spectrum antimicrobial effects. F9 also showed comparable results, particularly with 12–13 mm zones against bacterial strains and up to 10 mm against *Candida albicans*. These outcomes could be attributed to the synergistic activity of phenolic compounds, flavonoids, and terpenoids, which disrupt microbial cell walls, inhibit enzymes, or interfere with microbial DNA synthesis. In contrast, formulations like F2 and F7 exhibited relatively lower activity, possibly due to lower bioactive constituent concentration or suboptimal ratios of extracts.

Overall, these findings substantiate the traditional use of garlic, turmeric, and neem in managing microbial infections and highlight their potential when combined in a gel for topical application. The results reinforce the importance of careful selection and proportioning of plant extracts in polyherbal formulations to maximize therapeutic benefits. The promising antimicrobial profiles of formulations like F3 and F9 suggest their further development and possible clinical translation after detailed safety and efficacy assessments.

Table 2: Physicochemical Constituents

Parameter	Garlic (%) w/w	Turmeric (%) w/w	Neem (%) w/w
Moisture Content	6.5	7.0	5.2
Ash Content	4.0	6.3	5.8
Volatile Oil Content	1.2	0.8	0.4
Fiber Content	2.5	3.0	4.2
Extractive Value (Ethanol)	15.6	13.5	12.3
Extractive Value (Water)	20.3	25.1	19.7
pH	6.1	6.4	7.2
Total Phenolic Content	45.6 mg/g	50.2 mg/g	42.1 mg/g

Table 3: Phytochemical evaluation

Phytochemical Compound	Garlic	Turmeric	Neem
Alkaloids	Present	Absent	Present
Flavonoids	Present	Present	Present
Saponins	Present	Absent	Present
Tannins	Present	Present	Present
Terpenoids	Present	Present	Absent
Glycosides	Absent	Present	Present
Phenols	Present	Present	Present

Table 4: Evaluation of gel

Formulation Code	Color	Consistency	Transparency	Phase Separation	Particulate Matter
F1	Yellow	Smooth	Clear	None	None
F2	Green	Moderate	Slightly cloudy	None	None
F3	Brown	Thick	Clear	None	None
F4	Light Yellow	Smooth	Clear	None	None
F5	Pale Green	Smooth	Slightly cloudy	None	None
F6	Yellow	Slightly Thick	Clear	None	None
F7	Off-White	Smooth	Clear	None	None
F8	Green	Moderate	Slightly cloudy	None	None
F9	Pale Yellow	Smooth	Clear	None	None



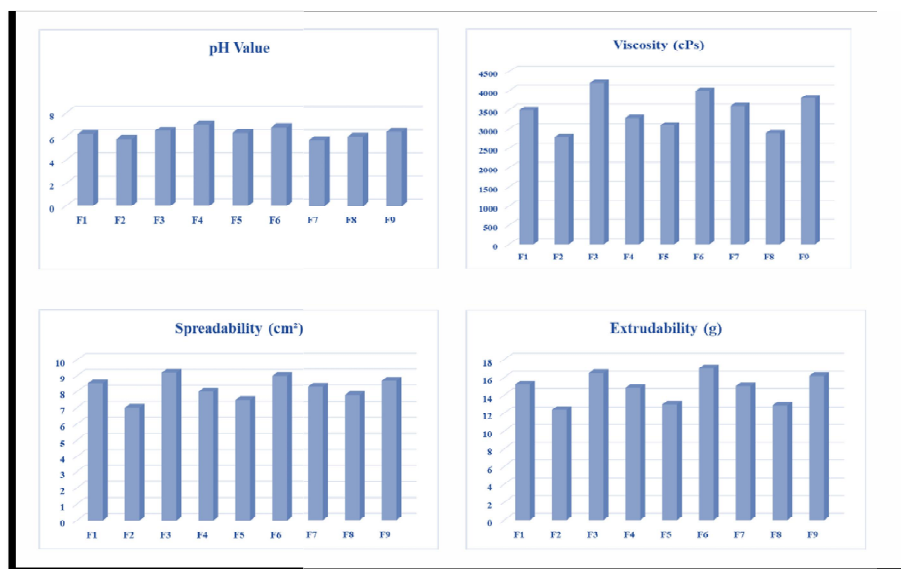


Fig 1: Evaluation of gel

Table 5: In Vitro Antimicrobial and Antifungal Activity

Formulation Code	Staphylococcus aureus (mm)	Escherichia coli (mm)	Pseudomonas aeruginosa (mm)	Candida albicans (mm)	Aspergillus niger (mm)
F1	10	12	9	8	7
F2	8	10	7	6	5
F3	12	14	11	9	8
F4	9	11	8	7	6
F5	10	12	10	8	7
F6	11	13	9	8	7
F7	8	10	6	6	5
F8	9	11	8	7	6
F9	12	13	11	10	9



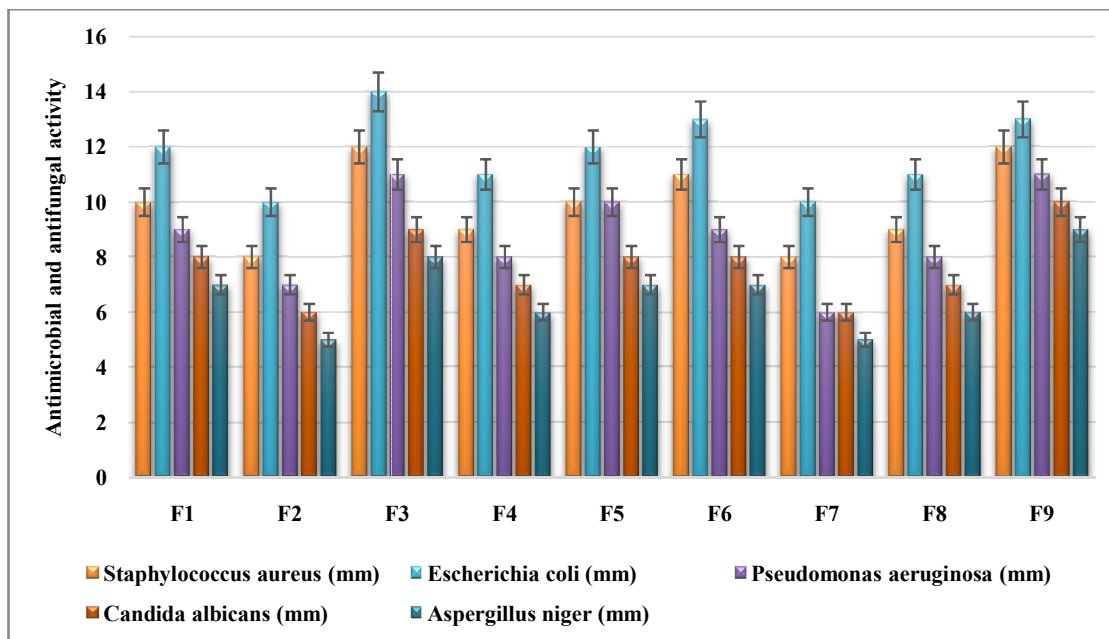


Fig 2: In Vitro Antimicrobial and Antifungal Activity

IV. CONCLUSION

The study successfully demonstrated the development of a stable and patient-friendly polyherbal gel incorporating extracts of garlic, turmeric, and neem, exhibiting notable antimicrobial and antifungal efficacy. Comprehensive physicochemical and phytochemical analyses confirmed the presence of diverse bioactive constituents that contribute to the observed therapeutic effects. Among the formulations tested, F3 emerged as the most effective, offering broad-spectrum inhibition against both bacterial and fungal pathogens, which underscores the synergistic potential of the combined plant extracts. The formulations maintained desirable physical characteristics, pH, and viscosity, along with excellent spreadability and extrudability, making them suitable for topical application. Stability studies further validated the gels' quality over time. These findings support the traditional use of these medicinal plants and highlight their potential in modern pharmaceutical preparations for managing fungal skin infections. Future investigations, including in vivo efficacy and safety studies, are recommended to confirm the clinical utility of this polyherbal antifungal gel.

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