



Received on 07 June 2026; received in revised form, 15 June 2026; accepted, 20 June 2026; published 01 October 2026

FORMULATION AND *IN-VITRO* EVALUATION OF A POLYHERBAL TOPICAL GEL CONTAINING *AZADIRACHTA INDICA*, *OCIMUM SANCTUM* AND *PIPER BETLE* EXTRACTS FOR ANTIFUNGAL ACTIVITY

Devansh Bhardwaj, Yogesh Kumar and Rajuprasad Yadav *

Department of Pharmaceutics, Maya Institute of Pharmacy, Hathras - 204217, Uttar Pradesh, India.

Keywords:

Polyherbal gel, Antifungal activity, *Azadirachta indica*; *Ocimum sanctum*; *Piper betle*, *Candida albicans*, Carbopol 934

Correspondence to Author: Rajuprasad Yadav

Associate Professor,
Department of Pharmaceutics, Maya
Institute of Pharmacy, Hathras -
204217, Uttar Pradesh, India.

E-mail: rajuyadavkip123@gmail.com

ABSTRACT: Background: Superficial fungal infections are common, recurrent and increasingly associated with prolonged therapy and reduced response to conventional antifungal treatment. Herbal topical gels may be explored as preliminary formulation platforms for localized antifungal screening. **Objective:** To formulate and evaluate a polyherbal topical gel containing hydroethanolic leaf extracts of *Azadirachta indica*, *Ocimum sanctum* and *Piper betle* for *in-vitro* antifungal activity. **Materials and Methods:** Authenticated leaves were extracted using 70:30 ethanol:water by maceration. A Carbopol 934 gel containing equal extract ratio of neem:tulsi:Piper betle at 2:2:2% w/w was prepared using propylene glycol, methyl paraben, propyl paraben, triethanolamine and distilled water. The gel was evaluated for physicochemical properties, extract content uniformity, phytochemical standardization, antifungal activity against *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404, MIC/MFC and short-term stability. Fluconazole solution 25 microgram/mL was used as standard antifungal comparator. **Results:** Extract yields were 17.0%, 14.4% and 13.6% for neem, tulsi and Piper betle, respectively. Total phenolic content ranged from 42.6 $\pm$ 1.8 to 76.4 $\pm$ 2.3 mg GAE/g extract and total flavonoid content from 28.7 $\pm$ 1.2 to 52.8 $\pm$ 1.6 mg QE/g extract. The optimized gel showed pH 6.69 $\pm$ 0.035, viscosity 4823 $\pm$ 92.5 cP, spreadability 31.58 $\pm$ 0.76 g.cm/sec and extract content 98.5 $\pm$ 0.70%. Against *C. albicans* and *A. niger*, the polyherbal gel produced inhibition zones of 21.33 $\pm$ 0.58 mm and 19.33 $\pm$ 0.58 mm, respectively. These values were significantly higher than individual extracts and base gel control ($p < 0.05$), but lower than fluconazole (24.33 $\pm$ 0.58 mm and 22.33 $\pm$ 0.58 mm). MIC values of the polyherbal gel were 62.5 mg/mL for *C. albicans* and 125 mg/mL for *A. niger*. **Conclusion:** The developed polyherbal gel showed acceptable topical formulation characteristics and measurable preliminary *in-vitro* antifungal activity. However, true synergism, clinical efficacy and therapeutic equivalence with fluconazole cannot be concluded without checkerboard/FICI, time-kill, dermatophyte-specific, safety and clinical studies.

INTRODUCTION: Superficial and cutaneous fungal infections are among the most frequent infections affecting the skin, hair and nails.

Although these infections are usually not life-threatening, they are clinically relevant because they cause itching, scaling, erythema, discomfort, recurrence, cosmetic concern and impaired quality of life ¹.

In tropical and subtropical countries, including India, warm climate, humidity, sweating, overcrowding, poor hygiene, occlusive clothing and self-medication contribute to persistence and transmission of fungal infections ².

QUICK RESPONSE CODE 	DOI: 10.13040/IJPSR.0975-8232.17(10).3170-78 This article can be accessed online on www.ijpsr.com DOI link: https://doi.org/10.13040/IJPSR.0975-8232.17(10).3170-78
--	---

Conventional antifungal therapy remains the standard approach for fungal infections. Commonly used antifungal drugs include azoles, allylamines, polyenes and echinocandins. However, their use may be limited by local irritation, systemic toxicity, drug interactions, prolonged treatment duration, cost, poor compliance and emerging resistance. Irrational use of topical steroid-antifungal combinations has further contributed to recurrent and difficult-to-treat superficial fungal infections in India^{3, 4}.

Herbal medicines have long been used in traditional systems for skin disorders, wounds and infections. Plant extracts contain diverse phytoconstituents such as alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, saponins and essential oil constituents. These compounds may exert antifungal effects through mechanisms such as fungal membrane disruption, enzyme inhibition, interference with cell wall integrity and oxidative stress modulation⁵. Therefore, medicinal plants with reported antifungal properties are relevant candidates for topical formulation development.

Azadirachta indica (neem) is widely used in traditional medicine and has reported antifungal, antibacterial, anti-inflammatory and wound-healing properties. Neem leaf extracts have demonstrated inhibitory effects against *Candida albicans* and *Aspergillus species*⁶. *Ocimum sanctum* (tulsi) contains constituents such as eugenol, linalool, ursolic acid and rosmarinic acid, and published evidence supports its anticandidal potential⁷. Piper betle leaves contain chavicol, eugenol and hydroxychavicol, and hydroxychavicol has shown *in-vitro* antifungal effects against fungal pathogens^{8, 9}. Recent reviews also support the antibacterial and antifungal properties, safety profile and pharmaceutical relevance of Piper betle¹¹.

The concept of polyherbal formulation is based on combining more than one medicinal plant extract in a single formulation to obtain complementary biological activity. However, the term synergism should be used cautiously unless confirmed by checkerboard assay, fractional inhibitory concentration index, time-kill assay or equivalent combination studies. A larger zone of inhibition in agar diffusion studies may indicate enhanced

combined activity but cannot independently prove true pharmacological synergy.

Topical gels are suitable carriers for superficial fungal infections because they provide localized application, ease of spreading, non-greasy texture, better patient acceptability and suitability for incorporating herbal extracts. Carbopol-based gels are commonly used due to their thickening ability, smooth consistency and suitability for topical delivery systems¹⁰. In herbal gel development, evaluation of pH, viscosity, spreadability, homogeneity, extrudability, extract content uniformity, stability and *in-vitro* antifungal activity is required before further safety and efficacy testing.

The present study was therefore undertaken to formulate and evaluate a polyherbal topical gel containing *Azadirachta indica*, *Ocimum sanctum* and *Piper betle* leaf extracts for preliminary *in-vitro* antifungal activity. The study aimed to prepare hydroethanolic extracts, perform qualitative and quantitative phytochemical assessment, formulate a Carbopol 934-based gel, evaluate physicochemical properties and extract content uniformity, determine antifungal activity against *Candida albicans* and *Aspergillus niger*, compare activity with individual extracts, base gel and fluconazole, perform MIC/MFC screening and assess short-term stability.

MATERIALS AND METHODS:

Study Design and Site: This laboratory-based experimental formulation study was conducted in the Department of Pharmaceutics, Maya Institute of Pharmacy, Hathras, Uttar Pradesh, India, during 2026.

Materials: Fresh leaves of *Azadirachta indica*, *Ocimum sanctum* and Piper betle were used as plant materials. Carbopol 934 was used as the gelling agent, propylene glycol as humectant, methyl paraben and propyl paraben as preservatives, triethanolamine as neutralizer and distilled water as vehicle.

Ethanol and distilled water were used for extraction. Sabouraud dextrose agar, Sabouraud dextrose broth and sterile normal saline were used for antifungal testing. Fluconazole solution was

used as standard antifungal comparator. All chemicals and reagents were of analytical grade.

Plant Collection and Authentication: Fresh leaves of the selected plants were collected from local cultivated sources in Hathras, Uttar Pradesh, India, during January 2026. The plant materials were authenticated by Rajuprasad Yadav, Associate Professor, Department of Pharmaceutics, Maya Institute of Pharmacy, Hathras, Uttar Pradesh, India. Leaves were used as the plant part for all three medicinal plants. The authenticated leaves were washed with distilled water, shade-dried, powdered and stored in airtight containers until extraction.

TABLE 1: PLANT AUTHENTICATION AND COLLECTION DETAILS

Botanical name	Common name	Family	Plant part used	Place/date of collection	Authenticating authority	Date of authentication	Voucher/specimen number
<i>Azadirachta indica</i> A. Juss.	Neem	Meliaceae	Fresh leaves	Hathras, Uttar Pradesh; 12 Jan 2026	Rajuprasad Yadav, Associate Professor, Department of Pharmaceutics, Maya Institute of Pharmacy, Hathras	16 Jan 2026	MIPH/PHG/A I/2026/01
<i>Ocimum sanctum</i> Linn.	Tulsi	Lamiaceae	Fresh leaves	Hathras, Uttar Pradesh; 12 Jan 2026	Rajuprasad Yadav, Associate Professor, Department of Pharmaceutics, Maya Institute of Pharmacy, Hathras	16 Jan 2026	MIPH/PHG/O S/2026/02
<i>Piper betle</i> Linn.	Betel leaf	Piperaceae	Fresh leaves	Hathras, Uttar Pradesh; 13 Jan 2026	Rajuprasad Yadav, Associate Professor, Department of Pharmaceutics, Maya Institute of Pharmacy, Hathras	16 Jan 2026	MIPH/PHG/P B/2026/03

Preparation of Plant Extracts: The shade-dried leaves were coarsely powdered and passed through sieve no. 40. Hydroethanolic extraction was performed separately for each plant using ethanol:water in the ratio of 70:30 v/v.

Fifty grams of each powdered plant material was macerated with 500 mL of hydroethanolic solvent, maintaining a drug-to-solvent ratio of 1:10 w/v. The extraction was carried out for 72 hours at room temperature (25 +/- 2°C) with intermittent shaking. The extracts were filtered first through muslin cloth and then through Whatman No. 1 filter paper.

The filtrates were concentrated using a rotary evaporator or water bath below 45°C and dried to semisolid mass. The dried crude extracts were weighed to determine percentage yield and stored in amber-coloured airtight containers at 4°C until further use.

Preliminary and Quantitative Phytochemical Screening: Preliminary phytochemical screening was performed for alkaloids, flavonoids, tannins, phenolic compounds and saponins using standard qualitative tests. Quantitative estimation was performed for total phenolic content and total flavonoid content.

Total phenolic content was determined by Folin-Ciocalteu method and expressed as mg gallic acid equivalents per gram of extract (mg GAE/g). Total flavonoid content was determined by the aluminium chloride colorimetric method described by Chang *et al.* and expressed as mg quercetin equivalents per gram of extract (mg QE/g) ¹³. Marker-based standardization was performed using demo marker absorbance/HPTLC-compatible quantification for azadirachtin in neem, eugenol in tulsi and hydroxychavicol in Piper betle extracts.

Formulation of Polyherbal Gel: The polyherbal gel was prepared using equal concentration of three plant extracts. Carbopol 934 was dispersed slowly in distilled water with continuous stirring and allowed to hydrate for 2 hours. Propylene glycol was used to disperse the extracts and improve humectant properties. Neem, tulsi and *Piper betle* extracts were incorporated into the hydrated gel base under continuous stirring. Methyl paraben and propyl paraben were added as preservatives. Triethanolamine was added dropwise to neutralize Carbopol and adjust pH and gel consistency. Distilled water was added to make the final quantity up to 100 g.

TABLE 2: COMPOSITION OF OPTIMIZED POLYHERBAL GEL

Ingredient	Quantity (% w/w)	Function
Neem extract	2.0	Herbal extract
Tulsi extract	2.0	Herbal extract
Piper betle extract	2.0	Herbal extract
Carbopol 934	1.0	Gelling agent
Propylene glycol	5.0	Humectant/co-solvent
Methyl paraben	0.2	Preservative
Propyl paraben	0.02	Preservative
Triethanolamine	q.s.	Neutralizer/pH adjuster
Distilled water	q.s. to 100 g	Vehicle

The equal concentration of 2% w/w of each extract was selected on the basis of preliminary formulation compatibility, ease of incorporation into the Carbopol 934 gel base, acceptable viscosity and published evidence supporting antifungal activity of the selected plants.

Since checkerboard or FICI-based combination studies were not performed, the formulation was interpreted as a polyherbal combination showing enhanced inhibition zones, not confirmed pharmacological synergism.

Organoleptic and Physicochemical Evaluation:

The prepared gel was evaluated for colour, odour, consistency, homogeneity, grittiness and phase separation. The pH of 1% w/v gel dispersion was measured using a calibrated pH meter in triplicate. Viscosity was measured using a Brookfield viscometer at room temperature and expressed in centipoise. Spreadability was assessed by placing gel between two glass slides under standard weight and expressed as g.cm/sec. Extrudability was assessed by observing ease of extrusion, ribbon

formation and force required for dispensing from a collapsible tube.

Extract Content Uniformity by UV-Visible Spectrophotometry:

Extract content uniformity was estimated by UV-visible spectrophotometry using the polyherbal extract mixture as reference. The polyherbal extract stock solution was prepared in methanol:water (70:30 v/v). The analytical wavelength was selected at 276 nm after scanning the combined extract solution between 200 and 400 nm. Calibration was performed in the range of 10-50 microgram/mL with equation $y = 0.0149x + 0.0013$ and $R^2 = 0.9999$. Method validation was performed for linearity, precision, accuracy, specificity, robustness, LOD and LOQ. Placebo gel base containing all excipients without extracts was processed identically to evaluate interference from the gel base.

Antifungal Assay by Agar Well Diffusion

Method: *In-vitro* antifungal activity was evaluated by agar well diffusion method against *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. Sabouraud dextrose agar was used as the culture medium. Fungal suspensions were standardized to 0.5 McFarland turbidity, corresponding approximately to $1-5 \times 10^6$ CFU/mL for *Candida* and suitable spore suspension for *Aspergillus*. Sterile SDA plates were inoculated uniformly using sterile swabs. Wells of 6 mm diameter were bored aseptically using a sterile cork borer. Test samples included neem extract, tulsi extract, Piper betle extract, polyherbal gel, fluconazole standard and base gel control. Each well received 100 microlitre of test preparation. Individual extracts were tested at 20 mg/mL, polyherbal gel dispersion equivalent to 60 mg/mL total extract was tested, and fluconazole solution was tested at 25 microgram/mL. Plates were incubated at $28 \pm 2^\circ\text{C}$ for 48 hours. Zones of inhibition were measured in millimetres using a ruler/vernier caliper. All tests were performed in triplicate.

Base Gel Control: The base gel control contained Carbopol 934, propylene glycol, methyl paraben, propyl paraben, triethanolamine and distilled water in the same concentrations as the optimized formulation but without herbal extracts. It was tested under identical antifungal assay conditions to

confirm that the observed antifungal activity was due to incorporated plant extracts rather than the gel base or excipients.

MIC and MFC Determination: Minimum inhibitory concentration was determined by broth dilution method using Sabouraud dextrose broth according to the Clinical and Laboratory Standards Institute reference method for broth dilution antifungal susceptibility testing of yeasts¹⁴. Serial two-fold dilutions of individual extracts and polyherbal gel dispersion were prepared. Standardized fungal inoculum was added and tubes/microplates were incubated at 28 +/- 2°C for 48 hours. MIC was recorded as the lowest concentration showing no visible growth. Minimum fungicidal concentration was determined by subculturing from tubes/wells showing no visible growth onto SDA plates and recording the lowest concentration showing no fungal growth after incubation.

Stability Study: Stability testing of the optimized gel was performed in laminated aluminium collapsible tubes with screw caps. Samples were stored at 25 +/- 2°C/60 +/- 5% RH and 40 +/- 2°C/75 +/- 5% RH for three months. At initial, 1 month, 2 months and 3 months, samples were evaluated for appearance, pH, viscosity,

spreadability, extract content, microbial limit and preservative efficacy observation. The study was considered preliminary and short-term; long-term stability in final packaging is required before product-level claims.

Statistical Analysis: Data were expressed as mean +/- standard deviation. One-way ANOVA followed by appropriate post-hoc comparison was used to compare antifungal activity among individual extracts, polyherbal gel, fluconazole and base gel control. A p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION:

Extract Yield and Phytochemical Screening: Hydroethanolic extraction produced measurable yields from all selected plant materials. Neem extract showed the highest percentage yield, followed by tulsi and *Piper betle*.

The variation in yield may be attributed to differences in plant matrix composition, polarity of phytoconstituents and solvent affinity. Preliminary phytochemical screening showed alkaloids, flavonoids, tannins, phenolic compounds and saponins in varying intensity across the three extracts.

TABLE 3: EXTRACT YIELD AND QUALITATIVE PHYTOCHEMICAL PROFILE

Parameter	Neem extract	Tulsi extract	Piper betle extract
Plant material used (g)	50	50	50
Extract obtained (g)	8.5	7.2	6.8
Percentage yield (%)	17.0	14.4	13.6
Alkaloids	++	+	+
Flavonoids	+++	++	++
Tannins	++	++	+
Phenolic compounds	+++	++	++
Saponins	++	+	+

TABLE 4: QUANTITATIVE PHYTOCHEMICAL AND MARKER-BASED STANDARDIZATION OF EXTRACTS

Parameter	Neem extract	Tulsi extract	Piper betle extract
Total phenolic content (mg GAE/g extract)	76.4 +/- 2.3	58.9 +/- 1.9	42.6 +/- 1.8
Total flavonoid content (mg QE/g extract)	52.8 +/- 1.6	44.2 +/- 1.4	28.7 +/- 1.2
Selected marker	Azadirachtin	Eugenol	Hydroxychavicol
Marker content (% w/w of extract)	0.42 +/- 0.03	1.18 +/- 0.06	2.36 +/- 0.08

Phenolic and flavonoid contents were highest in neem extract, while Piper betle showed the highest selected marker content for hydroxychavicol. These results support the presence of phytoconstituent groups relevant to antifungal activity. However, the marker data should be interpreted as

standardization parameters and not as proof of clinical antifungal efficacy.

Physicochemical Evaluation of Polyherbal Gel: The prepared gel showed acceptable organoleptic and physicochemical characteristics. The pH was

suitable for topical application, but pH alone cannot establish skin safety; therefore, skin irritation, patch testing and cytotoxicity studies are required in future. The viscosity and spreadability values

indicated suitable semisolid consistency and ease of application. Extract content uniformity indicated reasonable distribution of the polyherbal extract mixture within the gel base.

TABLE 5: PHYSICOCHEMICAL EVALUATION OF OPTIMIZED POLYHERBAL GEL

Parameter	Observation/value	Interpretation
Colour	Light greenish-brown	Due to herbal extracts
Odour	Mild herbal odour	Acceptable
Consistency	Smooth semisolid	Suitable for topical application
pH	6.69 +/- 0.035	Acceptable topical pH range
Viscosity	4823 +/- 92.5 cP	Suitable semisolid consistency
Spreadability	31.58 +/- 0.76 g.cm/sec	Easily spreadable
Homogeneity	Uniform; no lumps/grittiness	Acceptable
Extrudability	Smooth, continuous ribbon	User-friendly
Extract content	98.5 +/- 0.70%	Uniform extract distribution

UV Method Validation for Extract Content

Uniformity: The placebo gel base showed no significant absorbance interference at the selected wavelength after identical extraction and dilution. Therefore, the developed UV method was

considered suitable for estimating total extract content uniformity in the prepared gel. Since crude extracts were used, this method estimated combined extract content rather than individual active marker content.

TABLE 6: UV-VISIBLE METHOD VALIDATION FOR POLYHERBAL EXTRACT CONTENT ESTIMATION

Parameter	Result	Interpretation
Analytical wavelength	276 nm	Selected for combined extract estimation
Diluent	Methanol:water (70:30 v/v)	Suitable extract solubilization
Linearity range	10-50 microgram/mL	Linear response
Calibration equation	$y = 0.0149x + 0.0013$	Acceptable slope/intercept
Regression coefficient	$R^2 = 0.9999$	Excellent linearity
Accuracy at 80%	98.92 +/- 0.88%	Acceptable recovery
Accuracy at 100%	99.64 +/- 0.73%	Acceptable recovery
Accuracy at 120%	100.18 +/- 0.91%	Acceptable recovery
Intra-day precision	%RSD = 0.86	Precise
Inter-day precision	%RSD = 1.12	Precise
Specificity	No significant placebo gel interference	Specific under tested conditions
Robustness	%RSD = 1.24	Robust under minor variation
LOD	1.62 microgram/mL	Sensitive
LOQ	4.91 microgram/mL	Suitable for quantification

In-vitro Antifungal Activity: The polyherbal gel showed measurable antifungal activity against both fungal strains. Its inhibition zones were significantly higher than those of individual extracts and base gel control, but significantly lower than fluconazole. Therefore, the developed

gel should not be considered equivalent or superior to standard antifungal therapy. The base gel control showed negligible inhibition, suggesting that the observed activity was mainly associated with incorporated herbal extracts.

TABLE 7: COMPARATIVE ANTIFUNGAL ACTIVITY OF TESTED SAMPLES BY AGAR WELL DIFFUSION

Sample	Concentration applied	<i>C. albicans</i> ATCC 10231 zone (mm)	<i>A. niger</i> ATCC 16404 zone (mm)
Neem extract	20 mg/mL; 100 microlitre/well	16.33 +/- 0.58	15.33 +/- 0.58
Tulsi extract	20 mg/mL; 100 microlitre/well	14.33 +/- 0.58	13.33 +/- 0.58
Piper betle extract	20 mg/mL; 100 microlitre/well	12.67 +/- 0.58	11.33 +/- 0.58
Polyherbal gel	Gel dispersion equivalent to 60 mg/mL total extract; 100 microlitre/well	21.33 +/- 0.58*#	19.33 +/- 0.58*#
Fluconazole	25 microgram/mL; 100 microlitre/well	24.33 +/- 0.58	22.33 +/- 0.58
Base gel control	Equivalent gel base; 100 microlitre/well	Negligible	Negligible

Values are expressed as mean +/- SD; n = 3. *p < 0.05 compared with individual extracts and base gel control; #p < 0.05 compared with fluconazole, indicating lower activity than standard drug.

MIC and MFC Findings: The MIC/MFC findings supported the agar well diffusion results and showed that the polyherbal gel dispersion had lower MIC values than the individual extracts. However, true synergism cannot be concluded

because checkerboard assay and FICI calculation were not performed. These results should therefore be interpreted as preliminary evidence of enhanced combined *in-vitro* activity rather than confirmed synergy.

TABLE 8: MIC AND MFC VALUES OF TESTED EXTRACTS AND POLYHERBAL GEL

Sample	<i>C. albicans</i> MIC (mg/mL)	<i>C. albicans</i> MFC (mg/mL)	<i>A. niger</i> MIC (mg/mL)	<i>A. niger</i> MFC (mg/mL)
Neem extract	125	250	125	250
Tulsi extract	250	500	250	500
<i>Piper betle</i> extract	250	500	500	>500
Polyherbal gel dispersion	62.5	125	125	250
Fluconazole	0.025	0.05	0.025	0.05

Stability Study: The stability results suggest that the formulation retained acceptable appearance, pH, viscosity, spreadability and extract content during the short-term study. More noticeable changes occurred at accelerated conditions, particularly slight thinning and reduction in

viscosity and extract content. These findings support preliminary stability only. ICH-relevant long-term stability, validated microbial limit testing, preservative efficacy testing and packaging compatibility studies are required before product-level claims.

TABLE 9: SHORT-TERM STABILITY PROFILE OF OPTIMIZED POLYHERBAL GEL

Condition/time	Appearance	pH	Viscosity (cP)	Spreadability (g.cm/sec)	Extract content (%)	Microbial limit observation
Initial	Smooth, uniform	6.69 +/- 0.035	4823 +/- 92.5	31.58 +/- 0.76	98.50 +/- 0.70	Within acceptable preliminary limit
25 +/- 2°C/60 +/- 5% RH - 1 month	No change	6.67 +/- 0.041	4790 +/- 88.2	31.42 +/- 0.72	98.20 +/- 0.68	No visible contamination
25 +/- 2°C/60 +/- 5% RH - 2 months	No change	6.66 +/- 0.039	4771 +/- 90.4	31.21 +/- 0.70	98.05 +/- 0.71	No visible contamination
25 +/- 2°C/60 +/- 5% RH - 3 months	No change	6.65 +/- 0.044	4755 +/- 86.6	31.05 +/- 0.69	97.90 +/- 0.74	No visible contamination
40 +/- 2°C/75 +/- 5% RH - 1 month	Slight thinning	6.63 +/- 0.047	4680 +/- 96.2	30.68 +/- 0.81	97.60 +/- 0.77	No visible contamination
40 +/- 2°C/75 +/- 5% RH - 2 months	Slight thinning	6.60 +/- 0.050	4618 +/- 102.5	30.24 +/- 0.84	97.20 +/- 0.82	No visible contamination
40 +/- 2°C/75 +/- 5% RH - 3 months	Slight thinning	6.58 +/- 0.052	4550 +/- 108.4	29.86 +/- 0.88	96.80 +/- 0.86	No visible contamination

TABLE 10: PRESERVATIVE EFFICACY OBSERVATION OF OPTIMIZED GEL

Test parameter	Initial	3 months at 25°C	3 months at 40°C	Interpretation
Bacterial growth observation	No visible growth	No visible growth	No visible growth	Acceptable preliminary preservation
Fungal growth observation	No visible growth	No visible growth	No visible growth	Acceptable preliminary preservation
Physical contamination	Absent	Absent	Absent	No visible contamination

DISCUSSION: The present study demonstrated the feasibility of formulating a Carbopol 934-based polyherbal topical gel containing *Azadirachta indica*, *Ocimum sanctum* and *Piper betle* leaf extracts. The formulation was pharmaceutically acceptable in terms of pH, viscosity, spreadability, homogeneity, extrudability and extract content uniformity. The improved inhibition zones and

lower MIC values of the polyherbal gel compared with individual extracts suggest enhanced combined *in-vitro* antifungal activity.

However, this finding should not be interpreted as proven synergism because checkerboard assay, FICI calculation and time-kill studies were not performed.

Fluconazole remained superior to the polyherbal gel in agar diffusion and MIC/MFC testing. Therefore, the developed herbal gel cannot be considered a replacement for standard antifungal therapy on the basis of the present results.

The findings are preliminary and support further development, standardization and safety evaluation. Future work should include dermatophyte-specific testing, expanded fungal panels, checkerboard combination studies, *ex-vivo* skin permeation, skin irritation, cytotoxicity, validated microbial limit testing, preservative efficacy and clinical evaluation.

CONCLUSION: A polyherbal topical gel containing hydroethanolic leaf extracts of *Azadirachta indica*, *Ocimum sanctum* and Piper betle was successfully prepared using Carbopol 934 as the gelling agent and propylene glycol as humectant.

The formulation showed acceptable organoleptic and physicochemical characteristics, including suitable pH, viscosity, spreadability, homogeneity, extrudability and extract content uniformity. Quantitative phytochemical evaluation confirmed the presence of phenolic and flavonoid constituents, and marker-based standardization was included for preliminary extract characterization.

The polyherbal gel demonstrated measurable *in-vitro* antifungal activity against *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. The activity was higher than individual extracts and base gel control but lower than fluconazole. MIC/MFC findings supported the preliminary antifungal potential of the formulation.

However, true synergism, therapeutic superiority and clinical efficacy cannot be concluded from the present study. Further checkerboard/FICI analysis, time-kill studies, dermatophyte-specific testing, skin irritation studies, cytotoxicity studies, long-term stability and clinical evaluation are required before therapeutic application.

ACKNOWLEDGEMENT: The authors express their sincere gratitude to the Department of Pharmaceuticals, Maya Institute of Pharmacy, Hathras, Uttar Pradesh, India, for providing laboratory facilities, materials, instruments and

academic support required to carry out this research work. The authors are also thankful to the faculty members, laboratory staff and technical personnel for their cooperation and assistance during extraction, formulation development, physicochemical evaluation and *in-vitro* antifungal studies.

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

REFERENCES:

1. Havlickova B, Czaika VA and Friedrich M: Epidemiological trends in skin mycoses worldwide. *Mycoses* 2008; 51(4): 2-15.
2. Bongomin F, Gago S, Oladele RO and Denning DW: Global and multi-national prevalence of fungal diseases estimate precision. *J Fungi (Basel)* 2017; 3(4): 57.
3. Verma S and Madhu R: The great Indian epidemic of superficial dermatophytosis: an appraisal. *Indian J Dermatol* 2017; 62(3): 227-236.
4. Dalei SR, Behera SK, Mishra D, Sahoo AK, Sahu S and Sethy M: Current status of dermatophytosis: a hospital-based study. *J Family Med Prim Care* 2023; 12(11): 2654-2660.
5. Nazzaro F, Fratianni F, Coppola R and De Feo V: Essential oils and antifungal activity. *Pharmaceuticals (Basel)* 2017; 10(4): 86.
6. Mahmoud DA, Hassanein NM, Youssef KA and Abou Zeid MA: Antifungal activity of different neem leaf extracts and nimonol against some important human pathogens. *Braz J Microbiol* 2011; 42(3): 1007-1016.
7. Chandini R, Anuradha A, Rajeshkumar S, Bharathwaj VV and Shanmugam R: Anti-candidal effect of *Ocimum sanctum*: a systematic review. *J Pharm Bioallied Sci* 2022; 14(1): 37-43.
8. Ali I, Khan FG, Suri KA, Gupta BD, Satti NK and Dutt P: *In-vitro* antifungal activity of hydroxychavicol isolated from *Piper betle* L. *Ann Clin Microbiol Antimicrob* 2010; 9: 7.
9. Sivareddy B, Reginald BA, Sireesha D, Samatha M, Reddy KH and Subrahmanyam G: Antifungal activity of solvent extracts of *Piper betle* and *Ocimum sanctum* Linn on *Candida albicans*: an *in-vitro* comparative study. *J Oral Maxillofac Pathol* 2019; 23(3): 333-337.
10. Gupta A, Mishra AK, Singh AK, Gupta V and Bansal P: Formulation and evaluation of topical gel of diclofenac sodium using different polymers. *Drug Invent Today* 2010; 2(5): 250-253.
11. Naya NMDMW, Sasadara MMV, Sanjaya DA, Yuda PESK, Dewi NLKAA and Cahyaningsih E: *Piper betle* (L): recent review of antibacterial and antifungal properties, safety profiles, and commercial applications. *Molecules* 2021; 26(8): 2321.
12. Chang CC, Yang MH, Wen HM and Chern JC: Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 2002; 10(3): 178-182.
13. Clinical and Laboratory Standards Institute: Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard. CLSI document M27. Wayne, PA: Clinical and Laboratory Standards Institute.

How to cite this article:

Bhardwaj D, Kumar Y and Yadav R: Formulation and *in-vitro* evaluation of a polyherbal topical gel containing *Azadirachta indica*, *Ocimum sanctum* and *Piper betle* extracts for antifungal activity. Int J Pharm Sci & Res 2026; 17(10): 3170-78. doi: 10.13040/IJPSR.0975-8232.17(10).3170-78.

All © 2026 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)