

Preformulation Characterization, UV Spectrophotometric Method Development and Preliminary Antifungal Evaluation of Eucalyptus Oil for Topical Drug Delivery

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Abstract

Eucalyptus oil is a volatile, lipophilic natural product with promising antifungal potential; however, its hydrophobicity and volatility pose challenges for analytical standardization and topical formulation. This study aimed to establish a preformulation profile of eucalyptus oil, develop a UV spectrophotometric method for its quantitative estimation, assess concentration-dependent antifungal activity, and evaluate compatibility with selected excipients for subsequent topical organogel development. Eucalyptus oil was characterized for colour, clarity, odour, weight per millilitre, and solvent compatibility. UV spectrophotometric analysis was performed following wavelength optimization, and 210 nm was selected for quantitative estimation. Linearity was evaluated over 25–45 µg/mL. Preliminary antifungal activity was assessed at 1%, 2.5%, 5%, and 10% concentrations using a disc-diffusion assay, with nystatin as the reference standard. Drug–excipient compatibility was investigated using Fourier-transform infrared spectroscopy (FTIR) and UV-based assay following storage at 40°C/75% relative humidity for one month. The oil was pale yellow, clear and transparent, with a characteristic camphoraceous odour and a weight per millilitre of 0.9079 g/mL. It showed compatibility with methanol, ethanol, propylene glycol and Tween 80, whereas phase separation occurred with water. The developed UV method exhibited strong linearity over 25–45 µg/mL ($R^2 = 0.99950$), with the regression equation $Y = 0.01984X - 0.0158$. Antifungal screening demonstrated concentration-dependent inhibition, with zones of 10, 17, 25, and 33 mm for 1%, 2.5%, 5%, and 10% eucalyptus oil, respectively, compared with 24 mm for nystatin. FTIR profiles retained characteristic eucalyptus-oil bands, while assay values ranged from 98.90% to 100.57%. these findings provide a rational preformulation basis for further development and optimization of eucalyptus-oil-based topical antifungal delivery systems.

Keywords: Eucalyptus oil; Antifungal activity; Preformulation; UV spectrophotometry; Drug–excipient compatibility.

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Introduction

Superficial fungal infections represent an important global dermatological problem, particularly because of their recurrent nature, increasing treatment challenges and the need for effective local therapy. Topical antifungal therapy remains an important approach for localized cutaneous infections because it can deliver the active agent directly to the affected tissue while potentially reducing systemic exposure [1]. However, the effectiveness of topical therapy depends not only on the intrinsic antifungal potency of an active compound but also on its physicochemical properties, retention at the site of

infection, penetration through the stratum corneum and stability within the formulation. These considerations have stimulated increasing interest in plant-derived bioactive compounds and essential oils as potential alternatives or complementary agents for topical antifungal applications. [2]. A systematic review of essential oils as topical anti-infective agents reported encouraging activity against topical fungal infections, although considerable heterogeneity among studies emphasizes the need for better standardized and mechanistically supported investigations. Essential oils are complex mixtures of volatile secondary

metabolites, predominantly comprising monoterpenes, sesquiterpenes and their oxygenated derivatives. Their antimicrobial effects have been associated with several mechanisms, including disruption of fungal membrane integrity, alteration of membrane permeability, interference with cellular metabolism and induction of oxidative stress. A comprehensive review of essential oils and their constituents for skin fungal infections highlighted the considerable antifungal potential of monoterpene-rich oils while also emphasizing the importance of formulation strategies for improving their pharmaceutical applicability. More recent research similarly emphasizes that essential oils may provide promising antifungal activity but require improved standardization, mechanistic investigation and delivery approaches before their therapeutic potential can be reliably translated into pharmaceutical products [2]. Among medicinally relevant essential oils, eucalyptus oil has attracted considerable interest because of its rich terpenoid composition and broad biological activities. Depending on botanical species and geographical origin, eucalyptus essential oils may contain substantial quantities of oxygenated monoterpenes, particularly 1,8-cineole (eucalyptol). The chemical composition of eucalyptus oil is closely associated with its biological properties and may vary considerably between species and production conditions. For example, investigation of *Eucalyptus smithii* essential oil identified 1,8-cineole as the predominant constituent at 72.2% and demonstrated antifungal activity against several dermatophytes, with reported MIC and MFC values varying according to the fungal species tested. Morphological changes observed by scanning electron microscopy further supported direct effects of the oil on fungal cells [3]. Evidence for the antifungal potential of eucalyptus-derived preparations is also available from investigations involving other *Eucalyptus* species. A review of *Eucalyptus camaldulensis* reported substantial antimicrobial activity of its essential oil and extracts, including activity against several fungal models and *Candida albicans* [4]. In another investigation, eucalyptus essential oil demonstrated activity against *Candida albicans*, while microscopic analysis indicated structural damage following exposure to essential oils [5]. Collectively, these observations suggest that eucalyptus oil possesses a biologically relevant antifungal profile; nevertheless, differences in chemical composition, fungal strain, assay methodology and concentration make direct comparison between studies difficult. Therefore, characterization of the individual oil sample and standardized assessment of its biological activity are important prerequisites for pharmaceutical development. The pharmaceutical utilization of eucalyptus oil is complicated by its lipophilic and volatile nature. Poor aqueous

compatibility can restrict incorporation into conventional hydrophilic topical systems, while volatility may influence dose reproducibility, stability and residence time following topical administration. The principal constituent 1,8-cineole has also been investigated as a chemical penetration enhancer, demonstrating the ability to influence transport across the skin barrier. However, its penetration-enhancing behaviour must be balanced against appropriate formulation design and skin tolerability [6]. Consequently, a rational development strategy should begin with systematic physicochemical characterization, including solvent compatibility and basic quality attributes, before selection of an appropriate delivery platform. Recent formulation studies provide evidence that eucalyptus oil can be incorporated into advanced topical delivery systems. Eucalyptus-oil-based nanoemulsions have been investigated as vehicles for topical delivery, with eucalyptus oil incorporated into nanoemulsion systems containing ketoconazole. The optimized system demonstrated nanoscale droplets, high drug content, controlled release and enhanced antifungal performance against *Candida albicans*, together with acceptable skin tolerability in the reported experimental model [7]. Similarly, eucalyptus essential oil has been incorporated into nanoemulsion and nanogel systems, demonstrating the feasibility of using structured delivery platforms to improve the pharmaceutical handling of volatile essential oils [8]. These studies support the broader concept that formulation architecture can play an important role in translating the biological properties of essential oils into practical topical dosage forms. Despite this progress, an important research gap remains. Much of the existing literature focuses either on the biological activity of eucalyptus oil or on its incorporation into advanced delivery systems, whereas systematic integration of preformulation characterization, quantitative analytical standardization, preliminary concentration selection and drug-excipient compatibility within a single development pathway remains limited. [9]. An appropriate analytical method is essential for monitoring the active oil during formulation development and subsequent stability studies. UV spectrophotometry offers a simple and accessible analytical approach; however, because eucalyptus oil is a multicomponent mixture, its analytical response must be carefully characterized and subsequently supported by appropriate validation and, preferably, complementary chemical fingerprinting [10]. In the present investigation, eucalyptus oil was therefore subjected to a structured preformulation assessment encompassing organoleptic characterization, weight-per-millilitre determination and solvent compatibility. A UV spectrophotometric procedure was subsequently investigated to establish a quantitative response

suitable for preliminary pharmaceutical development.

Materials and Methods

Preformulation study:

Organoleptic Characterization

The procured eucalyptus oil was evaluated for colour, clarity and odour. A small quantity of the oil was transferred into a clean, dry glass vial and visually examined against a white background under adequate illumination. Clarity was assessed by observing the sample for turbidity, suspended particles and phase separation. The characteristic odour of the oil was evaluated by gently assessing the vapours from the opened vial [11].

Determination of Weight per Millilitre

The weight per millilitre of eucalyptus oil was determined using a pycnometric method. A clean, dry pycnometer was weighed, followed by weighing the pycnometer filled with distilled water and subsequently with the eucalyptus oil. The measurements were performed at room temperature. The weight per millilitre was calculated using the following equation [12].

Solubility Study

The solubility of eucalyptus oil was evaluated in methanol, ethanol, water, propylene glycol and Tween 80. Briefly, 1 mL of eucalyptus oil was gradually added to 5 mL of each solvent with gentle stirring at room temperature. The resulting mixtures were visually examined for clarity, turbidity, precipitation and phase separation. Formation of a clear and homogeneous mixture was considered indicative of solubility under the experimental conditions [13].

UV Spectrophotometric Method Development

Preparation of Standard Stock Solution

Accurately weighed 50 mg of eucalyptus oil was transferred into a clean, dry 100 mL volumetric flask. Approximately 30 mL of methanol was added, and the solution was sonicated for 2–3 min to facilitate complete dispersion. The volume was then made up to the mark with methanol to obtain a standard stock solution containing 500 µg/mL of eucalyptus oil [14].

Selection of Analytical Wavelength

An aliquot of 0.8 mL of the 500 µg/mL stock solution was transferred into a 20 mL volumetric flask and diluted to volume with methanol to obtain a 20 µg/mL solution. The prepared solution was scanned in the UV region to determine the wavelength of maximum absorption. Eucalyptus oil showed an absorption maximum at approximately 205 nm. However, 210 nm was selected for quantitative analysis considering solvent cut-off limitations at wavelengths below 210 nm [15].

Selection of Linearity Range

A concentration range of 25–45 µg/mL was selected based on the preliminary absorbance response, with 35 µg/mL considered the central test concentration. Standard solutions of 25, 30, 35, 40 and 45 µg/mL

were prepared by appropriate dilution of the stock solution with methanol [16].

Calibration Curve

Each concentration level was analysed in triplicate at 210 nm. The mean absorbance was plotted against the corresponding eucalyptus-oil concentration to construct the calibration curve. Linear regression analysis was performed to determine the slope, intercept and coefficient of determination (R^2) [17].

The calibration equation was:

$$Y = 0.01984X - 0.0158$$

with $R^2 = 0.99950$.

Selection of Formulation Excipients

The formulation excipients were selected based on their intended functional roles in the development of a eucalyptus-oil-based topical organogel. Soy lecithin was selected as the primary organogelator because of its amphiphilic nature and ability to form reverse micellar structures in non-aqueous systems, thereby contributing to formation of a three-dimensional gel network. Beeswax was incorporated as a secondary structuring agent to improve the mechanical strength and consistency of the organogel and to reduce mobility of the oil phase. Isopropyl myristate (IPM) was selected as a lipophilic solvent and penetration-enhancing component because of its compatibility with the oil phase and potential contribution to spreading and dermal delivery. Ethanol was selected as the polar component required for formation of the lecithin-based organogel system. Butylated hydroxytoluene (BHT) was incorporated as an antioxidant to minimize oxidative degradation of the volatile eucalyptus oil and thereby improve formulation stability [18].

Quantitative Compatibility Assessment

Quantitative drug–excipient compatibility was assessed by determining the eucalyptus-oil content in the prepared compatibility samples using the developed UV spectrophotometric method. Compatibility samples equivalent to 35 mg of eucalyptus oil were accurately weighed and transferred individually into 100 mL volumetric flasks. Approximately 70 mL of methanol was added, followed by sonication for 5–7 min to facilitate extraction of eucalyptus oil into the solvent. The volume was then made up to the mark with methanol. The resulting solution was filtered through a suitable 0.45 µm syringe filter, discarding the initial 3–5 mL of filtrate. Subsequently, 1 mL of the filtrate was diluted to 10 mL with methanol to obtain a nominal concentration of 35 µg/mL. The absorbance was measured at 210 nm using the developed UV spectrophotometric method [19].

Drug–excipient compatibility

The drug–excipient compatibility samples were subjected to Fourier-transform infrared (FTIR) spectroscopy to investigate potential chemical interactions between eucalyptus oil and the selected excipients. Following storage at 40°C/75% RH for

one month, the samples were analyzed using an Agilent Cary 630 FTIR spectrometer. The FTIR spectra of eucalyptus oil and the corresponding drug–excipient combinations were recorded over the appropriate infrared spectral region, and the characteristic absorption bands of eucalyptus oil were identified and compared with those observed in the compatibility samples. The principal absorption bands observed for eucalyptus oil were assigned to their corresponding functional groups. The characteristic peaks were subsequently compared between pure eucalyptus oil and each compatibility sample. Retention of the characteristic peaks without substantial disappearance or marked shifts was considered indicative of the absence of prominent chemical incompatibility under the investigated conditions. The reported compatibility samples included eucalyptus oil with soy lecithin (1:1.5), beeswax (1:1), isopropyl myristate (1:4) and BHT (1:0.25) [20].

Preliminary Antifungal Evaluation eucalyptus oil

The preliminary antifungal activity of eucalyptus oil was evaluated to investigate its concentration-dependent inhibitory effect and to identify a suitable concentration for subsequent topical formulation development. Eucalyptus oil was prepared at concentrations of 1%, 2.5%, 5% and 10% using methanol as the diluent. Methanol was used as the blank control, while nystatin was included as the reference antifungal standard. The antifungal activity was evaluated by the disc-diffusion method. Briefly, the fungal inoculum was prepared according to the procedure described in the experimental report and uniformly inoculated onto the appropriate agar medium. Sterile discs were impregnated with the respective eucalyptus-oil preparations and placed aseptically on the inoculated agar surface. A blank methanol disc and nystatin-containing disc were included as controls. The plates were incubated at $35 \pm 2^\circ\text{C}$, and the diameter of the resulting zones of inhibition was measured in millimetres after incubation [21].

Results

Organoleptic Characterization of Eucalyptus Oil

The procured eucalyptus oil was evaluated for colour, clarity and odour. The oil was observed to be pale yellow in colour, clear and transparent in appearance, with a strong camphoraceous odour. These observations confirmed the characteristic organoleptic properties of the procured eucalyptus oil.

Table 1. Organoleptic Characteristics of Eucalyptus Oil

Parameter	Observation
Colour	Pale yellow
Clarity	Clear, transparent
Odour	Strong camphoraceous

Determination of Weight per Millilitre

The weight per millilitre of eucalyptus oil was determined using the pycnometric method at room temperature. The empty pycnometer weighed 24.8952 g, while the weights of the pycnometer containing water and eucalyptus oil were 34.9121 g and 34.0256 g, respectively. Based on the psychometric calculation, the weight per millilitre of eucalyptus oil was found to be 0.9079 g/ml.

Solubility Study

The solubility of eucalyptus oil was evaluated in methanol, ethanol, water, propylene glycol and Tween 80. Eucalyptus oil produced clear and homogeneous solutions with methanol, ethanol, propylene glycol and Tween 80, indicating good solubility in these solvents. In contrast, the oil formed two separate layers with water, indicating practical insolubility. Thus, eucalyptus oil exhibited a predominantly hydrophobic character and showed better compatibility with the selected organic and surfactant-based solvents.

Table 2. Solubility Profile of Eucalyptus Oil in Different Solvents

Solvent	Observation	Result
Methanol	Clear homogeneous solution	Soluble
Ethanol	Clear homogeneous solution	Soluble
Water	Two separate layers formed	Insoluble
Propylene glycol	Clear homogeneous solution	Soluble
Tween 80	Clear homogeneous solution	Soluble

UV Spectrophotometric Method Development

The UV spectrophotometric method was developed using methanol as the solvent. A eucalyptus-oil stock solution of 500 µg/mL was prepared, and a 20 µg/mL solution was scanned in the UV region. Eucalyptus oil exhibited a maximum absorption at approximately 205 nm. However, 210 nm was selected as the analytical wavelength because wavelengths below 210 nm may be affected by solvent cut-off interference. At 210 nm, the 20 µg/mL solution showed an absorbance of 0.3924.

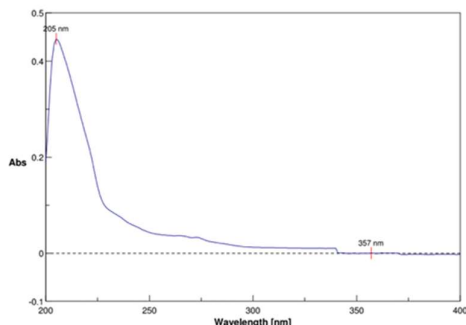


Figure 1. UV Absorption Spectrum of Eucalyptus Oil Showing λ_{max} at 205 nm

Linearity Study

The linearity of the developed UV spectrophotometric method was evaluated over the concentration range of 25–45 $\mu\text{g/mL}$ at 210 nm. Concentrations of 25, 30, 35, 40 and 45 $\mu\text{g/mL}$ were analysed in triplicate. The mean absorbance increased progressively from 0.4853 at 25 $\mu\text{g/mL}$ to 0.8811 at 45 $\mu\text{g/mL}$, demonstrating a concentration-dependent response.

Concentration ($\mu\text{g/mL}$)	Mean absorbance	% RSD
25	0.4853	0.063
30	0.5771	0.036
35	0.6715	0.054
40	0.7773	0.032
45	0.8811	0.034

Table 3. Linearity Data of Eucalyptus Oil by UV Spectrophotometric Method at 210 nm

The calibration curve showed excellent linearity with $R^2 = 0.99950$, a slope of 0.01984 and an intercept of -0.0158 . The regression equation was:

$$Y = 0.01984X - 0.0158$$

The obtained correlation coefficient was above the specified acceptance criterion of 0.98, confirming the linear response of eucalyptus oil over the investigated concentration range.

Quantitative Drug–Excipient Compatibility Study

The drug–excipient compatibility samples were prepared at the selected ratios and stored at $40^\circ\text{C}/75\% \text{ RH}$ for one month before analysis. The compatibility samples consisted of eucalyptus oil alone and combinations with soy lecithin, beeswax, IPM and BHT. The samples were analysed quantitatively by the developed UV spectrophotometric method at 210 nm. The absorbance values of the samples ranged from 0.6698 to 0.6777, indicating only minor variation among the compatibility samples. All samples showed assay values within the specified acceptance range of 95.0–105.0%. The highest assay was observed for the eucalyptus oil–beeswax combination (100.57%), whereas the eucalyptus oil–IPM combination showed the lowest assay

(98.90%). Overall, all compatibility samples complied with the predefined assay criteria.

Table 4. Composition and Ratios of Eucalyptus Oil–Excipient Compatibility Samples

Sample	Ratio	Absorbance	% Assay
Eucalyptus oil	As such	0.6698	98.98
EO + Soy lecithin	1:1.5	0.6752	99.83
EO + Beeswax	1:1	0.6777	100.57
EO + IPM	1:4	0.6712	98.90
EO + BHT	1:0.25	0.6722	99.05

FTIR Compatibility Study

FTIR analysis was performed after one month of storage at $40^\circ\text{C}/75\% \text{ RH}$. The characteristic absorption bands of eucalyptus oil were retained in the compatibility samples. The principal bands of pure eucalyptus oil included 2922.56 cm^{-1} for aliphatic C–H stretching, 1641.67 cm^{-1} for C=C stretching, 1464.37 cm^{-1} for C–H bending, 1375.24 cm^{-1} for CH_3 bending, 1214.51 cm^{-1} , 1166.58 cm^{-1} and 1078.94 cm^{-1} corresponding to ether-related C–O/C–O–C stretching, and 984.03 cm^{-1} for =C–H bending. The prominent characteristic peaks of eucalyptus oil were retained in the eucalyptus oil–soy lecithin, eucalyptus oil–beeswax, eucalyptus oil–IPM and eucalyptus oil–BHT compatibility samples. No substantial disappearance of the characteristic peaks was reported. The PDF therefore concluded that no prominent chemical incompatibility was observed between eucalyptus oil and the selected excipients under the investigated storage conditions.

Table 5. FTIR Spectral Characteristics of Eucalyptus Oil

Observed Frequency (cm^{-1})	Functional Group	Significance
2922.56	C–H stretching	Aliphatic terpene structure
1641.67	C=C stretching	Unsaturation in terpenes
1464.37	C–H bending	Hydrocarbon framework
1375.24	C–H bending	Methyl groups
1214.51	C–O stretching	Ether linkage
1166.58	C–O–C stretching	1,8-cineole
1078.94	C–O stretching	Ether group
984.03	=C–H bending	Substituted alkene

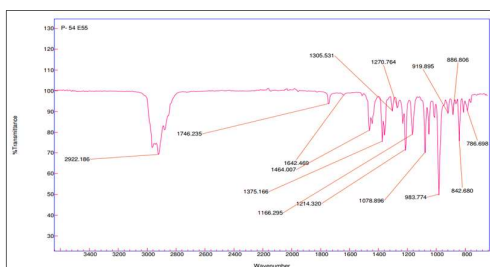


Figure 2. FTIR Spectrum of Eucalyptus Oil: Soy lecithin

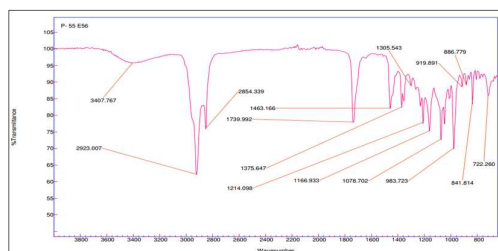


Figure 3. FTIR Spectrum of Eucalyptus Oil: Beeswax

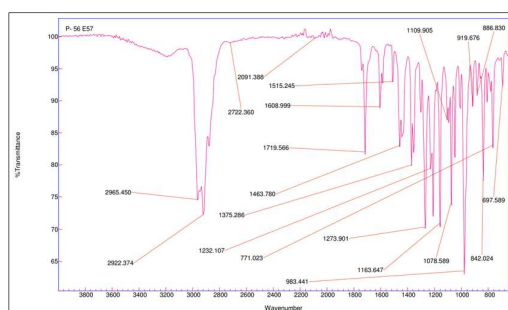


Figure 4. FTIR Spectrum of Eucalyptus Oil: Isopropyl myristate

Physical Observation During Compatibility Study

Following storage at 40°C/75% RH for one month, the compatibility samples showed no conformational change (NCC) in their physical appearance compared with the initial observations. Pure eucalyptus oil remained pale yellow, while the eucalyptus oil–soy lecithin, beeswax, IPM and BHT combinations retained their respective initial appearances.

Table 6. Physical Appearance of Eucalyptus Oil–Excipient Compatibility Samples Before and After Accelerated Storage.

Ingredient/Combination	Initial Observation	After 1 Month at 40°C/75% RH
Pure eucalyptus oil	Pale yellow	NCC
EO + Soy lecithin	Yellowish brown	NCC
EO + Beeswax	Light yellow	NCC
EO + IPM	Light yellow	NCC
EO + BHT	White to light yellow suspension	NCC

Preliminary Antifungal Activity

The antifungal activity of eucalyptus oil was evaluated at concentrations of 1%, 2.5%, 5% and 10% using methanol as the blank and nystatin as the standard. The samples were evaluated by the disc-diffusion method. The PDF reports that the antifungal testing was performed using a fungal culture on GMB medium, with incubation at 35 ± 2°C and examination after 20–24 h. A concentration-dependent increase in the zone of inhibition was observed with increasing eucalyptus-oil concentration. The recorded zones were 10 mm, 17 mm, 25 mm and 33 mm for 1%, 2.5%, 5% and 10% eucalyptus oil, respectively, compared with 24 mm for nystatin. The blank showed

a zone of 3 mm; the PDF specifically notes that the blank zone was subtracted from the results for the eucalyptus-oil concentrations.

Table 7. Preliminary Antifungal Activity of Eucalyptus Oil at Different Concentrations

Sample	Concentration	Zone of inhibition (mm)
Blank (Methanol)	—	3
Eucalyptus oil	1%	10
Eucalyptus oil	2.5%	17
Eucalyptus oil	5%	25
Eucalyptus oil	10%	33
Nystatin	Standard	24

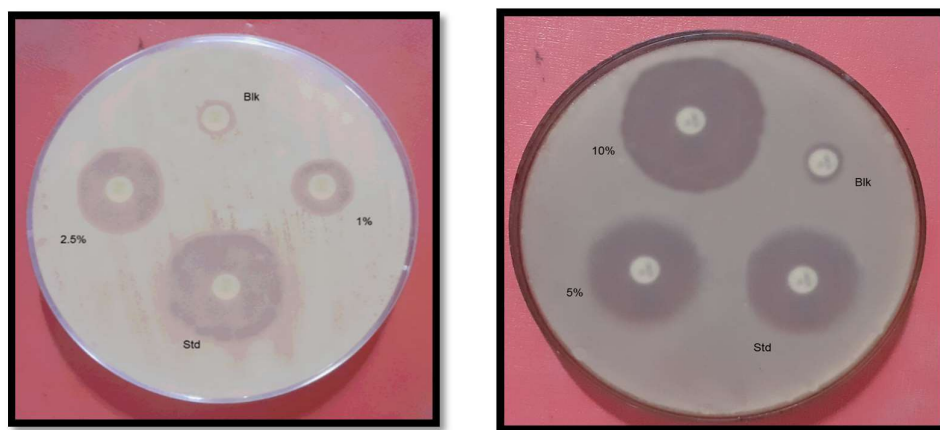


Figure 5 – Concentration-Dependent Antifungal Activity of Eucalyptus Oil

The results demonstrated a progressive increase in antifungal activity with increasing eucalyptus-oil concentration. The 5% eucalyptus-oil preparation produced a zone of inhibition of 25 mm, which was slightly higher than the nystatin standard (24 mm), whereas the 10% preparation exhibited the maximum zone of inhibition (33 mm). Based on the concentration-dependent antifungal response, eucalyptus oil demonstrated sufficient antifungal activity to support its further development

Discussion

The present study was undertaken to establish the preformulation characteristics, develop a simple UV spectrophotometric method for quantitative estimation, evaluate drug–excipient compatibility, and investigate the preliminary antifungal activity of eucalyptus oil for potential topical delivery. The procured eucalyptus oil showed a pale-yellow colour, clear and transparent appearance, and characteristic camphoraceous odour, indicating satisfactory organoleptic properties. Its weight per millilitre was found to be 0.9079 g/mL, providing an important preliminary quality-control parameter for subsequent formulation development. The solubility study demonstrated that eucalyptus oil formed clear and homogeneous mixtures with methanol, ethanol, propylene glycol and Tween 80, whereas phase separation was observed with water. This behaviour indicates the predominantly hydrophobic nature of

eucalyptus oil and highlights the importance of appropriate excipient selection for incorporation into topical formulations. The observed compatibility with propylene glycol and Tween 80 may facilitate uniform incorporation of eucalyptus oil into suitable delivery systems.

UV spectrophotometric investigation showed maximum absorption at approximately 205 nm; however, 210 nm was selected for quantitative analysis to minimize possible solvent cut-off interference. The developed method showed a progressive increase in absorbance with increasing concentration over the range of 25–45 µg/mL. The calibration curve exhibited excellent linearity with an R^2 value of 0.99950 and the regression equation $Y = 0.01984X - 0.0158$. This strong linear relationship demonstrates the suitability of the developed method for preliminary quantitative estimation of eucalyptus oil during formulation

development and compatibility studies. Drug–excipient compatibility studies were performed using soy lecithin, beeswax, IPM and BHT at selected ratios following storage at 40°C/75% RH for one month. The assay values ranged from 98.90% to 100.57%, remaining within the predefined acceptance range of 95–105%. The beeswax combination showed the highest assay value, whereas the IPM combination showed the lowest, although both remained within acceptable limits. FTIR analysis further supported compatibility, as the characteristic eucalyptus-oil absorption bands were retained in the combinations without substantial disappearance or marked shifts. Physical observations also revealed no reported conformational changes after accelerated storage. The preliminary antifungal study demonstrated a clear concentration-dependent response. Zones of inhibition increased from 10 mm at 1% to 17 mm at 2.5%, 25 mm at 5%, and 33 mm at 10% eucalyptus oil, compared with 24 mm for nystatin. The 5% preparation therefore showed slightly greater inhibition than the reference standard, while 10% exhibited the maximum activity. Overall, these findings provide a rational preformulation basis for further development and optimization of eucalyptus-oil-based topical antifungal delivery systems.

Conclusion

The present study successfully established the preliminary preformulation profile of eucalyptus oil for potential topical antifungal drug delivery. Eucalyptus oil exhibited satisfactory organoleptic characteristics, with a pale-yellow colour, clear and transparent appearance, characteristic camphoraceous odour and a weight per millilitre of 0.9079 g/mL. The oil showed good compatibility with methanol, ethanol, propylene glycol and Tween 80, while being practically insoluble in water. A simple UV spectrophotometric method was developed at 210 nm, showing excellent linearity over the concentration range of 25–45 µg/mL ($R^2 = 0.99950$). Drug–excipient compatibility studies demonstrated acceptable assay values ranging from 98.90% to 100.57%, while FTIR analysis showed retention of characteristic eucalyptus-oil peaks without prominent spectral changes. No significant physical changes were observed after storage at 40°C/75% RH for one month, supporting the suitability of soy lecithin, beeswax, IPM and BHT for further formulation development. The preliminary antifungal evaluation demonstrated a concentration-dependent increase in activity, with zones of inhibition of 10, 17, 25 and 33 mm at 1%, 2.5%, 5% and 10% concentrations, respectively. The 5% preparation produced a zone of inhibition slightly higher than the nystatin standard, while the 10% preparation showed the maximum inhibition. Overall, the findings provide a rational scientific basis for the further development and optimization

of eucalyptus-oil-based topical organogel formulations with potential antifungal application.

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Reference

1. Deyno, S., Mtewa, A. G., Abebe, A., Hymete, A., Makonnen, E., Bazira, J., & Alele, P. E. (2019). Essential oils as topical anti-infective agents: A systematic review and meta-analysis. *Complementary Therapies in Medicine*, 47, 102224. <https://doi.org/10.1016/j.ctim.2019.102224>.
2. Rashed, A. A., Rathi, D.-N. G., Nasir, N. A. H. A., & Rahman, A. Z. A. (2021). Antifungal properties of essential oils and their compounds for application in skin fungal infections: Conventional and nonconventional approaches. *Molecules*, 26(4), 1093. <https://doi.org/10.3390/molecules26041093>.
3. Baptista, E. B., Zimmermann-Franco, D. C., Lataliza, A. A. B., & Raposo, N. R. B. (2015). Chemical composition and antifungal activity of essential oil from *Eucalyptus smithii* against dermatophytes. *Revista da Sociedade Brasileira de Medicina Tropical*, 48(6), 746–752. <https://doi.org/10.1590/0037-8682-0188-2015>.
4. Sabo, V. A., & Knezevic, P. (2019). Antimicrobial activity of *Eucalyptus camaldulensis* Dehn. plant extracts and essential oils: A review. *Industrial Crops and Products*, 132, 413–429. <https://doi.org/10.1016/j.indcrop.2019.02.051>.
5. Tyagi, A. K., & Malik, A. (2010). Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: Microscopic observations and chemical characterization of *Cymbopogon citratus*. *BMC Complementary and Alternative Medicine*, 10, 65. <https://doi.org/10.1186/1472-6882-10-65>.
6. Dao, L., Dong, Y., Song, L., & Sa, C. (2024). The fate of 1,8-cineole as a chemical penetrant: A review. *Current Drug Delivery*, 21(5), 697–708. <https://doi.org/10.2174/1567201820666230509101602>.
7. Ahmad, I., Farheen, M., Kukreti, A., Afzal, O., Akhter, M. H., Chitme, H., Visht, S., Altamimi, A. S. A., Alossaimi, M. A., Alsulami, E. R., Jaremko, M., & Emwas, A.-H. (2023). Natural oils enhance the topical delivery of ketoconazole by nanoemulgel for fungal infections. *ACS Omega*, 8(31), 28233–28248. <https://doi.org/10.1021/acsomega.3c01571>.
8. Alipanah, H., Abdollahi, A., Firoozian, S., Zarenezhad, E., Jafari, M., & Osanloo, M. (2022). Nanoemulsion and nanogel containing *Eucalyptus globulus* essential oil: Larvicidal activity and

- antibacterial properties. *Interdisciplinary Perspectives on Infectious Diseases*, 2022, 1616149. <https://doi.org/10.1155/2022/1616149>.
9. Hou, G.-W., & Huang, T. (2024). Essential oils as promising treatments for treating *Candida albicans* infections: Research progress, mechanisms, and clinical applications. *Frontiers in Pharmacology*, 15, 1400105. <https://doi.org/10.3389/fphar.2024.1400105>.
 10. Li, K., Guo, J., Liang, R., Wang, X., Chen, Q., Wang, J., Ge, X., Chen, C., Sun, J., Zhao, C., Shi, H., Qiao, R., & Zhang, X. (2025). A review on antifungal activity of plant essential oils. *Phytotherapy Research*, 39(8), 3736–3761. <https://doi.org/10.1002/ptr.70038>.
 11. European Directorate for the Quality of Medicines & HealthCare. (n.d.). *Eucalyptus oil (Eucalypti aetheroleum)* (European Pharmacopoeia, Monograph 0390).
 12. Ghaffar, A., Yameen, M., Kiran, S., Kamal, S., Jalal, F., Munir, B., Saleem, S., Rafiq, N., Ahmad, A., Saba, I., & Jabbar, A. (2015). Chemical composition and in-vitro evaluation of the antimicrobial and antioxidant activities of essential oils extracted from seven *Eucalyptus* species. *Molecules*, 20(11), 20487–20498. <https://doi.org/10.3390/molecules201119706>.
 13. Li, K., Guo, J., Liang, R., Wang, X., Chen, Q., Wang, J., Ge, X., Chen, C., Sun, J., Zhao, C., Shi, H., Qiao, R., & Zhang, X. (2025). A review on antifungal activity of plant essential oils. *Phytotherapy Research*, 39(8), 3736–3761. <https://doi.org/10.1002/ptr.70038>.
 14. Alipanah, H., Abdollahi, A., Firoozian, S., Zarenezhad, E., Jafari, M., & Osanloo, M. (2022). Nanoemulsion and nanogel containing *Eucalyptus globulus* essential oil: Larvicidal activity and antibacterial properties. *Interdisciplinary Perspectives on Infectious Diseases*, 2022, 1616149. <https://doi.org/10.1155/2022/1616149>.
 15. International Council for Harmonisation. (2023). *ICH Q2(R2): Validation of analytical procedures*. International Council for Harmonisation.
 16. International Council for Harmonisation. (2023). *ICH Q14: Analytical procedure development*. International Council for Harmonisation.
 17. Kumar, R., & Katare, O. P. (2005). Lecithin organogels as a potential phospholipid-structured system for topical drug delivery: A review. *Pharmaceutical Technology*, 6(2), E298–E310. <https://doi.org/10.1208/pt06024>.
 18. Raut, S., Azheruddin, M., Kumar, R., Singh, S., Giram, P. S., & Datta, D. (2024). Lecithin organogel: A promising carrier for the treatment of skin diseases. *ACS Omega*, 9(9), 9865–9885. <https://doi.org/10.1021/acsomega.3c05563>.
 19. Bunaciu, A. A., Ho, V. D., & Aboul-Enein, H. Y. (2025). Fourier transform infrared spectroscopy used in drug excipients compatibility studies. *Applied Spectroscopy Reviews*, 60(5), 385–403. <https://doi.org/10.1080/05704928.2024.2438747>.
 20. Singh, C., Rao, K., Yadav, N., Bansal, N., Vashist, Y., Kumari, S., & Chugh, P. (2023). A review: Drug excipient incompatibility by FTIR spectroscopy. *Current Pharmaceutical Analysis*, 19(5). <https://doi.org/10.2174/1573412919666230228102158>.
 21. A. F. Feyaerts *et al.*, “Antifungal properties of essential oils and their compounds for application in skin fungal infections: Conventional and nonconventional approaches,” *Molecules*, vol. 26, no. 4, Art. no. 1093, 2021, doi: 10.3390/molecules26041093.