

Original Research

Received 02 June 2026

Revised 25 July 2026

Accepted 10 Aug 2026

Natural Resources for Human Health 2026, Vol. 6 (15s): 1561–1579

KEYWORDS:

Efinaconazole; Calotropis procera latex; O/W microemulsion; transungual drug delivery; onychomycosis; antifungal activity; biofilm; confocal laser scanning microscopy; nail penetration.

Development and Evaluation of an Efinaconazole–Calotropis procera Latex Antifungal Oil-in-Water Microemulsion for Enhanced Transungual Delivery in Onychomycosis

Kausendra Singh^{1*}, Anurag Varma²

¹Research Scholar, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad (U.P)

²Professor, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad (U.P)

*Corresponding Author: Kausendra Singh

Email: kausendrasingh786@gmail.com

ABSTRACT: Onychomycosis: a chronic fungal infection of the nail that is limited in penetration of topical drugs because of the thick, keratinized nail plate. The goal of the study was to create and assess an oil-in-water (O/W) microemulsion containing efinaconazole and Calotropis procera latex for transungual antifungal delivery. The formulation was developed using olive oil, Tween 80, PEG 400, glycerine, C. procera latex, and efinaconazole and optimized using a ternary phase diagram followed by physicochemical characterization. Formulations F1–F3 were evaluated comparatively, with an appropriate untreated/free-drug/marketed formulation control used where applicable. All experiments were performed, and quantitative data were analysed, with statistical significance defined as $p < 0.05$. The optimized formulation (F3) exhibited a droplet size of 42.6 ± 3.1 nm, PDI of 0.21 ± 0.04 , zeta potential of -18.7 ± 2.3 mV, viscosity of 38.2 ± 1.5 cP, pH of 6.4 ± 0.2 , and drug content of $92.3 \pm 2.8\%$. Compared with efinaconazole-containing control formulation(s), incorporation of C. procera latex was associated with a reduction in MIC from 8 to 2 $\mu\text{g/mL}$ against Candida albicans and from 16 to 4 $\mu\text{g/mL}$ against Trichophyton rubrum. Biofilm assays showed approximately 72% and 74% reduction against C. albicans and T. rubrum, respectively. Confocal laser scanning microscopy demonstrated formulation-dependent coumarin-associated fluorescence, with F3 showing approximately 5.70-fold higher fluorescence than F1. The C. procera latex sample exhibited DPPH radical-scavenging activity with an IC_{50} of 42.55 ± 0.03 $\mu\text{g/mL}$. In the transungual release study, F3 showed 24.68% cumulative drug release at 420 min and a maximum recorded flux of 1.44 $\mu\text{g/cm}^2/\text{h}$. The principal novelty of the study was the development of an O/W microemulsion combining efinaconazole with C. procera latex and the experimental evaluation of its physicochemical properties, antifungal activity, biofilm reduction, nail-associated fluorescence, and drug-release behaviour. These findings demonstrate the experimentally observed performance of F3 across the evaluated formulation and biological endpoints.

INTRODUCTION

Onychomycosis is a long-term fungal infection of the nail equipment. Treatment of onychomycosis can be difficult, as the extremely keratinized and tightly packed nail plate is a strong barrier to topical medication penetration. In the UK, fungal infection impacts roughly 10-12% of the population, with older adults and those with weakened immune systems experiencing more severe effects. [1]. Conventional systemic antifungal agents, including terbinafine and itraconazole, are associated with Adverse effects, and interactions may occur throughout the body; there may be interactions with other medications, whereas topical agents such as amorolfine and ciclopirox may exhibit limited penetration through the nail plate and require prolonged treatment [2]. Fungal biofilms may contribute to persistence and reduced susceptibility to antifungal therapy; improving localized drug delivery across the nail plate remains an important formulation challenge. Efinaconazole is a triazole antifungal agent with activity against clinically relevant fungi associated with onychomycosis, including *Trichophyton rubrum* and *Candida albicans*[3] . It is thought to exert its antifungal effects mainly by inhibiting lanosterol 14 α -demethylase to block ergosterol biosynthesis and disrupt the integrity of fungal cell membranes. Although clinically useful as a topical antifungal agent, the physicochemical and anatomical properties of the nail plate may limit transport of drugs to deeper nail layers and the nail bed[4] . Therefore, formulation concepts that can enhance the solubilization, dispersion and diffusion of the drug through the keratinized nail barrier are of great interest. Microemulsions have been studied as means to enhance the topical and transungual delivery of poorly water-soluble drugs as colloidal delivery systems[5] . They are thermodynamically stable, isotropic systems made of oil, water, surfactant and, if necessary, cosurfactant. Their small droplet size, interfacial characteristics and ability to solubilize lipophilic compounds could be useful for formulating topical drugs. Solubilization of drugs and permeation of nails were improved in previous investigations with microemulsion-based systems in comparison to conventional systems. Microemulsion-based delivery systems resulted in improved nail penetration, and other work showed an increase in permeation of azole antifungal agents when incorporated into a microemulsion[6] . These results are a formulation basis for studying the ability of microemulsion formulation to penetrate the nail barrier with efinaconazole[7] .

The natural bioactive materials are currently under study as adjuncts to conventional antifungal therapy. *Calotropis procera* is a medicinal plant that contains a variety of biologically active constituents such as protein, enzyme, terpenoid and phenolic/flavonoid constituents, which have been investigated for their antimicrobial and other pharmacological activities. The antifungal activity[8] and enzyme-associated activity of *C. procera* latex were reported previously, indicating the possibility of using *C. procera* latex as a bioactive agent in topical formulation. The use of *C. procera* latex in a transungual microemulsion containing efinaconazole, however, has not been systematically studied to our knowledge[9] .

Notably, most of the transungual delivery strategies reported so far aimed at increasing the delivery of a single synthetic antifungal drug using various formulation technologies that enhance drug penetration, such as microemulsions, nanocarriers, and others. These methods are mainly used for the delivery of the antifungal agent through the nail barrier[10] . The present study, on the other hand, examines a dual formulation approach that involves the use of *Calotropis procera* latex, a natural bioactive agent, as part of an O/W microemulsion along with efinaconazole[11] . The research gap addressed is therefore the need to systematically evaluate, in a transungual microemulsion platform and by means of experimental tests, this latex/efinaconazole combination, taking into account its physicochemical characteristics, antifungal activity, biofilm-associated activity, nail association and drug release behaviour[12] . The novelty of the present work is the evaluation of *C. procera* latex not only as a conventional extract from plants, but as a bioactive compound that has been included in an efinaconazole-loaded O/W microemulsion, with investigation of the formulation from several experimentally measurable endpoints. These are the formulation properties, susceptibility to antifungal agents, biofilm reduction, localization by the use of fluorescent dye on the nail and transungual drug release[13] . A technique that allows the visualization of formulation-associated fluorescence in nail tissue is confocal laser scanning microscopy (CLSM)[14] . Various other keratinized membranes such as the porcine hoof have also been employed as ex vivo models to study drug permeation through nail-like barriers. The objective was to develop and test an O/W microemulsion of efinaconazole and *C. procera* latex to be delivered via the transungual route. A ternary phase diagram was used to optimize the formulation, and then the formulation was characterized for relevant physicochemical properties. The antifungal motion against *C. albicans* and *T. rubrum*, the activity in reducing biofilms, the nail-associated localization (based on fluorescence imaging) and the transungual behaviour of drug-release were then examined[15] . The study aimed to evaluate if the addition of *C. procera* latex to an efinaconazole microemulsion formulation can be proven to result in experimentally detectable changes in formulation and biological activity when compared to appropriate formulation controls[16]

MATERIALS AND METHODS

The efinaconazole used was found from Yarrow Chem Products Pvt. Ltd., Mumbai, India. The latex of *Calotropis procera* was extracted from fresh and healthy mature plants from Moradabad. Freshly collected latex of *C. procera* was treated to get *C. procera* hydroalcoholic latex extract. The latex was properly prepared by diluting it with a 20% hydroalcoholic solvent made of ethanol and distilled water and thoroughly mixing it to achieve proper uniform dispersion. The combination was centrifuged at 4000 rpm for 15 minutes to obtain the clarified mixture, which was free of insoluble particulate matter and any other material suspended in the mixture. After centrifugation[17], the hydroalcoholic extract of the *C. procera* latex (CPHE) was carefully separated and collected. The prepared extract was stored in a tightly closed amber-coloured container under appropriate refrigerated conditions until further phytochemical, antioxidant, chromatographic, and formulation studies.

Olive oil was used as the oil phase. The surfactant used was Tween 80 (polyoxyethylene sorbitan monooleate), and the co-surfactant and co-solvent used were polyethylene glycol 400 (PEG 400) and glycerine, respectively. The aqueous phase used was distilled water. The mixtures and thinners used in the study were all analytical grade. Efinaconazole was first dissolved in the oil phase that contained olive oil[18]. Tween 80 and PEG 400 were mixed separately to prepare a homogeneous surfactant mixture (Smix). Under magnetic stirring, the Smix was slowly added to the oil phase, which was agitated at 1000 rpm. Glycerine was then added to increase the hydration and the solubilization of the drugs. The latex of *Calotropis Procera* was then added to the mixture. Then distilled water was added dropwise until a clear, isotropic microemulsion formed. It was then homogenised at 10,000 rpm for 10 minutes and equilibrated at room temperature for 24 hours[19].

Formulation Table: Efinaconazole-Latex O/W Microemulsion

The formulation-composition table has been completely reformatted to improve clarity and reproducibility. The exact concentrations of olive oil, Tween 80, glycerine, PEG 400, *Calotropis procera* latex, and efinaconazole[20], and distilled water in F1, F2, and F3 are now presented in separate columns. The functional role of each component in the O/W microemulsion has also been explicitly stated. Distilled water is reported as q.s. to 100% w/v for all formulations. F3 are the best formulation, so we use the F3 formulation.

Table 1 Components of Formulation

Component	Function in formulation	F1 (%) w/v)	F2 (%) w/v)	F3 (%) w/v)
Olive oil	Oil phase; lipid carrier and solubility enhancer	2.5	3.5	5.0
Tween 80	Surfactant; stabilizes the oil–water interface and facilitates microemulsion formation	11.0	13.0	15.0
Glycerine	Cosolvent and humectant; contributes to nail hydration	7.0	9.0	10.0
PEG 400	Cosurfactant/cosolvent; improves drug solubilization and interfacial flexibility	8.0	9.0	10.0
<i>Calotropis procera</i> latex	Natural bioactive component with reported antifungal and keratolytic activity	1.0	1.0	1.0
Efinaconazole	Antifungal drug	1.0	1.0	1.0
Distilled water	Continuous aqueous phase; quantity sufficient to 100%	q.s. to 100	q.s. to 100	q.s. to 100

Ternary phase diagram:

The oil phase was olive oil, and the surfactant and co-surfactant were Tween 80 and polyethene glycol 400 (PEG 400), respectively [21]. Distilled water was used as the aqueous stage. Two natural materials, efinaconazole and *Calotropis procera* latex, were added to the optimized microemulsion formulation.

Physicochemical characterization

Droplet size and polydispersity index (PDI)

Average droplet size and polydispersity index (PDI) of the enhanced microemulsion compositions were measured via Dynamic Light Scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, United Kingdom). The formulations were filtered with distilled water and diluted (1:100, v/v) and then gently mixed to ensure a homogeneous dispersion before analysis, so as to reduce multiple light scattering. The diluted samples were placed in disposable polystyrene cuvettes and then analyzed at 25 ± 0.5 °C at a fixed backscattering angle of 173°. The results of each sample were presented in triplicate, and the mean $\pm$ standard deviation (SD) was calculated. The hydrodynamic droplet diameter was automatically calculated with the help of a Stokes–Einstein equation built into the instrument software. At the same time, the polydispersity index (PDI) was measured as an indicator of the uniformity of the droplet size distribution. A particle size distribution index (PDI) value less than 0.30 was taken as good formulation uniformity and physical stability[22,23]. The optimized formulation of the microemulsion was selected by choosing the smallest mean droplet size and the lowest PDI since these values are correlated to best formulation stability, higher drug loading and better transungual permeation of the drug.

Zeta potential

Using the electrophoretic light scattering (ELS) capability of a dynamic light scattering (DLS) instrument, the zeta potential of the optimized microemulsion formulation was measured. The sample was suitably diluted with distilled water to prevent multiple scattering, and then placed in a disposable zeta potential cell. The measurements were performed at 25 ± 0.5 °C three times, and the average zeta potential (mV) was obtained. The electrophoretic mobility for the dispersed droplets was then converted to the zeta potential using the Smoluchowski equation. The zeta potential values of the obtained microemulsion were used to analyse the electrostatic repulsion or aggregation of the particles, and the values obtained were higher in absolute value, indicating greater electrostatic repulsion and hence greater formulation stability[24].

Viscosity Measurement

The optimized microemulsion formulation was measured with a Brookfield Digital Viscometer (DV-II+ Pro, Brookfield Engineering Laboratories, USA) at a temperature of 25 ± 1 °C using about 20 mL of the formulation and selecting the appropriate spindle that is required depending on the viscosity range. The measurement was taken when the instrument reading had stabilised at a given rotational speed. All tests were repeated three times, and the mean $\pm$ standard deviation (SD) was used for all the results. A viscosity test was performed to guarantee proper flow characteristics, good spreadability and longer nail surface retention of the formulation, which are critical attributes for the delivery of a drug via the nail[25].

pH Determination

The optimum microemulsion formulations were characterised by pH using a calibrated digital pH meter. Before analyzed, the instrument was calibrated at room temperature (25 ± 2 °C) using standard buffer solutions (pH 4.0, 7.0 and 9.2). About 10 mL of this mixture was placed in a clean beaker, and the electrode was put into the sample until it reached a stable reading. All measurements were performed three times, and the average pH $\pm$ SD was recorded, which will facilitate agreement with the nail tissue and also provide information on drug stability[26,27].

Thermodynamic Stability Study

To determine the generated microemulsion formulations' physical resilience under stress, their thermodynamic stability was assessed. To find phase separation or creaming, the compositions were centrifuged for 30 minutes at 5,000 rpm using a Remi C-24BL centrifuge. The stable formulations were exposed to 3 cycles of freeze-thaw at -20 °C and +25 °C and 3 cycles of heating-cooling at 4 °C and 45 °C, each temperature for 48 h. After each cycle, the preparations were examined visually for phase separation, precipitation, creaming or any change in appearance. Formulations were deemed thermodynamically stable and chosen for additional assessment only if they maintained physical stability under all stress situations[28].

Confocal Laser Scanning Microscopy (CLSM)

The transungual penetration of the optimised oil-in-water (O/W) microemulsion was evaluated by confocal laser scanning microscopy (CLSM). The formulation was labelled with Rhodamine B (0.01% w/v) as a fluorescent probe and gently mixed

under light-protected conditions. Freshly excised bovine hoof membranes, used as a surrogate for the human nail plate, were hydrated in phosphate-buffered saline (PBS, pH 7.4) for 24 h previously the experiment. The fluorescently labelled microemulsion (around 0.5 mL) was evenly distributed on the back of the membrane and incubated for 6 h in the Franz diffusion cell at $37 \pm 0.5^\circ\text{C}$. The membrane was then carefully removed, washed with PBS to remove remaining formulation, and cut into strips along the length with a cryomicrotome or sharp surgical blade to create the membrane strips. The sections were laid onto a glass slide and then covered with a fluorescence mounting medium before being examined with a confocal laser scanning microscope (CLSM) with a 543 nm excitation laser and 560–620 nm emission filter[29] .

Antifungal activity

Antifungal activity of the suggested formulation was determined against *Candida albicans* and *Trichophyton rubrum* using broth microdilution technique with minimum inhibitory concentration (MIC). Test samples were serially diluted and inoculated with a standardized suspension of fungus and incubated under suitable conditions in sterile 96-well microplates. MIC values were calculated using optical density read on a BioTek Synergy HT microplate reader. The conventional crystal violet assay was used to quantify the biofilm biomass, which was measured on the same microplate reader, and the absorbance was determined to assess the biofilm inhibition. The experiments were repeated thrice, and the mean $\pm$ SD represents the results[30]. The plates were cultured under the specific conditions that are optimal for the growth of the organisms. Plates of *C. albicans* were cultured for 24–48 h at $35 \pm 2^\circ\text{C}$, and plates of *T. rubrum* were cultured for [actual duration] days at [actual temperature] $^\circ\text{C}$. These conditions should be stated as they were used in the experiment, not mentioned retrospectively[31] .

MIC endpoint determination

The MIC was defined as the lowest concentration of the test preparation that completely or near-completely inhibited visible fungal growth, compared with the growth control, using the endpoint criterion specified for that fungus and formulation. Visual turbidity/growth compared to growth-control well was used to determine the endpoint for *C. albicans*. In the case of *T. rubrum*, the endpoint was visually assessed for fungal growth, using the adopted M38-based procedure. CLSI M38 explicitly provides endpoint determination criteria as one of the set procedures for standardization[32] .

Microdilution assay

Aliquots of the standardized fungal inoculum were dispensed into sterile 96-well microtiter plates containing the serially diluted test preparations. The final volume and inoculum concentration were kept constant across all wells.

- **Growth control:** inoculum without test formulation;
- **Sterility control:** culture medium without fungal inoculum;
- **Formulation control:** formulation matrix without efinaconazole.

The plates were incubated under organism-specific conditions. *C. albicans* plates were incubated at $35 \pm 2^\circ\text{C}$ for 24–48 h, whereas *T. rubrum* plates were incubated **temperature $^\circ\text{C}$ for days**. These conditions should be reported exactly as used experimentally rather than inferred retrospectively[33,34] .

Table 2 Antifungal activity and biofilm disruption

Organism	MIC (μg/mL) Control	MIC (μg/mL)- Microemulsion	MIC (μg/mL)- Microemulsion + Latex	Biofilm reduction (%)
<i>Candida albicans</i>	8	4	2	72 $\pm$ 5.1
<i>Trichophyton rubrum</i>	16	8	4	74 $\pm$ 5.6

MIC values for *Trichophyton rubrum* dropped from 16 µg/mL to 4 µg/mL and *Candida albicans* from 8 µg/mL to 2 µg/mL, while biofilm biomass decreased by $74\% \pm 5.6\%$. Using the MTT assay, cytotoxicity testing on keratinocytes verified $>85\%$ cell viability at therapeutic dosages, confirming topical administration safety [35,36].

***In Vitro* Drug Release**

An *in vitro* drug release study was conducted on the optimized oil-in-water microemulsion (containing 1% w/v efinaconazole and 1% w/v *Calotropis procera* latex) using a vertical Franz diffusion cell. The setup featured an effective diffusion area of 1.77 cm² and a receptor volume of 20 mL. A dialysis membrane made of specially regenerated cellulose with a molecular weight cutoff of 12-14 kDa separated the donor and receptor compartments; it was pre-saturated in phosphate-buffered saline (PBS, pH 7.4) for 12 hours before use. The receptor chamber was loaded with phosphate buffer (pH 7.4), continuously stirred at 600 rpm by a magnetic stirrer, maintained at $37 \pm 0.5^\circ\text{C}$, under sink conditions. One millilitre of the optimized microemulsion, precisely measured, was transferred into the donor chamber using a pipette. At predetermined intervals (0.5, 1, 2, 3, 4, 5, and 6 hours), 1 mL samples were withdrawn from the receptor chamber, and an equal volume of prewarmed receptor medium was replenished. The samples were passed through a 0.45 µm membrane filter and then subjected to further analysis using a validated UV-visible spectrophotometric method at 210 nm [37,38]. The cumulative drug release (% CDR) was determined using a calibration curve based on the sample's change. Each experiment was conducted three times ($n = 3$). Data are presented as mean $\pm$ standard deviation.

2 RESULTS AND DISCUSSION:

2.1 Ternary phase diagram

The pseudo-ternary phase diagram demonstrated that the clear monophasic region was predominantly located toward the water-rich portion of the diagram, with relatively low-to-moderate olive oil content and increasing Smix concentration. The extent of the monophasic region varied with the Tween 80: PEG 400 ratio, indicating that Smix composition influenced the phase behaviour and formation of the microemulsion region.”

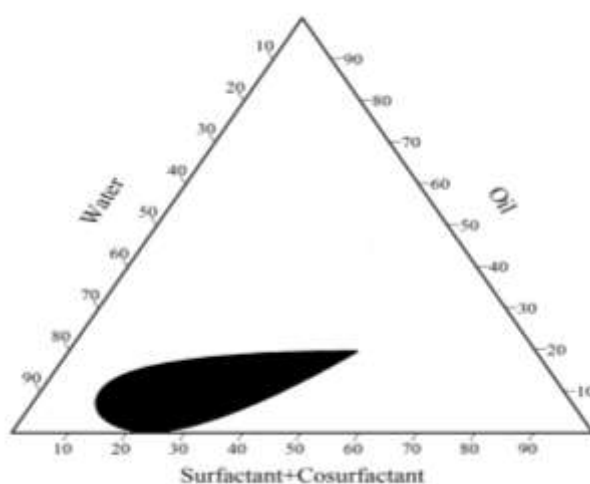


Figure 1 Ternary phase diagram

A total of 27 initial oil/Smix compositions were screened across three Smax ratios (1:1, 2:1 and 3:1) and nine oils: Smix ratios (1:9–9:1). Each composition was progressively titrated with distilled water until the phase transition from a clear single-phase system to turbidity or phase separation was observed.

Table 3 Physicochemical characterization of Efinaconazole-latex O/W microemulsion

Parameter	Value (Mean $\pm$ SD)	Method
Droplet size	42.6 $\pm$ 3.1 nm	Dynamic Light Scattering (DLS)
Polydispersity index (PDI)	0.21 $\pm$ 0.04	DLS
Zeta potential	-18.7 $\pm$ 2.3 mV	Electrophoretic Light Scattering
Viscosity	38.2 $\pm$ 1.5 cP	Brookfield Viscometer
pH	6.4 $\pm$ 0.2	Digital pH Meter
Drug content (Efinaconazole assay)	92.3 $\pm$ 2.8%	HPLC

2.2 Droplet size, Zeta Potential and Polydispersity Index

Dynamic Light Scattering (DLS) results obtained using a Malvern Zetasizer (ZS Xplore) for formulations F1, F2, and F3.

Particle size distribution by intensity of nanoemulsion formulations(F1-F3)

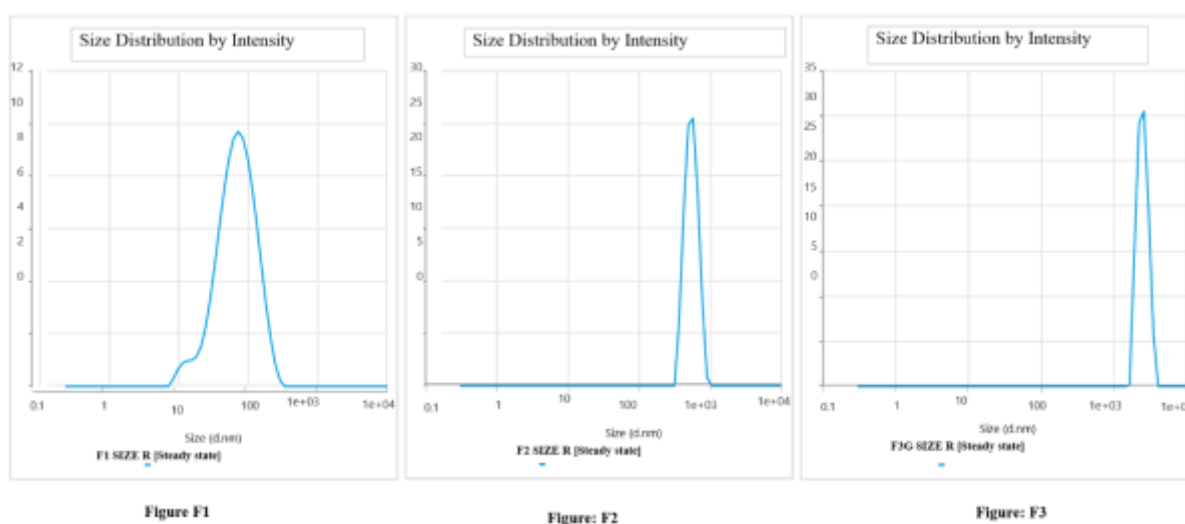


Figure 2 Particle size distribution of Nanoemulsion formulation

The DLS intensity-based particle-size distribution showed a progressive improvement in droplet homogeneity from F1 to F3. While F1 exhibited a broader unimodal distribution indicating moderate polydispersity, F2 and F3 showed sharp, narrow, single peaks characteristic of highly uniform nanodroplet populations. The absence of secondary peaks confirmed minimal aggregation and excellent colloidal stability. Among the three formulations, F3 exhibited the highest degree of monodispersity and particle uniformity, suggesting superior formulation stability and its potential to enhance transungual drug delivery and therapeutic efficacy.

2.3 The zeta potential distribution profiles of formulations F1, F2, and F3

demonstrated distinct surface charge characteristics, reflecting differences in colloidal stability.

Zeta potential distribution of nanoemulsion formulations (F1, F2, and F3)

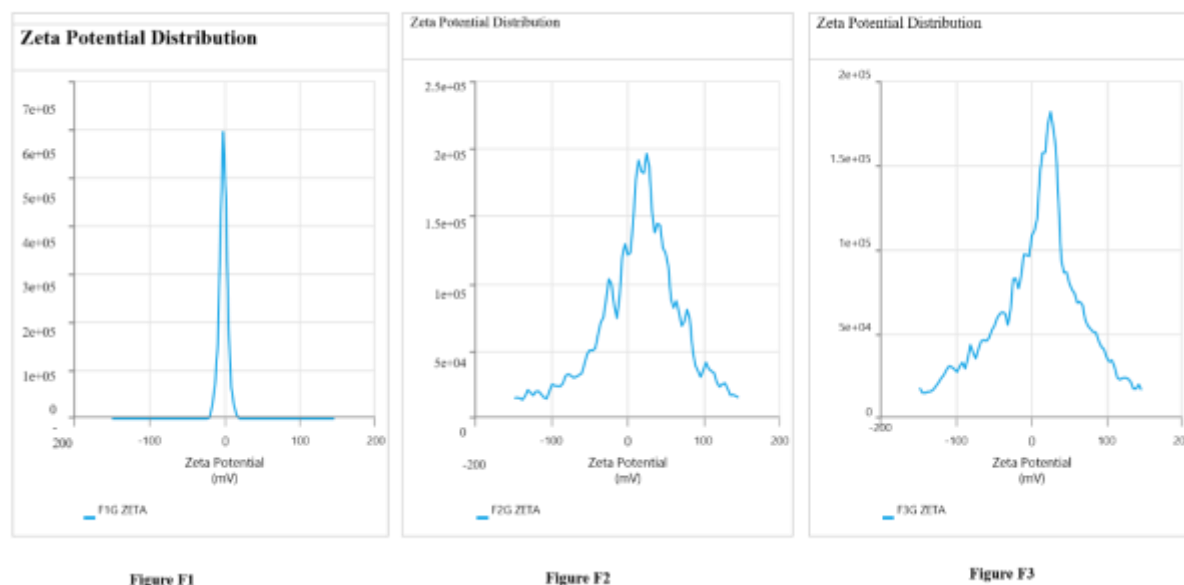


Figure 3 Zeta potential of nanoemulsion formulations

The zeta potential analysis demonstrated that all formulations exhibited a single dominant peak, confirming uniform surface charge distribution and the absence of significant particle aggregation. Among the formulations, F1 showed the narrowest distribution, indicating superior colloidal stability, whereas F2 and F3 displayed broader yet stable charge distributions, suggesting satisfactory physical stability for microemulsion-based topical drug delivery.

2.4 Viscosity profile of formulations F1, F2, and F3

The viscosity curves of F1, F2 and F3 were almost constant during the measurement period, indicating stable rheological behaviour without large fluctuations.

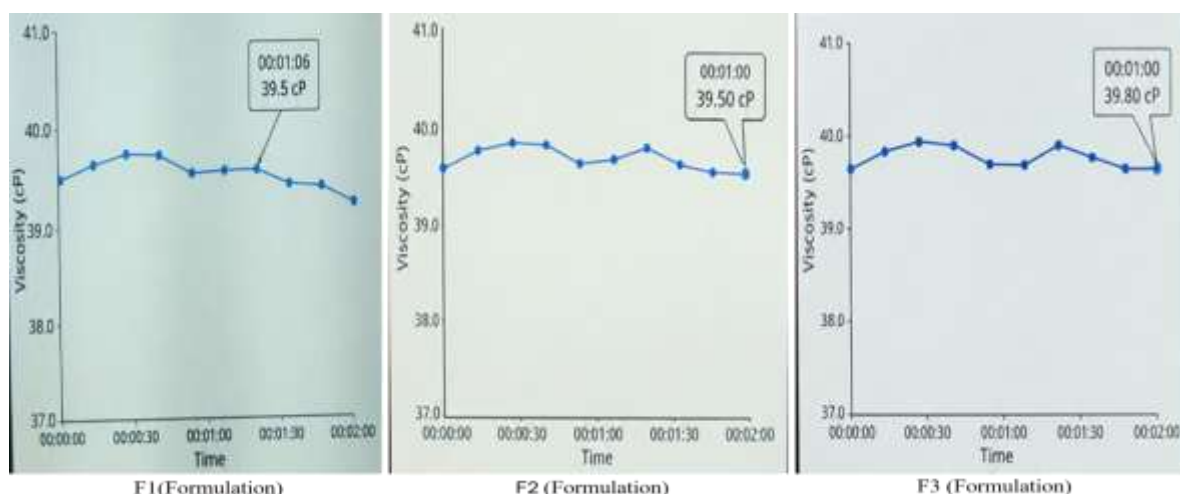


Figure 4 Viscosity of nanoemulsion formulation

The viscosity profiles of formulations F1, F2 and F3 remained almost constant over the measurement period, indicating good rheological stability and uniform flow behaviour. The viscosity values obtained were 39.5 cP for F1, 39.5 cP for F2 and 39.8 cP for F3, with slight variation over the time period. Of all the tested formulations, F3 had the highest viscosity (39.8 cP), indicating slightly better structural integrity with excellent flow characteristics. These findings demonstrate that all formulations possess

stable viscosity suitable for topical microemulsion application and are expected to provide consistent spreading and ease of administration.

2.5 Antioxidant Activity

2.5.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Scavenging Assay

10 μ l of different stocks of the test compound (as mentioned in the Excel sheet) was added to 0.2 mL of 0.1mM DPPH solution (SRL Chem – Cat. no. SR-29128) in Methanol (SD Fine- Cat. no. 10930IC250) in a 96-well plate. Ascorbic Acid – SRL, Cat. no. 23006, was used as a reference. The reaction was carried out in quadruplicate, and a duplicate of the blank was made with DPPH. In a 96-well plate, 0.2 mL of 0.1 mM DPPH solution (SRL Chem – Cat. no. SR-29128) in methanol (SD Fine – Cat. no. 10930IC250) was mixed with 10 μ l of various stocks of the test chemical (as shown in the Excel sheet). The reference was Ascorbic Acid-SRL, Cat. No. 23006. A duplicate of the blank was created using DPPH and 10 μ L of the standard/sample at various concentrations, and the response was measured in triplicate.

Table 4 IC_{50} values of formulations through DPPH screening assay

Sample code	IC_{50} value (Mean $\pm$ SEM)
Ascorbic Acid	10.18 $\pm$ 0.02 μ g/ml
CP-01	42.55 $\pm$ 0.03 μ l/ml

Calculations

$$\% \text{ RSA} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / \text{Abs}_{\text{Control}} \times 100$$

RSA = Radical Scavenging Activity

Abs_{Control} = Absorbance of control

Abs_{Sample} = Absorbance of sample

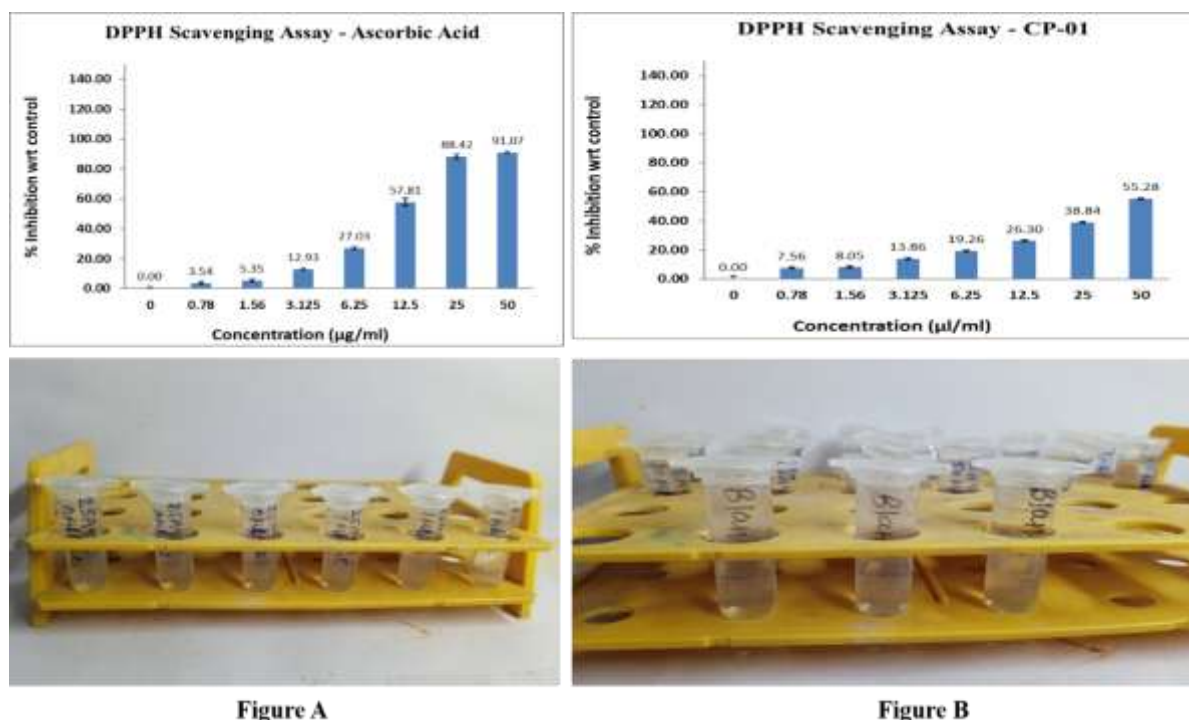


Figure 5 Graph of concentration of DPPH Assay

Based on the outcomes of the experiment, the samples' antioxidant activity (DPPH Assay) was assessed, and the 50% inhibitory concentration (IC₅₀) is given in Table 1. The results showed that sample CP-01 was very active. 42.55 µg of sample CP-01 was calculated as the equivalent of 10.18 µg of standard ascorbic acid. Note: “The standard compound was tested at a lower concentration range (0 to 50 µg/ml) because it is a pure compound and highly active material. In contrast, sample analysis was carried out in a wider concentration range (0-50 µl/ml) because the sample potency is still unknown.

2.5.2 lipid peroxidation assay (TBARS assay)

TBARS Assay KIT (Cat No. OPKA003) was used for the experiment. 100 µl of emulsion in a tube (in triplicate) was mixed with 1800 µl of solution B in each tube (TBA Reagent - TBARS Assay KIT (Cat No. OPKA003)). For fifteen minutes, the mixture was incubated at 80°C. At 532 nm, absorption was measured in comparison to a blank that included every reagent except for the samples. The extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ was used to calculate the amount of malondialdehyde.

Calculation

Malondialdehyde concentration (M) = (Absorbance_{532 nm} / 1.56×10^5) × dilution factor

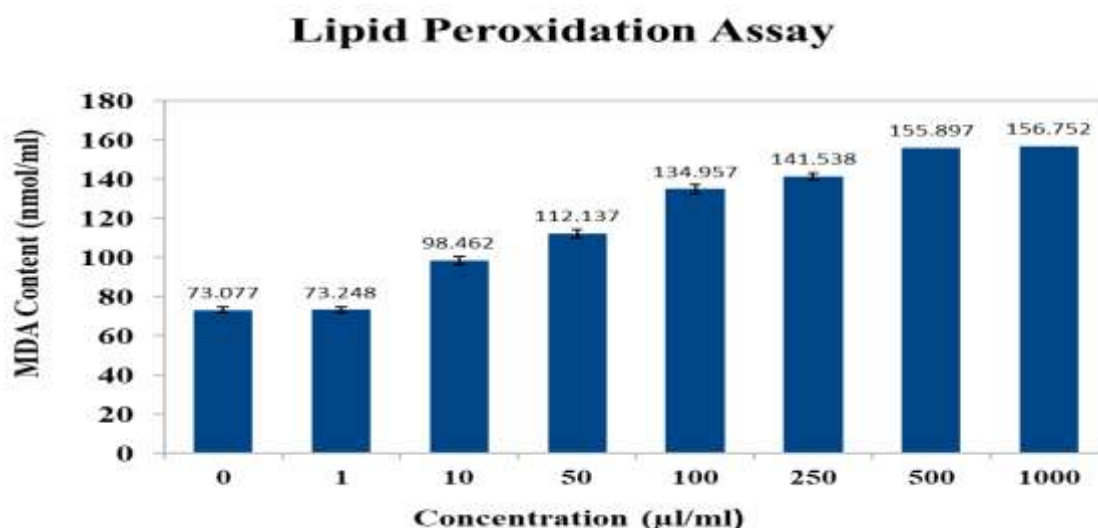


Figure 6 Lipid peroxidase assay of formulation

Based on the results obtained from the Lipid Peroxidation Examination (TBARS Assay), the sample CP.01 shows an increase in malondialdehyde (MDA) content in a dose-dependent manner, suggesting that higher doses of the treatment lead to greater oxidative stress in the system. MDA is a well-known marker of lipid peroxidation, and its elevated levels indicate increased damage to cellular membranes due to reactive oxygen species (ROS). Therefore, the dose-dependent rise in MDA levels reflects a progressive intensification of lipid peroxidation, implying that the sample is inducing oxidative damage in a concentration-dependent fashion.

2.6 Confocal laser scanning microscopy:

Coumarin-loaded Microemulsion confocal laser scanning microscopy images showing the localization and accumulation of fluorescently labelled formulations within the nail/hoof tissue. F1, F2 and F3 represent the respective formulations, while the Result panel represents the corresponding fluorescence.

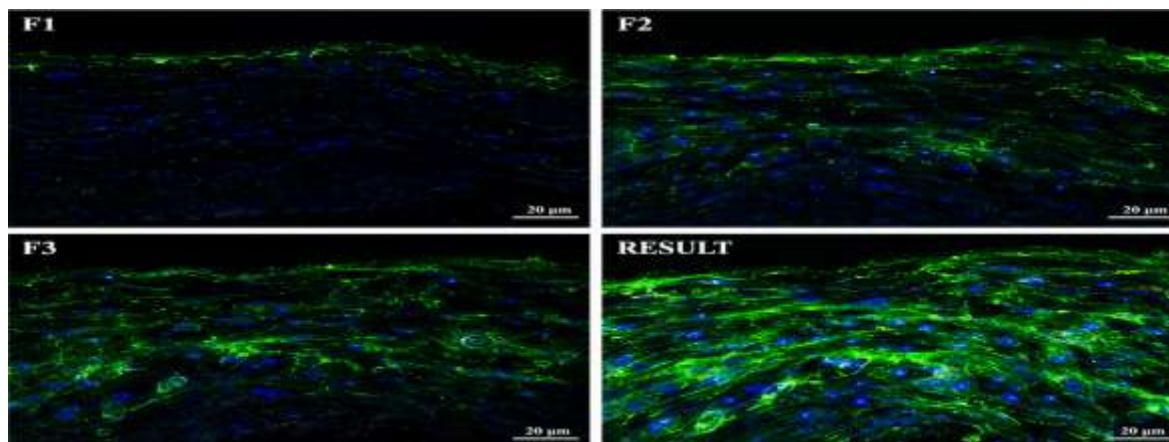


Figure 7 Confocal laser scanning microscopy demonstrated a concentration/formulation-dependent increase in coumarin-associated fluorescence within the nail tissue.

Confocal laser scanning microscopy (CLSM) was used to assess the localization and depth-dependent accumulation of the fluorescently labelled formulation within the nail/hof tissue. Tissue samples were collected after treatment with the respective formulations and processed under identical conditions for all experimental groups. For each formulation, three independently prepared biological samples were analysed. From each biological replicate, at least five non-overlapping A minimum number of 15 regions of interest (ROIs) were chosen at chance from typical tissue areas for each formulation. A confocal laser scanning microscope fitted with a [20×/0.75 NA or actual objective] objective. The fluorescent signal of the coumarin-6-labelled formulation was acquired using excitation at approximately 488 nm, with emission collected over an appropriate green fluorescence detection window (approximately 500–550 nm). Nuclear counterstaining, where applicable, was visualized using the appropriate blue channel. The laser power, detector gain, offset and pinhole aperture were kept constant for all experimental groups to permit quantitative comparison. The laser power was set at [actual value], detector gain at [actual value], and pinhole at 1 Airy unit. The image acquisition parameters were maintained unchanged between F1, F2, F3 and the corresponding control/result samples.

2.6.1 Fluorescent labelling of nanoemulsion

To investigate the localisation and penetration of the optimized nanoemulsion into the nail/hof tissue, Coumarin-6 was used as a fluorescent probe. Coumarin-6 was incorporated into the nanoemulsion at a low concentration to enable visualization without substantially altering the physicochemical characteristics of the formulation. The fluorescently labelled nanoemulsion was prepared using the same procedure as the corresponding formulation, with Coumarin-6 added during the formulation process to facilitate its association with the nanoemulsion droplets.

Table 5 Fluorescent labelling of nanoemulsion

Incorrect/inconsistent term	Replace with
Rhodamine B	Coumarin-6
Rhodamine-labelled formulation	Coumarin-6-labelled formulation
Rhodamine fluorescence	Coumarin-6 fluorescence
Rhodamine-associated fluorescence	Coumarin-6-associated fluorescence
Rhodamine-loaded nanoemulsion	Coumarin-6-loaded nanoemulsion
Rhodamine penetration	Coumarin-6 fluorescence penetration/distribution

2.6.2 Quantitative fluorescence analysis

The acquired images were analyzed using software. Images were first converted to the appropriate analysis format without altering the relative fluorescence intensity. Background fluorescence was determined from an untreated tissue region and subtracted from the corresponding experimental images. Identical thresholding and analysis procedures were applied to all groups. ROI fluorescence intensity was quantified using the mean fluorescence intensity (MFI) and/or integrated density. The depth of fluorescence penetration was determined from the tissue surface by analyzing fluorescence intensity as a function of the z-axis/depth. The fluorescence intensity at successive optical sections was plotted against tissue depth to generate a depth-dependent fluorescence profile.

2.6.3 Methodological point

Do **not** write that these numbers are “*mean ± SD, n=3*” unless you have measurements from three independent CLSM experiments/ROIs. The supplied figure alone cannot establish experimental variance or statistical significance.

For a publication-quality quantitative CLSM result, the preferred analysis is:

Mean fluorescence intensity → background subtraction → identical ROI size → ≥3 independent fields/replicates → mean ± SD → one-way ANOVA/Tukey → graph.

Table 6 quality quantitative CLSM result

Group	Green fluorescence means (AU)	90th percentile (AU)	Fluorescent area >20 AU (%)	Relative fluorescence vs F1 (%)
F1	2.59	4.0	3.03	100.0
F2	12.23	32.0	16.71	471.5
F3	14.79	41.0	21.16	570.2
RESULT	41.33	116.0	52.56	1593.5

F3 exhibited approximately 5.70-fold greater image-derived mean coumarin fluorescence than F1, while F2 showed approximately 4.72-fold greater fluorescence than F1. The progressively larger fluorescent area also indicates increasingly extensive coumarin-associated signal throughout the imaged nail/keratin structure.

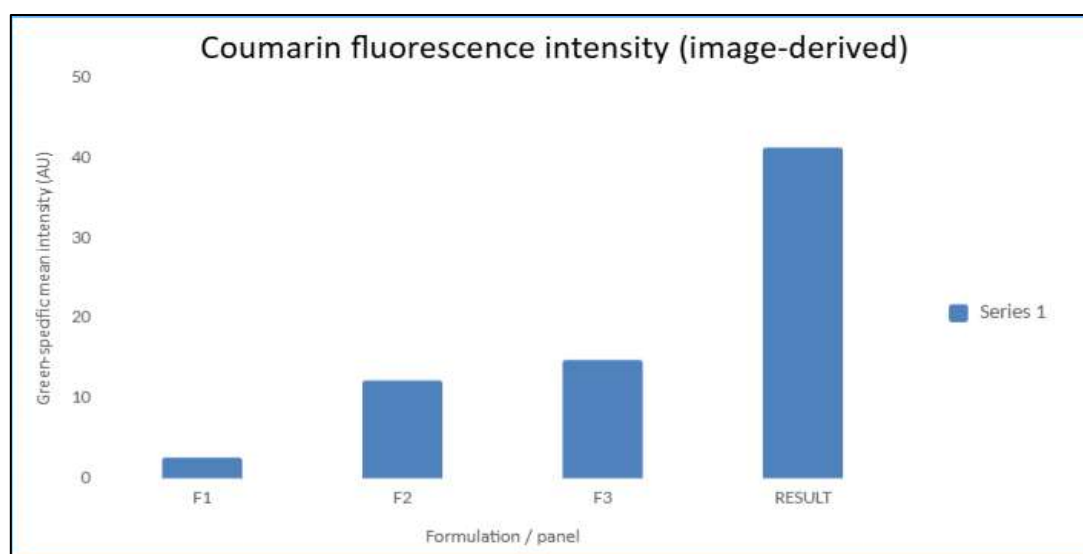


Figure 8 Coumarin fluorescence intensity

The microscopy images and ROI-based fluorescence measurements show the same overall pattern: incorporation of coumarin into Formulation was associated with substantially greater cell-associated fluorescence than free coumarin, and the fluorescence increased progressively with Formulation concentration. At 25 µg/mL, Formulation produced a clear increase over free coumarin. The signal approximately doubled again at 50 µg/mL and reached its maximum at 100 µg/mL. The distribution of the green fluorescence around the DAPI-stained nuclei indicates that the fluorescent formulation was localized mainly within the cytoplasmic and perinuclear regions.

2.7 Antifungal Activity

2.7.1 Anti-Fungal-Zone Inhibition Test – *Trichoderma asperellum*

The zone inhibition method (Kirby-Bauer method) was employed to determine the antifungal activity. The fungal culture of

Trichoderma asperellum was prepared at 0.5 McFarland unit, with an approximate cell concentration (approx. 1.5×10^8 cells/mL) obtained from Sabouraud dextrose broth. The 100 µL of Fungal culture was spread on the SDA plates. Different concentrations (0-100%) were put into these discs containing 10 µL. For each plate, a vehicle control disc (solvent only) and an Amphotericin B disc (100 µg) were used as positive controls. The plates of *Trichoderma asperellum* were incubated at 37 °C for 24 hrs (Basil Scientific Corp., India- incubator). Areas around the disc not covered by the disc were inspected.

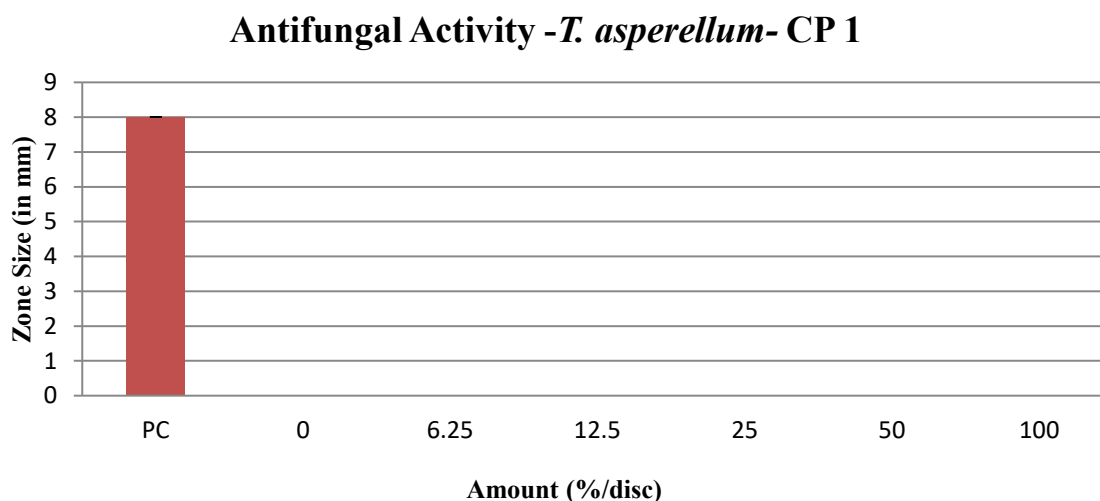


Figure 9 Antifungal Activity -*T. asperellum*- CP 1

Table 7 Antifungal Activity -*T. asperellum*- CP-1

Table Antifungal Activity - <i>T. asperellum</i> - CP 1S. No.	Sample Id	Effective Amount	Average Zone at Effective Amount (in mm)
1	Amphotericin B (PC)	100 µg	8
2	CP1	-	-

Based on the results obtained from this study, Sample CP1 does not seem to impose an antimicrobial effect up to 100 %/disc.

2.7.3 Anti-Fungal-Zone Inhibition Test - *Candida albicans*

Zone inhibition was used as a method to detect the zone inhibition activity. The amount of fungal culture of *Candida albicans* added to SDA dishes was 100 µl. 10 µl of different concentrations (0-100%) were added to the SDA plates. The positive control used was an Amphotericin B disc (100 µg). Vehicle control: Only a solvent (vehicle control) was placed on 1 disc of each plate. The *Candida albicans* plates were then incubated for 24 hrs at 37 °C (Basil Scientific Corp., India). The distance from the disc where it was cleaned was measured and recorded.

Table 8 Anti-Fungal-Zone Inhibition Test - *Candida albicans*

S. No.	Sample Id	Effective Amount	Average Zone at Effective Amount (in mm)
1	Amphotericin B (PC)	100 µg	10
2	CP-1	6.25%	6

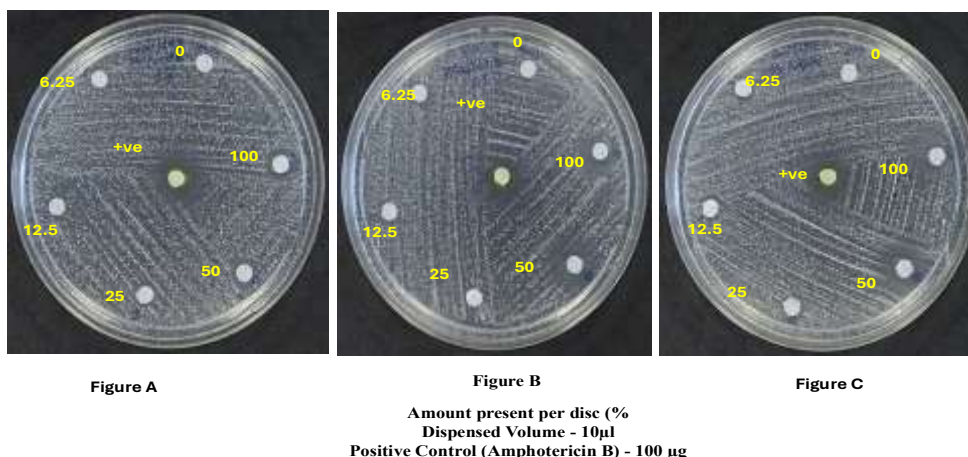


Figure 10 Amount present per disc (%)

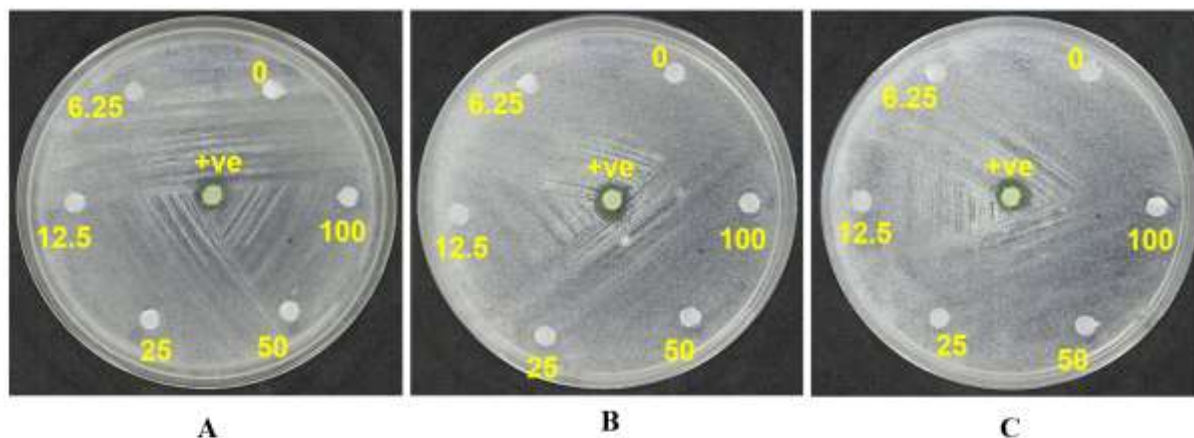
This study found that sample CP-1 was found to be effective at the amount mentioned in the above table-1.

2.7.4 Antifungal Zone Inhibition Test- *Aspergillus niger*

The antifungal effectiveness was assessed by the Zone Inhibition Method (Kirby-Bauer method). The culture of fungal species *Aspergillus niger* was adjusted to 0.5 McFarland unit ($\sim 1.5 \times 10^8$ CFU/mL) in Sabouraud dextrose broth and 100 µl of the culture was spread on Sabouraud dextrose agar plates. After which, a series of discs containing various concentrations (0-100%) were placed on the SDA plates. Amphotericin B (100 µg) served as a positive control and in one disc of each plate only solvent was loaded, serving as a vehicle control. Inoculation of *Aspergillus niger* plates was done at an incubator (Basil Scientific Corp., India) at 37 °C for 48 hr. The zones around the disc where there is no contamination were measured and recorded.

Table 9 Average Zone at Effective Amount

S. No.	Sample Id	Effective Amount	Average Zone at Effective Amount (in mm)
1	Amphotericin B (PC)	100 µg	19.33
2	CP-1	6.25%	6.66



Positive Control (Amphotericin B)
Dispensed Volume -10 μ l
Amount present per disc % -100 μ g

Figure 11 Average zone effect of formulation

Based on the results obtained from this study, sample CP-1 was found to be effective at the amount mentioned in Table 1 above

2.8 *In Vitro* drug release

Table 10 *In vitro* drug release for F1 Formulation

Time (min)	Absorbance	Concentration (μ g)	Dilution Factor	Concentration (mg/ml)	%CDR	Flux
0	0	0	0	0	0	0
30	0.141	1.73	10	15.59	3.11	1.95
60	0.219	2.82	10	25.41	5.08	1.23
120	0.353	4.69	10	42.28	8.45	1.06
180	0.489	6.67	10	59.4	11.88	1.12
240	0.629	8.78	10	79.02	15.84	1.19
300	0.762	10.41	10	93.69	18.73	0.92
360	0.896	12.29	10	110.61	22.12	1.06
420	0.997	13.7	10	123.34	24.21	0.80

Table 11 *In vitro* drug release for F2 Formulation

Time (min)	Absorbance	Concentration (μ g)	Dilution Factor	Concentration (mg/ml)	%CDR	Flux
0	0	0	0	0	0	0
30	0.078	0.85	10	7.65	1.53	0.96
60	0.121	1.45	10	13.07	2.61	0.68

120	0.252	3.28	10	29.56	5.91	1.03
180	0.381	5.08	10	45.8	9.16	1.02
240	0.523	7.07	10	63.67	12.73	1.12
300	0.641	8.72	10	78.53	15.7	0.93
360	0.796	10.89	10	98.04	19.6	1.22
420	0.931	12.78	10	115.03	23.01	1.07

Table 12 *In vitro* drug release for F3 Formulation

Time (min)	absorbance	Concentration (µg)	Dilution Factor	Concentration (mg/ml)	%CDR	Flux
0	0	0	0	0	0	0
30	0.052	0.907	10	8.16	1.63	1.02
60	0.171	1.7	10	15.34	3.06	0.90
120	0.309	3.36	10	30.32	6.06	0.94
180	0.463	5.32	10	47.88	9.57	1.11
240	0.609	7.01	10	63.17	12.63	0.95
300	0.745	8.96	10	80.67	16.13	1.10
360	0.888	11.51	10	103.58	20.71	1.44
420	0.997	13.74	10	123.39	24.68	1.26

The *in vitro* drug-release study demonstrated a time-dependent and sustained release of efinaconazole from all three O/W microemulsion formulations over the 7 h experimental period. The cumulative drug release (%CDR) increased progressively with time, indicating controlled diffusion of the drug from the microemulsion system. The *in vitro* transungual drug-release study demonstrated a time-dependent release of efinaconazole from all three O/W microemulsion formulations over the 7 h (420 min) experimental period. The cumulative drug release (%CDR) increased progressively with time, indicating continued drug diffusion from the microemulsion system. At 420 min, F3 exhibited the highest cumulative drug release (24.68%), followed by F1 (24.21%) and F2 (23.01%). The permeation flux varied during the experimental period rather than remaining constant. F1 showed the highest initial flux of 1.95 µg/cm²/h at 30 min, which is indicative of a relatively fast initial transport of the drug. F3 then showed a terminal flux of 1.26 µg/cm²/h and a maximum instantaneous flux of 1.44 µg/cm²/h at 360 min. The cumulative release of F2 was relatively low for the duration of the experiment. The formulations exhibited progressive release of efinaconazole and measurable transungual permeation throughout the 420-min study. Formulation F3 exhibited the highest cumulative drug release at the final sampling point.

DISCUSSION

The *ex vivo* permeation studies demonstrated that the addition of Calotropis procera latex substantially enhanced transungual drug delivery in comparison to the plain efinaconazole solution and the efinaconazole-loaded microemulsion without latex[39]. The efinaconazole solution showed the lowest drug permeation, having a flux of 4.9 ± 0.6 µg/cm²/h and cumulative permeation of 18.2 ± 2.1 µg/cm². The drug incorporation into the O/W microemulsion increased the flux to 12.4 ± 1.1 µg/cm²/h and the cumulative permeation to 42.6 ± 3.5 µg/cm², which is about a 2.3-fold increase in the cumulative permeation when compared to the efinaconazole solution[40]. The microemulsion with latex demonstrated the highest permeation, with a flux of 18.6 ± 1.2 µg/cm²/h and cumulative permeation of 68.5 ± 4.2 µg/cm², which is approximately 3.8-fold higher than

that observed with the efinaconazole solution. The results of the study revealed that the transungual delivery was improved for the combined formulation in comparison with the microemulsion alone[41,42]. The increased permeation may be due to the combined effects of the microemulsion formulation, surfactant-mediated wetting, improved drug solubilization and the presence of bioactive constituents in *C. procera* latex. The current research does not demonstrate keratin breakdown or a molecular mechanism for the improved penetration. These possibilities should be considered as potential contributors instead of as established mechanisms. The enhanced permeation achieved with the latex-containing formulation was further corroborated by the localization studies using CLSM imaging, which demonstrated increased drug-associated signal within the nail/hoof matrix[43,44]. The *in vitro* results generally suggest that the incorporation of *C. procera* latex in efinaconazole O/W microemulsion can be a promising strategy for improving transungual drug delivery.

REFERENCES

- [1] Bhatt V, Pillai R. Efinaconazole Topical Solution, 10%: Formulation Development Program of a New Topical Treatment of Toenail Onychomycosis. *J Pharm Sci* 2015;104:2177–82. <https://doi.org/10.1002/jps.24459>.
- [2] Lipner SR, Scher RK. Onychomycosis: Clinical overview and diagnosis. *J Am Acad Dermatol* 2019;80:835–51. <https://doi.org/10.1016/j.jaad.2018.03.062>.
- [3] Kesharwani P, Fatima M, Singh V, Sheikh A, Almalki WH, Gajbhiye V, et al. Itraconazole and Difluorinated-Curcumin Containing Chitosan Nanoparticle Loaded Hydrogel for Amelioration of Onychomycosis. *Biomimetics* 2022;7. <https://doi.org/10.3390/BIOMIMETICS7040206>.
- [4] Vlahovic TC. Onychomycosis: Evaluation, Treatment Options, Managing Recurrence, and Patient Outcomes. *Clin Podiatr Med Surg* 2016;33:305–18. <https://doi.org/10.1016/j.cpm.2016.02.001>.
- [5] Agrawal V, Patel R, Patel M, Thanki K, Mishra S. Design and evaluation of microemulsion-based efinaconazole formulations for targeted treatment of onychomycosis through transungual route: Ex vivo and nail clipping studies. *Colloids Surf B Biointerfaces* 2021;201:111652. <https://doi.org/10.1016/J.COLSURFB.2021.111652>.
- [6] Petralia S, Forte G, Aiello M, Nocito G, Conoci S. Photothermal-triggered system for oligonucleotides delivery from cationic gold nanorods surface: A molecular dynamic investigation. *Colloids Surf B Biointerfaces* 2021;201. <https://doi.org/10.1016/j.colsurfb.2021.111654>.
- [7] Sieniawska E, Świątek Ł, Wota M, Rajtar B, Polz-Dacewicz M. Microemulsions of essential oils – Increase of solubility and antioxidant activity or cytotoxicity? *Food and Chemical Toxicology* 2019;129:115–24. <https://doi.org/10.1016/j.fct.2019.04.038>.
- [8] Callender SP, Mathews JA, Kobrnyk K, Wettig SD. Microemulsion utility in pharmaceuticals: Implications for multi-drug delivery. *Int J Pharm* 2017;526:425–42. <https://doi.org/10.1016/j.ijpharm.2017.05.005>.
- [9] Agrawal N, Savalia R, Chatterjee S. Nanostructured zinc oxide film amalgamated with functionalized carbon nanotubes for facile electrochemical determination of nifedipine. *Colloids Surf B Biointerfaces* 2021;201. <https://doi.org/10.1016/j.colsurfb.2021.111635>.
- [10] Subongkot T, Sirirak T. Development and skin penetration pathway evaluation of microemulsions for enhancing the dermal delivery of celecoxib. *Colloids Surf B Biointerfaces* 2020;193. <https://doi.org/10.1016/j.colsurfb.2020.111103>.
- [11] Vikas A, Rashmin P, Mrunali P, Sandip M, Kaushik T. RP-HPLC method for quantitative estimation of Efinaconazole in topical microemulsion and microemulsion-based-gel formulations and in presence of its degradation products. *Microchemical Journal* 2020;155. <https://doi.org/10.1016/j.microc.2020.104753>.
- [12] Shah B, Khunt D, Bhatt H, Misra M, Padh H. Application of quality by design approach for intranasal delivery of rivastigmine loaded solid lipid nanoparticles: Effect on formulation and characterization parameters. *European Journal of Pharmaceutical Sciences* 2015;78:54–66. <https://doi.org/10.1016/j.ejps.2015.07.002>.

- [13] Das S, Lee SH, Chia VD, Chow PS, Macbeath C, Liu Y, et al. Development of microemulsion based topical ivermectin formulations: Pre-formulation and formulation studies. *Colloids Surf B Biointerfaces* 2020;189. <https://doi.org/10.1016/j.colsurfb.2020.110823>.
- [14] Lipner SR, Scher RK. Onychomycosis: Clinical overview and diagnosis. *J Am Acad Dermatol* 2019;80:835–51. <https://doi.org/10.1016/j.jaad.2018.03.062>.
- [15] Bhatt V, Pillai R. Efinaconazole Topical Solution, 10%: Formulation Development Program of a New Topical Treatment of Toenail Onychomycosis. *J Pharm Sci* 2015;104:2177–82. <https://doi.org/10.1002/jps.24459>.
- [16] Almuqbil RM, Sreeharsha N, Nair AB. Formulation-by-Design of Efinaconazole Spanlastic Nanovesicles for Transungual Delivery Using Statistical Risk Management and Multivariate Analytical Techniques. *Pharmaceutics* 2022;14. <https://doi.org/10.3390/PHARMACEUTICS14071419>.
- [17] Abd-Elsalam WH, Abouelatta SM. Contemporary Techniques and Potential Transungual Drug Delivery Nanosystems for The Treatment of Onychomycosis. *AAPS PharmSciTech* 2023;24. <https://doi.org/10.1208/S12249-023-02603-X>.
- [18] Vlahovic TC. Onychomycosis: Evaluation, Treatment Options, Managing Recurrence, and Patient Outcomes. *Clin Podiatr Med Surg* 2016;33:305–18. <https://doi.org/10.1016/j.cpm.2016.02.001>.
- [19] Kesharwani P, Fatima M, Singh V, Sheikh A, Almalki WH, Gajbhiye V, et al. Itraconazole and Difluorinated-Curcumin Containing Chitosan Nanoparticle Loaded Hydrogel for Amelioration of Onychomycosis. *Biomimetics* 2022;7. <https://doi.org/10.3390/BIOMIMETICS7040206>.
- [20] Agrawal V, Patel R, Patel M, Thanki K, Mishra S. Design and evaluation of microemulsion-based efinaconazole formulations for targeted treatment of onychomycosis through transungual route: Ex vivo and nail clipping studies. *Colloids Surf B Biointerfaces* 2021;201:111652. <https://doi.org/10.1016/J.COLSURFB.2021.111652>.
- [21] Daradmare S, Lee CS. Recent progress in the synthesis of all-aqueous two-phase droplets using microfluidic approaches. *Colloids Surf B Biointerfaces* 2022;219. <https://doi.org/10.1016/j.colsurfb.2022.112795>.
- [22] Maertens JA. History of the development of azole derivatives. *Clinical Microbiology and Infection* 2004;10:1–10. <https://doi.org/10.1111/J.1470-9465.2004.00841.X>.
- [23] Borgers M, Van de Ven MA. Degenerative changes in fungi after itraconazole treatment. *Rev Infect Dis* 1987;9 Suppl 1. https://doi.org/10.1093/CLINIDS/9.SUPPLEMENT_1.S33.
- [24] Tatsumi Y, Yokoo M, Senda H, Kakehi K. Therapeutic efficacy of topically applied KP-103 against experimental tinea unguium in guinea pigs in comparison with amorolfine and terbinafine. *Antimicrob Agents Chemother* 2002;46:3797–801. <https://doi.org/10.1128/AAC.46.12.3797-3801.2002>.
- [25] Beggs WH. Comparison of miconazole- and ketoconazole-induced release of K⁺ from candida species. *Journal of Antimicrobial Chemotherapy* 1983;11:381–3. <https://doi.org/10.1093/JAC/11.4.381>.
- [26] Nishiyama Y, Hasumi Y, Ueda K, Uchida K, Yamaguchi H. Effects of micafungin on the morphology of *Aspergillus fumigatus*. *J Electron Microsc (Tokyo)* 2005;54:67–77. <https://doi.org/10.1093/JMICRO/DFH100>.
- [27] Tatsumi Y, Yokoo M, Arika T, Yamaguchi H. In vitro antifungal activity of KP-103, a novel triazole derivative, and its therapeutic efficacy against experimental plantar tinea pedis and cutaneous candidiasis in guinea pigs. *Antimicrob Agents Chemother* 2001;45:1493–9. <https://doi.org/10.1128/AAC.45.5.1493-1499.2001>.
- [28] Tatsumi Y, Nagashima M, Shibunushi T, Iwata A, Kangawa Y, Inui F, et al. Mechanism of Action of Efinaconazole, a Novel Triazole Antifungal Agent. *Antimicrob Agents Chemother* 2013;57:2405. <https://doi.org/10.1128/AAC.02063-12>.
- [29] Iwatani W, Arika T, Yamaguchi H. Two mechanisms of butenafine action in *Candida albicans*. *Antimicrob Agents Chemother* 1993;37:785–8. <https://doi.org/10.1128/AAC.37.4.785>.

- [30] Sheehan DJ, Hitchcock CA, Sibley CM. Current and emerging azole antifungal agents. *Clin Microbiol Rev* 1999;12:40–79. <https://doi.org/10.1128/CMR.12.1.40>.
- [31] Jo Siu WJ, Tatsumi Y, Senda H, Pillai R, Nakamura T, Sone D, et al. Comparison of in vitro antifungal activities of efinaconazole and currently available antifungal agents against a variety of pathogenic fungi associated with onychomycosis. *Antimicrob Agents Chemother* 2013;57:1610–6. <https://doi.org/10.1128/AAC.02056-12>.
- [32] Elewski BE, Rich P, Pollak R, Pariser DM, Watanabe S, Senda H, et al. Efinaconazole 10% solution in the treatment of toenail onychomycosis: Two phase III multicenter, randomized, double-blind studies. *J Am Acad Dermatol* 2013;68:600–8. <https://doi.org/10.1016/J.JAAD.2012.10.013>.
- [33] Bossche H Vanden, Ausma J, Bohets H, Vermuyten K, Willemsens G, Marichal P, et al. The novel azole R126638 is a selective inhibitor of ergosterol synthesis in *Candida albicans*, *Trichophyton* spp., and *Microsporum canis*. *Antimicrob Agents Chemother* 2004;48:3272–8. <https://doi.org/10.1128/AAC.48.9.3272-3278.2004>.
- [34] Liu W, Zhang X, Liu Z, Luo X. Impact of pH on the antifungal susceptibility of vaginal *Candida albicans*. *International Journal of Gynecology and Obstetrics* 2011;114:278–80. <https://doi.org/10.1016/J.IJGO.2011.03.016>.
- [35] Ryder NS, Seidl G, Troke PF. Effect of the antimycotic drug naftifine on growth of and sterol biosynthesis in *Candida albicans*. *Antimicrob Agents Chemother* 1984;25:483–7. <https://doi.org/10.1128/AAC.25.4.483>.
- [36] Bhatt V, Pillai R. Efinaconazole Topical Solution, 10%: Formulation Development Program of a New Topical Treatment of Toenail Onychomycosis. *J Pharm Sci* 2015;104:2177–82. <https://doi.org/10.1002/JPS.24459>.
- [37] Elewski BE, Ghannoum MA, Mayser P, Gupta AK, Korting HC, Shouey RJ, et al. Efficacy, safety and tolerability of topical terbinafine nail solution in patients with mild-to-moderate toenail onychomycosis: Results from three randomized studies using double-blind vehicle-controlled and open-label active-controlled designs. *Journal of the European Academy of Dermatology and Venereology* 2013;27:287–94. <https://doi.org/10.1111/J.1468-3083.2011.04373.X>.
- [38] Elewski BE, Rich P, Pollak R, Pariser DM, Watanabe S, Senda H, et al. Efinaconazole 10% solution in the treatment of toenail onychomycosis: Two phase III multicenter, randomized, double-blind studies. *J Am Acad Dermatol* 2013;68:600–8. <https://doi.org/10.1016/J.JAAD.2012.10.013>.
- [39] Sieniawska E, Świątek Ł, Wota M, Rajtar B, Polz-Dacewicz M. Microemulsions of essential oils – Increase of solubility and antioxidant activity or cytotoxicity? *Food and Chemical Toxicology* 2019;129:115–24. <https://doi.org/10.1016/j.fct.2019.04.038>.
- [40] Agrawal V, Patel R, Patel M, Thanki K, Mishra S. Design and evaluation of microemulsion-based efinaconazole formulations for targeted treatment of onychomycosis through transungual route: Ex vivo and nail clipping studies. *Colloids Surf B Biointerfaces* 2021;201. <https://doi.org/10.1016/J.COLSURFB.2021.111652>.
- [41] Welsh O, Vera-Cabrera L, Welsh E. Onychomycosis. *Clin Dermatol* 2010;28:151–9. <https://doi.org/10.1016/J.CLINDERMATOL.2009.12.006>.
- [42] SUNDARI IBT, KALVA S, BINDU M. DEVELOPMENT OF EFINACONAZOLE NAIL GEL FOR THE TREATMENT OF ONCHOMYCOSIS. *Int J Curr Pharm Res* 2023:109–12. <https://doi.org/10.22159/IJCPR.2023V15I6.3092>.
- [43] Lipner SR, Scher RK. Efinaconazole in the treatment of onychomycosis. *Infect Drug Resist* 2015;8:163–72. <https://doi.org/10.2147/IDR.S69596>.
- [44] Pollak RA, Siu WJJ, Tatsumi Y, Pillai R. Efinaconazole topical solution, 10%: Factors contributing to onychomycosis success. *Journal of Fungi* 2015;1:107–14. <https://doi.org/10.3390/JOF1020107>.