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Research Article

# Formulation, Development and Evaluation of Topical Emulgel of Luliconazole Using Essential Oils

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## I N F O

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## A B S T R A C T

*Introduction:* Luliconazole, an antifungal agent, is characterized by low solubility and high permeability. The study was conducted to develop emulgel formulations of luliconazole using essential oils as penetration enhancers and to compare their properties with those of plain luliconazole emulgel.

*Methods:* Emulgels were prepared by incorporating luliconazole emulsions into a Carbopol 940 gel base. Penetration enhancers such as clove oil and tea tree oil were used at various concentrations. The formulations were evaluated for their physical properties, in vitro drug release, ex vivo permeation, antifungal activity, and stability.

*Results:* The evaluation revealed that the luliconazole emulgel containing clove oil demonstrated superior in vitro drug release, ex vivo permeation, antifungal activity, and skin permeability compared to the plain luliconazole emulgel and the formulation containing tea tree oil.

*Conclusion:* The combination of luliconazole with clove oil in the emulgel formulation enhances skin permeability and potentiates antifungal activity, making it a promising approach for improving luliconazole's therapeutic efficacy.

**Keywords:** Luliconazole, Emulgel, Tea Tree Oil, Clove Oil, Essential Oil

## Introduction

Gels are currently receiving increasing attention, especially hydrogel formulations, for topical application of drugs since they have an attractive appearance and develop a pleasant cool feeling. They are easy to apply and remove and generally provide faster drug release compared with ointment and cream.[1]Emulgel is a mixture of emulsion and gel technology. The emulsion and gel preparation each have their characteristics, but the gel has some drawbacks in terms of hydrophobic drug delivery. The emulsion may be formulated as an emulgel using a gelling agent.[2]

Luliconazole is a broad-spectrum antifungal agent which acts by inhibiting the fungal enzyme alpha lanosterol demethylase. It has low solubility and more permeability.[3]Enulgel was prepared using Carbopol 934, clove oil, and tea tree oil to improve the solubility and permeability of luliconazole for topical application.[4]

Luliconazole is a synthetic antifungal agent of the imidazole class; it works by slowing the growth of fungi that cause infection. It is used to treat fungal infections. Triazole drug targets the fungal-specific synthesis of membrane lipids. Luliconazole inserts preferentially into fungal membranes and disrupts their function. 5-fluorocytosine targets fungal-specific DNA replication.[5]

Incorporation of hydrophobic drugs, better loading capacity, better stability, controlled release, no intensive sonication, avoiding first pass metabolism, avoiding gastrointestinal incompatibility, more selective for a specific site, improved patient compliance, convenient and easy to apply.

### Emulgel Advantages

- **Increased bioavailability:** Clove oil enhances luliconazole's skin permeation and bioavailability.
- **Prolonged antifungal activity:** Sustained release of luliconazole and clove oil's antimicrobial effects
- **Reduced systemic absorption:** Minimises potential side effects and improves safety profile
- Clove oil's antimicrobial properties overcome fungal resistance.

### Safety Advantages

- **Natural and safe ingredients:** Clove oil and luliconazole are well-tolerated and minimally toxic.
- **Low risk of side effects:** Emulgel formulation reduces systemic absorption and potential side effects.
- **Hypoallergenic:** Reduced risk of allergic reactions and skin irritation

## Application of the Formulation

- **Tinea pedis (athlete's foot):** Effective in treating fungal infections of the foot, including interdigital spaces
- **Tinea cruris (jock itch):** Treats fungal infections of the groin area, inner thighs, and buttocks
- **Tinea corporis (ringworm):** Effective against fungal infections of the body, including arms, legs, and trunk
- **Tinea versicolor:** Treats fungal infections causing skin discolouration and patches
- Cutaneous candidiasis: Effective against fungal infections caused by Candida species
- **Dermatophytosis (fungal skin infections):** Treats various fungal skin infections, including those caused by *Trichophyton, Microsporum,* and *Epidermophyton species.*[4,5]

## Materials and Methods

### Materials

Luliconazole was obtained from Glenmark Pharmaceutical Ltd., Nashik, India. Tea tree oil and clove oils were obtained from Baeyork Nashik, India.All other compounds were analytical grade and were utilised without further chemical modification.

### Preparation of Luliconazole Emulgel

Carbopol 934 was dissolved in water. The oil phase was prepared with span 20 and light liquid paraffin. Luliconazole drug was dissolved separately in ethanol andintroduced in the oil phase. Tween 20 was dissolved in water to make the aqueous phase. Methyl paraben and propyl paraben were dissolved separately in propylene glycol and added to the aqueous phase.The aqueous and oil phases were heated to 70-80 °C separately. The phase was poured into the aqueous phase with constant stirring to form an emulsion. Emulgel was prepared adding emulsion and gel in equal ratios and stirring. Tea tree oil and clove oil were incorporated into the emulgel at the polymer dispersion step. Seven formulations were prepared as shown in Table 1.

**Table 1.Formulations of Luiconazole Emulgel with Essential Oils**

| Ingredients | Quantity (g/100 mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
| Luliconazole | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Carbopol 934 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Tween 20 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Span 20 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Light liquid paraffin | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| Ethanol | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Propylene glycol | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Methyl paraben | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Propyl paraben | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Clove oil | --- | 1.0 | 2.0 | 3.0 | --- | --- | --- |
| Tea tree oil | --- | --- | --- | --- | 1.0 | 2.0 | 3.0 |
| Purified water q.s. | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

## $2^3$ Factorial Design

A $2^3$ full factorial design containing 3 factors was evaluated at two levels and the experimental trials were performed at all possible combinations.

Independent Variables

The three evaluated independent formulation variables included:

1. **Control (X1):** F1
2. **Essential oil type (X2):** Tea Tree Oil (TTO) and Clove Oil (CO)
3. **Essential oil concentration (X3):** 1%, 2%, and 3%

## Dependent Variables

The dependent variables included pH (Y1), spreadability (Y2), emulsion stability (Y3), drug release (Y4), and anti-fungal activity (Y5).

## Experimental Domain

The Table 2 describes Experimental domain, the Actual and Coded values in Control(X1), Essential oil type (X2) and Essential oil concentration (X3)

**Table 2.Experimental domain**

| Model Factor | Actual Values | | Coded Values | |
|---|---|---|---|---|
| | Low Level | High Level | Low Level | High Level |
| X1 | 1 | 1 | - | + |
| X2 | 1 | 3 | - | + |
| X3 | 1 | 3 | - | + |

Figure 1 describes the Comparative Analysis of Drug Content,Extrudability and *In Vitro* Diffusion of all the batches (F1,F2,F3,F4,F5,F6 and F7). Based on the optimisation study, all the batches demonstrated optimal results.Among those three formulations - F1 (control), F4 (containing 3 mL of clove oil), and F7 (containing 3 mL of tea tree oil) - were selected for further investigation due to their distinct composition, specifically the presence and absence of the volatile oils (clove oil and tea tree oil).

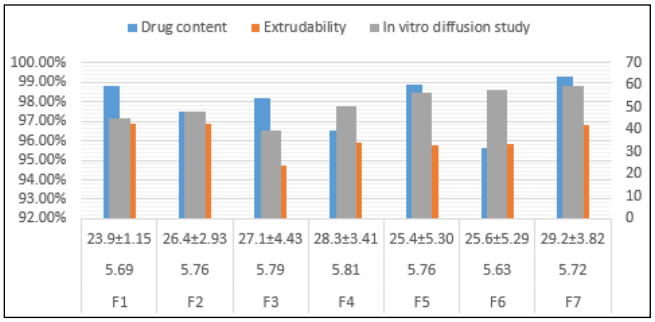


**Figure 1.Comparative Analysis of Drug Content, Extrudability, and *In Vitro* Diffusion**

## Evaluation of Luliconazole Emulgel with Essential Oils

### Physical Appearance

The prepared luliconazole emulgel with essential oils was inspected for their colour, odour, homogeneity, consistency, phase separation, texture and grittiness visually.[4,6]

### pH

The pH of 1% aqueous solutions of the produced emulgels was measured using a pH meter.

### Spreading Coefficient

The spreading coefficient was calculated using Mutimer's instrument. The spreading coefficient was calculated using the emulgel's 'Slip' and 'Drag' features. A ground glass slide was kept on the wooden block, about 2 g emulgel was placed on this slide, second glass slide with a hook was placed on it. Air was evacuated and time taken (in seconds) by the top slide to travel a 5 cm distance was recorded. The higher the coefficient of spreading, the shorter the interval. A formula is used to determine spreadability[7]-

Spreadability = $\frac{Weight \times Distance}{Time}$

### Rheological Studies

The viscosity of luliconazole emulgel formulations at 25°C was measured by Brookfield Viscometer with Spindle no. 18.[6]

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## Drug Content

The drug content of luliconazole in emulgel was determined by dissolving a known amount of the emulgel formulation (1.0 g) in 100 mL ethanol, diluting appropriately, using a UV spectrophotometer at 297 nm.[56]

## Extrudability

Extrudability measures the force required to extrude material from a tube. The weight in grams required to extrude at least a 0.5 cm ribbon of emulgel in 10 seconds was used to estimate extrudability in this investigation. Extrudability improves as the amount of material extruded increases >90% extrudability- excellent, >80% extrudability good, >70% extrudability fair.[8]

## *In vitro* Diffusion Study

A modified diffusion cell with an egg membrane with a capacity of 20 mL was used to evaluate the *in vitro* diffusion of different batches of emulgel.[9]Phosphate buffer (pH 7.4) was utilised as a diffusion medium. The egg membrane was immersed in the diffusion medium for 12 hours. Throughout the investigation, the temperature was kept at 37°C ± 0.5°C. An emulgel containing 2 mg of luliconazole was placed in the donor chamber, and aliquots were removed from the receptor compartment every 4 hours until the same volume was replaced with new diffusion media. The samples were filtered via Whatman filter paper, and drug content was measured using a UV spectrophotometer at 297 nm after appropriate dilution. At each time point, the total amount of drug released was computed, and a graph of % drug release vs time was plotted.[10]

## *Ex vivo* Diffusion Study

*Ex vivo* diffusion of the emulgel was studied using a modified diffusion cell and the same approach as *in vitro* diffusion tests, with the exception that mice skin was utilized as the diffusion membrane. Freshly excised mice skin was soaked in phosphate buffer pH 7.4 for 1 hour and then mounted on cells.[11]

## Antifungal Test

The disc diffusion method was used to test the antifungal activity of luliconazole emulgel formulations plain and with essential oils and commercialised gel against *Candida albicans* (ATCC 10231) strains. The strains were grown on nutrient agar medium and utilised to test the antifungal activity of formulations. The volume of 10 µL (1000 µg/ disc) of each batch was soaked in paper disc. The disc was dried and kept on the agar media. Nystatin was utilised as a standard for comparison. The plates were incubated at 37°C for 24 hours and observed for the zone of inhibition.[12]

## Stability Studies

The produced luliconazole emulgel with essential oils was packaged in aluminium tubes (5 g) and evaluated for stability testing at 25°C /60 % relative humidity (RH) and 40°C /75 % RH. Physical appearance, pH, rheological characteristics, drug content, and drug release were all assessed in samples taken at 15-day intervals for three months.[13]

# Results and Discussion

## Physical Appearance

Table 3 shows the physical appearance of all batches, including colour, odour, homogenicity, consistency, phase separation, texture, and grittiness. All emulgel trials were found to be white, with a characteristic odour and acceptable homogenicity, consistency, and texture. There was no grit and no phase separation in any of the formulations.

**Table 3.Physical Appearance of Different Emulgel Formulations**

| S. No. | Parameter | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|---|---|---|---|---|---|---|---|---|
| 1 | Colour | White | White | White | White | White | White | White |
| 2 | Odour | Chara-cteristic | Chara-cteristic | Chara-cteristic | Chara-cteristic | Chara-cteristic | Chara-cteristic | Chara-cteristic |
| 3 | Homogeneity | Good | Good | Good | Good | Good | Good | Good |
| 4 | Consistency | Good | Good | Good | Good | Good | Good | Good |
| 5 | Phase separation | None | None | None | None | None | None | None |
| 6 | Texture | Good | Good | Good | Good | Good | Good | Good |
| 7 | Grittiness | Non-gritty | Non-gritty | Non-gritty | Non-gritty | Non-gritty | Non-gritty | Non-gritty |

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## pH

The pH of the emulgel batches was found to be in the range of 5.65.8, which is equivalent to the pH of the skin (Table 4).

## Spreading Coefficient

Table 5 represents the spreading coefficients of emulgel formulations. It was determined that all the created formulations had adequate spreadability, with F7 and F4 formulations having more spreadability than the others i.e. 29.2±3.82 and 28.3±3.41 respectively.

## Rheological Study

Table 6 represents the viscosity of formulations. Formulations F2 and F5 had the highest viscosity. It could be due to low concentrations of essential oils. For all formulations, the viscosity range was 12,500-14000 Cps.

## Drug Content

Table 7 represents the drug content of emulgel formulations. The drug release of formulations was found to be ranging between 95.6% and 99.3%.

## Extrudability

Table 8 represents the emulgel's ability to extrude from the tube,which is crucial during application and patient acceptance. All of the formulations had outstanding and satisfactory extrudability results.

## *In Vitro* Diffusion Study

Drug release from emulsified gel formulations was found to be in the following order: F7 > F6 > F4 > F5 > F2> F1> F3. Figure 2 represents the *in vitro* drug release of luliconazole emulgel plain and with essential oils. The drug release from the emulgel formulation (Batch-F1) without essential oils was slower, reaching up to 44.77% after 4 hours. Those containing 1%, 3%, and 5% tea tree oil released 47.99%, 39.66%, and 50.71% of the drug, respectively; compared to batches containing no essential oil. Clove oil emulgel studies have shown greater drug release in all concentrations examined than the tea tree oil emulgel trials. With 3% clove oil, the maximum drug release was 59.6% at the end of 4 hours. Further ex vivo investigations were conducted after observing a significant increase in drug release from an emulgel containing 3% tea tree oil and 3% clove oil.

## *Ex vivo* Diffusion Study

*Ex vivo* drug diffusion is represented in Figure 3. It was found that it followed the same pattern as the *in vitro* drug release study, with 13.39%, 11.52%, and 8.95% drug diffused in 4 hours from emulgel batches with 3% clove oil, tea tree oil, and no essential oil, respectively. The significant difference in efficiency between batches containing or not containing essential oils emphasise the need for a penetration enhancer in the luliconazole emulgel. In addition, emulgel formulation aids in enhancing solubility and extending medication release, which is especially significant in the case of fungal infections.

## Antifungal Study

The plain luliconazole emulgel produced a zone of inhibition of around 29 mm, whereas the emulgel prepared with 3% clove oil (F7) created a zone of inhibition of around 33 mm, and the emulgel formulated with 3% tea tree oil produced a zone of inhibition of around 31 mm (Table 9). Figure 4 represents the zone of inhibition.When compared with the marketed formulation, the emulgel made with 3% clove oil showed a greater zone of inhibition (Figure 5). This could be due to the inclusion of clove oil in the emulgels which may have promoted penetration through diffusion membranes and through the fungal cell wall, resulting in effective antifungal action.[14]

**Table 4.pH of emulgel formulation**

| F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|---|---|---|---|---|---|---|
| 5.69 | 5.76 | 5.79 | 5.81 | 5.76 | 5.63 | 5.72 |

**Table 5.Spreading Coefficient of the Formulation F1–F7 (Mean ± SD)**

| F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|---|---|---|---|---|---|---|
| 23.9 ± 1.15 | 26.4 ± 2.93 | 27.1 ± 4.43 | 28.3 ± 3.41 | 25.4 ± 5.30 | 25.6 ± 5.29 | 29.2 ± 3.82 |

**Table 6.Rheological Study of Emulgel Formulation**

| F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|---|---|---|---|---|---|---|
| 13,400 Cps | 13,700 Cps | 13,100 Cps | 12,700 Cps | 13,800 Cps | 13,300 Cps | 12,900 Cps |

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**Table 7.Drug Content of Emulgel Formulation**

| F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|---|---|---|---|---|---|---|
| 98.8% | 97.5% | 98.2% | 96.5% | 98.86% | 95.6% | 99.3% |

**Table 8.Extrudability of Emulgel Batches**

| F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|---|---|---|---|---|---|---|
| 96.89% | 96.84% | 94.7% | 95.87% | 95.78% | 95.83% | 96.80% |

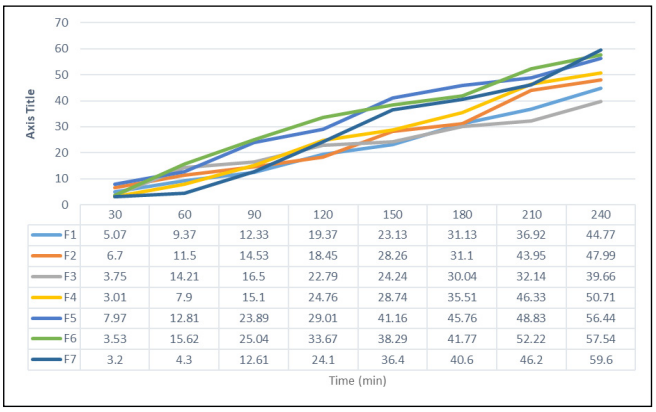


**Figure 2.*In Vitro* Drug Release Profiles of Luliconazole Emulgel Batches**

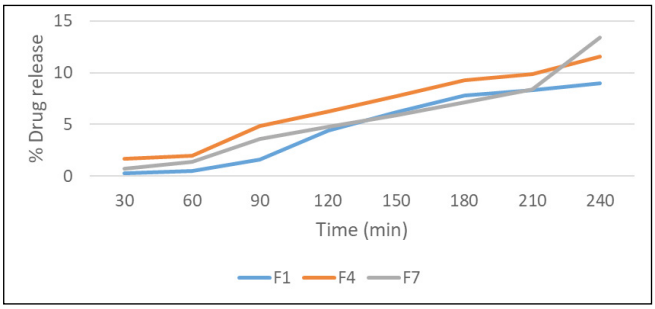


**Figure 3.Ex Vivo Diffusion Profiles of Luliconazole Emulgel Batches**

**Table 9.Antifungal Study of Luliconazole Emulgel with Essential Oils**

| Batches | Zone of Inhibition (mm) |
|---|---|
| F1 | 29 |
| F4 | 31 |
| F7 | 33 |
| Marketed formulation | 31 |
| Standard - nystatin | 22 |

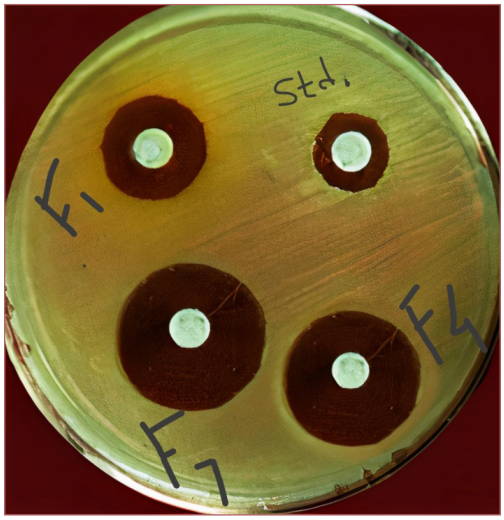


**Figure 4.Antifungal Activity of Optimised Batches**

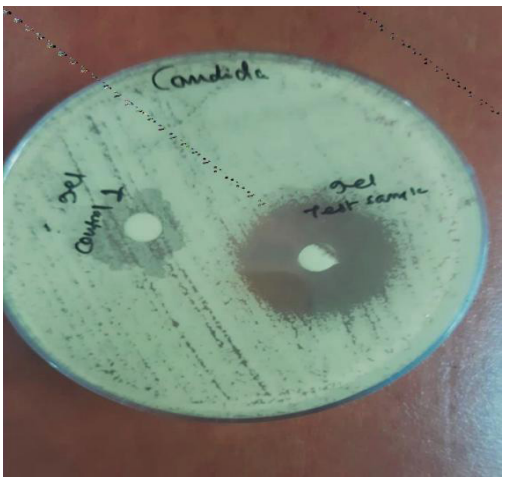


**Figure 5.Marketed Formulation Antifungal Activity**

## Conclusion

Emulgel formulations of Luliconazole were developed with clove oil and tea tree oil. An emulgel containing 3% clove oil(F4) and 3% tea tree oil(F7) was found to possess improved *in-vitro* drug release, *ex-vivo* diffusion and *in-vitro* antifungal activity. *In vivo* studies in relevant animal models may further highlight a detailed investigation of the efficacy and applicability of this drug delivery mechanism.

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Essential oils are identified as potent permeation enhancers, paving the way for future formulations. Novel luliconazole emulgel formulations with naturally occurring clove oil and tea tree oil have properties that make it a penetration enhancer demonstrating enhanced solubility, permeability, and antifungal efficacy. This synergistic combination overcomes the limitations of existing topical treatments, offering improved therapy for fungal infections.

The developed emulgel formulation of luliconazole, clove oil, and tea tree oil presented a synergistic combination, yielding high drug loading efficiency (>95%) and enhanced *in vitro* release (>40%). Notably, the pH range (5.635.81) was optimized for cutaneous delivery.

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**Conflict of Interest:** None

**Source of Funding:** None

**Authors' Contribution:** SK was responsible for the conception, constructing the hypothesis for the research and manuscript. SK and SW collaborated on the design, planning methods to generate the hypothesis and reach the conclusion. RP and MT supervised the project, organizing and overseeing its course. SW led data collection, processing, and reporting. SK, SP, and SW handled the analysis and interpretation of results. SW also conducted the literature search. The manuscript was written by SW, DP, and AB, while SP and MT performed the critical review.

**Declaration of Generative AI and AI-Assisted Technologies in the Writing Process:** Generative AI, including Copilot, was employed in drafting, editing, and refining the manuscript. All content has been reviewed and validated by the authors to ensure accuracy and integrity. AI tools were used to support, not replace, the intellectual contributions of the authors.

## References

1. Sreevidya V. S. An overview on emulgel Int Pharm Phytopharmacol Res. 2019;9(1):92-7
2. Sharadha M, Gowda DV, Vishal Gupta N, Akhila A-R. An overview on topical drug delivery system-updated review, Int J Res Pharm Sci 2020;11(1):368-85.
3. Kumar M,Shanthi N, Mahato AK. Qualitative and quantitative methods for determination of drug luliconazole. Int J Res Advent Technol. 2018;6(10):2764-70. [Google Scholar]
4. Yadav S, Waikar S, Invally M, Ranade S. Topical emulgel of tolnaftate with penetration enhancer: development, characterisation and antifungal activity. Indian J Med Res Pharm Sci. 2017;4(10):28-35. [Google Scholar]
5. Shekar B, Ramesh K, Vijaykumar G. Formulation and evaluation of luliconazole emulgel. Int J Pharm Res Appl. 2024;8:2675.
6. Dhobale S, Shelke G, Jadhav S, Gaikwad D.Formulation and evaluation of luliconazole emulgel for topical drug delivery. Int Res J Sci Eng. 2018;A3:85-90. [Google Scholar]
7. Naga S D,Malyadri, Saibabu Formulation development and evaluation of luliconazole topical emulgel.Int J Indig Herbs Drugs. 2021;6(3):79-87. [Google Scholar]
8. Avinash S, Gowda DV, Suresh J, Aravind Ram A. S, Srivastava A, Osmani, RA. Formulation and evaluation of topical gel using *Eupatorium glandulosum* michx. for wound healing activity.Pharma Lett. 2016;8(8):255-66. [Google Scholar]
9. Salunke P, Shinde C, Chavan S, Mohite N. Design and characterisation of emulgel of an antifungal drug. J Pharm Sci Res. 2019;11(6):2357-61. [Google Scholar]
10. Kaur LP, Garg R, Gupta GD. Development and evaluation of topical gel of minoxidil from different polymer bases in application of alopecia, Int J Pharm Pharm Sci. 2010;2(3):437.
11. Rasool BK, Abu-Gharbieh E, Fahmy S, Saad H, Khan S. Development and evaluation of ibuprofen transdermal gel formulations. Trop J Pharm Res. 2010;9(4):355-63. [Google Scholar]
12. Manna AK, Nanda U, Kar S. Preparation and evaluation of antimicrobial herbal formulation of *Pterospermum acerifolium* Willd, Int J Pharm Sci Res. 2017;8(12):5308-14.
13. ICH Harmonized Tripartite Guidelines,Stability testing of new drug substances and products. ICH Committee; 200, https://database.ich.org/sites/default/files/Q1A-%28R2%29%20Guideline.pdf 22/02/2024
14. Rajan R, Vasudevan DT. Effect of permeation enhancers on the penetration mechanism of transfersomal gel of ketoconazole J Adv Pharm Technol Res. 2012;3(2):112. [PubMed] [Google Scholar]

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